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Research paper

Do cinnamylideneacetophenones have antioxidant properties and a protective effect toward the oxidation of phosphatidylcholines?

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ABSTRACT

Cinnamylideneacetophenones (CA) are an important group of α,β,γ,δ-diunsaturated ketones that have been widely used in a variety of synthetic transformations. Biological studies concerning these compounds are scarce and refer mainly to antiviral and antibacterial evaluations. Curcumin (**CR**), a natural polyphenol, is a yellow pigment extracted from the plant *Curcuma longa*, which is one of the major spices used in the Indian culinary. It has been reported that **CR** has cancer chemopreventive properties in a range of animal models of chemical carcinogenesis, along with antioxidative and anti-inflammatory properties. Inspired by the biological activity shown by **CR** and their structural resemblance with CA, it was considered to study the ability of the latter molecules to inhibit lipid oxidation induced by the hydroxyl radical (Fenton reaction) by electrospray ionization (ESI) mass spectrometry (MS) using phosphatidylcholine (PC) liposomes as a model of cell membrane. Compound **4**, holding a methylated hydroxy group in the position R^2, and **CR** showed similar effects in inhibiting lipid peroxidation. In the presence of **7**, the extension of oxidation was higher than the one verified in all other compounds. Other methodologies, namely DPPH radical scavenging and oxygen radical absorption capacity (ORAC) assays, were performed to complement and clarify the results attained by oxidation of PC monitored by ESI-MS and to evaluate the antioxidant profile of compounds. For both assays, compound **7** showed to be rather efficient due to its specific structure. This derivative can form a quite stable allylic radical by abstraction of a hydrogen atom which accounts for these results.

1. Introduction

Oxidative stress by free radicals drives damage into important biomolecules namely proteins, lipids, and DNA, changing their structure and thus being responsible for loss of function or even resulting in the formation of new deleterious species. Lipids are one of the major targets of oxidation in biological systems [1]. The development and progression of several diseases namely age-related illnesses such as Alzheimer, Parkinson, multiple sclerosis, diabetes, cardiovascular, liver and lung diseases have been associated with lipid peroxidation (LPO) [2,3].

LPO can be triggered by reactive oxygen species (ROS) that comprise both free radical namely $O_2^{\bullet-}$ (superoxide radical), $OH^{\bullet}$ (hydroxyl radical), $HO_2^{\bullet}$ (perhydroxyl radical) and $RO^{\bullet}$ (alkoxy radicals) as well as non-radical forms such as H_2O_2 (hydrogen peroxide) and $^1O_2$ (singlet oxygen). The overall process of free radical-mediated LPO comprises three distinct stages: initiation, propagation and termination steps. Initiation of LPO in a membrane takes place mainly by formation of $OH^{\bullet}$ capable of hydrogen atom abstraction in an unsaturated fatty acyl chain of a polyunsaturated fatty acid (PUFA), which are incorporated in the outermost layer of cells in the form of phospholipids. The formation of a carbon-centered lipid radical gives rise to a $LOO^{\bullet}$ by addition of oxygen. $LOO^{\bullet}$ can further propagate the peroxidation reaction chain by abstracting a hydrogen atom from adjacent PUFA side chains leading to the formation of a variety of oxidized phospholipids [4].

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Since phospholipids, namely phosphatidylcholines (PC), are one of the main constituents of the cell membranes, the formation of such products is responsible for the modification of membrane important features [5]. The formation of oxidized phospholipid derivatives may result in the increase of phospholipid polarity, disordering of the phospholipid bilayer leading to loss of structural and chemical properties or even total disruption of its integrity [6,7].

The adverse effects of ROS excessive production towards biomolecules namely lipids can be balanced via an oxidant and antioxidant regulation. It is rather important to find this balance since the antioxidant protection system must minimize the levels of most harmful ROS while allowing enough ROS to remain available for important processes such as cell signaling and redox regulation. The ingestion of antioxidant supplements may help the organism to correct the elevated levels of oxidative stress that cannot be controlled by the endogenous antioxidants and reduce, consequently, the damaging effects of ROS by delaying many events that contribute to cellular aging. It is therefore, for the reasons mentioned above, quite important to assess the efficiency of novel antioxidants using effective methodologies.

Curcumin (**CR**), 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-hepta-3,5-dione (Fig. 1), is extracted as a yellow pigment from the rhizomes of the plant *Curcuma longa* [8] and is one of the major species used in the Indian cuisine. This natural polyphenol is considered to be a safe phytochemical showing important pharmacological properties such as anti-inflammatory [9], anticancer [10], and antioxidant activities [9,11]. The antioxidative properties of **CR** are thought to be related to its ability to capture peroxyl radicals by hydrogen atom transfer from the hydroxy group of the aromatic ring being considered a *phenolic chain-breaking* antioxidant [12]. Some researchers have proposed differently; that hydrogen atom abstraction from the methylene group of the diketone moiety rather than the hydroxy group would be responsible for the antioxidant properties of **CR** [13].

Inspired by the biological activity shown by **CR** and their structural resemblance with cinnamylideneacetophenones (CA) it was considered to study the ability of the latter molecules to inhibit lipid oxidation induced by the hydroxyl radical (Fenton reaction). CA (Fig. 1) are an important group of α,β,γ,δ-diunsaturated ketones that have been widely used in a variety of synthetic transformations [14]. However, studies concerning the biological activity of these compounds are scarce and refer mainly to antiviral and antibacterial evaluations [15]. Recently, Brantley and co-workers reported the evaluation of a series of cinnamylideneacetophenones for cytotoxicity against breast cancer cells using the Alamar Blue™ assay [16]. It was found in these studies that the two most active derivatives bore a 2-nitro group on the B-ring. Both of these agents exhibited anticancer activity in the nanomolar to sub-micromolar range yet exhibited substantially less cytotoxicity in the MCF-10A cells. Also, these derivatives appeared to have a selectivity index superior to that observed for the established chemotherapeutic agent doxorubicin [16].

PC liposomes are widely used as a model of cell membrane and studies concerning the impact of synthetic and natural oxidants have been performed for a greater understanding of the mechanism that rule the LPO processes. It was envisioned the use of mass spectrometry (MS) and electrospray (ESI) to evaluate the protective effect of these antioxidants candidates by identifying the possible formation of PC oxidation products. It was equally considered to measure the antioxidant activity for this family of compounds using classical methods namely the radical scavenging capacity against 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) and the peroxyl radical scavenging capacity (oxygen radical absorbance capacity assay, ORAC). It was aimed to evaluate the relationship established between the results obtained with these two methods and the ability of these compounds to inhibit phosphatidylcholine oxidation induced by the hydroxyl radical. Also, a validation of the MS methodology towards the qualitative and quantitative evaluation of the antioxidant properties would be, if possible, an important result.

The question aimed to be answered with these studies is: *Do cinnamylideneacetophenones have a protective effect toward the oxidation of phosphatidylcholines?* To answer this question, the chosen compounds were tested for their ability to inhibit PLPC (1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine) peroxidation induced by the hydroxyl radical (Fenton reaction). The dMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) was used as internal standard to evaluate the extension of PLPC oxidation since it is a phospholipid with two saturated fatty acyl chains and therefore resistant to oxidation. Oxidation was monitored by electrospray ionization mass spectrometry (ESI-MS) and oxidation products

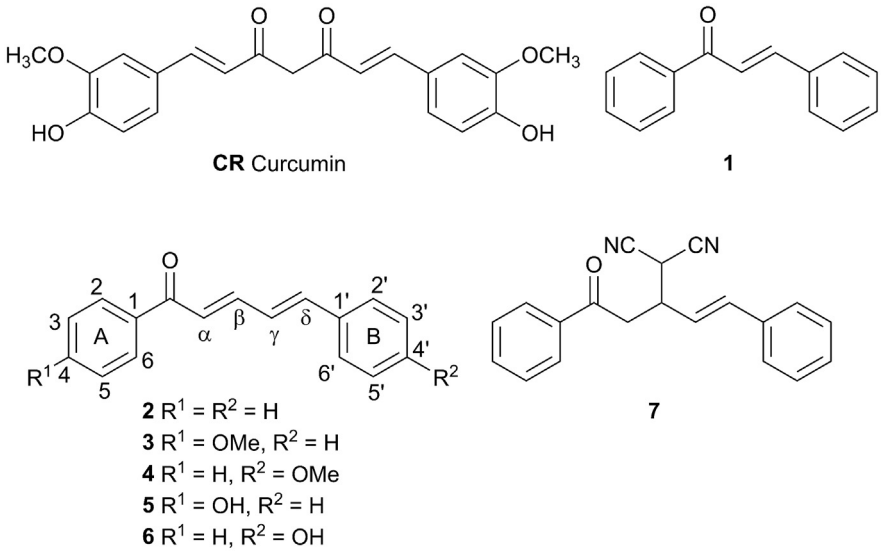

Fig. 1. Structure of curcumin (**CR**), chalcone (**1**), cinnamylideneacetophenones (**2**–**6**), and the 1,4-Michael addition CA derivative (**7**).