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Antioxidant Activity and Anti-Hyperglycemic Effect of *Lagenaria Siceraria* Fruit Extract

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Abstract: *Lagenaria siceraria* (Molina) Standl., known as bottle gourd is widely available throughout Vietnam as an edible vegetable. Bottle gourd is a commonly fruit but there are little reports about its biological activities. The aim of the present study was to evaluate antioxidant activity and anti-hyperglycemic potential of ethanolic extract of bottle gourd. The ethanolic extract was tested for its antioxidant activity on chelating power, the ferric reducing/antioxidant power assay (FRAP) and the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay. The results showed that chelating activity was 80.02%, FRAP value was 92.84 μmol Fe^{2+}/g and active radical scavenging which showed an IC_{50} was 49.50 μg/ml. The five different doses of ethanolic extract were treated by oral tolerance to mice body before oral glucose tolerance and blood glucose level was measured using glucose oxidase method. The result indicated that inhibition percentage of ethanolic extract of bottle gourd at the dose of 400 mg/kg in glucose-induced hyperglycemic mice was effectively similar to standard drug glibenclamide. The results obtained indicate that ethanolic extract from bottle gourd is the potential antioxidant and anti-hyperglycemic agent.

Key words: *Lagenaria siceraria* fruit, ethanolic extract, chelating power, *FRAP*, *DPPH*, anti-hyperglycemic.

1. Introduction

Oxidative metabolism is an essential process for survival. On the other side, this process produces free radicals, a potential damage risk to cells. The free radicals and reactive species cause excess oxidative stress, the imbalance between oxidation and antioxidation. Oxidation occurs in food causing chemical spoilage which results in deterioration of nutrition and safety of food. In human body, excess oxidative stress is the major cause of aging and many diseases such as cancer, cardiovascular diseases, diabetes mellitus... [1]. Oxidative stress plays a role in pathogenesis of diabetes mellitus leading to hyperglycemia and other complications such as heart attack, kidney failure, retinotherapy. Antioxidants, which can slow or prevent oxidation, are the key to solve this problem. Synthetic antioxidants have been used in food industry to increase food shelf life but a risk of toxicity and carcinogen still exists. Some of synthetic antioxidants are used to treat human with high efficiency, but also cause undesirable effects on patients. Natural antioxidants from fruits, vegetables, medicinal herbs or plants, which are safe and inexpensive, seem to be the best option.

Lagenaria siceraria (Molina) Standl also known as bottle gourd is a common fruit in Vietnam as well as other countries. *Lagenaria siceraria* (LS) fruit is rich of healthy nutrients such as 18 amino acids, a lot of minerals (mostly potassium and calcium) and a lot of vitamins (mainly choline—good for nerve and brain function maintenance, vitamin C and some vitamin B [2, 3]. LS fruit is known to be effective in diuretic, anti constipation, weight loss, diabetes, detoxification, analgesic, immune system conditioning and anti-cancer [4, 5]. Despite of its uasge, LS fruit is still not widely researched in Vietnam. The aims of this study were examined antioxidant activity and the anti-hyperglycemic potential of ethanolic extract from LS fruit in oral glucose tolerance tests carried out with glucose-loaded mice.

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2. Materials and Methods

2.1 Chemicals and Reagents

2,4,6-tripyridyl-s-triazine (TPTZ), ethylene diamine tetra acetic acid (EDTA), hydrochloric acid and ethanol, were obtained from Merck (Darmstadt, Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxy toluene (BHT), were purchased from Sigma Chemicals Co (St. Louis, MO, USA). The test drug glibenclamide (Flamingo Pharm., Ltd, India) was purchased from a registered local medical store. All other chemicals used in the experiments were of analytical grade.

2.2 Plant Material and Extraction

The fresh fruits of *L. siceraria* which were collected from district 2, Ho Chi Minh City in December, 2014 were cut into pieces and dried in oven at not higher than 60 °C. Dried samples were pulverized to medium size powders (1 × 1 mm) and were exhaustedly extracted by maceration with ethanol under room temperature (29 $\pm$ 2 °C) to obtain ethanolic extract. The ethanolic extract was dried under reduced pressure at a yield of 5.9% (w/w). This extract was used for tests.

2.3 Animals

Male albino mice weighing 20-25 g (6-8 weeks old), were provided by Ho Chi Minh City Pasteur Institute. Mice were divided into different groups and were fed for one week in standard conditions (70%-80% relative humidity and 12 h photoperiod) for acclimatizing to experimentation condition.

2.4 Preliminary Phytochemical Investigation

Identification of phytochemical groups in the sample was carried out using the process described by Ciulei [6] and modified by University of Pharmacy and Medicine Ho Chi Minh City [7].

2.5 Ferric Reducing Antioxidant Power Assay (FRAP)

The total antioxidant potential of sample was determined using a ferric reducing ability of plasma FRAP assay as measure of "antioxidant power" [8] with slight modification.

The working FRAP reagent was prepared by mixing 10 volumes of 300 mM acetate buffer, pH 3.6, with 1 volume of 10 mM TPTZ in 40 mM hydrochloric acid and with 1 volume of 20 mM ferric chloride. Freshly prepare FRAP reagent was warmed to 37 °C. Subsequently, 50 µl of sample were added to 1.5 ml of the FRAP reagent. Absorbance readings were taken after 30 minute [9]. Standard curve was prepared using different concentrations (100-1,000 µM) of $FeSO_4.7H_2O$. All solutions were used on the day of preparation. FRAP assay measures the change in absorbance at 595 nm owing to the formation of a blue colored Fe^{II}-tripyridyltriazine compound from colourless oxidized Fe^{III} form by the action of electron donating antioxidants [8, 10]. All tested samples were performed in triplicate. The result was expressed as µM Fe^{2+}/g dry weight of plant material.

2.6 Free Radical Scavenging Activity Assay

The DPPH assay was determined using the method proposed by Von Gadow, Joubert, and Hansmann (1997) [11]. Aliquots (50 µl) of the tested samples were mixed with 2 ml of 6.10-5 M methanolic solution of DPPH• radical. A methanolic solution of pure compound was tested too. Absorbance measurements commenced immediately. The decrease in absorbance at 515 nm was determined after 30 min for all samples. Methanol was used to zero spectrophotometer.

All tested samples were performed in triplicate. The percentage of inhibition of the DPPH• radical by the samples was calculated according to the formula [12]:

$$\% \text{ Inhibition} = [(A_{C(0)} - A_{A(t)})/A_{C(0)})] \times 100,$$

where, $A_{C(0)}$ is the absorbance of the control at t = 0 min and $A_{A(t)}$ is the absorbance of the antioxidant at t = 16 min. For determination of IC_{50}, BHT is used as reference.

2.7 Ferrous Ion Chelating Activity

The ferrous ions chelating ability of the extract was

estimated by the method of Dinis et al. [13]. Each extract with different concentration in water (1 ml) was added with 3.7 ml of methanol and 0.1 ml of 2mM $FeCl_2$. 0.2 ml of 5 mM ferrozine was added for the reaction initiation. After 10 min of incubation at room temperature, the absorbance of the mixture was measured at 562 nm against a blank. EDTA was used as a standard. All determinations were performed in triplicate.

2.8 Anti-hyperglycemic Activity Test

The study was carried out as previously described by Rahman et al. [14] with slight modification. The male albino mice were divided in to 7 groups of 6 animals each. Group I (blank control group) was administered normal saline. Group II served as control group (hyperglycemic mice) with received vehicle alone. Group III received standard drug glibenclamide, 10 mg/kg weight body. Group IV, V, VI and VII were treated with ethanolic extract of bottle gourd at doses of 100, 200, 300 and 400 mg extract/kg, respectively. All of the treatments were orally administered to mice. After one hour, all mice were orally treated with 2g/kg of glucose with the exclusion of group I. The blood samples were collected two hours after glucose administration. Serum was separated and blood glucose levels were measured immediately by glucose oxidase method [15].

2.9 Statistical Analysis

Results were expressed as mean $\pm$ SD. The differences between the test groups and control were determined by least significant difference method at $p < 0.05$ confidence levels.

3. Results and Discussion

The identification of preliminary phytochemical groups in the plant sample revealed that the ethanolic extract of bottle gourd containing carotenoids, saponins, steroids and phenolic compounds (Table 1).

3.1 Free Radical Scavenging Activity

Free radicals are known to be a major factor in biological damage and DPPH method has been used to evaluate the free radical scavenging activity of natural antioxidants. As shown in Fig. 1, the ethanolic extract of bottle gourd exhibited free radical scavenging activity that increased with direct proportion to concentrations.

The antioxidant effect of LS fruit extract was comparable to that of BHT which was used as reference compound. The IC_{50} value of LS fruit extract was 49.50 μg/ml, 4.62 times lower than 10.72 μg/ml of BHT.

DPPH is a stable free radical that accepts an electron of hydrogen radical to become a stable diamagnetic molecule. The reduction of DPPH radical was estimated by the decrease in its absorbance at 517 nm induced by antioxidants. The result indicates the free radical scavenging capacity of LS fruit extract was potential.

3.2 Antioxidant Capacity Determined by FRAP

FRAP assay was performed to examine the reducing

Table 1 Preliminary qualitative phytochemical analysis of ethanolic extract of bottle gourd.

Plant phytochemicals	Testing methods	Presence
Carotenoid	Carr-Price reaction	+
Alkanoids	Mayer/Wagner	-
Flavonoid	Mg/HCl	++
Polyphenols	$FeCl_3$ 5%	++
Saponin	Foam test	++
	Fontan-Kaudel test	+
Steroid	Lieberman-Burchard reaction	+
Reducing compounds	Fehling reagent	++

Note: ++: moderately present, +: low, -: absent.

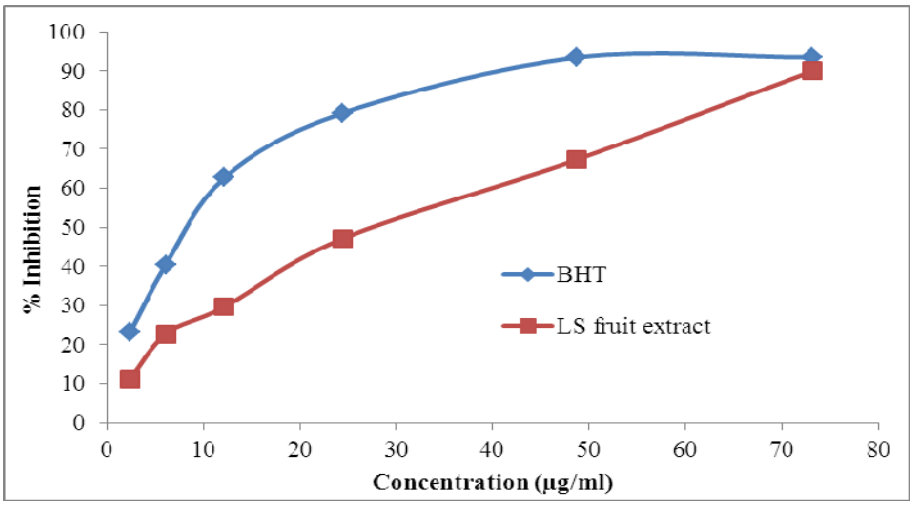

Fig. 1 DPPH scavenging activity of ethanolic extract of LS fruit.

capacity of LS extract. The antioxidant capacity of extract of LS fruit was 92.84 µmol $Fe^{2+/}$g or the FRAP value was 472 ± 7 µmol Fe^{2+}/L of 1mg/ml concentration of sample. The ethanolic extract of LS fruit had antioxidant potential and was 4.29 times lower that of BHT (2,023 ± 45 Fe^{2+}/L) at same concentration.

FRAP assay was performed to estimate the capacity of LS ethanolic extract to reduce Fe^{3+}/Fe^{2+} by the increased absorbance based on the formed ferrous iron. The reducing properties are associated with the presence of compounds that exert their action by breaking the free radical chain by donating a hydrogen atom [8]. The result obtained shows that LS extract has reducing capacity.

3.3 Chelating Effect of Ferrous Ion

In the present of chelating agent, the complex formation is disrupted. Reduction therefore permits estimation of chelating ability of coexisting chelator [16]. The ferrous ion chelating effect of ethanolic extract of LS fruit and standards at different concentrations, 10, 20, 30, 40, and 50 µg/ml (final concentration) were presented in Fig. 2. The percentages of metal scavenging capacity at the concentration of 30 µg/ml of ethanolic extract were found to be 80.02%. The chelating effect of LS fruit extract was comparable to that of EDTA which was used as reference compound. The IC_{50} value on chelating effect of LS fruit extract was 14.31 µg/ml.

The ethanolic extract of LS extract demonstrated a strong chelating activity on ferrous ion with increased concentration. Measurement of colour reduction allows an estimation of the metal chelating activity of the coexisting chelator. Chelating agents are considered as secondary antioxidants they reduce redox potential thereby stabilizing the oxidized form of the metal ion [17].

Antioxidants are divided into two classes: primary or chain-breaking antioxidants and secondary or preventative antioxidants [17]. Results from DPPH and FRAP demonstrate the primary antioxidant activity with free radical scavenging activity at different concentration and the amount of antioxidants in the sample, while chelating activity demonstrates the preventative property. With the combination of different methods, more precise evaluation of antioxidant activities will be obtained. A test for total phenolic capacity may be performed to estimate the correlation between antioxidant activities and total phenolic compounds.

3.4 Anti-Hyperglycemic Activity of Ethanolic Extract

The Table 2 showed the changes in the levels of blood glucose in normal and experimental groups. The results indicated that the fasting blood glucose levels

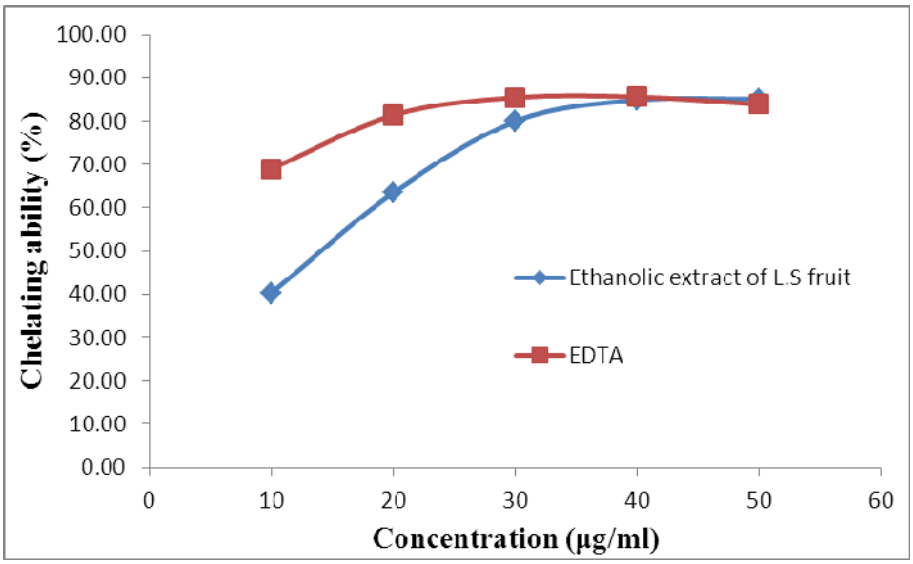

Fig. 2 Chelating ability of ethanolic extract of LS fruit.

Table 2 Effects of ethanolic extract of LS FRUIT on serum.

Group	Dose	Serum glucose (mmol/l)	% inhibition
Group I (blank control)	-	6.02 ± 0.60^{a}	-
Group II (control)	-	15.07 ± 1.34^{e}	-
Group III (glibenclamide)	10 mg/kg	5.74 ± 0.81^{a}	61.91
Group IV (bottle gourd)	100 mg/kg	12.78 ± 0.44^{d}	15.20
Group V (bottle gourd)	200 mg/kg	10.32 ± 0.87^{c}	31.52
Group VI (bottle gourd)	300 mg/kg	8.63 ± 0.45^{b}	42.73
Group VII (bottle gourd)	400 mg/kg	6.54 ± 0.94^{a}	56.60

Data were mean ± SD of values from 6 mice, values with the different superscript letters were significantly different at $p < 0.01$.

of group II (hyperglycemic mice) was significantly higher than others. The groups were received the ethanolic extract from bottle gourd had significant glucose lowering capacity at all doses (examined in a dose-dependent manner). The results shown that at dose of 400 mg/kg (group VII) possessed stronger pharmacological effects than with other doses. Maximum anti-hyperglycemic activity of ethanolic extract of bottle gourd in glucose-induced hyperglycemic mice was 56.60%, while the standard drug, glibenclamide (group III) reduced 61.91%. Blood glucose level of group VII and group III were equivalently when compared with that of blank control group ($p > 0.05$). Reduction of serum glucose levels of ethanolic extract by several factors. The extract of bottle gourd may influence in a positive manner the pancreatic secretion of insulin, or the extract may increase the glucose uptake [18], thus reducing the presence of glucose in serum.

Oxidative stress plays a role in β-cell dysfunction and insulin resistance leading to diabetes mellitus and hyperglycemia [1]. The result above shows that LS ethanolic extract at high dose has similar anti-hyperglycemic activity as glibenclamide. According to the result obtained on antioxidant activities, LS ethanolic extract can prevent hyperglycemia and diabetes mellitus complications.

The data as shown above indicated that the ethanolic extract of LS fruit possessed both potential antioxidant and anti-hyperglycemic activities. It may be due to the presence of respective secondary metabolites such as flavonoid, phenolic compounds…in the LS fruit. Therefore, this fruit can be considered as a healthy food.

4. Conclusions

The results of the study demonstrated that LS fruit is a promising source of natural antioxidant and anti-hyperglycemia. Therefore, LS fruit can provide therapeutic as well as daily nutrient values. These findings encourage further investigations including bioassay of the fractionated extract may lead to the isolation of compounds that are responsible for high antioxidant and anti-hyperglycemic effects.

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