

Quality of turmeric powder in herbal stores: pharmacognostical investigations on turmeric powders obtained from herbal stores in Istanbul, Turkey

Ebru Kuruldak¹ , Fatmanur Yılmaz² , Gizem Gülsoy Toplan^{1,3} , Berna Özbek Çelik² , Afife Mat¹ 

¹Istanbul University, Faculty of Pharmacy, Department of Pharmacognosy, Istanbul, Turkey

²Istanbul University, Faculty of Pharmacy, Department of Microbiology, Istanbul, Turkey

³Istinye University, Faculty of Pharmacy, Department of Pharmacognosy, Istanbul, Turkey

ORCID IDs of the authors: E.K. 0000-0003-2041-8810; F.Y. 0000-0001-8442-8538; G.G.T. 0000-0002-0544-2532; B.Ö.Ç. 0000-0001-8909-8398; A.M. 0000-0002-9225-8572

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ABSTRACT

Background and Aims: *Curcuma longa* L., known as Turmeric, has been traditionally used in Asian culture since ancient times to treat several disorders. With the increase in studies on turmeric and its major compounds, it became popular. The study aims to investigate the safety and efficacy of powdered samples collected from 15 different herbal stores in Istanbul.

Methods: Macroscopical, microscopical, bacteriological, and some physicochemical methods were used to evaluate the turmeric powder samples and the extracts of the samples. Additionally, the qualitative determination of curcumin in extracts was carried out by thin-layer chromatography (TLC).

Results: The results of the study show that the powdered samples contain curcuminoids and are also of a moderate quality. The microbiological assay showed us the existence of high levels of pathogens.

Conclusion: Turmeric powder should be consumed carefully, the storage period, and also the origin of the Turmeric is significant in consumption.

Keywords: *Curcuma longa*, turmeric, curcuminoids, TLC, antibacterial activity, ash content

INTRODUCTION

Plants have been used to protect from disorders, treat many illnesses, and also for wellbeing, throughout human history. Today, many of them are still used in phytotherapy, while their secondary metabolites are increasingly gaining attention in the preparation of standardised herbal products or drugs. *Curcuma longa* L., known as Turmeric, has been traditionally used in Asian culture since ancient times to treat several disorders and also as a spice. Turmeric is the most commonly used plant in Ayurvedic medicine and has become popular all over the world by crossing the borders of Asia, with recent studies on its chemical composition and biological activities (Prasad & Aggarwal, 2011). With the growing interest, it is easy to reach turmeric products via pharmacies, the internet, and herbal stores.

Curcuma longa is one of 133 species of *Curcuma* which belong to the Zingiberaceae family. This perennial herb is native to India and Southeast Asia. Its rhizomes and oil have significant value (Prasad & Aggarwal, 2011). Ayurvedic remedies are used for many disorders such as: the improvement of digestion problems, irritable bowel syndrome, some liver diseases and the dissolution

Address for Correspondence:
Afife MAT, e-mail: afifemat@gmail.com

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of gallbladder stones, some respiratory diseases such as runny noses, coughs, and sinusitis. They are also used for increasing the general energy of the body, regulating the menstrual cycle, the treatment of asthma, relieving arthritis, the calming of allergies and the treatment of diabetic wounds. Turmeric is also commonly used as a spice in South Asian and Middle Eastern food culture (Prasad & Aggarwal, 2011). Additionally, it is used as a dyeing agent in the textile and cosmetic industries due to possessing a bright yellow colour.

In recent years, several studies showed that the extracts and secondary metabolites of *Curcuma longa* possess remarkable anti-microbial, antifungal, antiviral, anti-inflammatory, anti-diabetic, neuroprotective, cardioprotective, gastroprotective, and especially anti-cancer effects (Aamon & Wahi, 1991; Labban, 2014; Gounder, & Lingamallu, 2012). Although some mechanisms for its biological activity have been elucidated, many mechanisms are not yet known and studies need to be conducted (Aamon & Wahi, 1991). The main mechanisms of these biological activities are based on increasing the level of antioxidant enzymes in the blood, the inhibition of lipid peroxidation, the scavenging effect of oxygen radicals, stimulation of COX2 activation, inhibition of TNF-alpha and nitric oxide secretion, and increasing collagen secretion (Araújo & Leon, 2001; Becit, Aydın, & Başaran, 2017)

In previous studies, more than 235 compounds, primarily phenolic compounds, and terpenoids were identified in *Curcuma* species (Li et. al., 2011). The chemical composition of *Curcuma longa* has been extensively studied revealing two major groups, curcuminoids and volatile oil, which are explored as essential components for biological activities. Curcumin, demethoxycurcumin, and bisdemethoxycurcumin are the major curcuminoids isolated from turmeric (Jayaprakasha, Rao & Sakariah, 2005). These curcuminoid pigments are responsible for the yellow colour in plants. The essential oil of Turmeric shows variety in its chemical composition which gives its aromatic odor and taste. According to studies, the major components of essential oil were determined as *ar*-turmerone, alpha-turmerone, beta-turmerone, zingiberene, zingiberone, and curlone (Jayaprakasha, Rao & Sakariah, 2005; Singh et. al., 2010). The essential oil also contains germacrone, sabinene, eucalyptol, borneol, and sesquiphellandrene (Jayaprakasha, Rao & Sakariah, 2005, Raina et al., 2002). As a result of many studies, having the great potential of anti-inflammatory and anti-cancer activities of turmeric is attributed to curcuminoids and volatile oil.

Due to the increase in scientific studies on turmeric and understanding of its potential, the demand for its products has increased around the world (Pothitirat & Gritsanapan, 2006). Today, it is easy to find turmeric products such as powdered and fresh rhizomes, tablets, capsules, and also liquid forms. The usage of powdered turmeric is quite popular and also easily accessible in Turkey. It is commercially available and is sold in open or packed forms, both in herbal stores and in markets, but without quality, safety, and also bacteriological control.

This study aims to investigate the safety and efficacy of powdered turmeric samples by macroscopical, microscopical, bacteriological, chemical, and some physical methods. 15 differ-

ent powdered turmeric samples were collected from several herbal stores in both Asian and European sides of Istanbul and their quality was evaluated according to Turkish pharmacopeia (Turkish Pharmacopoeia Journal, 2016).

MATERIALS AND METHODS

Plant material

Turmeric samples were purchased from different herbal stores in Istanbul. The analysis was conducted on 15 different samples.

Macroscopical evaluation

Evaluations were made for each turmeric sample in terms of colour, odour, taste, and appearance. The expected colour of the powder is orange-yellow. Spicy is described as scented. It tastes an aromatic, slightly bitter taste of turmeric. In terms of appearance, it should not contain macro particles.

Microscopical evaluation

When examined with the Sartur reagent under a microscope, it usually contains starch grains gelatinised and collected in starch paste; rarely it is possible to observe ovoid starch granules. When examined with chloral hydrate solution, specific characteristics of turmeric can be observed. These are as follows:

- a. Fragments of parenchyma containing secretory cells containing brown-yellow lipid masses
- b. Reticulated or dimpled xylem
- c. Rare pieces of the epidermis, traces of cover hairs covering the walls with light and irregular thickened cells; rarely long and warped, thick-walled, single-celled trichomes shredded or attached to free or epidermal cells
- d. Rarely long and curved, thick-walled, single-celled trichomes; shredded, free, or attached to epidermal cells
- e. Rare periderma pieces, sometimes covered with the epidermis (Turkish Pharmacopoeia Journal, 2016).

Physicochemical analysis methods

Preparation of the extracts

10 mL of ethanol (96%) was added to 1 g of a powdered sample, shaken in the ultrasonic water bath which was allowed to stand for 30 minutes at room temperature. It was then filtered by cotton. The filtrate was used for the analysis (Turkish Pharmacopoeia Journal, 2016).

Reference solution

Novasol Curcumin Licaps (liquid-filled encapsulation) capsule was used as a reference. The liquid capsule containing 20 mg of curcuminoids is diluted with 10 mg of ethanol (96%) (check again) .

Thin-layer chromatography

The thin-layer chromatographic method was used to determine the existence of curcuminoids in the samples. The turmeric samples and diluted reference solutions were applied to the silica gel plate in 10 mm bands. The combination of glacial acetic acid and toluene (20:80) was used as a mobile phase. Af-

ter the saturation of the chromatogram tank with the solvent system at least 30 min. TLC plates were developed until the solvent front was ± 1 cm from the top of the plate. Later, the plate is allowed to dry on the fume hood. Dry plaque is examined at 245 and 365 nm under ultraviolet light. It is then treated with anisaldehyde solution and heated at 100-105°C for 10 minutes; afterward, it is examined under ultraviolet light again (Turkish Pharmacopoeia Journal, 2016).

Determination of water content

A glass weighing container for each sample was passed through ethanol and allowed to dry in the etuve. Weighing dried vessels were left in the desiccator for 30 minutes. Then, each container is weighed first empty, and then with 1 g of substance. Each weighed sample was left to the desiccator to wait until it is taken to the oven. After the weighing of all samples has been completed, it was left in the oven at 100-105°C for 2 hours. At the end of 2 hours, the samples are taken and left to desiccate again for 30 minutes. Then each sample is weighed again. Yield calculation is made. As a result of the determination of the amount of water with 15 gr of the powdered herbal drug is detected at a maximum of 120 mL/kg (Baytop T., 1980).

Determination of ash content

For each sample, a porcelain crucible is passed through ethanol and allowed to dry in the oven. The crucible dried in the oven is left in the desiccator for 30 minutes. Each crucible is then weighed empty and with 1 g of sample. Each weighed crucible is taken to the oven. It is left in the oven for 1 hour at 200°C and then for 3 hours at 600°C. At the end of 4 hours, the samples in the oven are left in the desiccator again for 30 minutes to cool down. Weigh the cooled samples and calculate the yield. As a result of ash determination, total ash should be at most 7.0% (Baytop T., 1980).

Bacteriological tests

Antimicrobial activities of the extracts

Microorganisms: The American Type Culture Collection (ATCC) standard strains of *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352 and as a representative of fungi, the yeast, *Candida albicans* ATCC 10231 were used in the experiments. Inoculums of bacteria and *C. albicans* were prepared with overnight cultures, for producing a concentration of 1×10^8 colony-forming units (cfu/ml) and 1×10^7 cfu/ml, respectively.

Media: Cation-adjusted Mueller-Hinton broth (CAMHB, Difco Laboratories) and RPMI-1640 medium (Sigma) buffered to pH 7.0 with morpholine propane sulfonic acid (MOPS, Sigma) were used to determine the minimum inhibitory concentration (MIC) of bacteria/spore suspension and yeast, respectively, and tryptic soy agar (TSA, Difco Laboratories) was used for colony counts.

Determination of minimum inhibitory concentrations

(MIC): *In vitro* antibacterial activities of 14 different turmeric extracted samples against *S. aureus* ATCC 29213, *E. faecalis* 29212, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *K. pneumoniae*

ATCC 4352; antifungal activities against *C. albicans* ATCC 10231 were investigated. MICs of compounds were determined by the micro broth dilution technique as described by the Clinical and Laboratory Standards Institute (CLSI 2000 & 2006). Molecules were dissolving in Dimethyl sulfoxide (DMSO, Sigma), and serial two-fold dilutions of molecules ranging from finally 5000 to 5 µg/mL or 500 to 0.5 µl/mL were prepared in Mueller-Hinton broth (MHB) for bacteria or spores, and RPMI-1640 medium for yeast. Each well was inoculated with 50 µL of a 4-6 h broth culture that gave a final concentration of 5×10^5 cfu/mL for bacteria or spores, and 5×10^3 cfu/mL for yeast in the test tray. The trays were covered and placed in plastic bags to prevent evaporation. The trays containing MHB were incubated at 37°C for 18-24 h, those containing RPMI-1640 medium at 37°C for 48 h. The MIC was defined as the lowest concentrations of compounds producing complete inhibition of visible growth. DMSO was used as a negative control in assays. Levofloxacin and fluconazole were used as reference antibiotics for bacteria and yeast, respectively.

Microbiological content determination

Turmeric samples collected from various transfers and numbered from 1 to 15 were weighed 1 gram and dissolved with 10 ml sterile distilled water. Then, ten-fold dilutions were made using sterile saline. 10 µl of dilutions were made, the Tryptic Soy Agar (TSA) for bacteria and Saboroud Dextrose Agar (SDA) for fungi spread on the solid broth surface and was left for incubation at 37°C for bacteria and 25°C for fungi in the oven. The colonies formed the following day were counted to determine the total number of live bacteria, and the number of total aerobic bacteria and fungi in one gram of turmeric was calculated in terms of the colony-forming unit (CFU) taking into account the dilution factor (Omurtag, 1966).

RESULT AND DISCUSSION

Macroscopical evaluation

The samples, which is shown in Figure 1 are numbered from 1 to 15. The appearance, odor, aromatic taste, and colour were determined for each sample. All turmeric samples possess an orange-yellow colour, aromatic, slightly bitter taste. The visible particles were not detected in most of the samples, only four samples (3, 9, 12, 14) contained minor black dirties.

Microscopical evaluation

Microscopical characters of each sample were evaluated by Sartur reagent and chloral hydrate solution. Examination under the microscope showed us the presence of gelatinised starch grains, reticulated or dimpled xylem, and fragments of parenchyma containing secretory cells containing brown-yellow lipid masses. Additionally, phelloderm pieces covered with epidermis were identified in some samples. The microscopical appearance of samples is shown below.

Physicochemical analysis results

TLC profiles of the extracts

Thin-layer chromatography plaque was examined under daylight and also 245 nm and 365 nm ultraviolet light. Consequently, three different curcumin compounds were identified

in each sample. Comparing the reference solution and extracts it is revealed that all samples contain significant curcuminoids which are generally used for standardising turmeric products (Figure 2).

Determination of water content

The percentage of total water was calculated from the amount of the sample before drying and the remaining amount after drying. The percentage of total water for turmeric is 15 g. for the sample it should be a maximum of 120 mL/kg. This corresponds to 10.71%. It was observed that all of the samples gave results below the maximum value (Table 1).

Determination of ash content

The total ash percentage was calculated over the amount of the sample before burning and the remaining amount after burning. The total ash percentage for turmeric should be a maximum of 7.0%. When evaluated according to this, it was observed that five samples (2, 3, 6, 11 and 14) left ash more than this percentage.

Sample No	Macroscobical Assessment	Microscobical Assessment
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		

Figure 1. The macroscobical and microscobical appearances of 15 powdered turmeric samples. a. parenchyma pieces including brown-yellow lipids, b. reticulated or dimpled xylem, c. single-celled trichomes attached to epidermis, d. periderma pieces, sometimes covered with parenchyma.

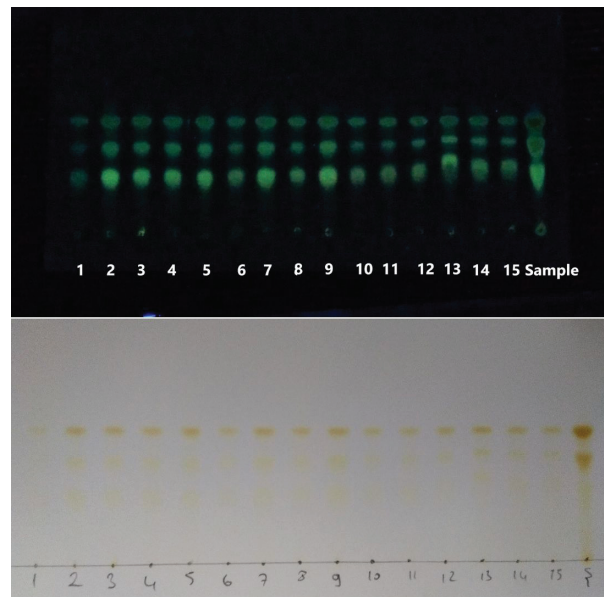


Figure 2. TLC chromatograms of samples under daylight (below) and 365 nm ultraviolet light (above).

Anti-microbiological activity

The MIC values of the extracted samples against the microorganisms which are representative for common infectious agents were obtained from susceptibility testing using the micro broth dilution technique, and they are summarised in Figure 3 below. The MIC values of the levofloxacin and fluconazole were within the accuracy range (CLSI, 2014) throughout the study.

Microorganisms	1	2	3	4	5	6	7	8	9	10	11	12	14	15
<i>S. aureus</i> ATCC 29213	312,5	312,5	312,5	625	625	312,5	312,5	312,5	312,5	312,5	312,5	156,25	156,25	312,5
<i>E. faecalis</i> ATCC 29212	625	625	312,5	156,2	312,5	78	156,2	156,2	78	78	625	625	1250	625
<i>E. coli</i> ATCC 25922	312,5	625	312,5	312,5	1250	625	625	625	312,5	312,5	312,5	625	625	156,2
<i>K. pneumoniae</i> ATCC 4352	625	625	312,5	312,5	625	312,5	312,5	156,2	312,5	312,5	625	625	625	312,5
<i>P. aeruginosa</i> ATCC 27853	625	312,5	312,5	312,5	625	312,5	625	312,5	312,5	312,5	312,5	625	312,5	625
<i>C. albicans</i> ATCC 10231	625	312,5	312,5	625	312,5	312,5	625	625	312,5	625	312,5	625	625	625
<i>S. epidermidis</i> ATCC 12228	1250	1250	1250	625	1250	1250	625	625	625	1250	625	625	1250	625

Figure 3. Antimicrobial activities of substances (µg/mL).

Microbiological content determination evaluation

Microbial count results are calculated. The number of bacteria in 1 gram of turmeric should not exceed 10⁴ CFU/gr and the amount of mould should not exceed 10³ CFU/gr (USP DSC, 2012). Only two samples were in the normal range for the fungi count results, and the rest of the samples had values above normal. All samples had shown above-normal results in bacterial count results.

CONCLUSION

Recently, turmeric has gained attention all around the world and that makes its products quite popular and accessible. However, determinations of the quality of these turmeric

Table 1. Ash content determination, water content determination, bacterial count and fungi count values of 15 samples.

Sample no	Ash content determination values	Water content determination values	Fungi count values (CFU/gr)	Bacterial count values (CFU/gr)
1	4,86 %	9,02 %	2,6*10 ⁴	3,1*10 ⁸
2	% 7,65 %	9,43	1,75*10 ⁴	9,5*10 ⁷
3	7,22	8,92 %	4*10 ³	2,85*10 ⁶
4	6,12 %	8,89 %	6*10 ³	2,7*10 ⁶
5	6,91 %	9,19 %	5*10 ²	1,15*10 ⁶
6	7,55 %	9,16 %	2,2*10 ⁵	2,95*10 ⁷
7	5,91 %	9,56 %	5*10 ³	3,2*10 ⁶
8	6,01 %	7,91 %	0	1,2*10 ⁶
9	5,64 %	9,76 %	6*10 ³	4,3*10 ⁶
10	2,51 %	9,60 %	1,7*10 ⁴	1,35*10 ⁶
11	7,59 %	9,11 %	5*10 ³	1,35*10 ⁶
12	5,18 %	8,80 %	4,5*10 ³	2,4*10 ⁶
13	4,90 %	9,54 %	1,05*10 ⁴	1,95*10 ⁶
14	7,80 %	9,02 %	4*10 ³	1,15*10 ⁷
15	4,79 %	8,37 %	1,5*10 ⁴	3,5*10 ⁷

products are insufficient, hence pharmacognostical studies on these samples should be carried out to evaluate the reliability and effectiveness.

The most used form of turmeric in Turkey is the powdered form due to its cheap price and easy consumption. The most important factors determining the medicinal effect are the source of the plant, usage form (fresh or dried), and its chemical composition. The macroscopical and microscopical evaluations confirm the powdered samples belong to the rhizome of turmeric. The presence of major curcuminoids is also detected by TLC. According to total ash and water content value, samples were found almost within acceptable limits, no adulteration or foreign materials were observed. Although physicochemical evaluations have confirmed that the powdered samples contain curcuminoids and are also of medium quality, microbiological tests have shown us the presence of high levels of bacteria and fungi. Our microbiological identification studies have shown that these microorganisms are spore bacteria belonging to *Bacillus* species from saprophytic bacteria. The fungi that cause microbial contamination in plant samples were determined as mould fungi. For products offered in open packaging, this pollution is an expected result. Turmeric is not cultivated in our country; the contamination might have consisted of import stages or storage conditions in herbal stores.

As a result of this study in which 15 different samples were evaluated, a big question mark has occurred about the consumption of the open turmeric powders neither as a spice nor for medicinal purposes.

In conclusion, we may say that it is recommendable to buy turmeric rhizomes and to powder it before using it at home.

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