



Curcumin: Natural Antimicrobial and Anti Inflammatory Agent

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Authors' contributions

This work was carried out in collaboration among all authors. Author PB designed the study, performed the experiment and wrote the first draft of the manuscript. Author ČK manage the analyses of the study. Authors LD, ON, ŽSN, ŠS and BF manage the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The pursuance of novel antimicrobial and anti-inflammatory agents has been expanding due to a significant need for more efficient pharmacotherapy of various infections and chronic diseases. During the last decade, pharmacokinetics, pharmacodynamics and pharmacological properties of curcumin have been extensively studied. The aim of the present study was to evaluate the antibacterial activity of curcumin against both Gram-positive and Gram-negative bacteria as well as its antifungal activity by using *in vitro* agar well diffusion assay. Moreover, the anti-inflammatory activity of curcumin was determined with *in vitro* assay of inhibition of protein denaturation. Results demonstrated wide antimicrobial activity of curcumin upon all of the test bacteria and fungi. The strongest activity of curcumin was observed at a concentration of 0.50 mg/ml against *S. aureus*, *L. monocytogenes*, *E. coli*, *P. aeruginosa* and *C. albicans*, resulting in a maximum zone of inhibition of 14.7 mm, 14.3 mm, 13.7 mm, 10.7 mm and 10.7 mm, respectively. Findings suggested that the

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antimicrobial activity of curcumin is dependent upon the concentrations. Furthermore, results demonstrated high effectiveness of curcumin compared to standard acetylsalicylic acid in inhibiting heat-induced protein denaturation, which activity is also dependent upon the concentrations. The present study emphasises the potential application of curcumin as a natural antimicrobial and anti-inflammatory agent. However, findings of this study are restricted to *in vitro* assays and consideration should be given to conducting a study involving wider dose range test substances as well as including further research on *in vivo* models.

Keywords: Curcumin; *in vitro*; antimicrobial agent; anti-inflammatory agent.

1. INTRODUCTION

Rational phytotherapy implies the use of natural products, which therapeutic efficiency and safety are based on scientific evidence [1]. Various natural products and phytochemicals have been investigated for different pharmacological properties in order to serve as a potential source for novel medicines [2]. Lately, due to the continuing increase of infections and chronic diseases worldwide, the detection of potential agents is of crucial importance [3]. The pursuance of novel antimicrobial and anti-inflammatory agents has been expanding due to a significant need for more efficient pharmacotherapy of various infections and chronic diseases. Numerous *in vitro* and *in vivo* studies have shown that phytochemicals possess a wide range of pharmacological activity including antimicrobial and anti-inflammatory activity [4-6]. Investigation of phytochemicals with potential antimicrobial and anti-inflammatory activity is rapidly growing and therefore leads to more frequent substitutions of synthetic medicines due to the development of resistance and risk of side effects [7].

Curcumin or diferuloylmethane (1*E*,6*E*)-1,7-bis (4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione) is bioactive phytochemical present in the rhizomes of plant *Curcuma long L.*

Zingiberaceae (Fig. 1). Curcumin belongs to a group of curcuminoids – natural phenols responsible for yellow colour. It has been used as a spice and traditional medicine in Ayurvedic medicine [8,9]. During the last decade, pharmacokinetics, pharmacodynamics and pharmacological properties of this yellow-orange polyphenolic compound have been extensively studied. Significant antitumor, antioxidant, antiviral, lipid-lowering, chemopreventive, hepatoprotective and neuroprotective properties of curcumin have been confirmed [10-13]. Recent studies have also confirmed the pharmacological activity of curcumin as anti-inflammatory and immunomodulatory agent [14]. It is established that mechanism of anti-inflammatory activity of curcumin occurs through the inhibition of cyclooxygenase-2 (COX-2) and lipoxygenases (LOX), and the induction of nitric oxide synthase (iNOS). Also, it is suggested that curcumin inhibits the action of inflammatory cytokines, such as interleukins and chemokines [15,16]. Due to its pronounced anti-inflammatory activity, curcumin is considered to be a potential mediator of accelerating the healing process of acute and chronic wounds and may inhibit the production of tumor necrosis factor-alpha (TNF- α) and TNF- α -mediated cellular signalling pathway [16]. Antimicrobial potential of curcumin has been evaluated against a wide range of microorganisms, including both Gram-positive

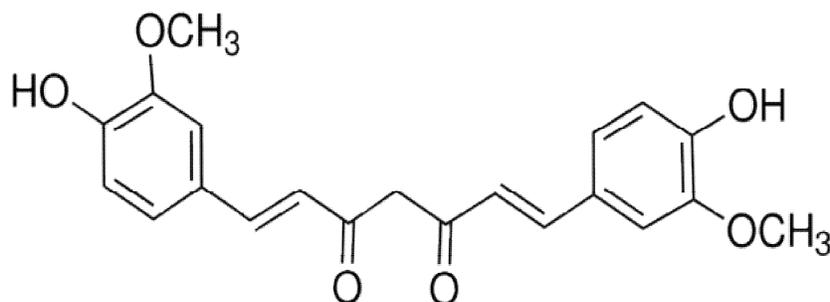


Fig. 1. Curcumin – structural formula

and Gram-negative bacteria as well as the fungi [14,17,18]. Curcumin has been described as a promising antifungal of clinical interest with stronger antifungal activity than fluconazole [19]. A recent study has suggested a possible mechanism of antifungal activity of curcumin through photodynamic technology and oxidative stress [20]. Still, the mechanisms of antimicrobial activity of curcumin have not been precisely determined. Some authors suggested that antibacterial mechanisms of curcumin are mediated by inhibition of bacterial cell proliferation, resulting in blocking Z-ring formation dynamics [21]. Others implied that its activity is due to the effects on virulence and biofilm propagation [22]. The mechanisms by which curcumin exhibits its antimicrobial activity differ depending on the strain and therefore can not be generalised [18,23].

The aim of the present study is to evaluate the antibacterial activity of curcumin against both Gram-positive and Gram-negative bacteria as well as its antifungal activity by using *in vitro* agar well diffusion assay. Moreover, the present study estimated anti-inflammatory activity of curcumin with *in vitro* assay of inhibition of protein denaturation.

2. MATERIALS AND METHODS

Curcumin was obtained from Sigma-Aldrich (CAS No: 458-37-7; St. Louis, MO, USA). Ethanol (puriss. p.a., $\geq 99.8\%$) was purchased from Sigma-Aldrich; dimethylsulfoxide (DMSO) (99.5% Ph. Eur., M=78.13) from Semikem d.o.o. (BiH); nutrient agar and antibiotic discs were purchased from Laboratorios Conda S.A. All other chemicals used were of the highest analytical grade available. Spectrometer Lambda 25 UV/VIS, Perkin Elmer, was used for measuring absorbance and incubator Lab-Line Imperial III, Barnstead, USA, for incubation of Petri plates.

2.1 *In vitro* Antimicrobial Activity

Antibacterial activity of curcumin was evaluated with *in vitro* agar well diffusion assay, against both Gram-positive and Gram-negative bacteria, as well as the antifungal activity. The following strains were obtained from American Type of Culture Collection (ATCC): *S. aureus* (ATCC6538), *L. monocytogenes* (ATCC 35152), *P. aeruginosa* (ATCC 9027), *E. coli* (ATCC8739) and *C. albicans* (ATCC10231). During the preparation of inoculums, suspensions of microorganisms were adjusted on approximately

1.5×10^8 CFU/ml and cultured on nutrient agar. In every Petri plate 4 wells of 6 mm diameter were created with sterile borer. Curcumin was dissolved in DMSO and prepared in form of solutions at the following concentrations: 0.10, 0.25, 0.35 and 0.50 mg/ml. In each well, a total volume of 100 μ l of test curcumin solutions was applied. The volume of 100 μ l of DMSO was used as negative control and applied into the wells. Following standard antibiotics were applied as positive control: Chloramphenicol (30 μ g/disc), Cefprozime (30 μ g/disc), Amikacin (30 μ g/disc) and Nystatin (100 μ g/disc).

After application of test substances, Petri plates containing bacteria were incubated at 37 °C for 18–24h and plates with fungi at 25°C for 48h. After the incubation period, the diameter of zone of inhibition (mm) was measured and recorded for each plate. The experiment was performed in triplicate.

2.2 *In vitro* Anti-Inflammatory Activity

Anti-inflammatory activity of curcumin was evaluated with *in vitro* assay of heat-induced inhibition of protein denaturation. Curcumin was dissolved in DMSO and prepared as test solutions at the final concentrations: 100, 200, 300, 400 and 500 μ g/ml acetylsalicylic acid was used as positive control and prepared at the same concentrations as the test curcumin solutions. The reaction mixture contained 2.0 ml of the test solution, 2.8 ml of phosphate buffer saline with adjusted pH 6.4 and 0.2 ml of egg albumin. Control mixture contained 2.0 ml of distilled water instead of the test solution. The mixtures were incubated for 15 min at $37 \pm 2^\circ\text{C}$ and then heated for 5 min at 70°C . After cooling down at the room temperature, absorbance was measured at 660 nm [24]. Measurements were performed in triplicate. Anti-inflammatory activity was expressed as the percentage of inhibition of protein denaturation and calculated by using the following formula:

$$\text{Inhibition (\%)} = 100 \times (1 - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}})$$

3. RESULTS AND DISCUSSION

3.1 *In vitro* Antimicrobial Activity

Due to increased development of multidrug-resistance microorganisms, there is a constant need for novel antimicrobial agents derived from different sources [3]. So far, phytochemicals have remained the leading source for potential

antimicrobial agents, whether investigated independently or in a combination with another substance [2,25]. Recent studies have demonstrated a wide range of antimicrobial properties of curcumin mediated by different mechanisms, such as alteration of gene expression, inhibition of bacterial DNA replication and disruption of the bacterial cell membrane [26-28]. Literature data demonstrate the beneficial and preventive role of curcumin against various microorganisms [29-31]. Despite various studies evaluating the antimicrobial activity of curcumin, further research is required with different concentrations against different strains of microorganisms. In this study, an *in vitro* agar well diffusion assay was used to assess the antimicrobial activity of test curcumin solutions at different concentrations. Antimicrobial activity of curcumin solutions was determined by the presence and size of zones of inhibition against test strains of bacteria and fungi. Results of the measurement of diameters of zones of inhibition (mm) of curcumin solutions are given in Table 1. For negative control, no zones of inhibitions were observed while positive control, standard antibiotics, demonstrated the most efficient inhibition of microbial growth (Table 2).

Test curcumin solutions showed antimicrobial activity upon all of the test bacteria, both Gram-positive and Gram-negative, as well as the fungi. Compared to the positive control, curcumin solutions were less efficient in suppressing microbial growth. However, the strongest activity of curcumin solutions was observed at a concentration of 0.50 mg/ml against *S. aureus*, *L. monocytogenes*, *E. coli*, *P. aeruginosa* and *C. albicans*, resulting in a maximum zone of inhibition of 14.7 mm, 14.3 mm, 13.7 mm, 10.7mm and 10.7 mm, respectively. Our results demonstrated a stronger antimicrobial activity of curcumin solutions against Gram-positive than Gram-negative bacteria. These findings are in accordance with a recently published study by Adamczak et al. [27]. Significantly higher antimicrobial activity of curcumin against Gram-positive than Gram-negative bacteria was also reported by other authors [30,31]. This is explained with the difference in the structure of bacterial cell walls as the cells of Gram-positive bacteria are not surrounded by an outer membrane. The cells of Gram-negative bacteria are more resistant due to the presence of an outer membrane that prevents antimicrobial agents to reach and damage the inner membrane of the cell wall [31]. Furthermore,

results of this study implied that the application of higher concentrations of curcumin solutions results in a larger diameter of zones of inhibition.

Therefore, antimicrobial activity of curcumin, both antibacterial and antifungal, is dependent upon the concentrations. These findings consist of a recently reported study, which emphasises that an increase in curcumin dose results in increased antibacterial activity [32]. Results of the previous study imply that the antifungal activity of curcumin against *C. albicans* generally increases by increasing the dose [33]. However, investigation of antifungal activity is restricted to only one positive control and it is mandatory to conduct further research on a wider range of fungi. A recent study by Narayanan et al. [34] revealed the variable antifungal activity of curcumin against planktonic and biofilm phase of different *Candida* species and therefore implied that curcumin could be considered a therapeutic alternative to conventional antifungals [34].

3.2 *In vitro* anti-Inflammatory Activity

Previous studies have reported curcumin as a potential anti-inflammatory agent, which activity is mediated via different mechanisms [14-16]. The present study was designed to evaluate the anti-inflammatory activity of curcumin solutions at different concentrations by applying *in vitro* assay of heat-induced inhibition of protein denaturation [24]. This *in vitro* assay is applicable for preliminary screening of potential anti-inflammatory drugs derived from plant sources [32]. Results of this study demonstrated the high effectiveness of test curcumin solutions compared to standard acetylsalicylic acid in inhibiting heat-induced protein denaturation (Fig. 2).

Test curcumin solutions showed mean inhibition of egg albumin denaturation of 87.51%, 81.36%, 78.56%, 69.87% and 63.14% at the concentration of 1.00 mg/ml, 0.75 mg/ml, 0.50 mg/ml, 0.25 mg/ml and 0.10 mg/ml, respectively. Acetylsalicylic acid, which served as positive control, showed inhibition of egg albumin denaturation of 96.84%, 91.27%, 87.59%, 85.12% and 85.03% at the concentration of 1.00 mg/ml, 0.75 mg/ml, 0.50 mg/ml, 0.25 mg/ml and 0.10 mg/ml, respectively. Maximum egg albumin inhibition was detected at the concentration of 1.00 mg/ml for both curcumin and acetylsalicylic acid. Therefore, test curcumin

Table 1. Antimicrobial activity of test curcumin solutions

Test curcumin solutions (mg/ml)	The diameter of zone of inhibition (mm)				
	<i>Escherichia coli</i> (ATCC8739)	<i>Pseudomonas aeruginosa</i> (ATCC9027)	<i>Staphylococcus aureus</i> (ATCC6538)	<i>Lysteria monocytogenes</i> (ATCC 35152)	<i>Candida albicans</i> (ATCC10231)
0.10	10.7	9.7	12.0	12.3	9.0
0.25	11.1	10.0	12.3	12.3	9.3
0.35	12.3	10.3	13.0	14.0	10.7
0.50	13.7	10.7	14.7	14.3	10.7

Table 2. Antimicrobial activity of testpositive and negative controls

Test controls	The diameter of zone of inhibition (mm)				
	<i>Escherichia coli</i> (ATCC8739)	<i>Pseudomonas aeruginosa</i> (ATCC9027)	<i>Staphylococcus aureus</i> (ATCC6538)	<i>Lysteria monocytogenes</i> (ATCC35152)	<i>Candida albicans</i> (ATCC10231)
CHL (30µg/disc)	14.7	8.0	10.0	18.0	NT
ZOX (30µg/disc)	10.3	ND	10.3	11.0	NT
AMK (30µg/disc)	10.3	18.3	20.0	18.7	NT
Nystatin(100 µg/disc)	NT	NT	NT	NT	15.7
DMSO	ND	ND	ND	ND	ND

*CHL=Chloramphenicol; ZOX=Ceftizoxime; AMK=Amikacin; DMSO=Dymethylsulfoxide; ND=not detected; NT=not tested

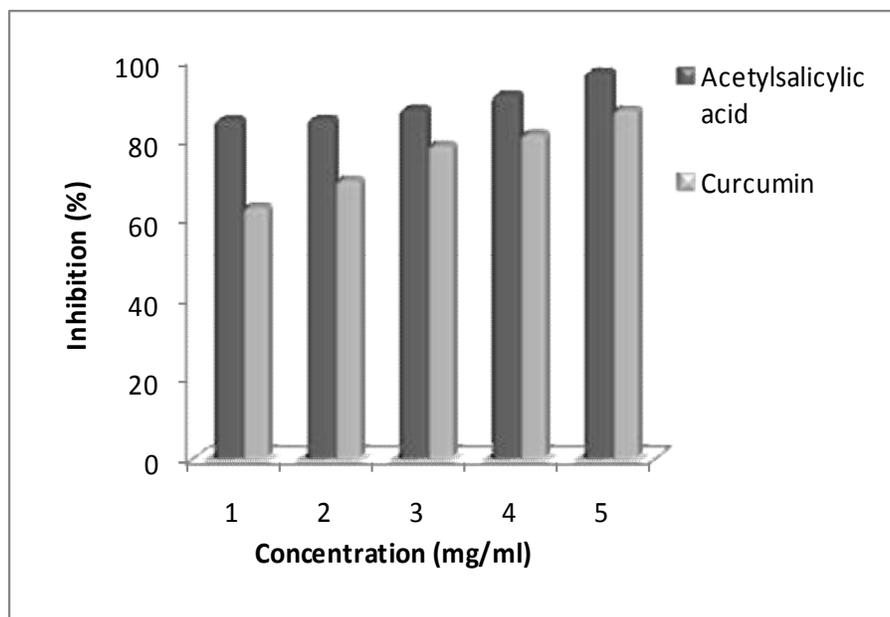


Fig. 2. Anti-inflammatory activity of test curcumin solutions

1=0.10 mg/ml; 2=0.25 mg/ml; 3=0.50 mg/ml; 4=0.75 mg/ml; 5=1.00 mg/ml

solutions demonstrated noticeable anti-inflammatory activity. Furthermore, our results indicated that an increase in curcumin concentration, as well as the concentration of

acetylsalicylic acid, leads to an increase in percentage of inhibition of protein denaturation. Therefore, anti-inflammatory activity of curcumin is dependent upon the concentrations. Similar findings were reported in previously published study by Ullah et al. [24].

4. CONCLUSION

Various studies, conducted as *in vitro* assays, have revealed different therapeutic applications of curcumin due to its different molecular mechanisms. Findings of the present *in vitro* study confirmed consideration of curcumin as a natural antimicrobial and anti-inflammatory agent. Test curcumin solutions demonstrated strong antibacterial, antifungal and anti-inflammatory activity. With an increase of curcumin concentration antimicrobial and anti-inflammatory activity increased, which implied that observed activity is dependent upon the concentration of curcumin. However, findings of this study are restricted to *in vitro* assays and consideration should be given to conducting a study involving wider dose range of test substances, as well as including further research on *in vivo* models. In conclusion, the present study emphasises the potential application of curcumin as a natural antimicrobial and anti-inflammatory agent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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