Research Article

Reducing power and superoxide radical scavenging activity of a triterpene derivative isolated from root bark of *Zizyphus nummularia* Aubrev

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Abstract

Objective: The aim of the study is to investigate the reducing power and superoxide radical scavenging activity of the ethanolic extract (EE) and an identified lead compound (LC) which is a triterpene derivative isolated from root bark of *Zizyphus nummularia*. **Material and methods**: The *in vitro* evaluation of antioxidant activities of ethanolic extract (EE) and an identified lead compound (LC) was done by measuring reducing power, superoxide radical. **Results and conclusion**: The result showed that the reducing power and superoxide radical scavenging activity of EE, LC followed a dose dependent pattern. The IC_{50} values for LC are comparatively lower than EE. So, the study showed that EE and LC of *Z. nummularia* have potent reducing power and superoxide radical inhibitory activity.

Keywords: *Zizyphus nummularia*, reducing power, superoxide radical, Octadecahydro-picene-2,3-14-15-tetranone

Introduction

Free radicals are chemically unstable atoms that cause damage to lipid cells, proteins and DNA as a result of imbalance between the generation of reactive oxygen species (ROS) and the antioxidant enzymes (Manian et al., 2008). Examples of these radicals include superoxide anions, hydroxyl, nitric oxide and hydrogen peroxide radicals. These radicals can be scavenged by the protective role of natural and synthetic antioxidant agents. Recently, there has been a worldwide trend towards the use and ingestion of natural antioxidants present in different parts of plants due to their phytochemical constituents (Mathew and Abraham, 2006; Abalaka et al., 2011).

Zizyphus nummularia (*Z. nummularia*) Aubrev (Rhamnaceae) is one of the most commonly occurring branched thorny shrub species in the sandy soil. The various parts of the plant have different medicinal activity because of presence of different phytoconstituent (Dey Ray and Dewanjee, 2015). We have reported the presence of a new triterpene derivative containing three basic rings of steroidal moiety and two diketone groups which are reported for the first time and the study concluded that anticancer activity of the compound may be inhibiting free radicals due to donation of the active hydrogen (Dey Ray and Dewanjee, 2015). Considering the above findings the present study was designed to investigate the reducing power and superoxide radical scavenging activity of the ethanolic extract (EE) and an identified lead compound (LC).

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Materials and methods

Plant material and reagents

Root bark of *Zizyphus nummularia* were collected in September 2010 from Durgapur, India and authenticated by the Taxonomists of Botanical Survey of India (Ref. CNH/I-I/20/2010/Tech.II/171). A voucher specimen (BCRCP/DP/PT/02/06) has been deposited at our laboratory for future reference. All the reagents used in the assay are of AR grade.

Isolation of triterpene derivative

A new triterpene derivative, Octadecahydro-picene-2,3-14-15-tetranone (represented here as lead compound) was isolated and reported in our earlier work (Dey Ray and Dewanjee, 2015).

Assay of reducing power

The reducing power was determined according to the method of (Oyaizu, 1986) with some modifications. Reaction was carried out in a mixture containing 2.5 mL of EE and LC

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(0.05–0.2 mg/mL) (as test sample), 2.5 ml of 0.1 M sodium phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1%, w/v) by incubating at 50 ºC for 20 min. After addition of 2.5 ml trichloroacetic acid (10%, w/v), the mixture was centrifuged at 5000g for 10 min. The upper layer (5 ml) was mixed with 0.5 ml of fresh $FeCl_3$ (0.1%, w/v), and the absorbance at 700 nm was measured against a blank. PCG was used as the positive control. The IC_{50} values were calculated by probit analysis.

Assay of superoxide radical scavenging activity

The superoxide radical scavenging activity was performed by the method of (Jing and Zhao, 1995) with some modifications. Reaction was carried out in a mixture containing 4.5 ml of 50 mMTris–HCl buffer (pH 8.2), 0.4 ml of 25 m Mpyrogallol solution and 1 ml of EE and LC (0.5–5 mg/mL) by incubating at 25 ºC for 5 min. Finally, 1 ml of 8 m MHCl solution was dripped into the mixture promptly to terminate the reaction. The absorbance of the mixture was measured at 420 nm. PCG was used as the positive control. The superoxide radical scavenging activity was calculated by the following formula:

$$\text{Scavenging activity (\%)} = [1 - (A_1 - A_2)/A_0] \times 100$$

where A_0 is the absorbance of the control (water instead of sample), A_1 is the absorbance of the sample, and A_2 is the absorbance of the sample only (Tris–HCl buffer instead of pyrogallol solution). The IC_{50} values were calculated by probit analysis.

Results and discussion

Figure 1 showed that the reducing power of ethanolic extract, LC and positive control group (PCG) increased with the increase of concentrations. However, the reducing power of ethanolic extract was lower than that of PCG and LC ($p < 0.05$). At the concentration of 0.2 mg/mL, the reducing power of ethanolic extract, LC and PCG were 0.3, 0.69 and 1.65, respectively. Accordingly, the IC_{50} values for ethanolic extract, LC and PCG were 35.48, 33.88 and 17.78 µg/mL, respectively. The results indicated that LC had higher reducing capacity than ethanolic extract of *Z. nummularia* but lower than PCG.

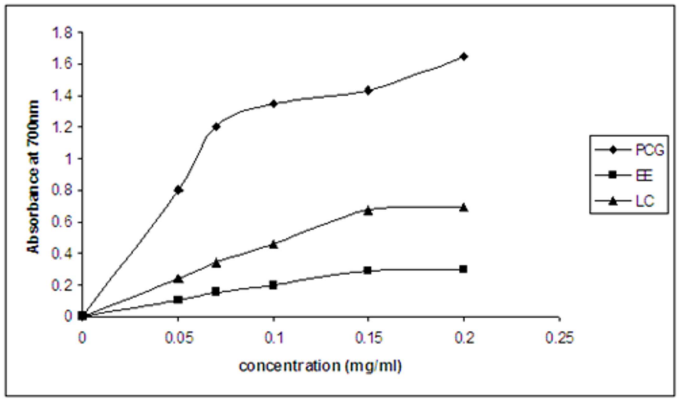

Figure 1. The reducing power of ethanolic extract (EE), lead compound (LC) of Z. nummularia and PCG

The antioxidant activities of compounds have been attributed to various mechanisms, such as prevention of chain initiation, binding with transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reducing capacity and radical scavenging ability (Liu et al., 2007). Among them, the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

The superoxide radical scavenging activities of ethanolic extract, LC and PCG are shown in figure 2. The scavenging activities of all samples were correlated well with the increase of concentrations, and the scavenging activity of PCG was higher than that of ethanolic extract and LC ($p < 0.05$). At the concentration of 5 mg/mL, the scavenging activity for EE, LC and PCG was 41.97%, 84% and 92.65%, respectively. And the IC_{50} values for EE, LC and PCG were 112.20, 79.43 and 89.12 µg/mL, respectively. The results indicated that LC had higher scavenging capacity than ethanolic extract of *Z. nummularia* but lower than PCG.

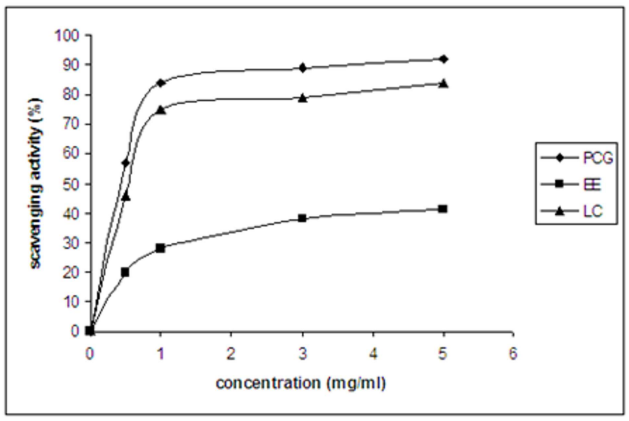

Figure 2. The scavenging activity on superoxide radical of ethanolic extract (EE), lead compound (LC) of *Z. nummularia* and PCG

Superoxide radical, arising either through metabolic process or from oxygen activation by physical irradiation, is considered as the primary ROS. Superoxide radical can further interact with other molecules to generate secondary ROS (e.g., hydroxyl radical, hydrogen peroxide and singlet oxygen), either directly or prevalently through enzyme or metal catalyzed processes (Valkoet al., 2007). As a result, the formation of superoxide radical could induce oxidative damage in lipids, proteins and DNA.

Conclusions

The results indicate that EE and LC of *Z. nummularia* have potent antioxidant activity on reducing power and inhibition of superoxide radical. However LC has better activity than EE.

Conflict of interest

The author declares no conflicts of interest

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