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Antioxidant Activity of Glycerol Derivatives of Fatty Acids from the Fruits of *Lycium chinense* Miller

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Lycium chinense, a famous Chinese medicinal herb, has long history of use as a traditional remedy for many diseases. Two compounds 1-oleo-2,3-dilinoleioglyceride (**1**) and 1,2-dioleo-3-linolenioglyceride (**2**) were isolated from the ethyl acetate extract of fruits of *L. chinense*, the antioxidant activity of glycerol derivatives of fatty acids were evaluated by three established methods namely, 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{-}$) radical scavenging, reducing power and the phosphomolybdenum activities. The antioxidant activity of glycerol derivatives of fatty acids (**1** and **2**) was evident as it showed significant natural antioxidants.

Key Words: *Lycium chinense*, Solanaceae, Constituents, Antioxidant activity.

INTRODUCTION

Lycium chinense Miller fruits (Fructus Lycii) known as "Gou-Qi-Zi" in Chinese, has long history of application as a valuable tonic and health food supplement for improving vision and maintaining good health. It is reputed to have the properties of nourishing the blood, enriching the yin, tonifying the kidney and liver, moistening the lungs[1,2]. Fruits of *L. chinense* (Solanaceae), distributed in northeast Asia, specially China, Japan, Korea and Taiwan, have been widely used as a tonic in traditional medicine. Numerous physiological and biochemical process in the human body may oxygen centered free radicals and other reactive oxygen species as by products. Overproduction of such free radicals can cause oxidative damage to biomolecules (*e.g.* lipids, proteins *etc*.) eventually leading to many common disease and other degenerative disease in humans. Plants may contain a wide range of free radical scavenging molecules and some other endogenous metabolites, which are rich in antioxidant activity. Antioxidant compounds possess several class of biological activities to greater or lesser extent. The intake of natural antioxidants has been associated with reduce of several disesaese in human body[3].

As resources of natural antioxidants much attention have been paid to plants and other organism. *L. chinense* is a famous traditional Chinese herbal medicine which has functions of nourishing the kidney, lever and brighting eyes, reducing blood glucose level and serum lipids, anti-aging, immuno-modulating, anticancer, antifatigue[4-10]. The plant was reported to possess antioxidant properties[11]. Evaluation of antioxidant and other activities of compounds from *L. barbarum* and *L. chinense* has been reported[10,12]. This paper describes the three antioxidant activites, 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{-}$) radical scavenging, reducing power and the phosphomolybdenum activities of two glycerol derivatives of unsaturated fatty acids as 1-oleo-2,3-dilinoleioglyceride (**1**) and 1,2-dioleo-3-linolenioglyceride (**2**) from the fruits of *L. chinense*. Antioxidant activity of glycerol derivatives of fatty acids (**1** and **2**) was evident as it showed significant natural antioxidants. Compounds **1** and **2** spectroscopic data were already reported in literature[13].

EXPERIMENTAL

Free radical scavenging activity: The antioxidant activity of the compounds (**1** and **2**), based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{-}$) free radical, was determined by the method described by Katerere and Eloff[14]. Different concentrations (100, 200, 300, 400 and 500 µg) of the tested compounds (0.2 mL) were taken in different test tubes with 4 mL of a 0.006 % MeOH solution of $DPPH^{-}$. Water (0.2 mL) in place of the compound was used as control. Butylated hydroxytoluene was used as standard. Absorbance at 517 nm was determined after 0.5 h of incubation at 37 ºC. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula, % radical scavenging activity = $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the compound/standard.

Assay of reductive potential: The reductive potential of the compounds (**1** and **2**) were determined according to the method of Dorman and Hiltunen[15]. The reaction mixture containing varying concentrations of the compound (100, 200, 300, 400 and 500 µg) in 1 mL of distilled water, phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1 %). The mixture was incubated at 50 ºC for 20 min. A portion (2.5 mL) of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged at 1000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1 %) and the absorbance was measured at 700 nm in a spectrophotometer. Butylated hydroxytoluene was used as the standard. Increased absorbance of the reaction mixture indicated increased reductive potential. All analysis were run in triplicate and averaged.

Evaluation of antioxidant capacity by phosphomolybdenum method: The total antioxidant capacity of the compounds (**1** and **2**) were evaluated by the method of Prieto *et al.*[16]. An aliquot of 0.1 mL of sample solution (100 µg/mL) was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95 ºC for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank. A typical blank solution contained 1 mL of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions as rest of the sample. The results are expressed as equivalents of α-tocopherol (mg/g of compound).

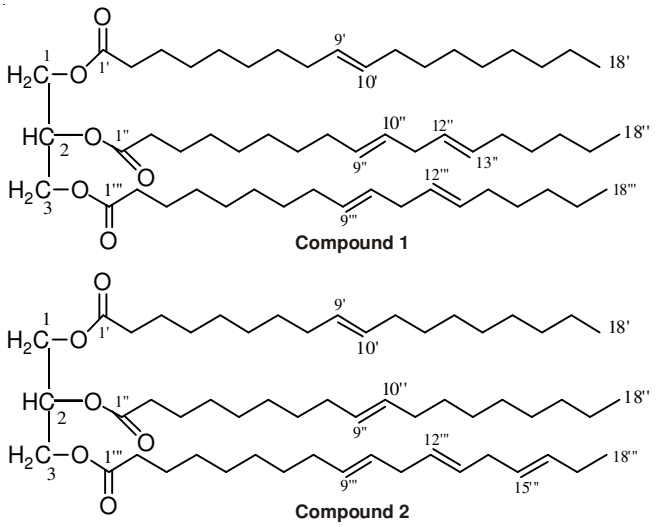

Fig. 1. Chemical structures of compounds **1** and **2**

RESULTS AND DISCUSSION

Free radical scavenging activity: The free radical-scavenging activity of the compounds (**1** and **2**) were tested through DPPH method of Katerere and Eloff[14] and the results were compared with butylated hydroxytoluene. DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants. The method is based on the reduction of methanolic $DPPH^-$ solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form $DPPH^-H$ by the reaction. The compound was able to reduce the stable radical $DPPH^-$ to the yellow-coloured diphenylpicrylhydrazine. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (*e.g.*, hydroquinone, pyrogallol, gallic acid) and aromatic amines (*e.g.*, *p*-phenylene diamine, *p*-aminophenol), reduce and decolourize 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability[17]. Figs. 2 and 3 shows the concentration dependent antioxidant activity of the compounds (**1** and **2**) at different concentration levels as measured by the $DPPH^-$ scavenging assay. The IC_{50} value of the compounds **1** and **2** were 387.89 and 251.00 µg/mL. The DPPH activity of butylated hydroxytoluene showed higher degree of free radical-scavenging activity than that of the compound at low concentration points. The DPPH activity of butylated hydroxytoluene exhibited 92.04 % at 50 µg/mL concentration with an IC_{50} value of 27 µg/mL (data not shown). Li *et al.*[10] reported that the polysaccharide fraction from the fruits of *L. barbarum* exhibited a weak DPPH activity. This is similar to other studies wherein they have reported that only 0.3 mg/mL tocopherol, 0.23 mg/mL butylated hydroxytoluene and 0.1 mg BHA exhibited a free radical scavenging activity equivalent to 3.9 mg/mL of red bean and 10 mg/mL of sesame coat extract[18,19].

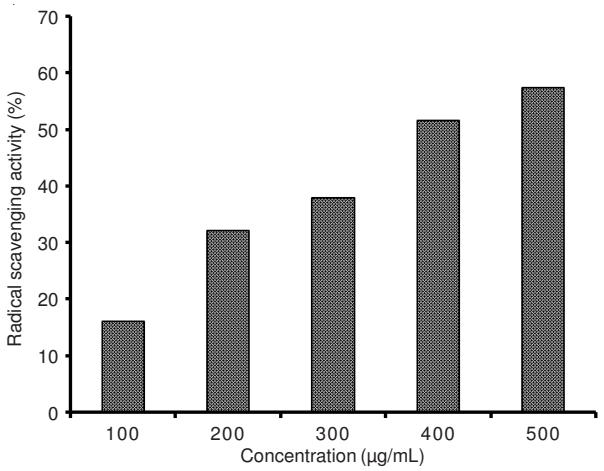

Fig. 2. Antioxidant activity of the compound (**1**) at different concentration levels as measured by DPPH radical scavenging activity

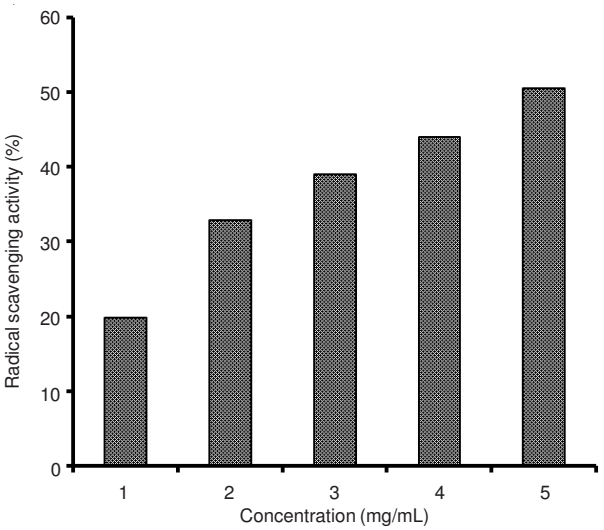

Fig. 3. Antioxidant activity of the compound (**2**) at different concentration levels as measured by DPPH radical scavenging activity

Reducing power: Antioxidant effect exponentially increases as a function of the development of the reducing power, indicating that the antioxidant properties are concomitant with the development of reducing power[20,21] have reported that the reducing power of tannins from medicinal plants prevents liver injury by inhibiting formation of lipid peroxides. Reductones are believed not only to react directly with peroxides but also prevent peroxide formation by reacting with certain precursors. As seen in Figs. 4 and 5 reducing power of the compounds **1** and **2** from the ethyl acetate extract of lycium fruit increased with increasing concentration from 100 to 500 µg/mL and from 200 to 1000 µg/mL respectively. The activity of butylated hydroxytoluene was higher than the test samples at each concentration points (Data not shown). This is in line with the observations of several other workers wherein the reducing power of butylated hydroxytoluene and tocopherol[19] and BHA[22] was higher than the extracts. In the present study, though the compounds from the ethyl acetate extract of lycium fruits exhibited a weak reducing power they did have an activity that reveals that the compounds from the ethyl acetate/ methanol extract of lycium fruit are electron donors and can react with free radicals and convert them to stable products thus terminating the free radical chain reactions.

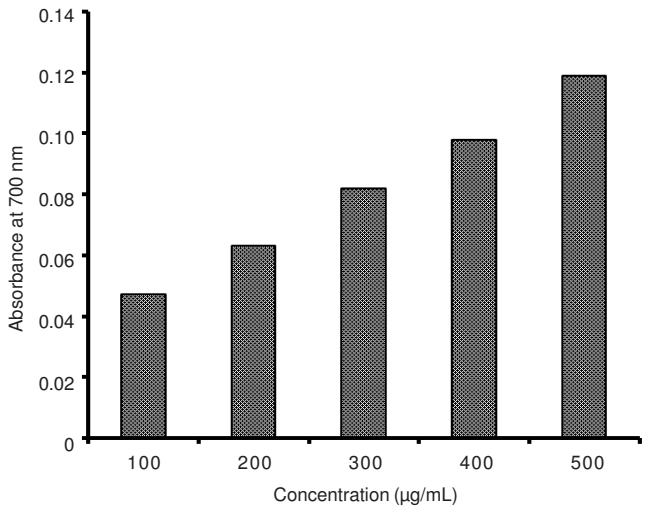

Fig. 4. Reducing power of the compound (**1**) at different concentration levels

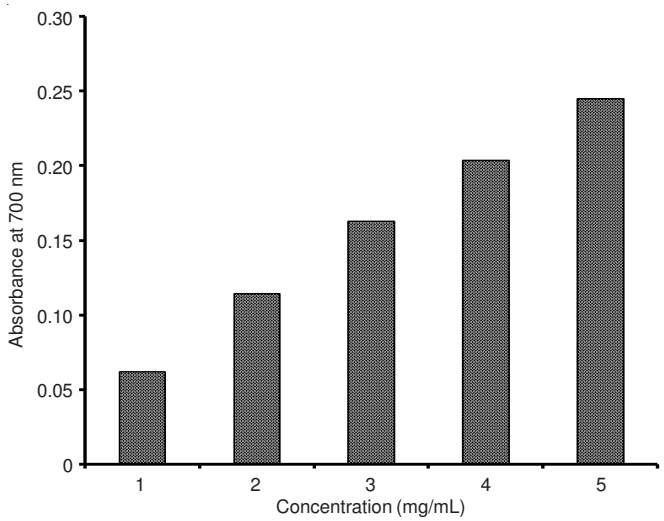

Fig. 5. Reducing power of the compound (**2**) at different concentration levels

Antioxidant capacity by phosphomolybdenum method: The antioxidant capacity of the compound (**1** and **2**) was measured spectrophotometrically through phosphomolybdenum method, which is based on the reduction of Mo(IV) to Mo(V) by the sample analyte and the subsequent formation of green phosphate/Mo(V) compounds with a maximum absorption at 695 nm. The antioxidant capacity of the compounds were found to be 47.31 and 190.10 mg/g of the extract).

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