

## Research Article

# Antioxidant Activities of Plumbagin and Its Cu (II) Complex

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Plumbagin and its Cu (II) complex [Cu (plumbagin)<sub>2</sub>]-H<sub>2</sub>O have been synthesized, and their antioxidant activities towards the inhibitory effect on DPPH free radical, reducing power, total antioxidant capacity, and inhibition on lipid peroxidation were investigated. Plumbagin and its Cu (II) complex were found to exhibit scavenging activities on DPPH radical with the inhibitory rate of 41% and 24%, respectively. The reducing power of plumbagin was outstanding at the concentrations of 1.0, 1.5, and 2.0 mg/mL, compared to Cu (II) complex and synthetic antioxidant 2,6-di-ter-butyl-4-methylphenol (BHT); the highest level reached 1.333 for plumbagin and 0.581 for Cu (II) complex. Also, the inhibition on lipid peroxidation of plumbagin was higher than that of Cu (II) complex and BHT, 46.4% for plumbagin and 24.5% for Cu (II) complex. The results give a strong impact for designing anticancer drugs, combined with their potential cytotoxic and antioxidant activities, which can be targeted selectively against cancer cells and increase their therapeutic index and additional advantages over other anticancer drugs.

## 1. Introduction

Recently, a large number of metal complexes have been designed and tested for anticancer activity and for supportive therapy in cancer patients, including the anticancer drugs doxorubicin, mitoxantrone, bleomycin, and hydroxyurea [1–3]. There is a major debate at present that natural products with diverse bioactivities are becoming an important source of potential (pro)drug chelators [4, 5]. Many naturally occurring molecules such as flavonoids, phenols, and quinones have metal chelating properties, cytotoxic, and antioxidant properties, including the inhibitory effect on free radicals and other damaging oxygen activated products such as the hydroxyl radical, superoxide, hydrogen peroxide, and lipid peroxides [6, 7]. Thus, the prospect of designing chelating prodrugs can be targeted selectively and activated against cancer cells combined with their potential cytotoxic and antioxidant activities increasing their therapeutic index and provides additional advantages over other anticancer drugs [8–10].

Our earlier studies have reported the synthesis and anticancer activities of plumbagin (Scheme 1) and its Cu (II) complex (Scheme 2). Plumbagin structurally derived from

naphthoquinone was extracted from *Plumbago zeylanica* L, a Chinese traditional medicine. It was found that both plumbagin and its Cu (II) complex exhibited significant antitumor activities against seven human tumour cell lines (BEL-7404, NCI-H460, CNE-2, 786-O, MCF-7, HCT-116, and Hep-G2), and Cu (II) complex possessed significantly enhanced antitumor with respect to the free ligand Cu (II) ion plays an important role [11]. All of these results make it interesting to study the antioxidant activities of plumbagin and its Cu (II) complex.

## 2. Materials and Methods

UV-Vis absorption spectra were performed on a Varian Cary100 UV-Visible spectrophotometer. 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Chemicals Co. All the metallic salts were purchased from Alfa Co. Ltd. The solvents were analytical grade. All the materials were used as received without further purification unless noted specifically.

Plant material, roots of *P. zeylanica*, was collected in Guangxi province and identified by Prof. Tang (Institute of Life Science, Guangxi Normal University).



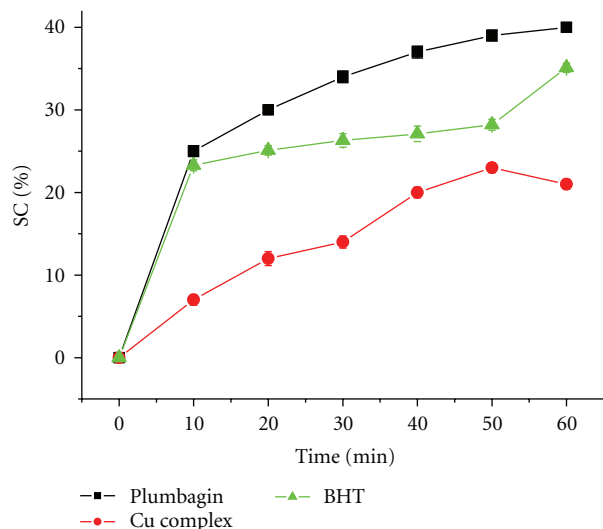


FIGURE 1: DPPH free radical scavenging activity of plumbagin, Cu (II) complex, and BHT in different reaction times (10, 20, 30, 40, 50, and 60 min).

### 3. Results and Discussion

**3.1. Scavenging Activity on DPPH Radical.** Antioxidant properties, especially radical scavenging activities, are very important due to the deleterious role of free radicals in biological system. DPPH is a kind of stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [6], which was widely used to investigate radical scavenging activity now for its advantage of ease and economy. In DPPH radical scavenging assay, antioxidants are able to reduce the stable DPPH radical to yellow-coloured, and the antioxidant power is indicated by the degree of discoloration which could be determined by measuring of a decrease in the absorbance at 515 nm.

As Figure 1 shows, at the concentration of 1 mg/mL of plumbagin or Cu (II) complex possessed scavenging activities on DPPH radical with the inhibitory rate of 41% and 24%, respectively, their scavenging effect increased with increasing reaction time, and plumbagin scavenging activities are pretty, more higher than its Cu (II) complex and BHT, indicating that the presence of Cu (II) decreased the scavenging effect of DPPH radical.

**3.2. Reducing Power.** The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [16]. In the assay, the presence of reductants in the antioxidant sample causes the reduction of the  $\text{Fe}^{3+}$ /ferricyanide complex to the  $\text{Fe}^{2+}$ /ferrous form [17], so the reducing power of the sample could be monitored by measuring the formation of Perl's Prussian blue at 695 nm [18]. As Figure 2 shows, the reducing properties of plumbagin and Cu (II) complex at different concentration exhibited correlation with increasing concentration. The reducing power of plumbagin was outstanding at the concentration of 1.5 and 2.0 mg/mL compared to Cu (II) complex and BHT. At the concentration of 2.0 mg/mL, the reducing power of

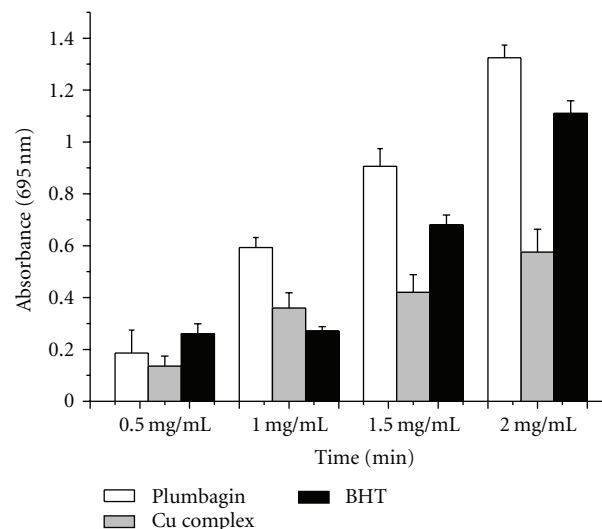


FIGURE 2: The reducing power of plumbagin, Cu (II) complex and BHT treated with different solution concentrations (0.5, 1.0, 1.5, and 2.0 mg/mL).

plumbagin and Cu (II) complex reached 1.333 and 0.581, respectively. It was speculated that the decreased antioxidant power of Cu (II) complex, compared to plumbagin, may be the absence of phenolic group due to forming the complex, thus the presence of the active hydrogen in phenolic hydroxyl group was likely to contribute considerably to the observed antioxidant effects.

**3.3. Total Antioxidant Capacity Assay.** The assay is based on the reduction of Mo (VI) to Mo (V) by plumbagin and Cu (II) complex in different reaction times (20, 40, 60, 80, 100, and 120 min) and subsequent formation of a green phosphate/Mo (V) complex at acid pH. The high absorbance values indicated that the sample possessed significant antioxidant activity. As Figure 3 shows, plumbagin and Cu (II) had significant total antioxidant activities compared to BHT, and the effects increased with increasing reaction time, while total antioxidant capacity of Cu (II) complex was superior to plumbagin.

**3.4. Lipid Peroxidation in Peanut Oil.** The level of 2-thiobarbituric acid-reactive substances (TBARS), products of lipid peroxidation, is often measured in order to assess the extent of oxidation that occurs in biological systems. Peanut oil was treated with plumbagin, Cu (II), and BHT complex at the concentration of 1 mg/mL in different reaction times (1, 2, 3, 4, 5, and 6 days). As shown in Figure 4, the inhibition rate of lipid oxidation between plumbagin and Cu (II) complex had significant effects and was enhanced with increasing reaction time, the highest level reached 46.4% for plumbagin and 24.5% for Cu (II) complex after 6 days, and the lipid oxidation inhibition of Cu (II) complex is lower than that of PLN and BHT at the same period.

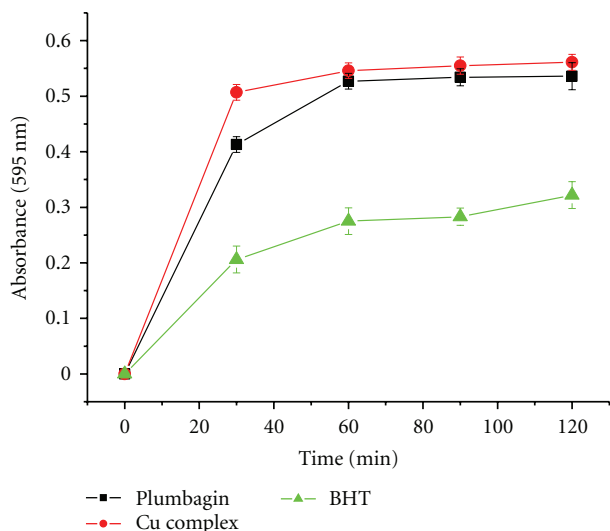


FIGURE 3: The total antioxidant power of plumbagin, Cu (II) complex, and BHT in different reaction times (20, 40, 60, 80, 100, and 120 min).

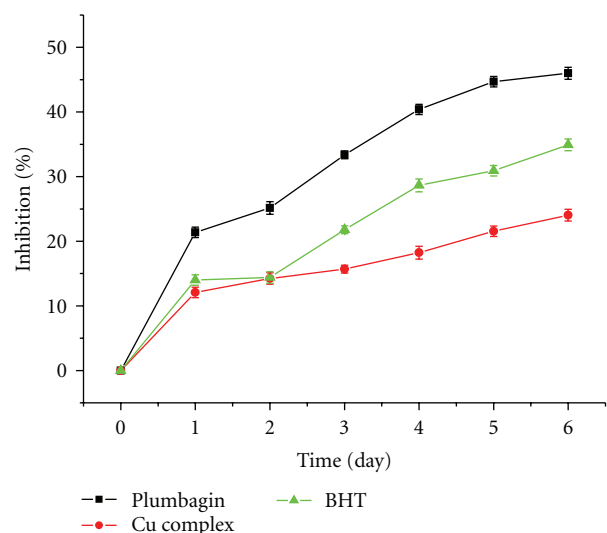


FIGURE 4: Lipid oxidation inhibition by plumbagin, Cu (II) complex, and BHT in peanut oil in different reaction times (1, 2, 3, 4, 5, and 6 days).

#### 4. Conclusions

In this study, we have investigated the antioxidant possibility of plumbagin and its Cu (II) complex. The results indicated that both the compounds exhibited some antioxidant activities, including the inhibitory effect on DPPH free radical, reducing power, total antioxidant capacity, and lipid peroxidation, which plumbagin possessed higher abilities than that of Cu (II) complex and BHT at the same condition towards DPPH free radical, reducing power, and lipid peroxidation. It was speculated that the decreased antioxidant power of Cu (II) complex compared to plumbagin may be the absence of phenolic group due to forming the complex, thus the presence of the active hydrogen in phenolic hydroxyl

group was likely to contribute considerably to the observed antioxidant effects. Also, this study gives a strong impact for designing anticancer drugs, combined with their potential cytotoxic and antioxidant activities, which can be targeted selectively against cancer cells and increase their therapeutic index and additional advantages over other anticancer drugs.

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