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Free Radical Scavenging Activities of the Extracts from *Taxus chinensis var. mairei*

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Under ultrasound, *Taxus chinensis var. mairei* were extracted with ethanol at 80 ºC to offer ethanol extract. Ethanol extract was further extracted with chloroform and separated by column chromatography to offer *paclitaxel*. Their free radical scavenging activity were evaluated and compared with the common antioxidant butylated hydroxytoluene, employing $DPPH^{\bullet}$ assay, $ABTS^{+\bullet}$ assay, $O_2^{\bullet-}$ assay and $OH^{\bullet}$ assay. The results showed that ethanol extract exhibited better scavenging activity than the common antioxidant butylated hydroxytoluene in these four assays, with IC_{50} of 13.19, 2.65, 25.33 and 343.00 µg/mL, while *paclitaxel* showed less scavenging activity than butylated hydroxytoluene, with IC_{50} of 1.49, 46.14, 3.52 and 2.17 mg/mL, respectively. The antioxidant activity screening results demonstrated that ethanol extract displayed better antioxidant activity than that of *paclitaxel* and the excellent antioxidant activity of ethanol extract may be attributed to other compositions of ethanol extract.

Key Words: *Taxus chinensis var. mairei*, *Paclitaxel*, Free radical scavenging activity, Ultrasound-assisted extract.

INTRODUCTION

Free radicals and reactive oxygen species play an important role in the pathogenicity of numerous diseases[1-5], such as various chronic and age-related diseases. To protect against the destruction caused by free radicals and reactive oxygen species, nature has created an antioxidant defense system composed of group of compounds and enzyme potent enough to remove free radicals and reactive oxygen species before they cause tissue damage. However, only endogenous antioxidant defenses are not completely efficient. It is necessary to supple some exogenous antioxidants. In addition, antioxidants are currently forged as the drug candidates to counter these diseases including cancer, carcinogenesis and heart diseases. Therefore, more and more studies has been attracted to explore new and efficient antioxidants. Screening for natural antioxidant from plants is one of the important and efficient methods.

Taxus chinensis var. mairei, a kind of *Taxus mairei* which is mainly distributed in Fujian, Taiwan, Zhejiang and Guangxi province of China, is largely used in the treatment of diuresis detumescence, diabetes mellitus and kidney *etc.*[6]. *Taxus chinensis var. mairei* is well known for its component *paclitaxel*, which is considered as a highly effective anticancer drug[7,8]. Previous studies have demonstrated that various diseases including cancer and carcinogenesis are characteristically associated with free radicals and reactive oxygen species, it is thus to expect that the extract of *Taxus chinensis var. mairei*, as well as its component *paclitaxel* may have good antioxidant activity. Therefore, *Taxus chinensis var. mairei* is chosen in the present work to extracted and evaluated for their antioxidant activities.

EXPERIMENTAL

Taxus mairei were collected from Guilin city of Guangxi Province in Octorber, 2011 and they were cut and crushed to offer brown powder. Under ultrasound, *Taxus mairei* (200 g) were extracted with ethanol at 70 ºC for 2 h and filtered through Whatman No. 4 filter paper. The extract solution was vacuum evaporated at 50 ºC to dryness to offer ethanol extract with 20.3 % yields. Ethanol extract was then extracted with chloroform and separated by column chromatography to offer white crystals of *paclitaxel* in 0.8 % yield, using chloroform-methanol as eluent, according to the previous literature[9]. The obtained *paclitaxel* in the experimental was confirmed by TLC method and its R_f value and melting point (m.p. 216-218 ºC) were consistent with the standard sample.

Ethanol extract and *paclitaxel* were dissolved in chloroform to the concentrations 1×10^{-3} mg/mL, respectively and then their UV/visbile spectra were determined by TU-1901 ultraviolet spectrophotometer. As showed in Fig. 1, it can be found that in the UV/visible spectra of ethanol extract there were two main peaks, while there was only a peak in 272 nm in the UV/visbile spectra of *Paclitaxel*. The peak around 200-250 nm in UV/visible spectra

of ethanol extract may be ascribed to alkaloids, while the peak 260-300 nm may be attributed to *paclitaxel*.

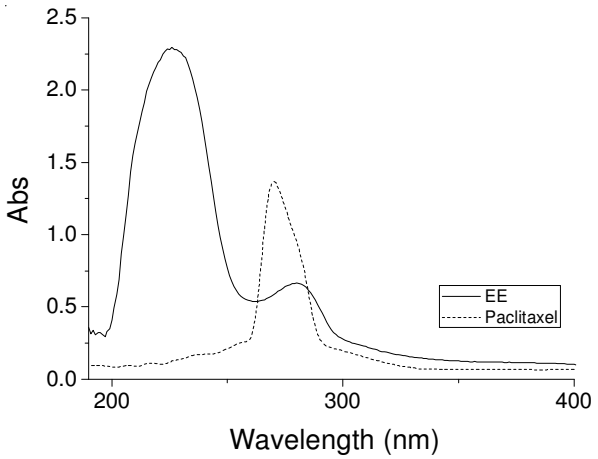

Fig. 1. UV/visible spectra of ethanol extract (EE) and *paclitaxel*

RESULTS AND DISCUSSION

The free radical scavenging activities of ethanol extract and *paclitaxel* were determined as shown in Figs. 2 and 3 according the literatures[10]. The values of IC_{50}, the effective concentration at which 50 % of the radicals were scavenged, were calculated to evaluate the radical scavenging activities (Table-1). A lower IC_{50} value indicated greater radical scavenging activity. IC_{50} values of less than 10 mg/mL usually indicated effective activities in antioxidant properties[11].

It can be seen in Figs. 2 and 3 that ethanol extract and *paclitaxel* showed evident radical scavenging activities in $DPPH^{\bullet}$ assay. As showed in Table-1, IC_{50} values of ethanol extract and *paclitaxel* were found to be 13.19 μg/mL and 1.49 mg/mL, respectively. Since that their IC_{50} values were much less than the standard value 10 mg/mL[11], it could concluded that ethanol extract and *paclitaxel* exhibited potent inhibition of DPPH radical. Moreover, ethanol extract even displayed better scavenging activity than the common antioxidant BHT in this assay.

Figs. 2 and 3 showed that ethanol extract and *paclitaxel* exhibited clear radical scavenging activities in $ABTS^{+\bullet}$ assay. Table-1 displayed that IC_{50} values of ethanol extract and

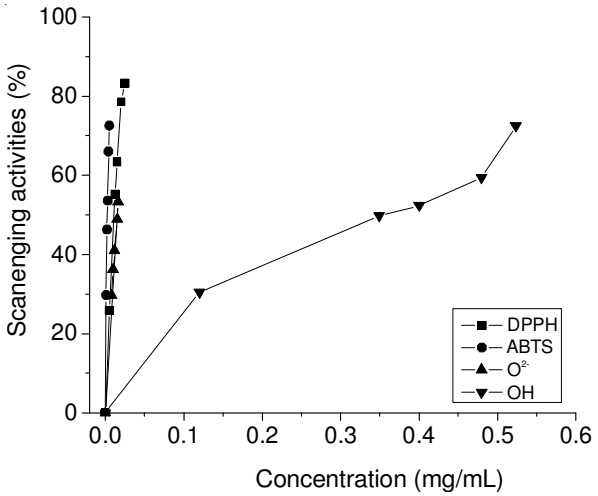

Fig. 2. Radical-scavenging activities of ethanol extract

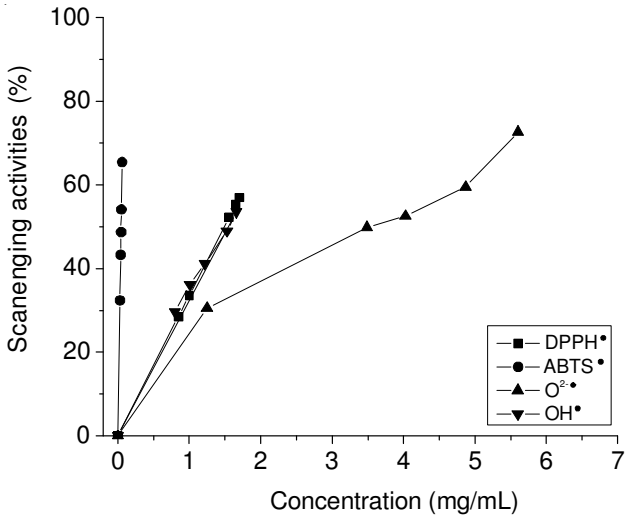

Fig. 3. Radical-scavenging activities of *paclitaxel*

TABLE-1
IC_{50} OF THE EXTRACTS AND *paclitaxel*

Entry	IC_{50} Exact (μg/mL)	IC_{50} *Paclitaxel* (mg/mL)	IC_{50} BHT (μg/mL)
$DPPH^{\bullet}$	13.19	1.49	14.50
$ABTS^{+\bullet}$	2.65	0.04614	8.15
$O_2^{-\bullet}$	25.33	3.52	55.85
$OH^{\bullet}$	343.00	2.17	5438.69

paclitaxel were found to be 2.65 μg/mL and 46.14 μg/mL, respectively. Obviously, their IC_{50} values were much lower than the standard value 10 mg/mL[11], it was thus to conclude that ethanol extract and *paclitaxel* showed good inhibition of $ABTS^{+}$ radical. It was important to note that ethanol extract even displayed better scavenging activity than the common antioxidant BHT in this assay.

As shown in Figs. 2 and 3, ethanol extract and *paclitaxel* exhibited marked radical scavenging activities in superoxide anion assay. Table-1 showed that IC_{50} values of ethanol extract and *paclitaxel* were 25.33 μg/mL and 3.52 mg/mL, respectively and they were less than the standard value 10 mg/mL[11], showing their good inhibition of superoxide anion radical. It was also important to note that ethanol extract even displayed better scavenging activity than the common antioxidant BHT in this assay.

As can be seen in Figs. 2 and 3, ethanol extract and *paclitaxel* displayed good activity in an amount dependent manner. It can be seen from Table-1, ethanol extract showed the best hydroxyl radical scavenging activity in this assay with IC_{50} of 343.00 μg/mL, while IC_{50} values of *paclitaxel* was found to be 2.17 mg/mL, respectively. It was important to point out that ethanol extract showed better hydroxyl radical scavenging activity than the common antioxidant BHT.

On the basis of the above observation, it could be concluded that ethanol extract and *paclitaxel* exhibited good antioxidant activities, which may contribute to their good antitumour activities. Since that ethanol extract showed better radical scavenging activity than *paclitaxel* in all the fours assays, so it could be concluded that the excellent antioxidant activity of ethanol extract may be ascribed to other composition of ethanol extract.

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