INVESTIGATIONS ON *CAPSICUM ANNUUM* L. SAMPLES PURCHASED FROM KAYSERI PROVINCE OF TURKEY

Ayşe BALDEMİR [a*], Esra KÖNGÜL [b], Nilay ILDIZ [c], Selen İLGÜN [a]

[a] Erciyes University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Kayseri, Turkey
[b] Erciyes University, Faculty of Pharmacy, Department of Pharmacognosy, Kayseri, Turkey
[c] Erciyes University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Kayseri, Turkey

Abstract

Capsicum annuum L. (Solanaceae) originates from Central America (native of Mexico). It is cultivated throughout Turkey as a condiment and vegetable, in fields or greenhouses, for its unripe green or ripe red fruits (in Turkish, "biber"). In this study, microscopical, physico-chemical analysis of 10 red pepper samples bought from Kayseri market were investigated if they conform to Capsicum annuum monograph in the European Pharmacopoeia. For this purpose, organoleptic and microscopic analyses, thin layer chromatography (TLC), tests on foreign matters, loss on drying, and total ash quantities were conducted on 10 samples, respectively. Additionally, antibacterial activities of Capsicum annuum methanol extracts and capsaicin (standard) against Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), and Staphylococcus aureus (ATCC 25923) were examined by using disc diffusion method.

Keywords: *Capsicum annuum, Red pepper, Anti-bacterial activity, Microscopical, Physico-Chemical, TLC, Solanaceae.*

Türkiye'nin Kayseri İlinden Satın Alınan Capsicum anuum L. Örnekleri Üzerinde Araştırmalar

Capsicum annuum L. (Solanaceae) türü Orta Amerika-Meksika kökenlidir. Türkiye'de baharat ve sebze olarak tüketilmek üzere tarlalarda, seralarda olgunlaşmamış yeşil ya da olgun kırmızı meyveleri için kültürü yapılmaktadır. Bu çalışmada, Kayseri piyasasından satın alınan 10 adet kırmızı biber örneğinin Avrupa Farmakopesi'nde yer alan C. annuum monografında belirtilen mikroskobik, fiziko-kimyasal analizler açısından uygunluğu araştırılmıştır. Bu amaçla, 10 örnek üzerinde sırasıyla Avrupa Farmakopesi'nde belirtilen organoleptik ve mikroskobik analizler, ince tabaka kromatografisi (İTK), yabancı madde tayini, kurutmada kayıp ve total kül miktar tayini analizleri yapılmıştır. Ek olarak, C. annuum metanol ekstreleri ve kapsaisin standartının Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853) ve Staphylococcus aureus (ATCC 25923'e karşı antibakteriyal aktiviteleri disk difüzyon metodu kullanılarak araştırılmıştır.

Anahtar Kelimeler: *Capsicum annuum, Kırmızı biber, Antibakteriyal aktivite, Mikroskobik, Fiziko-kimyasal, İTK, Solanaceae.*

***Correspondence:** E-mail: aysebaldemir@gmail.com; aysebaldemir@erciyes.edu.tr

INTRODUCTION

Red pepper (*Capsicum annuum* L.) is grown in almost every area in the world. It is widely cultivated in Turkey and consumed as a condiment and vegetable (1). Red pepper from the Solanaceae family is an annual agricultural plant that is grown in tropical and sub-tropical regions. Its fruits are berry, a circumscissile or septicidal capsule. This fruit is used as a spice after it has been dried and turned into the red pepper flakes; it is also used in its fresh form and as a sauce in cooking for its color and flavor. The spice-related properties of red peppers originate from their color components and pungency components that are called "capsaicinoids" and have an irritant and stimulating effect (2-6). Capsaicin is a pungent that is found in red peppers and is commonly used as a food additive; and it is also considered to be an antimicrobial agent (7). In addition, *Capsicum* species are used worldwide to treat gastric ulcers, alopecia, rheumatism, toothache and diabetes in conventional medicine; since they are good sources of vitamins C and E, provitamin A, carotenoids and various phenolics and flavonoids (8).

Red pepper ranks first among the spices that are used in Turkey so it has an important place in the economy of our country (2). Pepper (*C. annuum* L.) is an important agricultural crop, not only because of its economic importance, but also due to the fact that it plays an important role in human health since it contains high concentrations of various biofunctional and antioxidant compounds, including ascorbic acid and carotenoids (9, 10). However, some quality deteriorations occur in cultivation, drying, processing and storage of red pepper (4, 11). Additionally, a large number of red pepper preparations are sold on the market without proper scientific evaluation, mandatory safety and toxicological studies (especially for losing weight).

Nowadays, consumers are able to buy non-prescription herbal medications without being aware of the potential hazards of a poor quality product. A well-defined and constant composition of the drug is a very important prerequisite for the production of a quality drug (12). For the purpose of this study, red pepper samples bought from herbalists or from the bazaar in Kayseri were examined in terms of their microscopical, physico-chemical and anti-bacterial activities. *Capsicum annuum* monograph in the European Pharmacopoeia 6.0 (EP) was used to compare the results (13).

MATERIALS AND METHODS

Plant metarial

In this study, 10 red pepper samples were purchased from Kayseri market (herbalists, bazaar) between June to November 2013 (Table 1).

Table 1. Data of the samples used in the study.

Sample Code	Locality	Usage
C-1	27 Mayıs Street	As a spice (unpackaged)
C-2	Hunat Neighborhood	As a spice (unpackaged)
C-3	Shopping center	As a spice (unpackaged)
C-4	Sivas Street	As a spice (unpackaged)
C-5	Sivas Street	As a spice (unpackaged)
C-6	27 Mayıs Street	As a spice (unpackaged)
C-7	27 Mayıs Street	*Capsule to loose weight
C-8	Market	As a spice (Packaged)
C-9	Barbaros Street (bazaar)	As a spice (unpackaged)
C-10	27 May Street (Delicatessen)	As a spice (unpackaged)

* Recommended use: One capsule should be taken on an empty stomach

Organoleptic Analyses

Red pepper samples were purchased in powder form. Each sample was checked for organoleptic analysis (general appearance, color, taste, odor) (Table 3).

Microscopic Analyses

In microscopic studies, the samples were primarily examined under microscope using *chloralhydrate solution* R as mentioned in the European Pharmacopoeia. Additionally, distilled water and Sartur reagent were used for microscopical analysis (14). The characteristic elements were determined, and microphotographs were taken using a ZEISS Primostar 415500 with AxioCam ICc3 camera.

Thin- Layer Chromatography (TLC)

All samples were purchased in powder form (Table 1). In TLC study, we made use of the the European Pharmacopoeia (6.0) and "Plant Drug Analysis Book" for comparisons (13, 15). The application details of this study are given in Table 2.

Table 2. Conditions of TLC analysis of samples

Conditions of TLC analysis	
Test Solution	0.5 g of samples was extracted by 5 ml *methanol* R, shaken for 5 minutes and filtered.
Reference solution	2 mg of capsaicin dissolved in 5 ml *methanol* R
Plate	Silica Gel 60 $F_{254,}$ Aluminum sheets, 20 x 20 cm
Mobile phase	Petroleum ether-acetate-methanol (7.5: 2: 0.5 v /v / v)
Application	As bands
Development	10 cm
Drying	In air
Detection	Vanillin / sulfuric acid

Foreign Matters

10 g each of the samples was spread in thin layers on a sheet of white paper. Foreign matters were identified by checking with the naked eye, separated and weighed (13).

Losses on Drying

1,000 g of each sample was placed in glass weighing bottles before being dried in an oven to constant mass. The samples were dried in an oven at 105ºC for 2 h and the percentages of weight loss were calculated (13).

Total Ash

10 samples of *C. annuum* were weighed (1,000 g) separately in silica crucibles which were burned by a furnace to a constant mass. Then, the samples were incinerated at 600 ± 20ºC and weighed. They were allowed to cool in desiccators (13).

Preparation of Extracts

Each 10 g sample was extracted with methanol using a shaker water bath three times for 8 h. Thereafter, the extracts were filtered and evaporated to dryness in vacuo at 37ºC.

Anti-bacterial activity

Anti-bacterial activities of capcaisin (standard) and the extracts were tested using disc diffusion method (16) to determine the inhibition zone of the extract by using standard strains of *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923. Standard capcaicin (Sigma- Aldrich, Germany) solution and 10 plant extracts inhibited microorganism growth at different rates (0-22 mm inhibition zones). Standard strains were used as follows: Amoxicillin clavulanic acid (20/10 mcg), Imipenem (10 mcg) and Cefoxitin (30 mcg) (Bioanalyse, Turkey) discs. Plant extracts of 25 µg/ml were impregnated on sterile paper blank discs (Oxoid, UK). Standard capcaisin solutions of 100 µg/ml and 25 µg/ml were prepared by diluting in DiMethylSulfOxid (DMSO) (MERCK, Germany). Standard strains were prepared from fresh cultures of the microorganisms according to 0.5 Mc Farland standard by diluting in normal saline and were inoculated on Mueller Hinton agar (MERCK, Germany). Discs which were impregnated with plant extracts and standard antibiotic discs were placed on Mueller Hinton agar plates. Petri dishes were incubated for 16-18 h at 35^0C and the inhibition zones were measured.

RESULTS

Organoleptic Analysis

Red pepper samples were different from each other in terms of general appearance, color and taste. All results of the samples are given in Table 3.

Table 3. Organoleptic analysis and photographs of samples

Sample code	General view	Color	Flavor	Photographs
C-1	Bright, homogeneous, finely powdered	Crimson	Hot	
C-2	Bright, homogeneous, finely powdered	Crimson	Hot	
C-3	A little dull, homogeneous, finely powdered	Reddish brown	Hot	
C-4	Dull, containing yellow fibrous structures in powdered form	Reddish brown	Sweet	
C-5	Dull, light-colored small particles	Light brown	Sweet	

Table 4. Organoleptic analysis and photographs of samples (continued).

Sample code	General view	Color	Flavor	Photographs
C-6	Rough powder form	Dark red	Hot	
C-7	Finely powdered	Off-white	-	
C-8	Bright, homogeneous, finely powdered	Crimson	Hot	
C-9	Bright, finely powdered, containing very small black beads	Crimson	Hot	
***C-10**	Dull, homogeneous, finely powdered	Light Brown-reddish	Hot	

* C-10's local name is "Cırgalan".

Microscopic Analyses

As a result of microscopic examination of samples, the characteristic structures of red pepper were identified as specified in the European Pharmacopoeia (13). This study was carried out separately for each sample. Characteristic elements of *C. annuum* shown in Figure 1. These elements have been identified in all samples except the C7 which include in trace amounts of *C. annuum* elements.

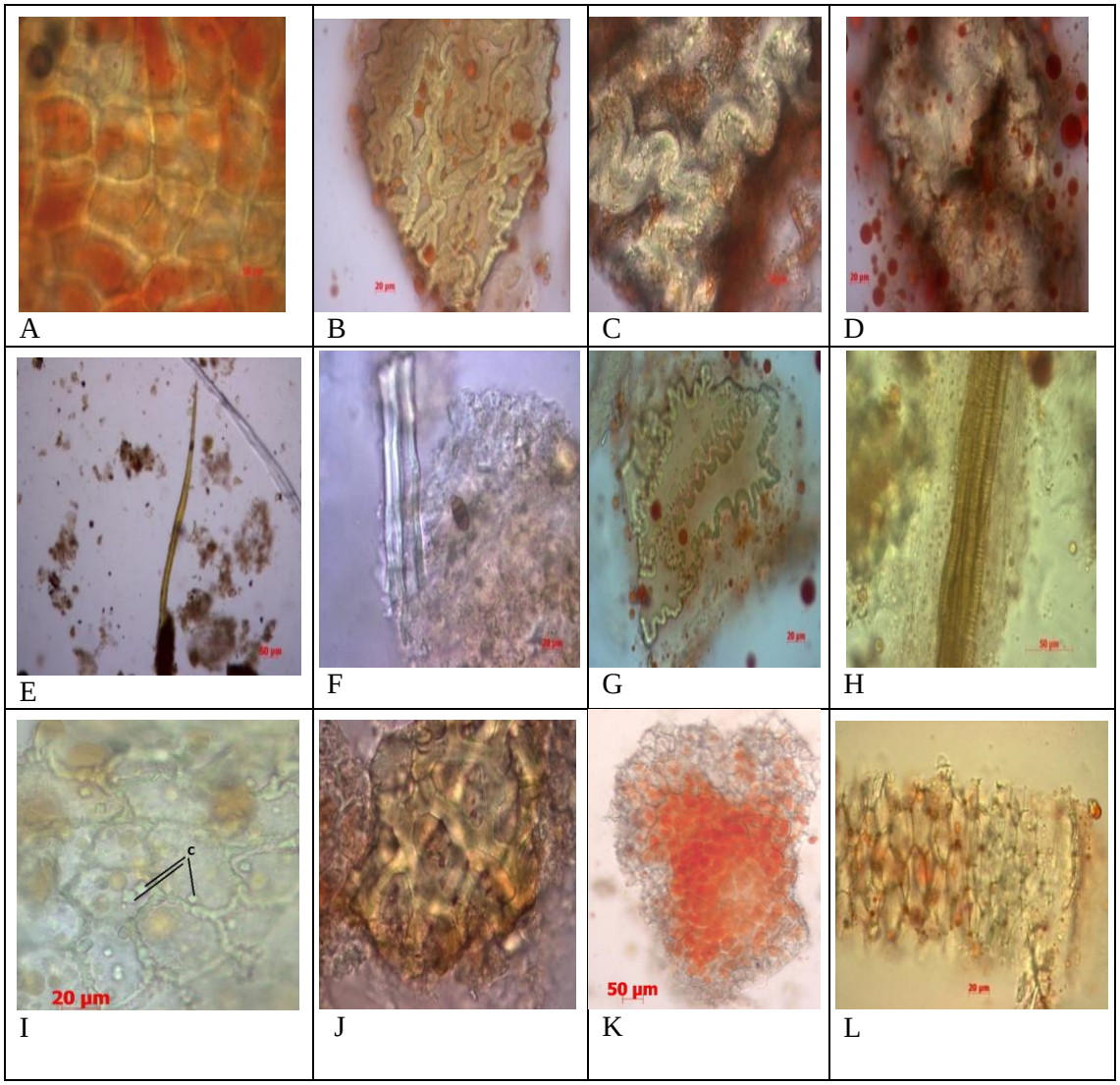


Figure 1. Some images from microscopic analysis of samples A- epicarp, B- sclereids of the endocarp, C- epidermis of the testa, D- droplets of red oil, E- trichomes, F- part of fiber, G- elongated sclereids of the endocarp, H- vessels, I- microsphenoidal calcium oxalate crystals, J- epicarp of the fruit, K- epidermis in surface view showing pigmented cells, L- parenchyma of the mesocarp (x40).

c: crystals.

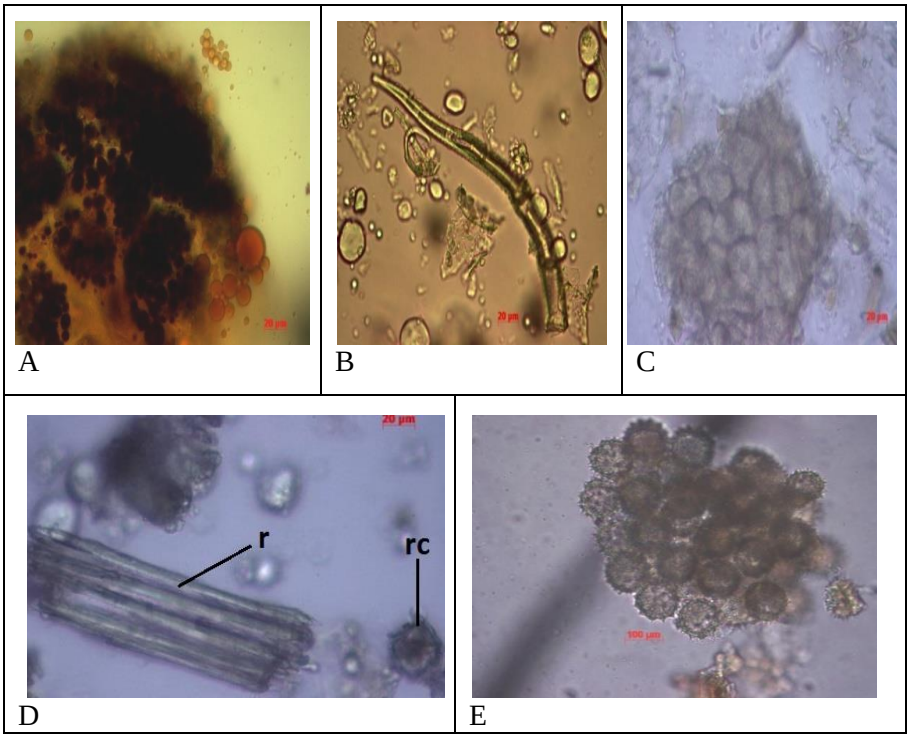
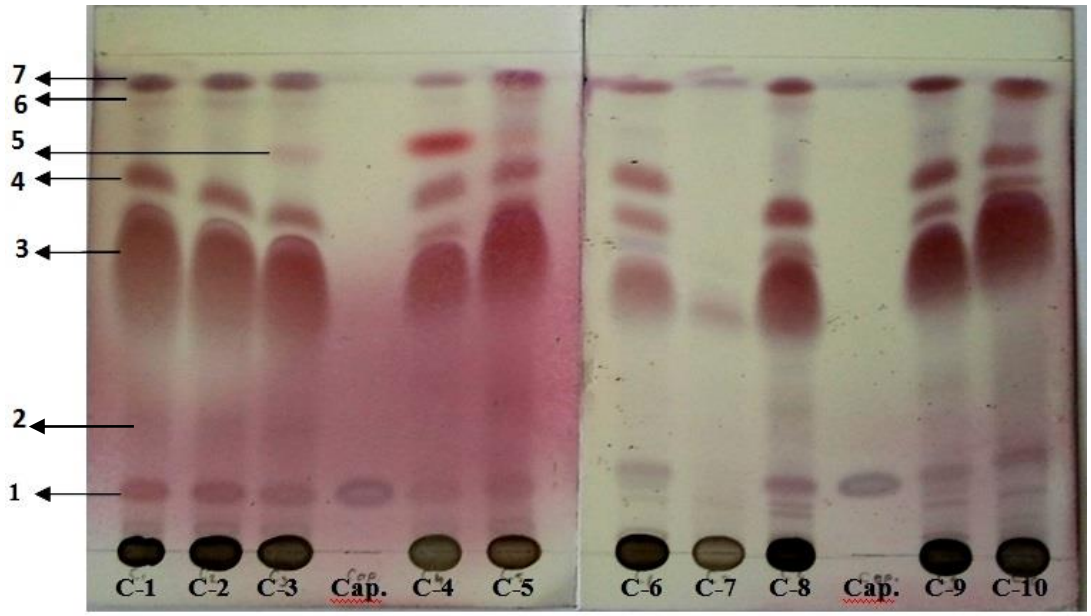


Figure 2. Some impurities detected in microscopic analysis of samples A- starch granules in sample C-4; B- starch granules in sample C-7; C- stone cells in sample C-7; D- raphide and rosette crystals in sample C-7; E-pollen grains in sample C-5 (x40).
r: rahide crystal, **rc:** rosette crystals

Thin- Layer Chromatography (TLC)

In TLC analysis, capsaicin was used as a reference substance and its Rf value was found to be ~ 0.13-0.21 (first band) and Rf of capsaicinoids (capsaicin and several related compounds are called capsaicinoids)~ 0.13-0.3. In the process, Rf values of all samples were calculated (third band (tailed) Rf ~ 0.4-0.75; fourth band Rf~ 0.65-0.7; fifth band (dyestuff ?) Rf~ 0.75-0.85; sixth band Rf~ 0.92 and seventh band Rf~ 0.97). Those of the band except the first, second and fifth are defined as carotenoids. Thin layer chromatogram of the samples and capcaisin standard is given in Figure 3. Our results are practically consistent with the literature (13, 15, 17).



1. Capsaicin; 2. Capsaicinoids; 3(Tailed band), 4, 6, 7. Carotenoids; 5. Dyestuff (?)

Cap: Capsaicin

Figure 3. Thin layer chromatogram of the samples and capcaisin standard.

Antibacterial activity

Antibacterial activity results of *Capsicum* extracts and capcaicin are shown in Table 5 and Figure 4. Inhibition zones of microorganisms were measured in milimeters (mm).

Table 5. Antibacterial activity results of *Capsicum* extracts and capcaicin.

Sample code and standard	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC 25923
C-1	[a] -	[b] 20±0.66	-
C-2	-	-	-
C-3	-	14±0.33	18±0.57
C-4	-	11±0.33	14±0.33
C-5	-	-	19±0.33
C-6	15±0.33	14±0.66	10±0.33
C-7	16±0.33	19±0.57	16±0.33
C-8	-	13±0.66	10±0.33
C-9	-	18±0.57	-
C-10	-	14±0.33	17±0.33
Capsaicin 25 µg/ml	-	-	13±0.33
Capsaicin 100µg/ml	-	12±0.57	21±0.33
Amoxicillin clavulanic acid (AMC)	18±0.66	-	-
Cefoxitin (FOX)	-	-	26±0.45
Imipenem (IPM)	-	24±0.49	-

[a] (-) inactive, [b]growth inhibition zones of microorganisms mm and data represent an average of three replicates (± SD).

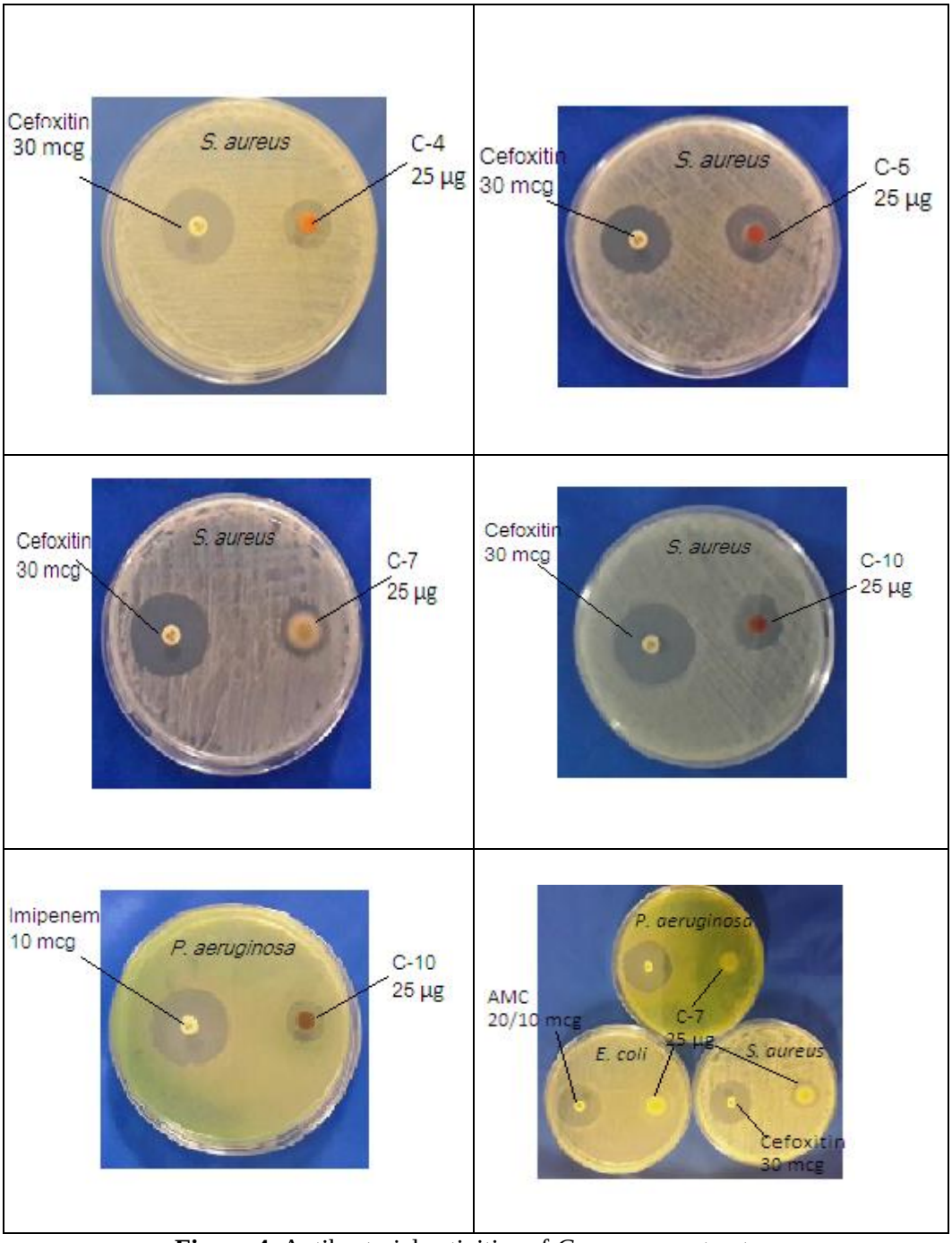


Figure 4. Antibacterial activities of *C. annuum* extracts

Physico-chemical tests

Foreign matters in the powdered samples were detected as less than 2%. The results of other tests are shown in detail in Table 6.

Table 6. Results of physico-chemical tests

Sample code	Loss on Drying (%)	Total Ash (%)
C-1	5.7±0.00	6±0.01
C-2	4±0.01	6.3±0.00
C-3	5.8±0.00	4.5±0.01
C-4	5.3±0.00	**13±0.00**
C-5	7.2±0.02	8.7±0.00
C-6	8±0.01	**11.5±0.02**
C-7	6.5±0.01	4.6±0.14
C-8	10±0.00	6.6±0.01
C-9	6±0.01	5.8±0.01
C-10	7±0.00	5.4±0.00
EP St.	**<11.0%**	**<10.0%**

DISCUSSION

In this study, red pepper (*Capsium annuum* L.) samples bought from different places in Kayseri were investigated in terms of microscopic, physico-chemical and antibacterial activities. For this purpose, tests specified in the European Pharmacopoeia were conducted on 10 samples.

As a result of the microscopic examination the characteristic elements of all the samples concerning *C. annuum* were determined and these findings were in accordance with those given in previous reports (18). In addition, some impurities were detected in microscopic analysis of samples. For instance, the C-4 sample was mixed with wheat starch and its total ash quantities did not conform to the European Pharmacopoeia (Figure 2 and Table 6). Sample C-7 had a trace amount of the characteristic elements of *C. annuum*. As a result of the microscopic analysis it was determined that the pepper capsule (sample C-7) contains a large amount of starch and parts of different plants. Söğüt et al. mentioned that the existence of different plant extracts in the capsule to loose weight. Results of this study are compatible with the literature (19).

In TLC study, zone of capsaicin was observed in all samples except of sample C-7. It was observed that the C-3, C-4 and C-5 samples especially have a pink-orange zone unlike the other samples in TLC analyses (Figure 3). These examples may contain some impurities. Microscopical results confirm this situation as well.

According to the the physico-chemical test results, loss on drying of samples were found between the ranges specified in the pharmacopoeia (<11.0%). For the amount of total ash, samples except for C-4 and C-6 were consistent with pharmacopoeia (<10.0%). It is possible to determine the quality of foodstuffs with ash content. Of its large amount of ash in food is not always considered as a positive result. For example, existence of high amount of ash in spices indicates the additive materials (20).

Anti-bacterial activities of 10 extracts and capsaicin against *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 25923) were investigated with disc diffusion method. While 100 µg/ml of capsaicin showed activity against *P. aeruginosa* (ATCC 27853), 25 µg/ ml of capsaicin showed no activity. Extracts of C-6 and C-7 inhibited growth of the three bacteria. The C-7 sample involved mixed plants, trace amounts of *Capsicum* and starch. Capsaicin (standard) has no inhibition effect on *E. coli* (ATCC 25922) and effective to *S. aureus* (ATCC 25923). *P. aeruginosa* (ATCC 27853) was the most susceptible microorganism to the C-1 sample. Similarly, *S. aureus* (ATCC 25923) was the most susceptible microorganism to the C-4 sample. Dorantes et al. were investigate inhibitory effects of extracts of three chilli peppers on the growth of four food borne pathogens and *S.aureus* was resistant to capsaicin but phenylpropanoids of capsaicin was showed good inhibitory action. Berber et al. had used different standard strains (*S.aureus* FRI-S6) and their findings showed that Gram negative microorganisms were susceptible than Gram positive. Keskin et al. *C. annuum* methanol extracts weren't susceptible other bacteria except *P.aeruginosa*. Location of plants, different climates, and extraction methods might be effective the antimicrobial and other activities. Our results are partially compatible with previous antimicrobial activity studies on *Capsicum annuum* (21-24).

CONCLUSION

In this study, Red pepper samples bought from Kayseri market were examined in terms of their anti-bacterial activities as well as microscopical, physico-chemical characteristics and TLC analyses. As a consequence, it was determined that some red pepper samples do not conform to European Pharmacopoeia standards. In addition, the Turkish Food Codex Communique states that starch (except for inherent starch), semolina, rasmol, bran and similar filler materials should not be included in the spices or spice mixtures. However, we have detected such impurities in some of the examples in this study. This issue is very important in terms of being a threat to human health. Inspections should be increased.

REFERENCES

1. Davis, PH, Calen J, Coode MJE. Flora of Turkey and The East Aegean Islands, University Press, Edinburgh, Vol 6, 437-443, 1967.
2. Akbay, C, Boz, I, Tiryaki, GY, Candemir, S, Arpacı, BB, Kahramanmaraş ve Gaziantep illerinde kırmızı biberin üretim yapısı ve kurutma yöntemleri. KSÜ Doğa Bil Derg 15 (2), 1-10, 2012.
3. Topak H, Erbil N, Dığrak M, Doğu Akdeniz ve Güneydoğu Anadolu Bölgesi'nde yetiştirilen biberlerin (*Capsicum annuum* L.) antimikrobiyal aktivitesinin araştırılması. Fırat Üniv Fen ve Müh Bil Derg 20 (2), 257-264, 2008.
4. Yalçın D, Kırmızı pul biber üretiminde kritik kontrol noktaları ve tehlike analizleri. KSÜ Fen ve Müh Derg 11 (2), 129-137, 2008
5. Duman AD, Zorlugenç B, Evliya B, Kahramanmaraş'ta kırmızı biberin önemi ve sorunları. KSÜ Fen ve Müh Derg 5 (1), 111-117, 2002.
6. Yemiş O, Bakkalbaşı E, Artık N, Kapsaisinoit kaynağı olarak kırmızı biberler. Gıda Müh Derg 18, 30-37, 2004.
7. Kurita S, Kitagawa E, Kim C-H, Momose Y, Iwahashi H, Studies on the Antimicrobial Mechanisms of Capsaicin Using Yeast DNA Microarray, Biosci Biotechnol Biochem 66(3), 532-536, 2002.
8. Tundis R, Menichini F, Bonesi M, Conforti F, Statti G, Menichini F, Loizzo MR, Antioxidant and hypoglycaemic activities and their relationship to phytochemicals in *Capsicum annuum* cultivars during fruit development, LWT- Food Sci and Technol 53, 370-377, 2013.
9. Paz OJJ, Chavez CLA, Bejar GAA, et al., Effect of heat treatment on the content of some bioactive compounds and free radical-scavenging activity in pungent and non-pungent peppers, Food Res Int p. 1-7, 2011.
10. Ghasemnezhad G, Sherafati M., Payvast A.G. variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (*Capsicum annum*) fruits at two different harvest times, J Funct Food 2011; 3: 44-49.
11. Kadakal Ç, Poyrazoğlu E, Yemiş O, Artık N, Kırmızı biberlerde acılık ve renk bileşikleri, Müh Bil Derg 7 (3), 359-366, 2001
12. Folashade KO, Omoregie EH, Ochogu AP, Standardization of herbal medicines- A rewiev, Int J Biodiver Conser 4(3), 101-112, 2012.

13. European Pharmacopoeia 6.0, Published in accordance with the convetion on the elaboration of a European Pharmacopoeia (European Treaty series No. 50.) European Directorate for the quality of Medicines and Healt Care, Council of Europe, Strasburg, Vol 2,1174-1175, 2008.
14. Çelebioğlu S, Baytop T, Bitkisel Tozların tetkiki için yeni bir reaktif, Farmakolog 1949, 19: 301.
15. Wagner H, Bladt S, Plant Drug Analysis, A Thin Layer Chromatography Atlas, 2nd ed., Honh Kong, 2001.
16. CLSI, 2012.
17. Perucka I, Oleszek W, Extraction and determination of capsaicinoids in fruit of hot pepper *Capsicum annuum* L. by spectrophotometry and high-performance liquid chromatography. Food Chem 71, 287-291, 2000.
18. Jackson BP, Snowdon, DW. Atlas of Microscopy of Medicinal Plants, Culinary Herbs and Spices, Published in London: Belhavan Press, Pp 39-41, 1990.
19. Söğüt Ö, Kaya H, Gökdemir MT et al., Zayıflama amacıyla acı biber hapı kullanımı sonrasında gelişen kardiyotoksisite: İki olgu sunumu. Türkiye Acil Tıp Derg 10 (3), 133-136, 2010.
20. Ağbaş B, Karakuş D, Adıgüzel R, Keser S, Demir E, Tunceli Sarımsağının (*Allium tuncelianum*) Toplam Antioksidan Özelliklerinin ve Kuru Madde İçeriğinin Normal Sarımsak (*Allium sativum*) ile Karşılaştırılması, BGD (Tunceli Üniversitesi) 1 (2), 51-62, 2013.
21. Berber I, Avşar C, Çine N, Bozkurt N, Elmas E, Sinop'ta Yetişen Bazı Bitkilerin Metanolik Ekstraktlarının Antibakteriyal ve Antifungal Aktivitelerinin Belirlenmesi, Karaelmas Sci Eng J 3(1), 10-16, 2013.
22. Dorantes L, Colmenero R, Hernandez H, Mota L, Jaramillo ME, Fernandez E, Solano C, Inhibition of growth of some foodborne pathogenic bacteria by *Capsicum annuum* extracts, Int J Food Mic, 57, 125–128, 2000.
23. Soetarno S, Sukrasno EY, Sukrasno S, Antimicrobial Activities of the Ethanol Extracts of *Capsicum* Fruits with Different Levels of Pungency, JMS 2(2), 57-63, 1997.
24.Keskin D, Toroglu S, Studies on antimicrobial activities of solvent extracts of different species, J.Environ. Biol, 32,251-256, 2011.