Revista Brasileira de Farmacognosia xxx (2017) xxx–xxx

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original Article

Phenolic-antioxidant capacity of mango seed kernels: therapeutic effect against viper venoms

Azza M. Abdel-Aty*, Walaa H. Salama, Mohamed Belal Hamed, Afaf S. Fahmy, Saleh A. Mohamed

Molecular Biology Department, National Research Centre, Dokki, Cairo, Egypt

ARTICLE INFO

Article history:
Received 21 March 2018
Accepted 13 June 2018
Available online xxx

Keywords:
Antioxidant
Anti-venom
Egyptian mango
Oxidative stress
Phenolic compounds

ABSTRACT

In this study, mango seed kernels Hindi cultivar extract contained a considerable amount of phenolics and flavonoids (17,400 and 3325 mg/100 g seed, respectively). The HPLC profiling revealed that hesperidin was the major phenolic compound of the mango seed kernels extract. This is the first report find hesperidin in mango extracts. The phenolic compounds of mango seed kernels extract were effective in scavenging free radicals of DPPH and ABTS with IC_{50} values of 47.3 and 7.9 μg/ml, respectively. The total antioxidant activity of mango seed kernels extract based on the reduction of molybdenum was also measured. The phenolic compounds of mango seed kernels extract potentially inhibited the protease, fibrinogenase, phospholipase A_2, L-amino acid oxidase, hyaluronidase, and hemolytic activities of the most dangerous *Cerastes cerastes* and *Echis coloratus* viper venoms. The phenolic compounds of mango seed kernels extract could completely neutralize the hemorrhage and lethality of both venoms in experimental animals. It could be concluded that the mango seed kernels extract phenolic compounds with potential antioxidant activity are considered as a new avenue in the viper bite treatment.

Introduction

Mango (*Mangifera indica* L., Anacardiaceae) is one of the most important tropical fruits in the world. Mango fruit occupies the second position as a tropical crop with a global production exceeding 35 million tons (Jahurul et al., 2015). In Egypt, over 4 million tons of mango fruits are produced (Abdalla et al., 2007). Mango fruits are processed in various products as puree, canned slices, syrup, nectar, leather, pickles, chutney and jam, resulting in a significant amount of wastes. Mango seed kernel represents about 20% of the whole mango fruit and includes rich levels of health-enhancing compounds and natural antioxidants (Sogi et al., 2013; Jahurul et al., 2015). Further, kernels have anti-tyrosinase, anti-inflammatory, anti-obesity, and hepato-protective effects (Kobayashi et al., 2013).

Vipers are the main cause (over 80%) of snake bite worldwide. Venoms of the vipers are plentiful sources of active molecules that affect a large number of physiological functions. These molecules include diverse hydrolytic enzymes that causing local harmful effects like hemorrhage, necrosis and edema end with tissue loss. They also cause systemic effects resulting in coagulopathy, cardiopathy, and neuropathy (Oussedik-Oumehdi and Laraba-Djebari, 2008). Furthermore, viper venom causes various complications as thrombocytopenia, renal abnormalities, hypopituitarism and permanent tissue damage. Oxidative stress plays a main role in viper bite pathophysiology and the constancy of the viper bite complications (Zengin et al., 2012; Sunitha et al., 2015). Insufficiency of anti-venom to reverse these complications leads to viper bite treatment still a challenge till now (Girish and Kemparaju, 2011). *Cerastes cerastes* (Linnaeus, 1758) (horn viper) and *Echis coloratus* Günther, 1878 (red carpet viper) are the most dangerous and medically important vipers in Egypt and are responsible for the greatest incidence of envenomation (Wahby et al., 2012).

The recovery and utilization of valuable compounds from mango by-products are an important challenge, hence, this study aims to determine the total phenolic, flavonoid contents and antioxidant capacity of Egyptian mango seed kernel Hindi cultivar extract (MSKE) and to assess its anti-venom properties against Egyptian *Cerastes cerastes* and *Echis coloratus* viper venoms.

Materials and methods

Plant

Egyptian mango Hindi cultivar (*Mangifera indica* L., Anacardiaceae) was obtained from Horticulture Institute Research, Agriculture Research Centre, Cairo, Egypt.

* Corresponding author.
E-mail: am.abdel-aty@nrc.sci.eg (A.M. Abdel-Aty).

https://doi.org/10.1016/j.bjp.2018.06.008

Animal

Cerastes cerastes (Linnaeus, 1758) and *Echis coloratus* Günther, 1878 adult vipers obtained from laboratory animal unit of Helwan Farm-VACSERA, Egypt. The *C. cerastes* and *E. coloratus* venoms were milked, lyophilized and stored at −20 °C.

Male Swiss-albino mice weighing (20 ± 2.4 g) were used for this study. All animals were housed at the animal house of the National Research Centre (NRC), under standardized conditions and diet. All the experimental protocols described in this study were performed in accordance with the recommendations of the ethical committee, NRC, Egypt (Protocol permit #15-097).

Plant extraction

Mango seed kernel Hindi cultivar (8 g) were chopped, grinded, soaked in 60 ml of 80% methanol and shaken overnight at 120 rpm and room temperature. The extract was centrifuged at 7200 × *g* for 10 min. The supernatant was evaporated under vacuum and dissolved in least volume of 0.1% DMSO and designated as a mango seed kernel extract (MSKE).

Total phenols measurement

The total phenolic content of MSKE was measured according to Velioglu et al. (1998). The reaction mixture includes: 0.1 ml methanol extract, 0.1 ml Folin-Ciocalteu reagent and 0.8 ml distilled water were incubated for 5 min at room temperature. Then 0.5 ml sodium carbonate (20%) was added and incubated at room temperature for 30 min. The absorbance was measured at 750 nm. The results were expressed as mg gallic acid equivalent (GAE)/100 g seed.

Total flavonoids measurement

The total flavonoid content of MSKE was measured according to Zhishen et al. (1999). Incubation of 0.25 ml methanol extract, 1.25 ml distilled water and 0.075 ml of 5% $NaNO_2$ for 6 min, then add 0.15 ml of 10% $AlCl_3$. After 5 min, 0.5 ml of 1.0 M NaOH and 0.275 ml distilled water were added. The absorbance was measured at 510 nm. The results were expressed as mg catechin equivalent (CE)/100 g seed.

HPLC analysis of phenolic compounds

The high performance liquid chromatography (HPLC) analysis was carried out for MSKE according to Kim et al. (2006). The separation and determination were performed on XDB-C18 column (150 × 4.6 μm). The column was eluted by acetonitrile (solvent A) and 2% acetic acid (solvent B) at a flow rate of 1 ml/min. The obtained peaks were monitored simultaneously at 280, 320 and 360 nm. Commercial phenolic compounds were used as standards.

Antioxidant assays

DPPH assay

1,1-Diphenyl-2-picrylhydrazyl (DPPH) method was used for determination of the antioxidant activity of MSKE (Ao et al., 2008). The reaction mixture includes: 0.1 ml methanol extract and 0.9 ml of 0.1 mM DPPH dissolved in methanol were incubated for 30 min at room temperature. The absorbance was measured at 517 nm. DPPH scavenging percent = [(O.D. control − O.D. sample)/O.D. control] × 100.

ABTS assay

ABTS (2,2′-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) reagent was prepared and used for determination of the antioxidant activity of MSKE (Re et al., 1999). The reaction mixture includes: 1 ml of ABTS reagent and 0.1 ml of the extract were incubated for 1 min at room temperature and the reduction of absorbance was measured at 734 nm. ABTS scavenging percent = [(O.D. control − O.D. sample)/O.D. control] × 100.

Phosphomolybdenum complex assay

The antioxidant activity of MSKE was also evaluated by formation of a phosphomolybdenum complex according to Prieto et al. (1999). The reaction mixture includes: 4 mM ammonium molybdate, 28 mM sodium phosphate, 600 mM sulfuric acid, and 50 μl of the extract were incubated at 95 °C for 90 min. After cooling, the absorbance was read at 695 nm. EC_{50} is defined as a concentration of the MSKE gives absorbance of 0.5.

Protease inhibition

Azocasein assay

The protease activity of crude venom was measured according to Lemos et al. (1991) using azocasein as a substrate. One ml reaction mixture includes: 0.2% azocasein, 10 μg of crude venom and 0.02 M Tris–HCl buffer, pH 7.0 were incubated at 37 °C. The reaction was stopped after 1 h by adding 100 μl of 20% TCA and precipitation removed by centrifugation at 5000 × *g* for 5 min. The change of absorbance (0.01 O.D.) was measured at 366 nm which considered as a one unit/h. Inhibition studies, the crude venom (10 μg) was pre-incubated with various concentrations of MSKE for 15 min at 37 °C and measurement of residual activity.

Gelatin zymography

Gelatin zymography of crude venom (30 μg) was performed in 12% native polyacrylamide gel co-polymerized with 0.2% gelatin according to Bee et al. (2001). After electrophoresis, the gel was incubated overnight in 0.02 M Tris–HCl buffer, pH 7.0 at 37 °C. After gel staining by Coomassie Brilliant Blue R-250, clear zones were appeared. Inhibition experiments, 30 μg of crude venom was pre-incubated with different concentrations of MSKE for 15 min at 37 °C and performed according to the method previously described.

Fibrinogen degradation inhibition

Fibrinogenase activity of crude venom was determined according to Ouyang and Teng (1976). One ml reaction mixture includes: Human plasma fibrinogen (2 mg), venom sample (2 μg) and 5 mM Tris–HCl buffer, pH 7.5 containing 5 mM $CaCl_2$ were incubated at 37 °C. The reaction was stopped after 2 h by adding 100 μl of stopping solution (4% SDS, 4% 2-mercaptoethanol and 10 M urea). The samples were electrophoresis on 10% SDS-PAGE according to Laemmli (1970). Inhibition experiments were performed by pre-incubated venom sample with different concentrations of MSKE for 15 min at 37 °C and performed according to the method previously described.

Phospholipase A_2 (PLA_2) inhibition

PLA_2 activity of crude venom was determined using egg yolk as a substrate according to Marinetti (1965). Egg yolk solution (1 ml) (1:5 w/v saline), crude venom alone (200 μg) or pre-incubated with different phenolic concentrations of MSKE at 37 °C for 15 min were mixed and reached to a final volume of 5 ml with saline. The absorbance was recorded each 5 min for 15 min at 900 nm. PLA_2 activity was also determined according to Gutierrez et al. (1988).

Please cite this article in press as: Abdel-Aty, A.M., et al. Phenolic-antioxidant capacity of mango seed kernels: therapeutic effect against viper venoms. Revista Brasileira de Farmacognosia (2017), https://doi.org/10.1016/j.bjp.2018.06.008