



## Comparison of the chemical composition and bioactive properties of extracts prepared from the mature Turkish and Brazilian banana peels

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### ABSTRACT

The banana (*Musa sp.*) plant grows in tropical and subtropical regions. The fruit of the banana is eaten and the peel of the banana is discarded. In this study, chemical analyzes (HPLC-TOF/MS ve GC-MS) and biochemical activities (antioxidant and antimicrobial) of ethyl acetate (Ea) and methanol/chloroform (Me/Ch) extracts prepared from banana peel (Bbp) grown in Brazil and banana peel (Tbp) grown in Turkey were investigated. Cetene, 4-hydroxybenzoic, palmitic, linoleic, stearic, gentisic and syringic acid are the most abundant compounds in the extracts. The total antioxidant activity of Tbp-Ea ( $A_{0.5}$ : 36.84  $\mu\text{g/mL}$ ), inhibition of lipid peroxidation of Bbp-Ea ( $IC_{50}$ : 3.22  $\mu\text{g/mL}$ ), reducing power of Tbp-Me/Ch ( $A_{0.5}$ : 4.96  $\mu\text{g/mL}$ ), DPPH<sup>\*</sup> scavenging activity of Bbp-Ea ( $IC_{50}$ : 8.54  $\mu\text{g/mL}$ ), metal chelating activity of Tbp-Ea ( $IC_{50}$ : 16.54  $\mu\text{g/mL}$ ) and  $H_2O_2$  scavenging activity of Tbp-Me/Ch ( $IC_{50}$ : 0.36  $\mu\text{g/mL}$ ) were found the most effective. It was exhibited that extracts were effective against gram-negative and gram-positive bacteria. In conclusion, the phytochemical and biochemical results of the Tbp and Bbp extracts will contribute further pharmacological, biochemical and pharmacological investigations.

**Key Words:** Banana (*Musa sp.*) peel extract, polyphenolics, fatty acids, volatile compounds, bioactivity.

### 1. INTRODUCTION

Polyphenolics are the most common secondary metabolites in plants and contain at least one aromatic ring. Phenolics form different chemical structures with the hydroxyl group of carbohydrates, lipids, organic acids, amines and cell wall components. Due to their natural antimicrobial and antioxidant bioavailability,

Olgun Türk ve Brezilya muz kabuklarından hazırlanan ekstraktların kimyasal bileşimi ve biyoaktivite özelliklerinin karşılaştırılması

### ÖZ

Muz (*Musa sp.*) bitkisi tropikal ve subtropikal bölgelerde yetişir. Muzun meyvesi yenir ve kabuğu atılır. Bu çalışmada Brezilya' da yetişen muz kabuğu (Bbp) ve Türkiye' de yetişen muz kabuğundan (Tbp) hazırlanan etil asetat (Ea) ve metanol/kloroform (Me/Ch) ekstraktlarının kimyasal analizleri (HPLC-TOF/MS ve GC-MS) ve biyokimyasal aktiviteleri (antioksidan ve antimikrobiyal) araştırıldı. Seten, 4-hidroksibenzoik, palmitik, linoleik, stearik, gentisik ve siringik asit ekstraktlarda en bol bulunan bileşiklerdir. Tbp-Ea'nın toplam antioksidan aktivitesi ( $A_{0.5}$ :36.84  $\mu\text{g/mL}$ ), Bbp-Ea'nın lipid peroksidasyonunun inhibisyonu ( $IC_{50}$ : 3.22  $\mu\text{g/mL}$ ), Tbp-Me/Ch'nin gücünü azaltma ( $A_{0.5}$ :4.96  $\mu\text{g/mL}$ ), Bbp-Ea'nın DPPH<sup>\*</sup> temizleme aktivitesi ( $IC_{50}$ : 8.54  $\mu\text{g/mL}$ ), Tbp-Ea'nın metal şelatlama aktivitesi ( $IC_{50}$ : 16.54  $\mu\text{g/mL}$ ) ve Tbp-Me/Ch'nin  $H_2O_2$  temizleme aktivitesi ( $IC_{50}$ : 0.36  $\mu\text{g/mL}$ ) en etkili bulundu. Ekstraktların gram negatif ve gram pozitif bakterilere karşı etkililiğini gösterdi. Sonuç olarak, Tbp ve Bbp ekstraktlarının fitokimyasal ve biyokimyasal sonuçları daha ileri farmakolojik, biyokimyasal ve farmakolojik araştırmalara katkı sağlayacaktır.

**Anahtar Kelimeler:** Muz (*Musa sp.*) kabuğu ekstresi, polifenolikler, yağ asitleri, uçucu bileşikler, biyoaktivite.

phenolic compounds in polymeric formulations present a wide range of applications in the fields of food, health and biotechnology. Consumption of products with high polyphenolics sources can slow, prevent or completely reverse the development of neurological cancer and cardiovascular diseases. Research of high polyphenolic content plants with the reuse of industrial fruit and vegetable wastes has increased the interest of researchers

in the processing of vegetables and fruits.<sup>1</sup> Research on the reuse of industrial fruit and vegetable debris containing high polyphenols has increased the interest of researchers in the recovery of by-products obtained during the processing stages of fruits and vegetables.<sup>2</sup> Banana (*Musa sp.*) is one of the most consumed fresh and processed tropical fruits in the world due to its high vitamin, mineral, nutritional value and also low cost.<sup>3</sup> Bananas are freshly consumed or processed to dry fruit ice-cream, jam and nectar to prepare functional foods, while peels constituting approximately 38% of fruit-weight are considered waste.<sup>4,5</sup> It was recorded that the peels are a waste product containing high dietary fibre and polyphenolic, and also have a strong antioxidant feature.<sup>6-8</sup> Recently, scientists have been in a lot of work to improve the nutritional value of food products and to reveal the value of food waste products.<sup>9,10</sup> Banana peels are large quantities annually, evidenced by household, market, restaurant and food processing waste. The disposal of banana peel is a major concern, but in recent research, it has been suggested that banana peel is a source of important bioactive compounds.<sup>11</sup> 1 ton dry banana peel is presented as a material balance for based on the valorization biorefinery and the results exhibit that protein (432 kg), citric acid (170 kg), ethanol (325 m<sup>3</sup>) and methane (220 m<sup>3</sup>) can be produced.<sup>12</sup> Banana peel has been used in different industrial field trials of organic fertilizer and refining agent. Gusmavartati et al. (2015) compared the banana peel and other organic natural product wastes in terms of carbon and nitrogen contents and reached very high results (C, 37.99% and %N, 1.37%). In addition, in this study, they obtained a very high yield of organic fertilizer by mixing with banana shells, house market and restaurant wastes at certain ratios.<sup>13</sup> Bp was determined to be a good absorbent for Cr(VI) ions in industrial wastewater.<sup>14</sup>

The climatic and geographical conditions of Turkey are suitable for growing bananas in the microclimate areas of the Mediterranean and Aegean regions as an agricultural product, and greenhouse cultivation is carried out. Imported bananas are preferred due to the size, colour difference and short shelf life of the banana grown in Turkey. However, Turkish people are highly preferred and consumed in the domestic market. In the literature, there are researches on the use of banana peel in different application areas. Although there are limited studies in the literature about Turkish banana, there is no research on the peel of Turkish banana. Thus, by comparing the phytochemical and bioactivities of the domestic and imported banana peel extracts, the content and activity of the domestic banana peels were revealed. In this work chemical contents of the ethyl acetate (Ea) and MeOH/CHCl<sub>3</sub> (Me/Ch) extracts of mature Turkish banana peel (Tbp) and mature Brazilian banana peel (Bbp) were analyzed with HPLC-TOF/MS and GC-MS, and their bioactivities were performed with antioxidant and antimicrobial tests.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

The ethyl acetate, methanol, chloroform, hexane are HPLC grade solvents and were purchased from Merck. Ammonium molybdate, sodium phosphate, K<sub>3</sub>Fe(CN)<sub>6</sub>, trichloroacetic acid, K<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>, NADH (nicotinamide adenine dinucleotide), NBT (nitroblue tetrazolium), PMS (ferrozine meta sulphate), FeCl<sub>3</sub> and DPPH· (2,2-diphenyl-1-picrylhydrazyl) were provided from Sigma. The other chemicals were purchased from local commercial sellers.

### 2.2. Sample

The mature Turkish (Tb) and Brazilian bananas (Bb) were purchased from the market. They were peeled and then their peels were dried in a dry and dark place at room temperature.

### 2.3. Extraction and phytochemical analysis

The dried Tb and Bb peels were extracted at room temperature and macerated with 10 g of banana peel MeOH/CHCl<sub>3</sub> (1:1) solvent mixture in a closed container and the dark.<sup>15</sup> At the end of the extraction period, the remains were filtered. Solvents of the extracts were removed in a vacuum on a rotary evaporator. Quantitative analysis and phytochemical content experiments were carried out with these extracted extracts. For GC-MS analyzes, the derivatization of the alcohols in the form of methoxy was carried out. The dried banana peel was added with a sufficient amount of purified water and subjected to boiling. After boiling, the extract was filtered with ordinary filter paper. The solvent mixture MeOH/CHCl<sub>3</sub> (Me/Ch) was added to the remaining waste from the filtrate and stored. Ethyl acetate (Ea) was added to the water portion obtained from the filtrate. The solvent of the Ea phase was completely evaporated.

The phenolic components of extracts were quantitatively determined with Agilent brand 1260 Infinity HPLC model and using Zorbax SB-C18 (4.6 x 100 mm-3.5 µm) column 6210 and Time of Flight LC/MS detector.<sup>16</sup> The acetonitrile and ultrapure water with formic acid (1%) were used as a mobile phase mixture.

The application program was applied with per flow rate (0.6 mL per minute), total injection volume (10 µL) and column temperature (35 °C). The program was carried out so that the pure solvent mixtures were passed through the column in the range of 0-1 minute, 10% B, 1-20 min, 50% B, 20-23 min, and 23-30 min [A: Water; B: acetonitrile] according to the solvent application programs.

## 2.4. Antioxidant activity

The activities of extracts were performed *in vitro* by six different methods<sup>17</sup> and compared with BHT.  $A_{0.5}$  ( $\mu\text{g/mL}$ ) values were found as an effective concentration at which the absorbance (A) was 0.5 value for total antioxidant activity and reducing power of extracts. And also  $IC_{50}$  values ( $\mu\text{g/mL}$ ) were performed with effective concentration scavenging 50% of radicals for lipid peroxidation, metal chelating,  $\text{H}_2\text{O}_2$  and free radical scavenging activities of extracts.

### 2.4.1. Total antioxidant activity by phosphomolybdate assay

The activity of extracts has performed the test described in literature<sup>18</sup> and based on the reduction of  $\text{Mo}^{+4} \rightarrow \text{Mo}^{+5}$  by the antioxidants. The formation of green  $\text{PO}_4^{3-}/\text{Mo}^{+5}$  compounds monitored at acidic pH. A 0.3 mL sample was added to ammonium molybdate, sulfuric acid and sodium phosphate, respectively. Subsequently, the mixture of reaction activity was incubated at 95 °C, and read absorbance at 695 nm.

### 2.4.2. Assay of reducing power

The reducing power of extracts was evaluated by Oyaizu Method with slight modification.<sup>19</sup> The extract was mixed with 0.20 M phosphate buffer (pH 6.6) and 10 g/L  $\text{K}_3\text{Fe}(\text{CN})_6$ , respectively. After the incubation period at 50 °C, 10% TCA (2.5 mL) was added to the reaction mixture and centrifuged at 3000 x g to obtain a clear solution. 2.5 mL of the reaction mixture was combined with 0.1%  $\text{FeCl}_3$  and water and read at 700 nm between 3 and 5 min after initiation of the reaction. It is indicated that the increased absorbance of the reaction depending on concentration has an effective reducing in the reaction condition that the reducing capacity had increased.

### 2.4.3. Assay of free radical scavenging activity

The activities of the samples were performed according to the reference.<sup>20</sup> The 1 mM of DPPH· solution was combined with a sample in the test tubes and incubated at room temperature for 30 min. The absorbance of the reaction mixture was monitored at 517 nm.

### 2.4.4. Assay of $\text{H}_2\text{O}_2$ scavenging activity

The activity of the sample was evaluated according to ref.<sup>21</sup> A 1 mL extract solution was added in a flask to 0.1 mM  $\text{H}_2\text{O}_2$  in phosphate buffer (pH 7.4), ammonium molybdate,  $\text{H}_2\text{SO}_4$  and KI. The reaction mixture was titrated with 5 mM  $\text{Na}_2\text{S}_2\text{O}_3$  until the yellow color was lighter and the volume was recorded.

### 2.4.5. Assay of metal chelating activity

The chelating activity was performed by inhibiting the

formation of ferrozine-  $\text{Fe}^{2+}$  complex after adding of extract with  $\text{Fe}^{2+}$ .<sup>22</sup> In the presence of sample, this complex is disrupted with sample-chelated complex. The rate of red colour reduction allows to measure level of the chelating activity. The chelating complex level of extract with  $\text{Fe}^{2+}$  was recorded at 562. Briefly, the sample was combined with 0.05 mL  $\text{FeCl}_2$ . After adding 0.2 mL ferrozine, the reaction medium was incubated at room temperature and then the absorbance of the mixture was measured at 562 nm.

### 2.4.6. Inhibition assay of lipid peroxidation

The assay was carried out using TBA method based on inhibition level of linoleic acid peroxidation according to the modification literature method.<sup>23</sup> Briefly, the extract was combined with linoleic acid, 100  $\mu\text{M}$  phosphate buffer (pH 7.4) and 20  $\mu\text{M}$  ascorbic acid. The reaction of peroxidation was initiated by adding of 10  $\mu\text{M}$   $\text{FeSO}_4$  (0.1 mL) and incubated for 60 min. The mixture was added to 10% TCA solution and kept in water bath (95 °C). After cooling at room temperature, butanol was mixed with the reaction medium and centrifuged at 4000xg. After removing the supernatant, the formation of TBA reactive substance was monitored at 532 nm.

## 2.5. Antimicrobial activity

The activity of extracts was performed three gram negative bacteria (*Pseudomonas aeruginosa* ATCC15442, *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* ATCC 10031) and three-gram positive (*Enterococcus faecalis* ATCC29212, *Bacillus cereus* CCM99, *Staphylococcus aureus* ATCC25213) bacteria. The microorganisms were cultured in Biochemistry Research Laboratory (Ondokuz Mayıs University, Department of Chemistry, Biochemistry Laboratory).<sup>24,25</sup>

The antibacterial activities of extracts were tested by disc diffusion.<sup>26</sup> The different concentrations of extracts were prepared a range of 1024-0.5  $\mu\text{g/mL}$ . For disc diffusion method, amoxicillin and tetracycline were used as a positive and solvent as negative control.<sup>27</sup> 6 mm sterile discs were impregnated with 20  $\mu\text{L}$  of extract materials and then incubated on MHA (Mueller Hinton Agar) medium for 16-18 hours at 37 °C on where 100  $\mu\text{L}$  of suspension containing 0.5 McFarland of bacteria spread on agar. The activity was evaluated by measuring the apparent transparent inhibition zones around the discs in millimeters.

## 3. Statistical Analysis

The data were presented as the mean  $\pm$  standard deviation (S.D.). The results were analyzed by one-way ANOVA of variance followed by Duncan's test and considered to be significant with  $p < 0.05$  confidence level, statistically.

## 4. RESULTS AND DISCUSSION

The amount of banana production in Turkey is increasing every year. In 2015, the banana production is 270500 tons. Bananas consumption has increased with the understanding of human health. This increase is not met by the climatic factors that limit banana breeding. Turkey has imported 235188 tonnes of bananas in 2013 to ensure that this deficiency.<sup>28</sup>

Dried banana peels were extracted with boiling water prior to extraction with organic solvents, which are an important part of the characterization of the phenolic contents, due to the solvent-free residue of the banana shell for different purposes.

The use of banana peel in different areas has been investigated in Turkey.<sup>29,30</sup> The water-soluble part of aqueous Turkish banana peel (Tbp) and Brazilian banana peel (Bbp) extracts can be an important source of raw materials such as phenolic and flavonoids for different applications and can be evaluated in different areas.

### 4.1. Chemical analysis

The analysis of the volatile compound and fatty acid components in the Tbp-Ea, Tbp-Me/Ch, Bbp-Ea and Bbp-Me/Ch extracts were made by GS-MS (Figure 1-4) and results were listed in Table 1. The 13,27-cycloursan-3-one (74.23%), 12-oleanen-3-yl acetate (16.47%) and 9,19-cyclolanost-23-ene-3,25-diol (6.29%) were found high volatile compound in Tbp-Ea extract. Cetene (3.39%), 15-heptadecenal (9.49%), 9-hexadecenol (9.74%), 1-nonadecene (6.12%) and 9,19-cyclolanost-23-ene-3,25-diol (3.66%) were the abundant volatile components in Bbp extract. Palmitic acid methyl, linoleic acid methyl and stearic acid methyl esters were the most common fatty acids in the extracts of Me/Ch and Ea in both banana samples, and their values ranged from 38.25 to 0.04%. HPLC-TOF/MS chromatograms of Tbp and Bbp extracts were obtained for quantitative and qualitative analysis as seen in Figure 5.

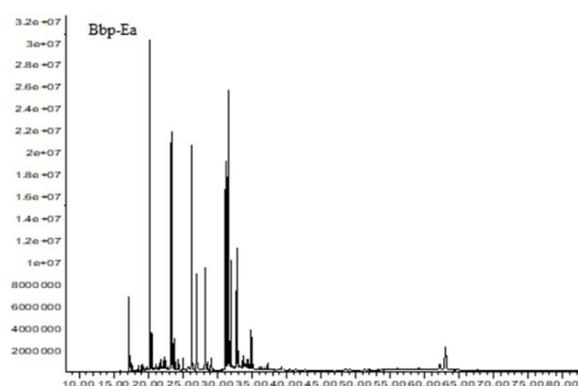


Figure 1. The GC analysis spectrum of Bbp-Ea extract

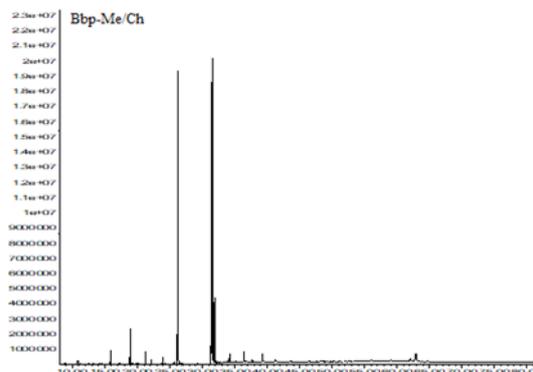


Figure 2. The GC analysis spectrum of Bbp-Me/Ch extract

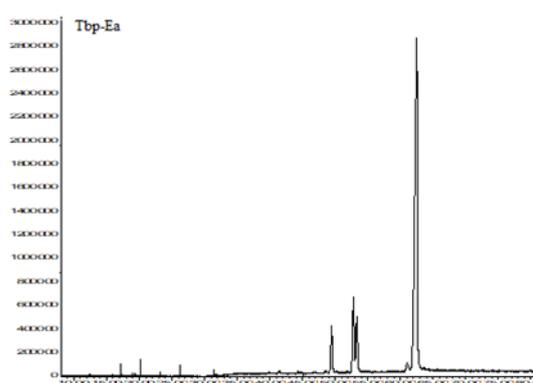


Figure 3. The GC analysis spectrum of Tbp-Ea extract

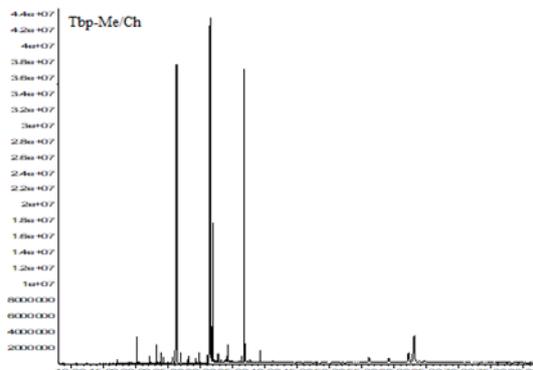


Figure 4. The GC analysis spectrum of Tbp-Me/Ch extract

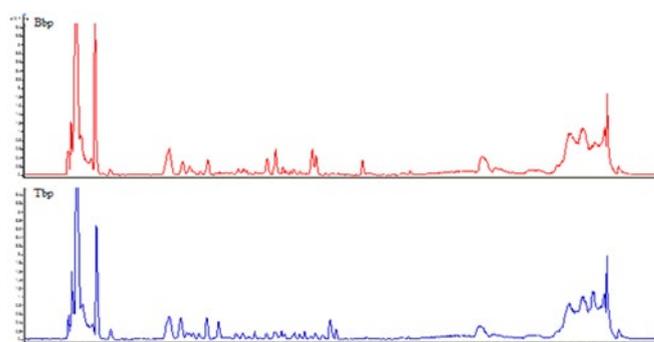


Figure 5. HPLC-TOF/MS chromatograms of extracts obtained from Bbp and Tbp

**Table 1.** The volatile compounds and fatty acid components (%) in the Tbp-Ea, Tbp-Me/Ch, Bbp-Ea and Bbp-Me/Ch extracts

No	Volatile Compounds, %	RT (min)	Bbp-Ea	Bbp-Me/Ch	Tbp-Ea	Tbp-Me/Ch
1	1-Tetradecene	17.180	3.33	-	0.35	-
2	3-Tetradecene	17.398	0.47	-	-	-
3	7-Tetradecene	17.591	0.42	-	-	-
4	2,6-Di-tert-butylbenzoquinone	18.598	0.22	-	-	-
5	Farnesane	18.925	0.28	-	-	-
6	2,4-di-tert-butyl-	19.185	0.24	-	-	-
7	2-Hexadecanol	19.814	0.14	-	-	-
8	Cetene	20.242	9.39	-	0.27	0.86
9	1-Decanol 2-hexyl-	20.318	0.35	-	-	-
10	7-Hexadecene	20.385	1.01	-	-	-
11	Benzophenone	21.031	0.40	-	-	-
12	Methyl tridecyl phthalate	21.492	0.14	-	-	-
13	1,3-di-iso-propylnaphthalene	21.643	0.20	-	-	-
14	1,7-di-iso-propylnaphthalene	21.727	0.36	-	-	-
15	Tetradecane	21.819	0.20	-	-	-
16	1-Hexadecanol	21.995	0.11	-	-	-
17	3-Eicosene	23.262	-	-	-	0.78
18	15-Heptadecenal	23.312	9.49	-	0.09	-
19	3-Octadecene	23.489	0.90	-	-	-
20	7-Octadecene	23.757	1.00	-	-	-
21	Hexahydrofarnesyl acetone	24.361	0.44	-	-	0.27
22	18-Norabietane	27.054	0.28	-	-	-
23	1-Nonadecene	28.228	6.12	-	-	0.59
24	2-Methyl-7-nonadecene	28.547	0.57	-	-	-
25	5-Eicosene	29.059	0.86	-	-	-
26	Methyl 2-ethylhexyl phthalate	29.839	-	-	-	0.82
27	1-Eicosanol	31.147	-	-	-	0.61
28	9-Hexadecanol	31.173	9.74	-	-	-
29	Behenic alcohol	32.775	2.93	-	-	0.39
30	Larixol	32.934	0.20	-	-	-
31	10-Heneicosene	33.060	0.36	-	-	-
32	Methyl dehydroabietate	34.587	0.16	-	-	-
33	12-Oleanen-3-yl acetate	52.798	-	-	16.47	-
34	Lupeol acetate	53.335	-	-	8.01	-
35	Stigmasterol	56.070	-	-	-	0.85
36	$\gamma$ -Sitosterol	59.157	-	-	-	0.69
37	13,27-Cycloursan-3-one	62.169	0.97	0.73	74.23	1.98
38	9,19-Cyclolanost-23-ene-3,25-diol	63.007	3.66	3.18	-	6.29
No	Fatty acids					
1	Undecanoic acid 10-methylmethyl ester	19.303	0.22	-	-	-
2	Myristic acid methyl ester	22.138	0.36	0.38	-	0.32
3	Pentadecanoic acid methyl ester	23.916	0.28	0.73	-	0.50
4	Palmitoleic acid methyl ester	25.670	0.27	-	-	0.52
5	Palmitic acid methyl ester	26.282	11.77	38.25	0.36	30.63
6	Benzenepropanoic acid 3.5-bis (1.1-dimethylethyl)-4-hydroxymethyl ester	26.953	4.77	-	-	0.65
7	Margaric acid methyl ester	29.327	-	-	-	0.41
8	Linoleic acid methyl ester	31.407	6.87	18.81	0.19	23.13
9	Linolenic acid methyl ester	31.550	11.83	30.77	-	22.27
10	Stearic acid methyl ester	31.902	3.37	4.78	0.04	5.56
11	Arachidic acid methyl ester	34.209	0.16	0.51	-	0.56
12	Behenic acid methyl ester	36.424	0.12	0.87	-	0.31
13	Tricosanoic acid methyl ester	37.732	-	-	-	0.16
14	Lignoceric acid methyl ester	39.293	0.18	0.99	-	0.71
15	Pentacosanoic acid methyl ester	41.163	-	-	-	0.14

**Bbp-Ea:** Brazilian banana peel ethyl acetate extract**Bbp-Me/Ch:** Brazilian banana peel methanol/chloroform extract**Tbp-Ea:** Turkish banana peel aqueous extract**Tbp-Me/Ch:** Turkish banana peel methanol/chloroform extract

According to screening library and revealing retention time (RT) of calibration standards, 131 components in the Bbp (Bbp-EA, Bbp-Me/Ch) and 171 components in the Tbp (Tbp-AE, Tbp-Me/Ch) were found. As a standard, organic acids and phenolic components were used in the quantitative determination and 36 components were identified. From these components, 4-hydroxybenzoic acid (Tbp-Ea: 68.41 mg, Tbp-Me/Ch: 50.26 mg), genestistic acid (Tbp-Ea: 0.47 mg, Tbp-

Me/Ch: 0.48 mg) and syringic acid (Tbp-Ea: 1.21 mg, Tbp-Me/Ch: 1.22 mg) were the most abundant in Tbp extracts (Table 2). The unknown compounds in Tbp and Bbp extracts were determined as seen in Figure 6. Bismuth subgallate (Bbp), trimethobenzamide (Tbp), jasmonic acid (Bbp), 5 $\beta$ -cholestanol (Tbp) and rhein glucuronide (Bbp) were the main unknown compound in extrats.

**Table 2.** Content analysis of organic acids and phenolic compounds in Tbp-Ea, Tbp-Me/Ch, Bbp-Ea and Bbp-Me/Ch extracts

No	Phenolics, (mg phenolic/kg)	Bbp- Ea	Bbp-Me/Ch	Tbp-Ea	Tbp-Me/Ch
1	Gallic acid	tr	tr	nd	nd
2	4-hydroxybenzoic acid	0.57	1.37	68.41	50.26
3	Gentisic acid	0.17	0.04	0.47	0.48
4	Protocatechuic acid	0.06	0.09	tr	nd
5	Caffeic acid	0.02	tr	nd	tr
6	Vanillic acid	0.27	0.03	0.43	nd
7	Syringic acid	0.07	0.10	1.21	1.22
8	Rutin	tr	0.01	0.50	0.38
9	4-hydroxybenzaldehyde	tr	tr	tr	tr
10	Polydatine	tr	tr	tr	nd
11	Ellagic acid	tr	nd	nd	nd
12	Scutellarin	0.04	tr	tr	nd
13	Quercetin-3- $\beta$ -D-glucoside	0.01	tr	tr	tr
14	Naringin	0.01	0.04	0.02	tr
15	Diosmin	0.18	0.20	nd	2.21
16	Taxifolin	0.07	tr	tr	nd
17	Hesperidin	0.03	tr	tr	tr
18	Apigetrin	tr	tr	nd	tr
19	Neohesperidin	0.20	tr	nd	nd
20	Myricetine	tr	tr	nd	tr
21	Baicalin	tr	tr	nd	nd
22	<i>p</i> -coumaric acid	tr	tr	tr	tr
23	Fisetin	tr	tr	nd	nd
24	Protocatechuic acid	tr	tr	tr	tr
25	Morin	0.03	0.06	nd	nd
26	Salicylic acid	tr	tr	tr	tr
27	Quercetin	tr	tr	nd	nd
28	Silibinin	tr	nd	tr	nd
29	Cinnamic acid	0.02	nd	nd	nd
30	Apigenin	0.07	tr	tr	tr
31	Naringenin	0.19	tr	nd	nd
32	Kaempferol	tr	nd	nd	nd
33	Eupatorin	tr	nd	nd	nd
34	Wogonin	0.03	nd	tr	tr
35	Galangin	tr	nd	nd	nd
36	Biochanin A	tr	nd	tr	tr

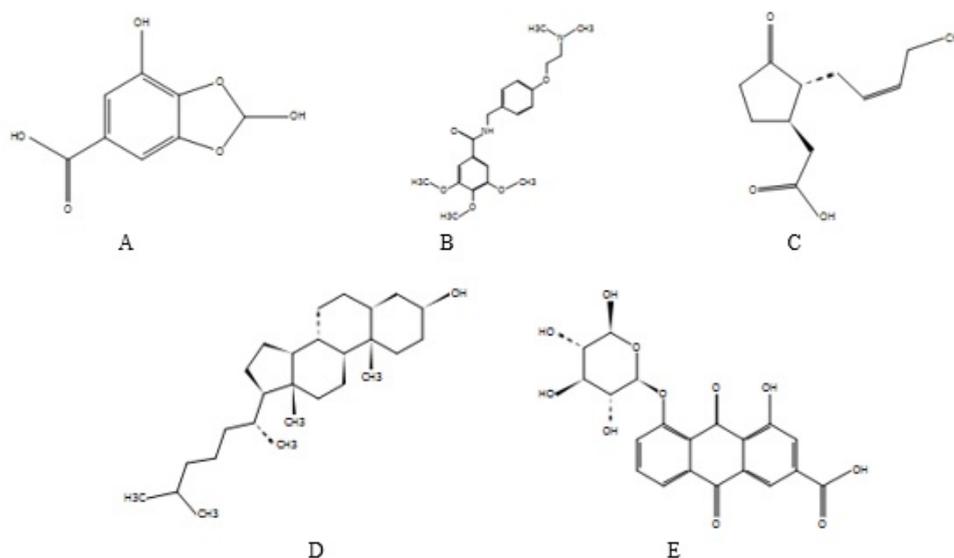
tr: trace; nd: not detected

**Bbp-Ea:** Brazilian banana peel ethyl acetate extract

**Bbp-Me/Ch:** Brazilian banana peel methanol/chloroform extract

**Tbp-Ea:** Turkish banana peel aqueous extract

**Tbp-Me/Ch:** Turkish banana peel methanol/chloroform extract



**Figure 6.** Unknown compounds from Bbp and Tbp extracts

## 4.2. Antioxidant activity

The antioxidant activities of Bbp-EA, Bbp-Me/Ch, Tbp-EA and Tbp-Me/Ch extracts were tested by total antioxidant activity, reducing power, inhibition of lipid peroxidation, metal chelating, free radical and  $H_2O_2$  scavenging activity and compared with BHT and EDTA as positive controls (Table 3).

### 4.2.1. Total antioxidant activity

The activity was evaluated spectrophotometrically by

phosphomolybdenum assay. In this assay, the extract reduced Mo(VI) to Mo(V) and the green-PO4-3/Mo+5 complex was formed.<sup>16</sup> The activities of Bbp-Ea, Bbp-Me/Ch, Tbp-EA and Tbp-Me/Ch extracts and BHA showed a significant increase depending on concentration ( $p < 0.05$ ). (Table 3). BHT, Tbp-Ea, Bbp-Me/Ch, Bbp-Ea and Tbp-Me/Ch showed the more effective  $A_{0.5}$  values, namely: 30.49, 36.84, 39.17, 49.93 and 71.45  $\mu\text{g/mL}$ . Tbp-Ea and Bbp-Me/Ch were good antioxidant sources and found to have high total antioxidant activity values which were correlated with their higher concentration of phenolics.

**Table 3.** Total antioxidant activity, reducing power, inhibition of lipid peroxidation, free radical scavenging and  $H_2O_2$  scavenging activities of the Tbp-Ea, Tbp-Me/Ch, Bbp-Ea and Bbp-Me/Ch extracts

Sample	Total antioxidant activity	Reducing power	DPPH <sup>•</sup> scavenging activity	Metal chelating activity	$H_2O_2$ scavenging activity	Inhibition of lipid peroxidation
	$A_{0.5}$ , $\mu\text{g mL}^{-1}$		$IC_{50}$ , $\mu\text{g mL}^{-1}$			
<b>Bbp-Ea</b>	49.93±3.07	9.43±0.78	10.50±2.73	17.35±1.97	0.71±0.15	3.22±0.85
<b>Tbp-Ea</b>	36.84±2.57	7.24±1.55	21.63±1.53	16.54±0.68	0.81±0.09	3.35±2.36
<b>Bbp-Me/Ch</b>	39.17±1.78	4.96±0.95	27.50±2.92	19.70±1.62	0.45±0.18	12.38±3.43
<b>Tbp-Me/Ch</b>	71.45±4.19	5.27±0.77	25.31±1.75	29.44±1.28	0.36±0.11	9.05±0.55
<b>BHT</b>	30.49±2.92	4.02±0.28	8.54±2.82	4.77±0.97	21.69±3.00	2.14±0.73

The values were represented means  $\pm$  SD ( $n = 3$ ),  $p < 0.05$

**Bbp-Ea:** Brazilian banana peel ethyl acetate extract

**Bbp-Me/Ch:** Brazilian banana peel methanol/chloroform extract

**Tbp-Ea:** Turkish banana peel ethyl acetate extract

**Tbp-Me/Ch:** Turkish banana peel methanol/chloroform extract

**BHT:** Butylated hydroxytoluene

### 4.2.2. Reducing power

This assay is used to determine the electron donating capacity of an antioxidant. In the presence of a reductive polyphenol,  $Fe^{3+}$ -ferricyanide leads to reduction of  $Fe^{2+}$ -ferricyanide. Then, the color conversion is monitored spectrophotometrically.<sup>31</sup> Bbp-Ea, Bbp-Me/Ch, Tbp-EA and Tbp-Me/Ch extracts exhibited effective reducing

power at 700 nm (Table 3). It has been determined that the different concentrations of the extract are gradually increased with increasing concentration, but exhibit lower reduction power than BHA. The Tbp-Me/Ch extract showed reducing capacity higher than that of Bbp-Ea, Bbp-Me/Ch and Tbp-EA significantly. The reducing potential ( $A_{0.5}$ ,  $\mu\text{g/mL}$ ) of the extracts and standard increased in the following order: BHT, 4.02 >

Bbp-Me/Ch, 4.96 > Tbp-Me/Ch, 5.27 > Tbp-Ea, 7.24 > Bbp-Ea, 9.43.

#### 4.2.3. Free radical (DPPH) scavenging

This assay was carried out with synthetic DPPH<sup>•</sup>, which is widely used in antioxidant activity tests in evaluating the radical scavenging activity of natural and synthetic antioxidants. When an antioxidant and DPPH<sup>•</sup> are mixed, the antioxidant gives an hydrogen to DPPH<sup>•</sup> and the purple color turns into a yellow color.<sup>16</sup> It was followed that the scavenging activities of Bbp-Ea, Bbp-Me/Ch, Tbp-EA and Tbp-Me/Ch extracts and BHT were changed as the concentrations increased. Table 3 shows the DPPH<sup>•</sup> scavenging capacities of the extracts. Extracts exhibited a lower free radical scavenging activity than BHT. Bbp-Ea and Tbp-Ea exhibited the effective IC<sub>50</sub> values, namely: 10.50 and 21.63 µg/mL.

#### 4.2.4. Metal chelating activity

The O<sub>2</sub><sup>•-</sup> and <sup>•</sup>OH radicals, which are the most active activators of oxidative stress, are of transition metal origin. These radicals transfer electrons, cause lipid peroxidation, damage proteins and accelerate lipid peroxidation.<sup>32</sup> The antioxidant inhibits oxidation, reduce redox potential and stabilize the formation of metal oxide. Bbp-Ea, Bbp-Me/Ch, Tbp-EA and Tbp-Me/Ch extracts exhibited effective in stabilizing binding to the active transition metal as natural chelator products (Table 3). It was found that the extracts had lower effect on the formation of Fe<sup>2+</sup>-extract complex according to the formation of Fe<sup>2+</sup>-EDTA. The chelating activity of Tbp-

Ea (IC<sub>50</sub>: 16.54 µg/mL) was found close to Bbp-Ea (IC<sub>50</sub>: 17.35 µg/mL).

#### 4.2.5. Inhibition of lipid peroxidation

Oxidation products resulting from oxidation can permanently affect or modify the defence mechanism through different chemical and physical processes. Among the target substances of oxidation are DNA, oxygen, polyunsaturated fatty acids and phospholipids cholesterol.<sup>33</sup> Lipid peroxidation is a radical chain process in which the primary products of lipid oxidation produced in the initial stage of the amount of peroxide and oxidation are measured.<sup>34</sup> The Bbp-Ea, Bbp-Me/Ch, Tbp-EA and Tbp-Me/Ch affected by inhibition of linoleic peroxidation, *p* < 0.05 and the results were exhibited in Table 3. The inhibition assays range from 10-100 µg mL<sup>-1</sup>. The outcomes were expressed exclusively by Bbp-Ea (3.22 µg/mL) and Tbp-EA (3.35 µg/mL) exhibited a closer inhibition of linoleic peroxidation than that of BHT. Bbp-Ea and Tbp-Ea exhibited more effective inhibition of linoleic acid peroxidation (IC<sub>50</sub>) than Bbp-Me/Ch and Tbp-Me/Ch.

#### 4.3. Antimicrobial activity

The activity results were exhibited in Table 4, where it can be seen that the Turkish banana peel extracts exhibited the best antibacterial activity against the *E. coli*, *E. faecalis* and *K. pneumoniae* tested in comparison with Brazilian banana peel extracts according to inhibition zone value.

**Table 4.** The antimicrobial activities of the Tbp-Ea, Tbp-Me/Ch, Bbp-Ea and Bbp-Me/Ch extracts

Samples/ Antibiotics	Test	Gram-negative bacteria		
		<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>
Tbp-Me/Ch	Inhibition zone, mm	10.8±1.13	8.75±0.49	12.15±4.03
Tbp-Ea		9.7±0.99	9.95±0.78	8.50±2.12
Bbp-Me/Ch		7.95±0.64	NA	8.85±1.06
Bbp-Ea		7.25±0.35	11.0±0.0	10.70±1.84
Tetracycline		25.25±0.35	15.65±2.05	20.30±0.28
Amoxicillin		25.35±1.91	NA	NA
Control		6.0±0.0	6.0±0.0	6.0±0.0
Samples/ Antibiotics	Test	Gram-positive bacteria		
		<i>E. faecalis</i>	<i>B. cereus</i>	<i>S. aureus</i>
Tbp-Me/Ch	Inhibition zone, mm	9.75±0.07	NA	7.0±0.00
Tbp-Ea		16.6±0.00	NA	8.20±0.85
Bbp-Me/Ch		8.70±0.42	NA	11.25±2.33
Bbp-Ea		8.00±0.57	NA	8.4±0.14
Tetracycline		17.90±0.90	47.00±4.24	20.40±0.57
Amoxicillin		25.7±0.99	34.75±3.89	26.95±0.78
Control		6.0±0.0	6.0±0.0	6.0±0.0

NA: no activity. The values were represented means ± SD (*n* = 3), *p* < 0.05

**Bbp-Ea:** Brazilian banana peel ethyl acetate extract

**Bbp-Me/Ch:** Brazilian banana peel methanol/chloroform extract

**Tbp-Ea:** Turkish banana peel ethyl acetate extract

**Tbp-Me/Ch:** Turkish banana peel methanol/chloroform extract

The Tbp-Ea was most active against *E. faecalis* in comparison to all the microorganisms tested and exhibited a broad spectrum of antibacterial activity. Specifically, the ethyl acetate and methanol/chloroform extracts showed different levels of antibacterial activity against gram positive and negative bacteria. This is supported by a previous study on water<sup>35</sup>, isopropyl alcohol<sup>36</sup> and ethyl acetate<sup>37</sup> extracts that exhibit close or greater activity against ethyl acetate and methanol/chloroform extracts against bacteria without cellular toxicity.

## 5. CONCLUSIONS

The agro-residues can be considered a valuable resource after recycling and reprocessing. The banana peel has a lot of antioxidant activity and can be a very cheap source of extracts rich in bioactive compounds. This study showed that it had important effects on the phenolic contents extracted by extraction with Ea and Me/Ch. Total antioxidant activities of Tbp-Ea and Bbp-Me/Ch were found to be close to BHT. Tbp-Ea, Bbp-Ea, Bbp-Me/Ch and Tbp-Me/Ch are promising natural additives that exhibit antibacterial, H<sub>2</sub>O<sub>2</sub> scavenging activity and the capacity to inhibit lipid peroxidation. However, the metal chelate capacity of the extracts was low. This research has compared the phytochemical and bioactivity potentials of Brazilian banana peel extract and Turkish banana peel extracts by chromatographic and spectroscopic analysis, and also revealed promising results.

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## Conflict of interests

The authors declare that there is no a conflict of interest with any person, institute, company, etc.

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