



# CHEMICAL STABILITY OF CURCUMINOIDS AND THEIR ESTROGENIC ACTIVITY IN ISHIKAWA CELLS



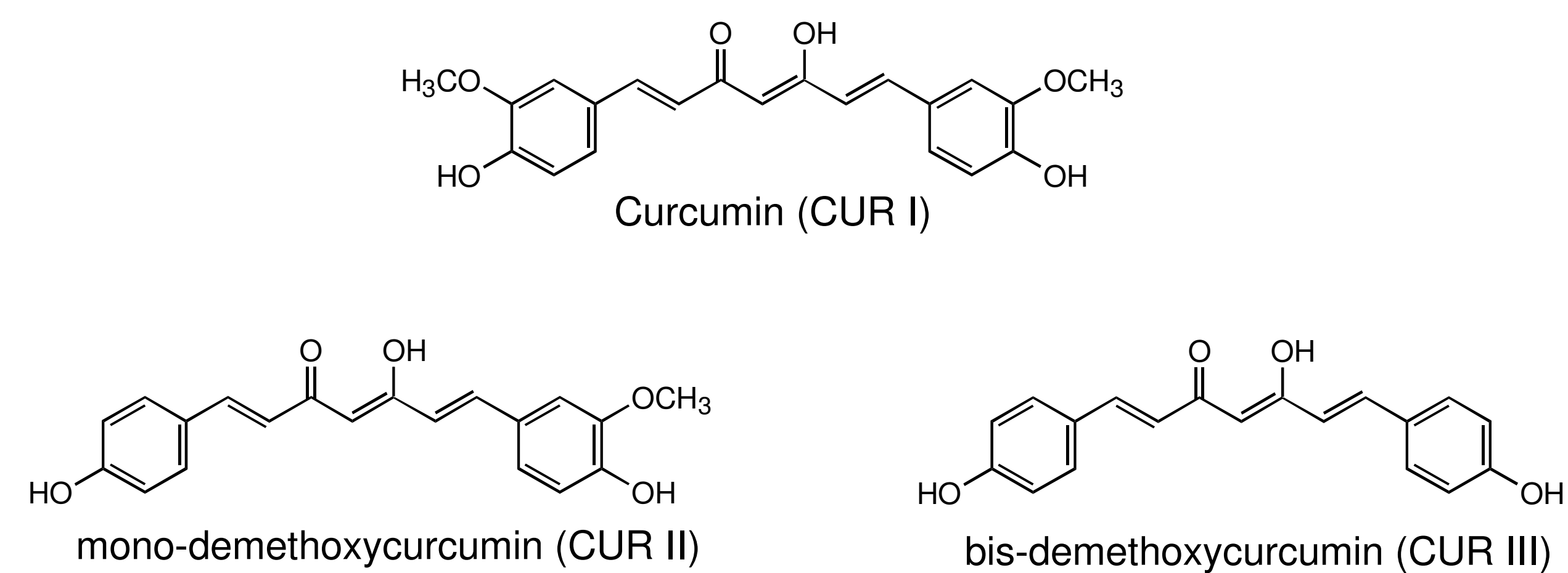
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## Introduction

The powdered dry rhizome of the plant *Curcuma longa*, commonly called turmeric, is widely used as a coloring agent and spice in many food items. In several Asian countries, it has also been used for centuries as a traditional remedy for the treatment of inflammation and other diseases. The yellow pigment of turmeric, which is composed of curcumin (CUR I), mono-demethoxycurcumin (CUR II) and bis-demethoxycurcumin (CUR III) has been reported to possess anti-oxidative, anti-inflammatory and anti-carcinogenic properties. Since these curcuminoids have a diphenolic structure similar to that of known phytoestrogens, we have studied the stability and the estrogenic activity of commercial curcumin (cCUR) and fractions of turmeric extract in Ishikawa cells, a cultured human endometrial adenocarcinoma cell line. Alkaline phosphatase (AP) activity in these cells is markedly stimulated by estrogens, and this enzyme can be easily quantified using p-nitrophenylphosphate as substrate.



## Methods

The assay for AP induction and cytotoxicity was carried out in 96-well-plates.  $2 \times 10^4$  cells per well were plated and cultured in 200  $\mu$ L medium (DMEM/F12) containing 5% CD-FCS (steroid-free fetal calf serum) at 37°C for 24 h. The medium was then removed, replaced by medium containing the test compounds, and further incubated for 48 h.

For measuring AP induction the medium was removed, the plates were placed on ice and 50  $\mu$ L/well of an alkaline buffer solution containing 5 mM 4-nitrophenylphosphate was added. The production of 4-nitrophenol (4-NP) was monitored periodically at 405 nm.

For the cytotoxicity assay 20  $\mu$ L per well of a MTT (5 mg/mL) solution was added and incubated for 3 h. After removing the medium, isopropanol was added and the reduction of MTT was monitored at 570 nm.

For the stability study the incubation medium was extracted with ethylacetate and the extract analyzed with HPLC.

## Acknowledgement

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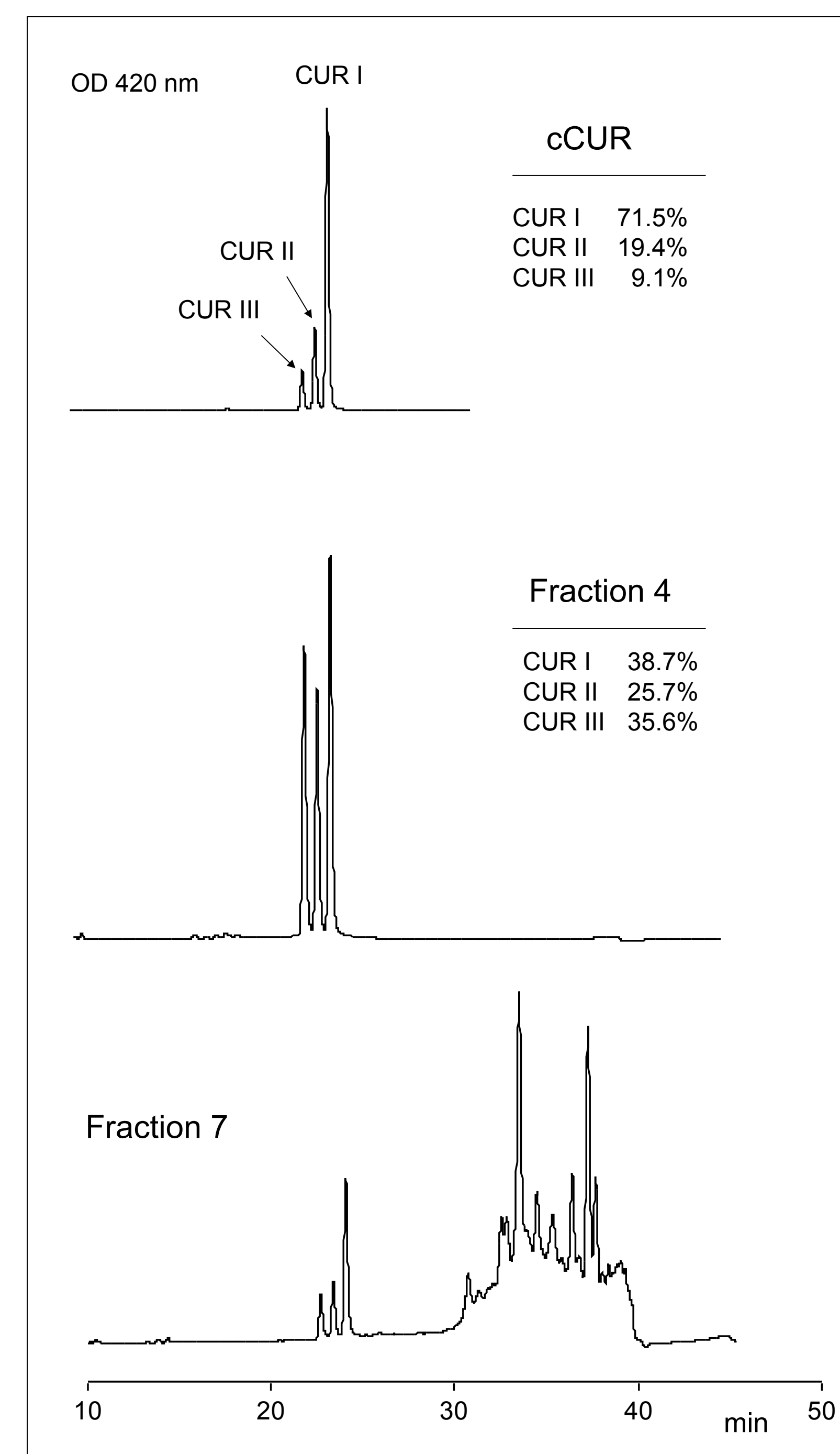


Fig. 1 HPLC profiles of cCUR and two turmeric fractions.

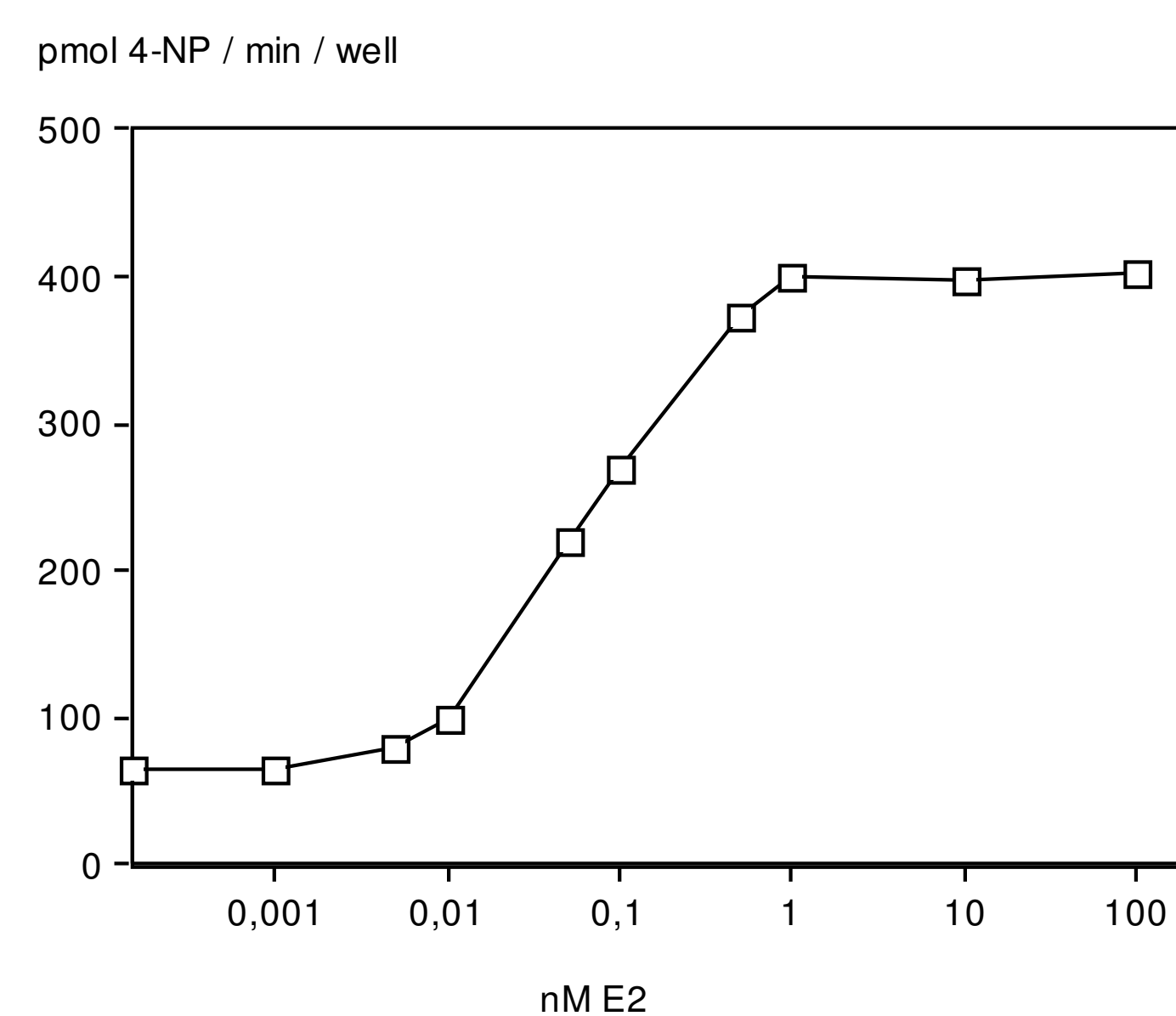


Fig. 4 Stimulation of AP in Ishikawa cells by E2.

## Results

The HPLC profiles of the investigated samples are depicted in Fig. 1. The samples of cCUR and the turmeric fraction 4 differ only in their quantitative composition of CUR I, II, and III. The structures of the compounds (retention time 30-40 min) of the turmeric fraction 7 are unknown, but all of them exhibit an UV/VIS spectrum similar to that of the curcuminoids.

When the stability in cell culture medium of cCUR (Fig. 2) and fraction 4 (data not shown) was studied, we observed that all three curcuminoids were unstable. Stability was markedly lower in the absence of serum in the medium. Furthermore, the three curcuminoids displayed different stabilities: CUR I was the least and CUR III the most stable curcuminoid. In contrast, the compounds of fraction 7 appear to be stable in medium without FCS, but disappear in the presence of serum.

The number of viable cells decreased markedly at concentrations higher than 5  $\mu$ g/mL of cCUR and fraction 4 but not fraction 7 (Fig. 3).

The stimulation of AP in Ishikawa cells by estradiol (E2) is depicted in Fig. 4. No enhanced AP activity was measured with the three samples (Fig. 5A). However, the curcuminoids markedly inhibited the AP activity stimulated by 1 nM E2 (Fig. 5B). This inhibition is not due to a direct effect on AP, since human placental AP was not affected by incubation with curcuminoids (Fig. 5C).

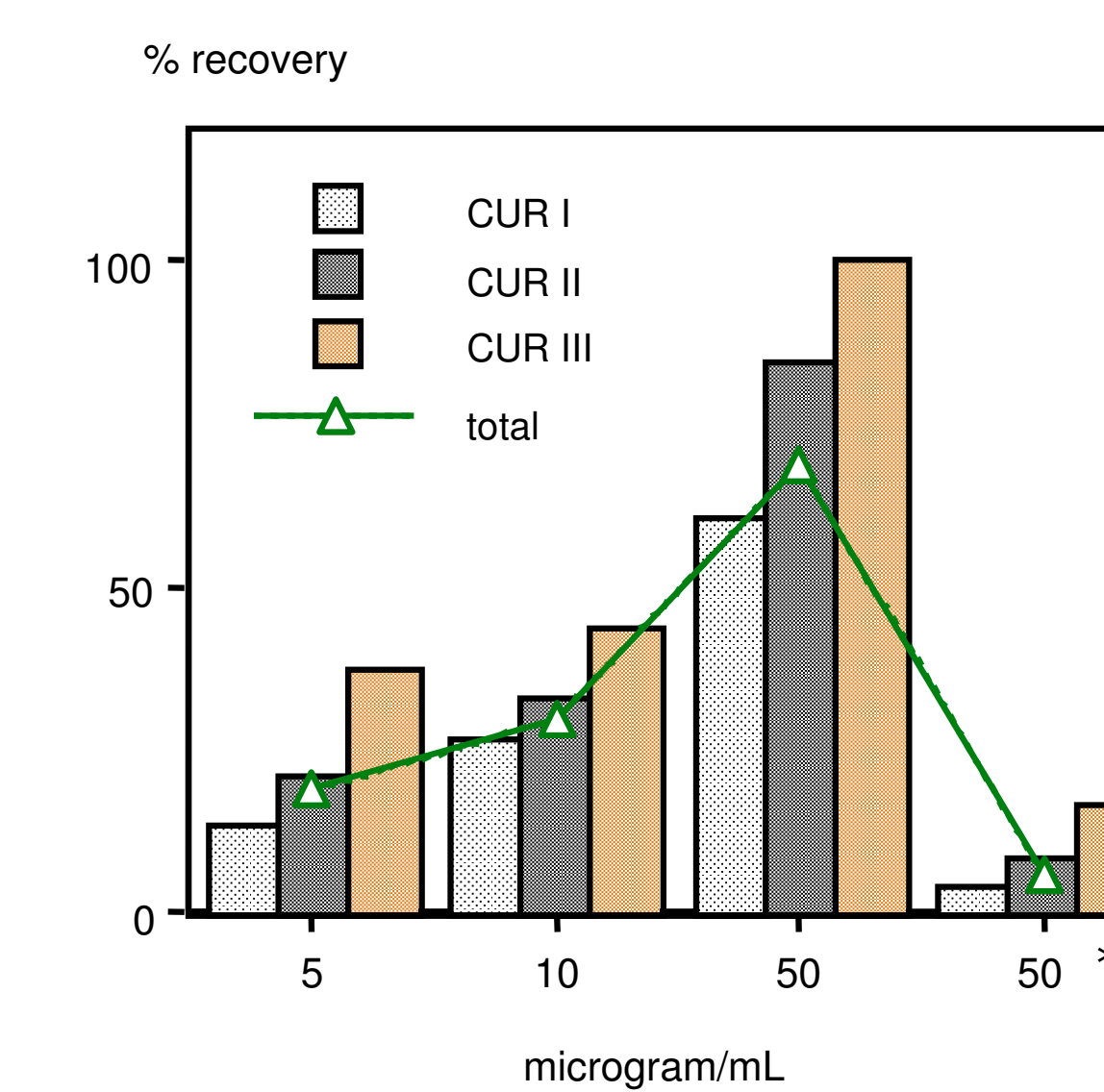


Fig. 2 Recovery of the curcuminoids after 18 h incubation of cCUR in medium with 5% FCS. \* = incubation without FCS and cells.

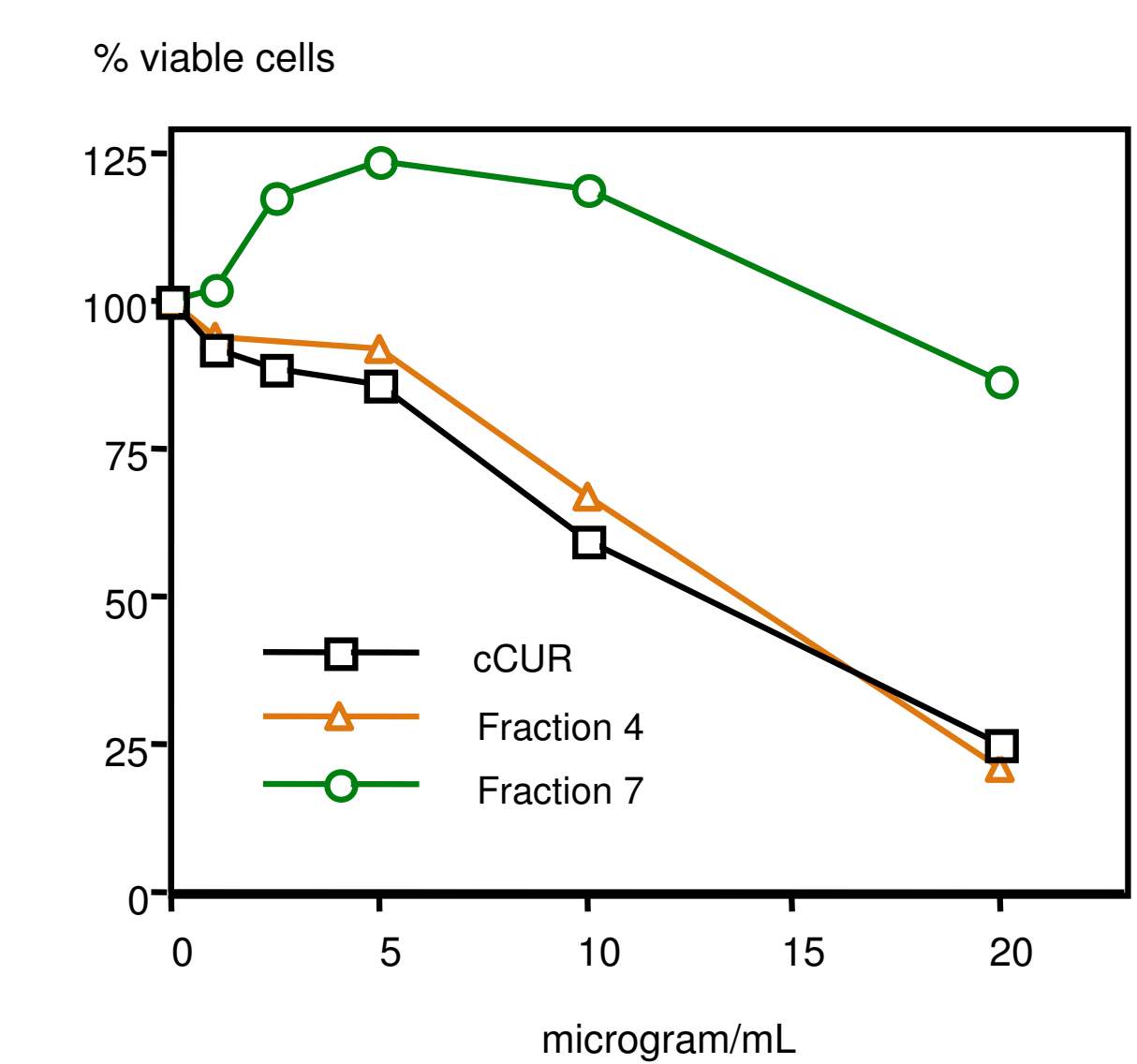


Fig. 3 Cytotoxicity of cCUR, fraction 4 and fraction 7.

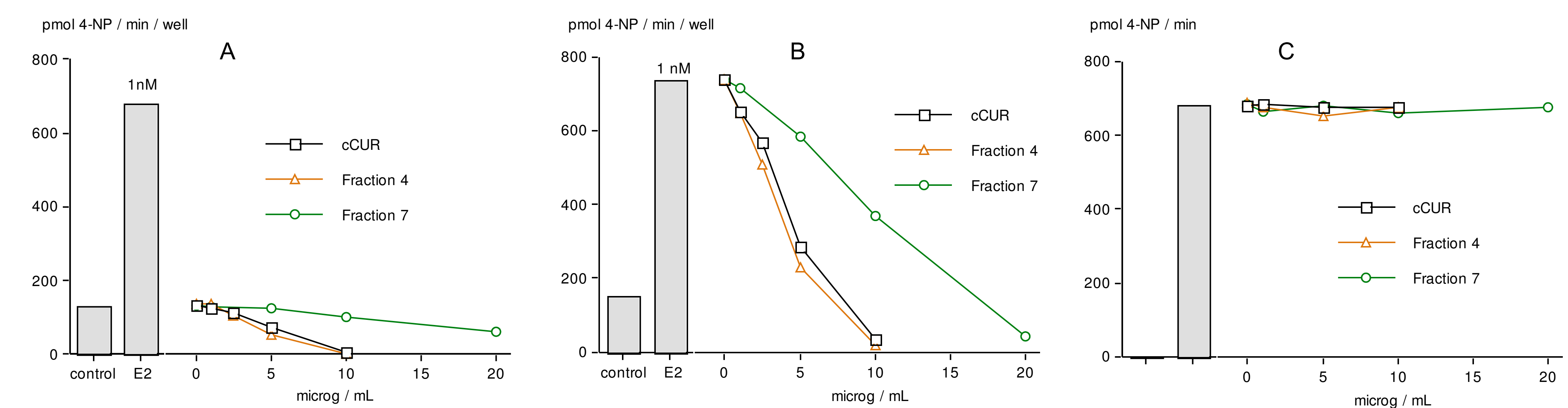


Fig. 5 Effect of curcuminoids on AP activity: in Ishikawa cells in the absence (A) and presence (B) of E2, in incubations of human placental AP (C).

## Conclusion

We conclude from our studies that curcuminoids are chemically unstable in aqueous media. In Ishikawa cells, curcuminoids are devoid of estrogenic activity but inhibit the estrogenicity of estradiol. The chemical structure of the major decomposition products and the mechanism of the putative anti-estrogenic effect of curcuminoids require further investigations.