Original article

Investigations on Capsicum Annuum L. Samples Purchased From Kayseri Province of Turkey

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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.

Key words: Capsicum annuum, Red pepper, Anti-bacterial activity, Microscopical, Physico-chemical, TLC, Solanaceae.

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).

MATERIAL AND METHODS

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

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.

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 105°C 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 ± 20°C 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 37°C.
weight). Proper scientific evaluation, mandatory safety awareness of the potential hazards of a poor prescription herbal medications without being drug (12). For the purpose of this study, red pepper is an important prerequisite for the production of a quality composition of the drug is a very important quality product. A well-defined and constant preparations are sold on the market without Additionally, a large number of red pepper chemical and anti-bacterial activities. Capsicum annuum monograph in the Peppers and is commonly used as a food additive; and it is also considered to be an antimicrobial agent (7). In addition, phenolics and flavonoids (8).

**Organoleptic analyses**

Red pepper samples were prepared from fresh cultures of the microorganisms according to 0.5 McFarland standard by diluting in normal saline and were inoculated on Mueller Hinton agar (MERCK, Germany). Discs which were prepared were impregnated with plant extracts and standard

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

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

**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\textsubscript{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 |

**Anti-bacterial activity**

Anti-bacterial activities of capsaicin (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 capsaicin (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 capsaicin 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 McFarland 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°C 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.

**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.

**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

| Sample code | General view | Color | Flavor | Photographs |
|-------------|--------------|-------|--------|-------------|
| C-1 | Bright, homogeneous, finely powdered | Crimson | Hot | ![Image](C-1.png) |
| C-2 | Bright, homogeneous, finely powdered | Crimson | Hot | ![Image](C-2.png) |
| C-3 | A little dull, homogeneous, finely powdered | Reddish brown | Hot | ![Image](C-3.png) |
| C-4 | Dull, containing yellow fibrous structures in powdered form | Reddish brown | Sweet | ![Image](C-4.png) |
| C-5 | Dull, light-colored small particles | Light brown | Sweet | ![Image](C-5.png) |
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 capsaicin standard is given in Figure 3. Our results are practically consistent with the literature (13, 15, 17).

**Table 3. Organoleptic analysis and photographs of samples (continued).**

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

* C-10’s local name is “Cırgalan”.

**Antibacterial activity**

Antibacterial activity results of *Capsicum* extracts and capsaicin are shown in Table 4 and Figure 4. Inhibition zones of microorganisms were measured in millimeters (mm).
Table 4. 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                     | -                    | 15±0.33                    | 10±0.33                |
| C-2                     | -                    | 14±0.33                    | 18±0.57                |
| C-3                     | -                    | 11±0.33                    | 14±0.33                |
| C-4                     | -                    | 19±0.33                    | -                      |
| C-5                     | -                    | 18±0.57                    | -                      |
| C-6                     | 20±0.66              | 14±0.66                    | -                      |
| C-7                     | -                    | 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).

Table 5. 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%        |
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- microphenoidal calcium oxalate crystals, J- epicarp of the fruit, K- epidermis in surface view showing pigmented cells, L- parenchyma of the mesocarp (x40).

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).

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.
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 5.

**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 5). 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.

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