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The Antioxidant Activity of Some Curcuminoids and Chalcones

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Abstract: The antioxidant properties of the synthetic compound (C₁)-(C₈), which comprised of 7 curcuminoids and a chalcone, were evaluated by two complementary assays, DPPH and β-carotene/linoleic acid. It was found that in general the free radical scavenging ability of (C₁)-(C₈) was concentration dependent. Compounds (C₁) and (C₄) were found highly potent antioxidants with higher antioxidant values than BHT suggesting that synthetic curcuminoids are more potent antioxidants than standard antioxidants like BHT. Using β-carotene-linoleic acid assay only the water soluble polyphenolic chalcone (C₅) showed 85.2% inhibition of the formation of conjugated dienes reflecting on its potent antioxidant activity.

Keywords: Curcuminoids; chalcones, antioxidant activity; polyphenols

1. Introduction

The phenolic compound curcumin (C₁) (also known as diferuloylmethane; [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione]) is the predominant biologically active component of turmeric, rhizomes of Curcuma longa that belongs to the ginger family, Zingiberaceae. Curcumin possesses a variety of pharmacological activities and therapeutic properties and is a potent antioxidant not only in food systems but also in biological systems. Recently curcumin has received considerable attention due to its various pharmacological activities [1-3]. Curcumin and the co-occurring compounds have been extensively investigated for their anti-inflammatory and anticancer activities. Curcumin and curcumin...
analogues therefore represent a novel class of highly selective COX-1 inhibitors and promising candidates for in vivo studies [4]. Curcumin has been shown to significantly affect the production of TNF. Thus suppression of TNF by curcumin leads to inhibition of NF-κB and cell proliferation. Using both the in vitro as well as in vivo models of inflammation various reports in the literature have shown that curcumin inhibits NF-κB in various tissues via different mechanisms such as the suppression of IL-1β induced NF-κB activation via inhibition of IκBα phosphorylation, IκBα degradation, p65 phosphorylation and p65 nuclear translocation which result in the down regulation of NF-κB targets including COX-2 and MMP-9 [5].

In this endeavor, many curcuminoids have been synthesized and their structure-activity relationship (SAR) has been reported many times over. The mechanism of action of natural as well as synthetic curcuminoids has been predicted through their antioxidant activity. Reactive oxygen species (ROS) are formed during normal cell aerobic respiration [6] and are the main cause of cell damage involved in chronic diseases like diabetes, cardiovascular and others [7]. Reactive oxygen species are also produced by neutrophils which are highly sophisticated cells that actively seek out, ingest, and destroy pathogenic microorganisms [8]. To achieve this essential role in host defence, neutrophils deploy a potent antimicrobial arsenal which includes ROS as oxidants. Antioxidants play an important role in neutralising (ROS) and protecting the cells from oxidative damage. Curcumin is an extremely potent lipid soluble antioxidant and has been suggested to act through its pro-oxidant/antioxidant effects because formation of ROS by curcumin and curcuminoids correlates with their apoptotic activity on tumor cells [9]. The free radical scavenging activity of curcumin can arise either from the phenolic OH group or from the CH2 group of the β-diketone moiety. A reactive free radical can undergo electron transfer or abstract H-atom from either of these two sites.

Some functional foods and plants are important sources of exogenous antioxidants, such as vitamins (Vitamin C and E), flavonoids and thiol compounds. It has been recognised that the mechanism of protection from damaging (ROS) by antioxidants depends upon the nature of the antioxidant [10, 11].

In an on-going work on curcumin, in our laboratories, we have synthesized a number of curcuminoids and have developed assays to assess their anti-inflammatory and antioxidant activities. In this endeavour, here we report the antioxidant activities of seven curcuminoids
(C1)-(C4), (C6)–(C8) with considerable structural diversity and the polyphenolic chalcone (C5) [12, 13].

Figure 1. Structures of compounds used in the anti-oxidant study

2. Experimental Section

2.1 Chemistry: Materials and Method

Melting points were recorded on Stuart SMP3 digital apparatus; IR spectra were recorded on Perkin-Elmer Spectrum 100 FTIR spectrophotometer with a universal ATR sampling accessory; ¹H NMR spectra were recorded on a Bruker AC 250 MHz and ¹³C NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometers. Mass spectra (MS) were obtained on VG 770E spectrometer operated in EI mode at 70 eV. TLC analyses were done using Merck aluminium coated silica gel sheets and flash chromatography was performed using BDH flash silica gel and the eluents are indicated in parenthesis for each compound. The ¹³C NMR spectral interpretation was done using the numbering system indicated on the structure for curcumin (C1) and the generalised structures in tables I and II [14].

2.1.1 The compounds 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (C1), (1,7-Bis(1-naphthyl)-1,6-heptadiene-3,5-dione (C6), 1,7-Bis(5-methylidenebutenolide)-1,6-
heptadiene-3,5-dione (C7), 1,7-Bis(3,4-methylenedioxyphenyl)-1,6-heptadiene-3,5-dione (C8) were made by the general procedure for curcuminoid synthesis [15] and are reported elsewhere [14]. The chalcone (E)-1-(4-methoxyphenyl)-3-(2,4,6-trihydroxyphenyl)prop-2-en-1-one (C5) was prepared according to literature method [12, 13] in (28% yield) as a dark maroon coloured solid, mp 110-112°C (Lit. m.p. 107–109°C [13]; FTIR (solid) 1645.26 (>C=O), 2400-3600 (broad peak, OH) cm⁻¹; ¹H NMR (250MHz, d⁴-methanol): δ 3.78 (3H, s, OCH₃), 5.80–7.70 (m, 6H, Ar–H), 7.70 (d, 1H, J = 15.7 Hz, >C=CH-CO-), 7.93 (d, 1H, J = 15.7 Hz, >CH=C-CO-), 10-12 (broad s, 3H, –OH).

2.2 DPPH assay
This assay spectrophotometrically measures the colour decay of the stable free radical diphenylpicrylhydrazyl (DPPH) by interaction with an antioxidant [16, 17]. Fifty μL of various concentrations of methanolic solution of the sample was added to 5 mL of a 101 μmol methanolic solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank at λ517 nm. Inhibition of free radical DPPH in percent (I%) was calculated in following way:

\[ I\% = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100 \]

Where \( A_{\text{blank}} \) is the absorbance of the control reaction (containing all reagents except the test compound), and \( A_{\text{sample}} \) is the absorbance of the test compound. Concentration providing 50% inhibition (IC₅₀) was calculated from the graph by plotting inhibition percentage against sample concentration. Assays were carried out in triplicate. Synthetic antioxidant butylated hydroxytoluene (BHT) was used as positive control.

2.3 β-Carotene-linoleic acid assay
In this assay antioxidant capacity of the compound is determined by measuring the conjugated dienes produced from linoleic acid oxidation [18]. A stock solution of β-carotene-linoleic acid mixture was prepared as following: 0.5 mg β-carotene was dissolved in 1 mL of chloroform (HPLC grade), 25 μL linoleic acid and 200 mg Tween 40 was added. The chloroform was completely evaporated using a vacuum evaporator. Distilled water (100 mL) saturated with oxygen (30 min, 100 mL/min.) was added with vigorous shaking. 2.5 mL of this mixture was added to three test tubes and ethanolic solution (350 μL) of the test compound (concentration 2 mg/mL) was added and the emulsion thus produced was incubated for up for 24 hours at room temperature. The same procedure was repeated with
positive control BHT and a blank. After completion of the incubation period absorbance of the mixture was taken at λ490 nm. Antioxidant capacities of the synthetic curcuminoids were compared with BHT and blank run under identical conditions.

3. Results and Discussion

In all of the synthetic curcuminoids (C2)-(C4) and (C6)-(C8), the heptadiene part has been kept unmodified with the the bis-aryl part of curcumin (C1) being modified. Compound (C5) is an example of a family of compounds known as chalcones and was chosen for the study because it has structural similarities to curcumin (C1). Curcuminooids have previously been studied for anti-inflammatory and antioxidant activities [19-21]. However, in our compounds (C1)-(C8) tested for antioxidant activity a great deal of structural diversity exists that includes phenolic as well as non-phenolic rings at the two ends of heptadiene chain.

Two complementary assays were employed for screening the antioxidative properties of the synthetic compounds (C1)-(C8). One of the assays measured the free radical scavenging activity using 2, 2-diphenylhydroxyl stable free radical (DPPH) and a second assay involved the inhibition of the lipid oxidation to determine antioxidant capacity of the samples. The inhibition of linoleic acid oxidation was determined by employing a modified β-carotene/linoleic acid assay [18]. In the absence of antioxidants, oxidation products (lipid hydroperoxides, conjugated dienes and volatile by-products) of linoleic acid bleach β-carotene in ethanolic solution. In the presence of antioxidants oxidation of β-carotene is scavenged preventing bleaching the colour of β-carotene.
Figure 2. Free radical inhibition percentage of C1 against increasing concentration in DPPH assay.

Figure 3. Free radical inhibition percentage of C2 against increasing concentration in DPPH assay.
Figure 4. Free radical inhibition percentage of C5 against increasing concentration in DPPH assay.

Figure 5. IC$_{50}$ values of active compounds in DPPH assay.
Figure 6. Relative antioxidant activity percentage (RAA%) of synthetic curcuminoids in β-carotene-linoleic acid assay.

Using the two assays, DPPH and β-carotene/linoleic acid, to evaluate the antioxidant properties of the synthetic compound (C1)-(C8) it was found that in general the free radical scavenging ability of curcuminoids (C2)-(C4), (C6)-(C8) and chalcone (C5) was concentration dependent. (Figs. 2-4).

Free radical scavenging activity of the active compounds (C1), (C4) and (C5) with 50% inhibition (IC50) was calculated from the inhibition curves and is shown in Figure 5. The inactive compounds (C2), (C3), (C6), (C7) and (C8), with no antioxidant or minimum antioxidant activity were not included in figure 5. In DPPH assay, the lower IC50] was interpreted as higher antioxidant activity of the compound. Compounds (C1) and (C4), both of which contain a phenolic group at position-4 of the aromatic ring, were found highly potent antioxidants with higher antioxidant values than BHT. Thus synthetic curcuminoids are more potent antioxidants than standard antioxidants like BHT.

Using β-carotene-linoleic acid assay only compound (C5), which contains phenolic groups at position-4 and 5, showed 85.2% inhibition of the formation of conjugated dienes reflecting on its potent antioxidant activity. This may be because of two important factors: Firstly compound (C5) is more polyphenolic in nature which augments the antioxidant activity of
the molecule. Secondly the polyphenolic nature of the compound enhances its water solubility thus amplifying its interaction with linoleic acid present in the emulsion and protecting it from oxidation to yield conjugated dienes. Since the other curcuminoids (C2), (C3), (C6), (C7) and (C8) did not show significant antioxidant activity by β-carotene-linoleic acid assay, it may be concluded that their interaction with linoleic acid was poor because of their insignificant solubility in aqueous solution. Our results demonstrate that β-carotene-linoleic acid assay is suitable only for water soluble antioxidants.

Compound (C7), synthesised in eight steps from L-ascorbic acid [21-23] (Fig. 7), which showed poor antioxidant activity is more likely to have good antioxidant activity in vivo because the methoxy would get cleaved in situ to yield the ene-diol system of ascorbic acid. Likewise compound (C8) which also showed insignificant antioxidant activity should also be expected to be better antioxidant in vivo due the in situ cleavage of the protected diphenolic system to yield free phenolic groups.

The curcuminoids (C1), (C6)-(C8) which have previously been studied for antiinflammatory activity [14] and compounds (C2) – (C4) which have been evaluated for their anticancer activity [24] were examined for anti-oxidant activity. It is known that the mechanism of anticancer and anti-inflammatory activities involves antioxidant activities of the molecules. Thus the nitrocurcuminoids (C2)-(C4) and the naphthyl curcuminoid (C6), that lack phenolic groups make interesting candidates for antioxidant study. Our results augument the anticancer activity of these compounds through antioxidant mechanism.
Figure 7. Synthesis of curcuminoid (C7) from L-ascorbic acid by the Pabon method.

4. Conclusions
It was found that in general the free radical scavenging ability of compounds (C1)-(C8) was concentration dependent and that antioxidant activity was related to the presence of phenolic groups in ortho and/or para positions of the aromatic rings in agreement with literature findings. Compounds (C1) and (C4), both of which contain the phenolic (4-OH) were found highly potent antioxidants with higher antioxidant values than BHT. These compounds offer optimism, as safe candidates, for their possible application in consumer products.

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References
1. Huang, M. T.; Ferraro, T. Phenolic compounds in food and cancer prevention. In Phenolic Compounds in Food and Their Effects on Health II. Antioxidants and Cancer Prevention; ACS Symposium Series 507; Houng, M. T., Ho, C. T., Lee, C. Y., Eds.; American Chemical Society: Washington, DC, 1992, pp 8-34.
2. Ruby, A. J.; Kuttan, G.; Babu, K. D.; Rajasekharan, K. N.; Kuttan R. Anti-tumour and antioxidant activity of natural curcuminoids. Cancer Lett., 1995, 94, 79-83.
3. Priyadarsini K.I; Maity D.K; Naik G.H; Kumar M.S; Unnikrishnan M.K; Satav J.G; Mohan H. Role of phenolic OH and methylene hydrogen on the free radical reactions and antioxidant activity of curcumin. Free Rad. Biol. Med., 2003, 35(5):475–484.
4. Handler N; Jaeger W; Puschacher H; LeisserK; Erker, T. Synthesis of novel curcumin analogues and their evaluation as selective cyclooxygenase (COX-1) inhibitors. Chem. Pharm. Bull. 2007, 55:(1), 64-71.
5. Shakibaei M; John T; Schulze-Tanzil G; Lehmann I; Mobasheri A. Suppression of NF-κB activation by curcumin leads to inhibition of expression of cyclo-oxygenase-2 and matrix metalloproteinase-9 in human articular hondrocytes: Implications for the treatment of osteoarthritis. Biochem. Pharmacol., 2007, doi: 10.1016/j.bcp.2007.01.005.
6. Gutteridge, J.M.C.; Halliwell, B. Free Radicals and Antioxidants in the Year 2000- a Historical Look to the Future, *Ann. N. Y. Acad.Sci.*, 2000, 899, 136-147.

7. Sugamura K., Keaney, J. F. Jr. Reactive oxygen species in cardiovascular disease. Free Radical Biology and Medicine, 2011, 51(5), 978-992.

8. Fialkow, L; Wang, Y; Downey, G.P. Reactive oxygen and nitrogen species as signaling molecules regulating neutrophil function. *Free Radical Biology and Medicine*, 2007, 42.2, 153–164

9. Mishra S; Kapoor N; Mubarak Ali A; Pardhasaradhi BV; Kumari AL; Khar A; Misra K. Differential apoptotic and redox regulatory activities of curcumin and its derivatives. Free Radic. Biol. Med. 2005, 38:1353–1360.

10. Koleva, I.I.; van Beek, T.A.; Linssen, J.P.H.; de Groot, A.; Evstatieva, L. N. “Screening of Plant Extracts for Antioxidant Activity: A Comparative Study on Three Testing Methods”, *Phytochem. Anal.*, 2002, 13, 8-17.

11. Ou, B.; Huang, D.; Hampsch-Woodill, M.; Flanagan, J. A.; Deemer, E. K. Analysis of Antioxidant Activities of Common Vegetables Employing Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP) Assay: A Comparative Study, *J. Agric. Food Chem.*, 2002, 50, 3122-3128.

12. Sui, Xin; Quan, Ying-Chun; Chang, Yue; Zhang, Rui-Peng; Xu, Yin-Feng; Guan, Li-Ping. Synthesis and studies on antidepressant activity of 2,4,6-trihydroxychalcone derivatives. *Med. Chem. Res.* 2012, 21, 1290–1296

DOI 10.1007/s 00044-011-9640-2

13. Zhao, Li-Ming; Jin, Hai-Shan; Sun, Liang-Peng; Piao, Hu-Ri; Quan, Zhe-Shan. Synthesis and evaluation of antiplatelet activity of trihydroxychalcone derivatives. *Bioorganic & Medicinal Chemistry Letters*, 2005, 15, 5027–5029.

doi:10.1016/j.bmcl.2005.08.039

14. Khan, M.A. ; El-Khatib, R.; Rainsford K.D.; Whitehouse, M.W. Synthesis and anti-inflammatory properties of some aromatic and heterocyclic aromatic curcuminoids, *Bioorganic Chemistry*, 2012, 40, 30–38.

15. Pabon, H.J.J. Synthesis of Curcumin and Related Compounds. *Rec.Trav. Chim. Pays-Bas.*, 1964, 83, 379-386.

16. Cuendet, M.; Hostettmann, K.; Potterat, O. Iridoid Glucosides With Free Radical Scavenging Properties from *Fagraea blumei.*, *Helv. Chim. Acta*, 1997,80,1144-1152.
17. Burits, M.; Bucar, F. Antioxidant Activity of Nigella sativa Essential Oil, *Phytother. Resear.*, 2000, 14, 323-328.

18. Dapkevicius, A.; Venskutonis, R.; van Beek, T. A.; Linssen, J. P. H. Antioxidant Activity of Extracts Obtained by Different Isolation Procedures from Some Aromatic Herbs Grown in Lithuania., *J. Sci. Food Agric.*, 1998, 77, 140-146.

19. Ruby, A.J.; Kuttan, G.; Dinesh Babub K., Rajasekharan, K.N.; Kuttan, R. Antitumour and antioxidant activity of natural curcuminoids, *Cancer Letters*, 1995, 94, 79-83.

20. Elise Portes, Christian Gardrat and Alain Castellan. A comparative study on the antioxidant properties of tetrahydrocurcuminoids and curcuminoids. *Tetrahedron*, 2007, 63, 9092-9099.

21. Khan, M.A.; Adams, H. Simple and Efficient Stereoselective Synthesis of (Z) and (E)-Alkylidene 2,3-Dimethoxybutenolides from L-Ascorbic Acid and D-Isoascorbic Acid. *Synthesis*, Thieme, 1995, 687-6929.

22. Khan, M.A.; Boyes S.A.; Adams , H. Synthesis of some substituted oxaspiro[4,5]decanenones by way of intermolecular Diels-Alder reaction of alkylidine 2,3-dimethoxybutenolides obtained from L-ascorbic acid. *Molecules*, Springer-Verlag,1, 1996, 27-36.

23. Abaza, M.S.; Khan, M.A.; Afzal, M. Chemistry, Biochemistry and Selective Cytotoxicity of Curcumin Analogues Against Human Cancer Cell Lines in *Curcumin : Biosynthesis, Medicinal Uses and Health Benefits*, Jun Sasaki and Masaki Kichida, Eds.; Nova Science Publishers Inc., N.Y., 2012.

24. Hahm, E-R; Cheon, G; Lee, J; Kim, B; Park, C; Yang, C-H. New and known symmetrical curcumin derivatives inhibit the formation of Fos-Jun-DNA complex. *Cancer Letters*, 2002, 184, 89–96