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Antioxidant activity of Knipholone anthrone

Solomon Habtemariam *

Pharmacognosy and Phytotherapy Research Laboratories, Department of Chemical and Pharmaceutical Sciences, Medway School of Science, The University of Greenwich, Chatham Maritime, Kent ME4 4TB, United Kingdom

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Abstract

Knipholone anthrone (KA), isolated from an Ethiopian medicinal plant, *Kniphofia foliosa*, Hochst (Asphodelaceae) has been shown to display a potent antiprotozoal activity and a relatively little cytotoxic effect on mammalin cells. In the present study, the antioxidant potential of KA was assessed using a variety of *in vitro* assay models. KA displayed a concentration-dependant scavenging effect against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. The IC_{50} values obtained for KA and the positive control (−)-epicatechin (EC) in the DPPH assay were 22 ± 1.5 and 8.7 ± 0.9 μM (mean ± SEM; $n = 3$ separate experiments), respectively. KA displayed a better activity than EC in scavenging superoxide anions and preventing deoxyribose degradation by hydroxyl radicals. KA appeared to form a complex with Fe^{2+}, displayed a concentration-dependant reducing power, and also protected (at concentrations of 4.4 μM and above) isolated DNA from damage induced by Fenton reaction-generated hydroxyl radicals.

Keywords: Knipholone anthrone; *Kniphofia foliosa*; Antioxidant activity; DPPH; Reducing power; Superoxide anion; Hydroxyl radical; Iron chelation; DNA damage

1. Introduction

Reactive oxygen species (ROS) such as superoxide anions ($O_2^{\cdot-}$) hydroxyl radicals ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2) are generated by normal physiological processes and various exogenous factors. Once generated, ROS initiate the peroxidation of membrane lipids as well as a wide range of biological molecules through a process that is believed to be implicated in the etiology of several disease conditions, including coronary artery diseases, stroke, rheumatoid arthritis, diabetes and cancer (Halliwell, Gutteridge, & Cross, 1992; Lefer & Grander, 2000).

Antioxidants are substances which can either directly scavenge ROS or prevent the generation of ROS. For many decades, there has been a growing interest in finding novel antioxidants that safely prolong the shelf life of foods as well as combating and/or preventing ROS-mediated diseases. As part of this search for antioxidants from natural sources, a study by Yen, Duh, and Chua (2000) on natural anthraquinons has lead in the identification of anthrone [**1**; see Fig. 1] but not anthraquinone [**2**] as a good antioxidant agent. In agreement with this observation, knipholone [**3**], an axially chiral phenylanthraquinone isolated from *Kniphofia foliosa*, Hochst (Asphodelaceae) (Bringmann, Kraus, Menche, & Messer, 1999a; Dagne & Steglich, 1984), is reported to have a very weak free radical scavenging and lipid peroxidation inhibitory activity (Wube et al., 2006). Knipholone [**3**] is also reported to have no metal-chelating activity (Wube et al., 2006). To the best of the author's knowledge, however, the antioxidant potential of knipholone anthrone [**4**] isolated from the same plant has not yet been reported. The aims of the present study were thus to establish the antioxidant properties of knipholone anthrone [**4**] by measuring scavenging activity against free radicals, chelating ability on ferrous ions, reducing capacity and protection of biological molecules from reactive oxygen species-induced damage.

* Tel.: +44 208 331 8302; fax: +44 208 331 9805.
E-mail address: S.Habtemariam@gre.ac.uk.

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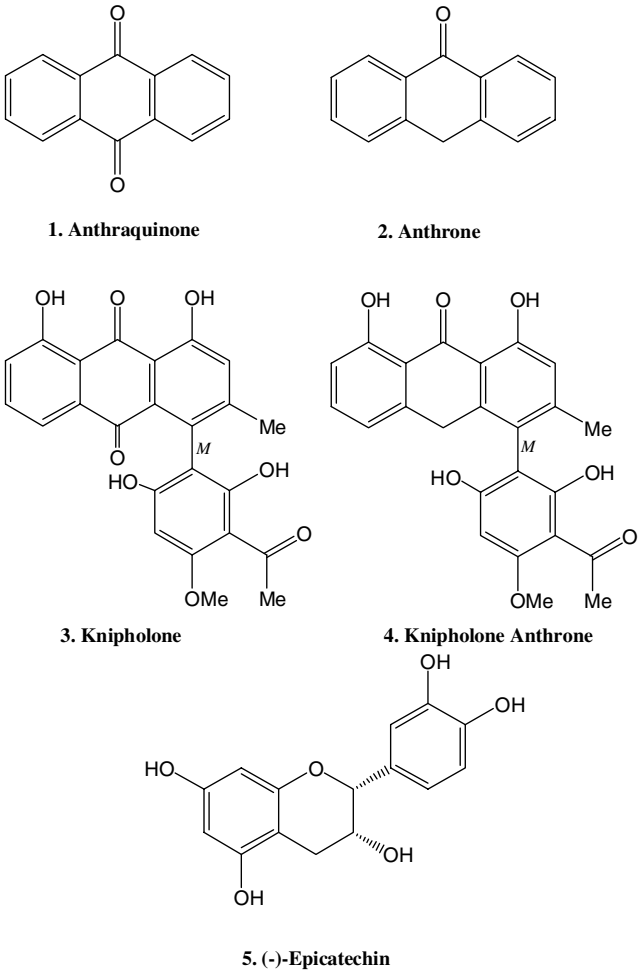

Fig. 1. Chemical structures of KA and related compounds.

2. Materials and methods

2.1. Materials

Agarose, ascorbic acid, 2-deoxy-D-ribose, 2,2-diphenyl-1-picrylhydrazyl (DPPH), DNA marker (lambda DNA Hind III digest), Dulbecco's phosphate buffer saline, ethylenediaminetetraacetic acid (EDTA), (−)-epicatechin (EC), ethidium bromide, $FeCl_2$, $FeCl_3$, hydrogen peroxide (H_2O_2), nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), thiobarbituric acid (TBA), tissue culture media and supplements (RPMI medium, heat inactivated foetal bovine serum, penicillin–streptomycin), trichloroacetic acid (TCA) and Tris were obtained from Sigma–Aldrich Chemical Company (Dorset, UK). Knipholone anthrone (KA) was kindly provided by Professor Ermias Dagne (Addis Ababa University, Ethiopia).

2.2. Scavenging of DPPH radical

The method of Blois (1958) was adopted for 96-well plate microtitre assay. Briefly, DPPH solution (0.1 mM, in methanol) was incubated with varying concentrations of test compounds made in threefold dilutions. The reaction mixture was incubated for 20 min at room temperature and the absorbance of the resulting solution was read at 550 nm against a blank using Multiscan EX Reader (Thermo Labsystems, Altrincham, UK). The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following equation:

$$\%\ \text{Scavenging effect} = (1 - A_{\text{Sample}}/A_{\text{Control}}) \times 100$$

where

A_{Control} is the absorbance value of DPPH alone and A_{Sample} is absorbance of DPPH and sample mixture minus sample absorbance alone.

2.3. Hydroxyl radical scavenging assay

The method of Halliwell, Gutteridge, and Aruoma (1987) was adopted. The reaction mixture, containing test compounds was incubated with deoxyribose (3.75 mM), H_2O_2 (1 mM), $FeCl_3$ (100 μM), EDTA (100 μM) and ascorbic acid (100 μM) in Dulbecco's phosphate buffer saline (pH 7.4) for 60 min at 37 °C. The reaction was terminated by adding 1 ml of TBA (1% w/v) and 1 ml of TCA (2% w/v) and then heating the tubes in a boiling water bath for 15 min. The contents were cooled and absorbance of the mixture was measured at 535 nm against reagent blank. Decreased absorbance of the reaction mixture indicates decreased oxidation of deoxyribose. The percentage inhibition of deoxyribose oxidation was calculated as $[1 - (T/C)] \times 100$, where:

C = control, malodialdehide produced by Fenton reaction alone,
T = test, malondiaaldehyde produced in the presence of test compounds.

2.4. Superoxide radical scavenging assay

Superoxide anion was generated in a non-enzymatic PMS–NADH system following modification of the method described by Robak and Gryglewski (1988). The reaction mixture, containing test compounds and PMS (30 μM), NADH (338 μM) and NBT (72 μM) in Dulbecco's phosphate buffer saline was incubated at room temperature for 5 min and the absorbance was read at 550 nm against a blank. The capability of scavenging superoxide radical was calculated using the equation similar with that described above for the DPPH assay.

2.5. Measurement of reducing power

The reducing power of test agents was quantified by the method described by Yen et al. (2000) with minor modifications. Briefly, 1 ml of the reaction mixture, containing

different concentrations of samples in Dulbecco's phosphate buffer saline (pH 7.0), was incubated with potassium ferricyanide (1%, w/v) at 50 °C for 20 min. Following the termination of the reaction by TCA solution (10%, w/v), ferric chloride (0.1%, w/v) was added to diluted (in distilled water) samples and the absorbance was measured at 700 nm. An increase in absorbance of the reaction mixture suggests a greater reducing power.

2.6. Fe^{2+} chelating assay

The absorption spectra (200–500 nm) of methanol solution of KA in the presence or absence of varying concentration of $FeCl_2$ was recorded using an UV–vis Spectrophotometer (VARIAN, Cary 100 Scan, Oxford, UK).

2.7. DNA extraction

RAW264.7 cells obtained from European Collection of Cell Culture (Porton Down, UK) were routinely maintained with RPMI 1640 medium supplemented with 10% heat inactivated foetal bovine serum, 50 IU/ml penicillin and 50 μg/ml streptomycin. Cells (1.5×10^9 cells) were washed twice with Dulbecco's phosphate buffer saline and treated with 2 ml of lysis buffer (150 mM NaCl, 10 mM Tris, pH 7.5, 10 mM EDTA and 0.5% SDS) for 10 min on ice. After adding RNAse (final concentration 50 μg ml^{-1}) to the lysate, the resulting suspension was incubated for 30 min at 37 °C. Proteinase K (final concentration 1000 μg ml^{-1}) was then added to the suspension and incubated for 1 h to dissolve the proteins. After extracting once with phenol and chloroform/isoamyl alcohol (24:1), respectively, DNA in the aqueous phase was precipitated at −70 °C (1 h) following addition of half volume of 7.5 M NH_4OAc and two volumes of absolute ethanol. The DNA taken by spooling was allowed to dry and dissolved in Tris-EDTA buffer (10 mM Tris, 1 mM EDTA).

2.8. Assessment of DNA damage induced by hydroxyl radical generated by Fenton reaction

The reaction mixture in 18 μl volume in TAE buffer (40 mM Tris, 20 mM sodium acetate, 1 mM EDTA, pH 8.2) contained 2.5 μg DNA, 0.5 mM $FeCl_2$, 3.3% H_2O_2 and various concentrations of the test compound. After 1 h of incubation at 37 °C, DNA samples were resolved in a 1% agarose gel containing ethidium bromide (0.5 μg/ml) in TAE buffer. The DNA in gel was visualized under a UV transilluminator (UVP BioImaging Systems, EpiChemi^3 Darkroom, Cambridge, UK).

3. Results and discussion

Knipholone anthrone [**4**, see Fig. 1] was originally isolated and characterized from an Ethiopian medicinal plant, *K. foliosa*, Hochst (Asphodelaceae) (Dagne & Steglich, 1984) and its structural identity with respect to its absolute configuration latter established by quantum chemical CD calculations (Bringmann et al., 1999a). KA has been shown to have a potent antiplasmodial effect and a relatively low level of cytotoxicity to mammalian cells (Bringmann, Menche, Bezabih, Abegaz, & Kaminsky, 1999b). Although knipholone [**3**], a structurally similar compound to KA, isolated from the same plant has been reported to have no significant antioxidant activity (Wube et al., 2006), anthrone [**1**] is reported to exhibit much higher antioxidant activity than anthraquinone [**2**] (Yen et al., 2000), suggesting that KA may also possess antioxidant activity. The primary aim of the present study was thus to investigate the antioxidant potential of KA by using a variety of *in vitro* assays.

The DPPH assay, which measures the ability of compounds to transfer labile H-atoms to radicals, is the commonest method of antioxidant activity evaluations (Brand-Williams, Cuvelier, & Berset, 1995). The abstraction of hydrogen by this stable free radical is known to lead to the bleaching of the absorption maxima at 517 nm and can easily be monitored spectrophotometrically. Based on the results obtained from this established assay system, it appears that KA showed a potent and concentration-dependent free radical scavenging effect (Fig. 2). The calculated IC_{50} value for KA and the positive control, EC in this assay were 22 ± 1.5 and 8.7 ± 0.9 μM (mean ± SEM; $n = 3$ separate experiments), respectively. This observed scavenging effect of the positive control, EC is also in close agreement with previous studies in the same assay system (Geetha, Garg, Chopra, & Kaur, 2004).

To date, the $OH^{\cdot}$ is one of the most reactive free radical species known with damaging effects to almost every biological molecule found in living cells. It can be generated *in vivo* in the presence of both $O_2^{\cdot-}$ radicals and transition metals, such as iron or copper via the Haber–Weiss

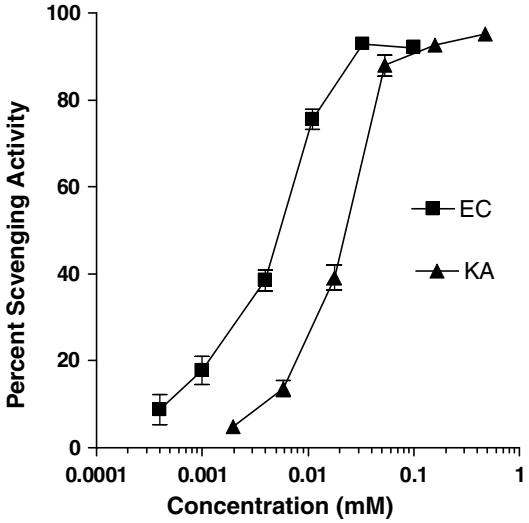

Fig. 2. DPPH scavenging activity of KA and EC. The percent scavenging activity of test compounds obtained from a typical representative experiment is shown. Data are mean values ± SEM ($n = 6$).

reaction (Castro & Freeman, 2001). In order to further substantiate the free radical scavenging capacity of KA, an *in vitro* Fenton-type assay system ($Fe^{2+} + H_2O_2 \rightarrow OH^{\cdot} + OH^- + Fe^{3+}$) that measures the generation of $OH^{\cdot}$ and its subsequent effect on oxidation of biological molecule (Halliwell et al., 1987) was used. The system involves autoxidation of iron(II)–EDTA complex in aqueous solution to form $O_2^{\cdot-}$, which is rapidly dismutated to H_2O_2 at pH 7.4, and H_2O_2 further interacting with iron(II), to form $OH^{\cdot}$ radicals in the presence of ascorbic acid as a catalyst (Caillet et al., 2005 and references there in). As shown in Fig. 3, the concentration-dependent scavenging effect by KA and the positive control, EC was evident. The activity range of the positive control EC in this assay system was comparable with previous studies (Zhao, Yang, Wang, Li, & Jiang, 2006) but appears (unlike the DPPH assay results) to be weaker than KA. As shown in Fig. 3, the order of potency observed in the DPPH assay was also reversed with KA (IC_{50} 0.03 ± 0.008 mM, $n = 3$ separate experiments) about two times more potent than EC (IC_{50} 0.07 ± 0.01 mM, $n = 4$ separate experiments).

Since the $OH^{\cdot}$ scavenging activity in Fenton assay system could be influenced both by electron-donating and metal-chelating properties, it was necessary to examine the metal-chelating potential of KA. It is well established that transition metals, in particular, iron have a major role in the generation of reactive oxygen species in living organisms. Iron exists in two distinct oxidation states – ferrous and the relatively biologically inactive form, ferric ion. Ferric ions, however, has been shown to be reduced to the active Fe^{2+}, depending on the conditions, particularly pH (Strlic, Radovic, Kolar, & Pihlar, 2002) and oxidized back through Fenton-type reactions, with production of $OH^{\cdot}$ or Haber–Weiss cycle reactions with superoxide anions (Kehrer, 2000). Iron chelating agents are thus expected to inhibit the metal-dependent oxidative processes and have potential in combating reactive oxygen species mediated diseases (Finefrock, Bush, & Doraiswamy, 2003). Metal-chelation properties of phenolics is commonly assessed by means of UV–vis absorption spectroscopy, analysing the shifts of UV bands. As shown in Fig. 4, the addition of Fe^{2+} to the UV–vis spectra of KA leads to a concentration-dependant red-shift suggesting complex formation between iron and KA. The observed protective effect of KA against the $OH^{\cdot}$ radical-induced deoxyribose oxidation could thus be in part due to chelation of iron ions.

In order to further establish the biological significance of antioxidant potential of KA, its protective effect to one of the major biological target of free radicals, DNA was assessed. It has been established that DNA damage induced by $OH^{\cdot}$ generated from Fenton reaction depends on the concentrations of Fe^{2+} and H_2O_2 (Tian & Hua, 2005). In the present study, 10% H_2O_2 produced a total destruction of DNA while lower concentrations in the presence of Fe^{2+} produced a concentration-dependent smearing (suggesting damage) of DNA on agarose gel (data not shown). In the presence of KA, the DNA damage in this system was inhibited in a concentration-dependant manner (Fig. 5). The results obtained from this qualitative assay also revealed a similar pattern of radical scavenging effect with the DPPH assay in that 4.4 μM and above concentrations of KA attenuated the $OH^{\cdot}$ radical-induced DNA damage (Fig. 5).

Different studies have indicated that the antioxidant effect of many compounds is related to the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain through donation of hydrogen atoms (Qi et al., 2005). The reducing capacity of a compound may thus further serve as a significant indicator of potential antioxidant activity. In the present study, the reductive ability of KA was assessed based on measurement of Fe^{3+}–Fe^{2+} transformation. The concentration-dependent reducing powers (absorbance at 700 nm) shown in Fig. 6 suggests that the compounds (KA and EC), as

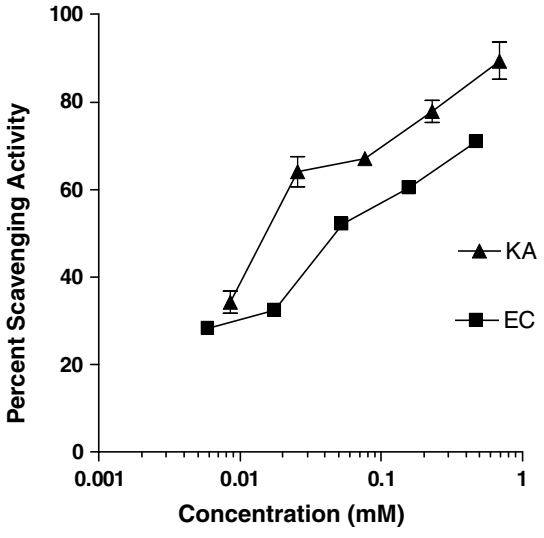

Fig. 3. Hydroxyl radical scavenging activity of KA and EC. The percentage inhibition of deoxyribose oxidation induced by hydroxyl radical generated from Fenton reaction is shown for KA and EC. Data are mean values ± SEM ($n = 4$).

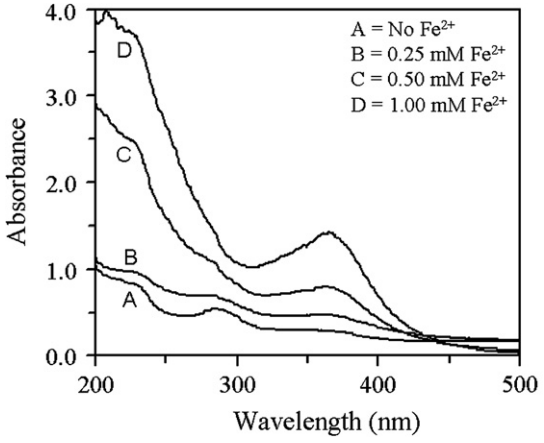

Fig. 4. UV–vis spectra of KA (50 μM) in the presence of several concentrations of Fe^{2+}.

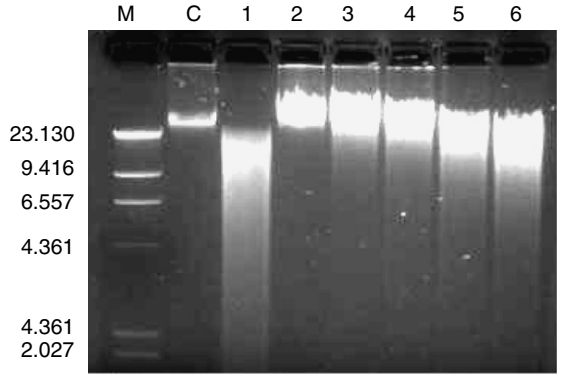

Fig. 5. Agarose gel electrophoresis pattern of isolated DNA damage by hydroxyl radical generated from Fenton reactions in the presence or absence of KA. DNA isolated from RAW264.7 cells was exposed to Fe^{2+} and 3.3% hydrogen peroxide as described in the materials and methods. Lane 1, λ Hind III fragment marker; lane 2, control (Fe^{2+} alone); lane 3, hydrogen peroxide and Fe^{2+} treatment; lanes 4–8, hydrogen peroxide and Fe^{2+} in the presence of 360, 120, 40, 13 and 4.4 μM KA respectively.

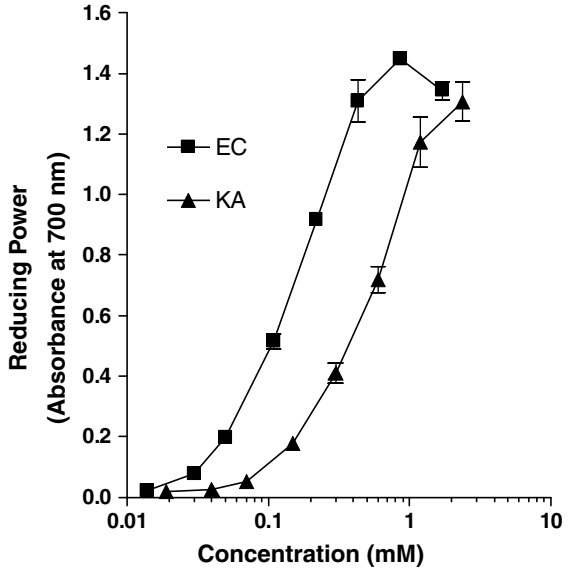

Fig. 6. Reducing power of KA and EC based on measurement of Fe^{3+}–Fe^{2+} transformation. Data points are mean values ± SEM ($n = 4$).

electron donors, can react with free radicals to convert them to more stable products leading to termination of radical chain reactions. This result was also similar with that obtained from the DPPH assay in that EC was more active than KA. As the oxidative deoxyribose damage assay system employed in the present study involves $OH^{\cdot}$ generation through ascorbate-catalyzed Fe^{3+}–Fe^{2+} transformation, the observed protective effect of KA could be due to its iron chelation and/or direct free radical scavenging effect.

Although the $O_2^{\cdot-}$ anion is relatively limited in its direct activity on oxidation of biological molecules, it is a well-recognized free radical species which is continuously formed by several cellular processes, including the mitochondrial electron transport systems. Based on the present assay system, which measures inhibition of $O_2^{\cdot-}$ radical-related formazan production, both KA and EC appeared to show a relatively weaker superoxide radical scavenging effect (Fig. 7) than their DPPH or $OH^{\cdot}$ scavenging effects. It is also evident from the results that KA appeared to be more potent than the positive control, EC at the concentrations tested (Fig. 7). It is worth noting that scavenging activity against $O_2^{\cdot-}$ radical is affected not only by concerted H-atom abstraction (electron donation) but also by catalyzing the dismutation of $O_2^{\cdot-}$ (protonation effect) (Murias et al., 2005) and hence compounds may exhibit differing level of scavenging effect against the various types of ROS.

The overall antioxidant activity of compounds have been shown to be attributed to various mechanisms, including prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Gülçin, Beydemir, Alici, Elmastas, & Büyükokuroglu, 2004). The results obtained from the variety of *in vitro* assay systems used in the present study revealed that KA showed a direct free radical scavenging effect, makes complex formation with iron, inhibit Fe^{3+}–Fe^{2+} transformation as evidenced in its potential reducing power and displayed a good protective effect against reactive oxygen species-induced damage of biological molecules, including isolated DNA. Since ROS are known to have deleterious effect on wound healing and enhancement of antioxidant defenses facilitate the wound healing processes (Aliyev, Glu, Eren, & Açıkgöz, 2004), the observed antioxidant activity of KA in the present study supports the traditional medicinal use of *K. foliosa* as a wound healing agent (Wube et al., 2006). In view of the observed biological activities of KA and known antioxidant (Kouam et al., 2005; Yen et al., 2000) and anticancer activities (Bondy et al., 1994; Hu & Zhou, 2004) of related

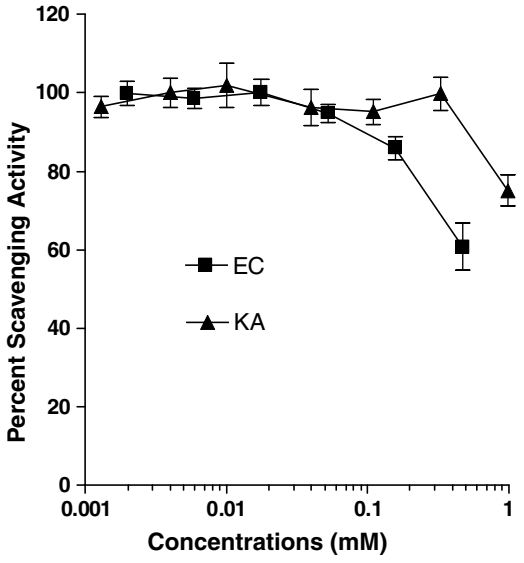

Fig. 7. Superoxide anion scavenging activity of KA and EC. The PMS–NADH and nitro blue colorimetric assay system is described in Section 2. Data are mean values ± SEM ($n = 6$).

anthrone-based compounds, further work is necessary to establish the wide-range therapeutic potential of KA.

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References

Aliyev, E., Glu, U. S., Eren, Z., & Açıkgöz, G. (2004). The effect of polylactide membranes on the levels of reactive oxygen species in periodontal flaps during wound healing. *Biomaterials, 25*, 4633–4637.

Blois, M. S. (1958). Antioxidant determination by the use of a stable free radical. *Nature, 181*, 119–200.

Bondy, G. S., Armstrong, C. L., Dawson, B. A., Héroux-Metcalf, C., Neville, G. A., & Rogers, C. G. (1994). Toxicity of structurally related anthraquinones and anthrones to mammalian cells *in vitro*. *Toxicology in Vitro, 8*, 329–335.

Brand-Williams, B., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology, 28*, 25–30.

Bringmann, G., Kraus, J., Menche, D., & Messer, K. (1999a). Elucidation of the absolute configuration of knipholone and knipholone anthrone by quantum chemical CD calculations. *Tetrahedron, 55*, 7563–7572.

Bringmann, G., Menche, D., Bezabih, M., Abegaz, B. M., & Kaminsky, R. (1999b). Antiplasmodial activity of knipholone and related natural phenylanthraquinones. *Planta Medica, 65*, 757–758.

Castro, L., & Freeman, B. A. (2001). Reactive oxygen species in human health and disease. *Nutrition, 170*, 161–165.

Caillet, S., Yu, H., Lessard, S., Lamoureux, G., Ajdukovic, D., Lacroix, M., (2005). Fenton reaction applied for screening natural antioxidants. *Food Chemistry*. Available from doi:10.1016/j.foodchem.2005.10.009.

Dagne, E., & Steglich, W. (1984). Knipholone: a unique anthraquinone derivative from *Kniphofia foliosa*. *Phytochemistry, 23*, 1729–1731.

Finefrock, A. E., Bush, A. I., & Doraiswamy, P. M. (2003). Current status of metals as therapeutic targets in Alzheimer's disease. *Journal of the American Geriatrics Society, 51*, 1143–1148.

Geetha, T., Garg, A., Chopra, K., & Kaur, I. P. (2004). Delineation of antimutagenic activity of catechin, epicatechin and green tea extract. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 556*, 65–74.

Gülçin, I., Beydemir, S., Alici, H. A., Elmastas, M., & Büyükokuroglu, M. E. (2004). *In vitro* antioxidant properties of morphine. *Pharmacological Research, 49*, 59–66.

Halliwell, B., Gutteridge, J. M. C., & Cross, C. E. (1992). Free radicals, antioxidants and human disease: where are we now? *Journal of laboratory and Clinical Medicine, 119*, 598–620.

Halliwell, B., Gutteridge, J. M. C., & Aruoma, O. I. (1987). The deoxyribose method: a simple test tube assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Biochemistry, 165*, 215–219.

Hu, W., & Zhou, W. (2004). Synthesis and antitumor activity of 10-substituted benzylidene anthrone. *Bioorganic & Medicinal Chemistry Letters, 14*, 621–622.

Kehrer, J. P. (2000). The Haber–Weiss reaction and mechanisms of toxicity. *Toxicology, 149*, 43–50.

Kouam, S. F., Ngadjui, B. T., Krohn, K., Wafo, P., Ajaz, A., & Choudhary, M. I. (2005). Prenylated anthronoid antioxidants from the stem bark of *Harungana madagascariensis*. *Phytochemistry, 66*, 1174–1179.

Lefer, D. J., & Grander, D. N. (2000). Oxidative stress and cardiac disease. *American Journal of Medicine, 109*, 315–323.

Murias, M., Jäger, W., Handler, N., Erker, T., Horvath, Z., Szekeres, T., et al. (2005). *Biochemical Pharmacology, 69*, 903–912.

Qi, H., Zhang, Q., Zhao, T., Chen, R., Zhang, H., Niu, X., et al. (2005). Antioxidant activity of different sulfate content derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta) *in vitro*. *International Journal of Biological Macromolecules, 37*, 195–199.

Robak, J., & Gryglewski, I. R. (1988). Flavonoides are scavengers of superoxide anions. *Biochemical Pharmacology, 37*, 837–841.

Strlic, M., Radovic, T., Kolar, J., & Pihlar, B. (2002). Anti- and prooxidative properties of gallic acid in Fenton-type systems. *Journal of Agricultural and Food Chemistry, 50*, 6313–6317.

Tian, B., & Hua, Y. (2005). Concentration-dependence of prooxidant and antioxidant effects of aloin and aloe-emodin on DNA. *Food Chemistry, 91*, 413–418.

Wube, A. A., Bucar, F., Asres, K., Gibbons, G., Adams, M., Streit, B., et al. (2006). Knipholone, a selective inhibitor of leukotriene metabolism. *Phytomedicine, 13*(6), 452–456.

Yen, G.-C., Duh, P.-D., & Chua, D.-Y. (2000). Antioxidant activity of anthraquinones and anthrone. *Food Chemistry, 70*, 437–444.

Zhao, M., Yang, B., Wang, J., Li, B., & Jiang, Y. (2006). Identification of the major flavonoids from pericarp tissues of lychee fruit in relation to their antioxidant activities. *Food Chemistry, 98*(3), 539–544.