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Biological Activity of Curcuminoids Isolated from *Curcuma longa*

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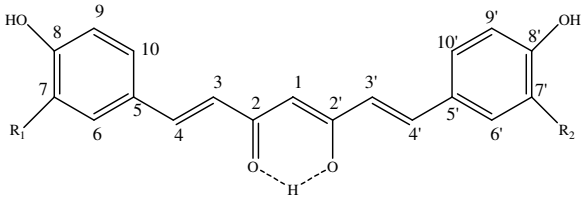
Abstract: Curcumin is the most important fraction of turmeric which is responsible for its biological activity. In this study, isolation and biological assessment of turmeric and curcumin have been discussed against standard bacterial and mycobacterial strains such as *E.coli*, *S.aureus, E.feacalis, P.aeuroginosa, M.smegmatis, M.simiae, M.kansasii, M. terrae, M.szulgai* and the fungi *Candida albicans.* The antioxidant activity of curcumin and turmeric were also determined by the CUPRAC method.

Keywords: Curcumin, turmeric, *Curcuma longa*, antimicrobial activity, antioxidant activity, CUPRAC method

1. Introduction

Turmeric is a spice which is obtained from rhizomes of plant *Curcuma longa*, a member of the family Zingaberaceae. Components of tumeric are named curcuminoids, which include mainly curcumin (diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin [1] (Figure 1).



Compound	R_1	R_2
Curcumin	OMe	OMe
Demethoxycurcumin	H	OMe
Bisdemethoxycurcumin	H	H

Figure 1. Chemical structures of curcuminoids.

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Turmeric consists of 3-5% curcuminoids. Curcumin is the most important fraction which is responsible for the biological activities of turmeric. The melting point of curcumin, $C_2H_{20}O_6$, is 184°C. It is soluble in ethanol and acetone, but insoluble in water [2]. Curcumin exists in solution as keto-enol tautomers [3]. Because of its biological activities, a large number of studies have been presented on curcumin. According to these studies, curcumin exhibits antiinflammatory [1] antioxidant [4, 5, 6, 7] anticarcinogenic [8] antiviral [9] antimicrobial activity [10, 11]. Beside these, curcumin has a variety of potentially therapeutic properties, such as antineoplastic, antiapoptotic, antiangiogenic, cytotoxic, immunomodulatory, [12] and antithrombotic, wound healing, antidiabetogenic, antistressor and antilithogenic actions[1].

In this study, isolation and biological assessment of turmeric and curcumin have been discussed against standard bacterial and mycobacterial strains such as *E.coli*, *S.aureus, E.feacalis, P.aeuroginosa, M.smegmatis, M.simiae, M.kansasii, M. terrae, M. szulgai* and the fungi *Candida albicans.* The antioxidant activity of curcumin and turmeric were also determined by the CUPRAC method.

2. Materials and Methods

2.1. Reagents and Equipment

Ammonium acetate, copper (II) chloride and neocuproine (2, 9-dimethyl-1,10-phenanthroline) were purchased from Sigma-Aldrich company. Preparation of the solutions has been reported in the literature [13, 14]. Beckman Coulter DTX 880 multimode optical spectrometer was used for the antioxidant activity assays. Mueller-Hinton Broth and Middlebrooke 7H9 Broth (purchased Salubris Inc. as prepared media.) were used for the antimicrobial activity assays. The different curry and "zerdaçal" (*C.longa*) powders were purchased from the local market. Turmeric 1 was extracted from curry powder bought from Istanbul, Turmeric 2 was extracted from "zerdaçal" (*C.longa*) powder bought from Gebze-Kocaeli, Turmeric 3 is commercial turmeric extract from UK and Turmeric 4 is commercial turmeric extract from India.

2.2 Extraction Procedure

Twenty grams of curry and "zerdaçal" (*C.longa*) powder was magnetically stirred in 50 mL of dichloromethane and refluxed for 1 hour. After filtration, the filtrate was concentrated in hot water bath at 50 °C. The reddish- oily residue was pulverized with 20 mL of hexane and the resulting solid was collected by suction filtration. The presence of three compounds was shown by TLC analysis (3 % methanol- 97 % dichloromethane)(R_f values are 0,49; 0,22; 0,085) [16]. This procedure was applied to different curry powders purchased from local market. ^{1}H NMR (DMSO): 3.90 (6H, s), 6.06 (1H, s), 6.76(2H, d), 6.82 (2H, d), 7.15 (2H, d), 7.32 (2H, s), 7.55 (2H, d), 9.70 (2H, br s)

2.3 *Biological Assessment*

2.3.1 *Antibacterial, antimycobacterial and antifungal activity:*

Extracts were tested against the following standard bacterial strains *S.aureus* ATCC 25923, *E.feacalis* ATCC 11700, *P.aeruginosa* ATCC 27853, *E.coli* ATCC 25922, *C.albicans* ATCC 10231 and *M.terrae*, *M.szulgai*, *M.kansasii*, *M.simiae*, *M.smegmatis* for the determination of their antimicrobial activies. The tube dilution procedure [15], outlined by National Committee for Clinical

Laboratory Standards, was used for the determination of antimicrobial activities of the compounds. The compounds were dissolved in 10 mL of ethanol and the solutions were transferred into 5 mL of Mueller Hinton Broth (Middlebrooke 7H9 for the mycobacteria) to give a final concentration of 200-25 μg/mL in test tubes. Then 100 μl of 10^6 colony forming units (cfu/mL) (according to McFarland turbidity standards) of standardized microorganism suspensions were inoculated into tubes. The same test was carried out with ethanol solution as a control. End-point was determined after incubation at 37 °C for 24 h. The complete absence of growth was considered as the minimum inhibitory concentration (MIC). Three replicates were used in the determination of MIC.

2.3.2 Total Antioxidant Capacity by CUPRAC Method

The CUPRAC method was used for the determination of antioxidant capacity of the compounds and described below briefly. Fresh solutions of the compounds were prepared in DMF (1 x 10^{-3} M). 1 mL of 10^{-2} M of $CuCl_2$, 1 mL of 7.5 x 10^{-3} M neocuproine and 1 M NH_4CH_3COO solution were added into the glass test tube. Then, 400 μL of freshly prepared solution was added and diluted to the final volume of 4.1 mL with deionized water. This procedure was repeated for 400 μL, 300 μL, 200 μL, 100 μL and 50 μL addition of freshly prepared solutions of the tested compounds. The prepared solutions mixed and incubated at room temperature for 30 minutes. After the incubation, 200 μL of solution from each tube was transferred to the 96 well plate. The absorbance at 450 nm was determined against a reagent blank by Beckman Coulter DTX 880 spectrometer.

The calculation of antioxidant capacity of compounds as Trolox equivalents (TEAC values) by the CUPRAC method has been reported in the literature, and briefly described herein. ε_{TR} values of compounds were determined from the linear regression equation as described in the literature [14].

3. Results and Discussion

In this study, turmeric was isolated from curry and "zerdaçal" (*C.longa*) powders that were bought from local market, extracted and the main compound was isolated. Their biological and antioxidant activities have been studied.

The moderate antibacterial and antifungal activity have been determined for the turmeric extracts and pure curcumin. None of the extracts and curcumin showed any activity against the gram negative bacteria *E.coli* and *P.aeuroginosa*. The results, found by MIC method were shown in Table1.

Table 1. Antibacterial and Antifungal Activities by MIC method (μg/ mL)

Samples	*E.coli*	*S.aureus*	*E.feacalis*	*P.aeuroginosa*	*C.albicans*
Turmeric 1	NA	256	512	NA	512
Turmeric 2	NA	256	512	NA	512
Turmeric 3	NA	128	512	NA	512
Turmeric 4	NA	128	1024	NA	512
Curcumin	NA	128	1024	NA	512
Gentamycin	4.0	0.48	3.2	1.0	-
Flucanozole	-	-	-	-	18

Non-tuberculosis mycobacteria (e.g. *M. kansasii, M.terrae ve M simiae, M.smegmatis*), that are isolated from urban water, are a frequent cause of epidemic diseases, particularly infections after surgical and dialysis operations. It is important to take caution against these common non-tuberculosis mycobacteria and also to find diagnosis methods and provide curing agents [19, 20]. In the literature, the antimycobacterial activities of curcumin were studied against *M. tuberculosis* and *Mycobacterium bovis* [17, 18], In this study, antimycobacterial activities of turmeric extracts and curcumin were evaluated against *M. smegmatis, M. simiae, M. kansasii, M. terrae and M. szulgai* by MIC method (Three replicates). All of the isolated turmeric extracts and curcumin showed very weak activity against the studied mycobacteria (Table 2)

Table 2. Antimycobacterial Activities by MIC method (µg/ mL)

Samples	*M.smegmatis*	*M.simiae*	*M.kansasii*	*M. terrae*	*M.szulgai*
Turmeric 1	512	512	128	512	256
Turmeric 2	1024	512	NA	256	256
Turmeric 3	1024	NA	64	256	128
Turmeric 4	1024	NA	NA	256	128
Curcumin	1024	NA	128	256	NA
Rifampicin	NT	NT	2	8	16

In the literature, the antioxidant activity of curcumin, was determined in two standart assays; TRAP assay (total radical trapping antioxidant parameter assay), FRAP assay (ferric reducing/antioxidant power assay) with Trolox as reference standard [21]. The calculation of antioxidant capacity of compounds as Trolox equivalents (TEAC values) by the CUPRAC(cupric reducing antioxidant capacity) method has also been reported in the literature, and briefly described herein. CUPRAC is a simple method for application of the antioxidant capacity assay for dietary polyphenols, vitamin C and vitamin E utilizing the copper (II)- neocuproine (Cu(II)-Nc) reagent as the chromogenic oxidant [14]. Then we decided to evaluate antioxidant activity of turmeric extracts and curcumin via CUPRAC method. All of the turmeric samples and the pure compound curcumin showed very good antioxidant activity (Table 3).

Table 3. Total Antioxidant Capacity by CUPRAC Method

Samples	$TEAC_{CUPRAC}$
Turmeric 1	0,7
Turmeric 2	0,7
Turmeric 3	0,6
Turmeric 4	0,7
Curcumin	0,8
α-Tocopherol	0,95
Hydroquinone	0,97

In conclusion, turmeric was extracted from different curry and "zerdaçal" (*C.longa*) powders that were bought from local market and the main components were isolated. Their biological and antioxidant activities have been studied. The moderate antibacterial and antifungal activity have been determined for the turmeric extracts and pure curcumin. Antimycobacterial activities have been evaluated against *M. smegmatis, M. simiae, M. kansasii, M. terrae and M. szulgai* by MIC method and the antioxidant activity have been evaluated via CUPRAC method. All of the isolated turmeric extracts and pure curcumin showed very weak activity against the studied mycobacteria but showed very good antioxidant activity.

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