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Original Article

Hepatoprotective and hypoglycemic effects of a tannin rich extract from *Ximenia americana* var. *caffra* root

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ABSTRACT

Background: Liver diseases and diabetes are serious health disorders associated with oxidative stress and ageing. Some plant polyphenols can lower the risk of these diseases.

Purpose: We investigated the phytochemical profiling of a root extract from *Ximenia americana* var. *caffra* using HPLC-PDA-ESI-MS/MS. The antioxidant activities *in vitro* were investigated. The hepatoprotective activities were studied in rat models with D-galactosamine (D-GaIN)-induced hepatotoxicity and the antidiabetic activities in STZ-diabetic rats were also investigated.

Materials and methods: HPLC-PDA-ESI-MS/MS was used to identify plant phenolics. The antioxidant activities *in vitro* were determined using DPPH and FRAP assays. The *in vivo* hepatoprotective activities were determined for D-GaIN-induced hepatotoxicity in rats. We determined the liver markers alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT), liver peroxidation product malondialdehyde (MDA), glutathione content (GSH), albumin and total bilirubin concentration. The histopathological changes in rat liver were also studied. The antidiabetic activities were also investigated in STZ-diabetic rats and serum glucose, serum insulin hormone, and lipid peroxides were determined.

Results: The root extract is rich in tannins with 20 compounds including a series of stereoisomers of (epi)catechin, (epi)catechin-(epi)catechin, (epi)catechin-(epi)catechin-(epi)catechin, and their galloyl esters. Promising antioxidant potential was observed *in vitro* in DPPH assay with EC_{50} of 6.5 µg extract / 26 µg raw material and in FRAP assay with 19.54 mM $FeSO_4$ compared with ascorbic acid (EC_{50} of 2.92 µg/ml) and quercetin ($FeSO_4$ 24.04 mM/mg), respectively. Significant reduction of serologic enzymatic markers and hepatic oxidative stress markers such as ALT, AST, MDA, GGT, and total bilirubin, as well as elevation of GSH and albumin were observed in rats with D-galactosamine-induced liver damage treated with the extract. These findings agree with a histopathological examination suggesting a hepatoprotective potential for the root extract. The root extract can mediate an antidiabetic effect by reducing elevated blood glucose and serum lipid peroxides levels and by increasing insulin in STZ-diabetic rats by −107, −31.1, +11.3%, respectively.

Conclusions: The results of this study suggest that the tannin-rich extract from *Ximenia americana* var. *caffra* could be an interesting candidate for the treatment of several health disorders associated with oxidative stress such as hepatocellular injury and diabetes.

Introduction

Production of reactive oxygen species (ROS) is an inevitable outcome of cellular metabolism and oxidative stress. Production of free radicals appears to be linked to the onset and progression of metabolic syndrome and serious disorders such as diabetes and liver diseases

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; D-GaIN, D-galactosamine; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; GGT, gamma-glutamyltransferase; GLB, Glibenclamide; GSH, reduced glutathione content; MDA, malondialdehyde; ROS, reactive oxygen species; STZ, streptozotocin

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(Halliwell et al., 1992). Several studies have shown that the use of polyphenolic compounds found in many medicinal plants can lower the risk of these diseases owing to their inherent antioxidant potential (Kinoshita et al., 2007; Simeonova et al., 2016; Youssef et al., 2017; Youssef et al., 2016).

The genus *Ximenia* represents one of 4 genera within the monophyletic group family Ximeniaceae (formerly Oleaceae) with ten known species. These species are cultivated as shrubs and trees and widely spread in tropical and subtropical regions. *X. americana* var. *caffra* (*X. a. caffra*) is a member of African origin belonging to this genus, grown as deciduous trees and known commonly as the large sour plum. It inhabits different territories in East Africa stretching from Tanzania to South Africa and crosses into some parts of Namibia and Botswana (Maroyi 2016).

As an herbal prescription, *X. a. caffra* has been in use for centuries in traditional medicine. The root of the plant has been used to treat leprosy, dysentery, and mental sickness. Both leaves and roots are used for wound healing, alleviating fever, treating infertility, diarrhea, abdominal pain, and bilharzia as well. Moreover, leaf decoctions are applied as a gargle for tonsillitis, a wash to soothe inflamed eyes, and also as a vermifuge. Traditional Malian healers have always used the plant against throat infection, amenorrhea, and also as a tonic (Maroyi 2016; Le et al., 2012).

Additionally, *X. a. caffra* stands out as a common traditional remedy against sexual transmitted diseases. Mulaudzi et al. (2011) have reported a notable activity of the plant leaves against *Neisseria gonorrhoeae* (the causative pathogen of gonorrhea) in Venda traditional medicine. A bio-guided fractionation of plant leaves has led to the identification of the bis-norsesquiterpene, vomifoliol, which could significantly inhibit *N. gonorrhoeae* (Nair et al., 2013). Geyid et al. (2005) have reported antibacterial and antifungal activities for leaf and root extracts. These activities were attributed to the presence of several classes of phytochemical constituents such as isoprenoids, sesquiterpenes, triterpenes, and steroids.

Fruits of *X. a. caffra* from Burkina Faso and Brazil are rich in phenols and flavonoids, and have exhibited appreciable antioxidant activities (Lamien-Meda et al., 2008; Almeida et al., 2016). Aside from the reported trypanocidal activities, aqueous extracts of the plant leaves from Tanzania have exhibited potent anticancer activity in a panel of 17 cell lines which was attributed to the presence of tannins (Voss et al., 2006; Nibret et al., 2010). Bioactivity investigations of the leaf extracts from Northeast of Namibia revealed antioxidant, anti-proliferation, and anti-inflammatory activities. The phytochemical investigation of these extracts suggested the presence of 10 polyphenols including gallic acid, catechin, quercetin, kaempferol, and their derivatives (Zhen et al., 2015).

In the same context, fractionation of an ethanol extract of the leaves from Mali led to the isolation and identification of the cyanogenic glucoside, sambunigrin as a main compound, quercetin, quercitrin, and avicularin. The antioxidant activity of the isolated compounds and subfractions as well as xanthine oxidase and 15-lipoxygenase inhibitory activities were also reported (Le et al., 2012).

As for the roots, two carboxylic fatty acids namely stearic and *trans*-4-octadecenoic acid along with β-sitosterol steroid were isolated (Almeida et al., 2016). So far, only Mwangi et al. (1994) have reported on the polyphenol constituents of *X. a. caffra* roots from Kenya.

In our study, we comprehensively identified and characterized the polyphenolic constituents of a methanol extract from *X. a. caffra* roots using HPLC-PDA-ESI-MS/MS. The potent antioxidant, hypoglycemic, and hepatoprotective activities of the root extract were investigated in corresponding rat models.

Materials and methods

Chemicals

Thiobarbituric acid was purchased from Fluka (Buchs, Switzerland). Glibenclamide (GLB), streptozotocin, silymarin and D-galactosamine were purchased from Sigma Chemicals (ST. Louis. Mo, USA). Solvents used for extraction and separation were all of analytical grade.

Plant material and extraction

Plant roots were collected from Lupaga Site in Shinyanga, Tanzania. The identity of the plant was confirmed by DNA barcoding using *rbc*L as a marker gene. Specimens of plant leaves and root were deposited under the accession number P7344 and P7346 at IPMB, Heidelberg, respectively.

The dried root was grinded and extracted with methanol at room temperature for 3 d (6 × 500 ml). The extracts were evaporated under vacuum until dryness. The obtained residue was further dissolved in methanol, centrifuged and only the methanol extract was further evaporated. After frozen at −70 °C, the samples were lyophilized yielding fine dried powder (25% w/w).

HPLC-PDA-MS/MS

For the phytochemical analysis, we used high performance liquid chromatography-mass spectrometry. The LC system was Thermofinigan (Thermo Electron Corporation, USA) coupled with an LCQ-Duo ion trap mass spectrometer with an ESI source (ThermoQuest). The separation was achieved using a C18 reversed-phase column (Zorbax Eclipse XDB-C18, rapid resolution, 4.6 × 150 mm, 3.5 µm, Agilent, USA). A gradient of water and acetonitrile (ACN) (0.1% formic acid each) was applied from 5% to 30% ACN in 60 min in flow rate of 1 ml/min with a 1:1 split before the ESI source. The MS was operated in the following conditions: capillary voltage (−10 V), the source temperature was set at 200 °C, and nitrogen was used as a sheath and auxiliary gas at a flow rate of 80 and 40 (arbitrary units), respectively in the negative mode. MS/MS fragmentation was recorded with collision energy of 35%. The ions were detected in a full scan mode and mass range of 50–2000 *m/z* and finally the machine was controlled using Xcalibur software (Xcalibur 2.0.7, Thermo Scientific).

Antioxidant activities in vitro

Determination of total phenolic contents by Folin–Ciocalteu method as previously described by Zhang et al. (2006) and antioxidant activities by DPPH and FRAP assays were done using the standard techniques described by Blois (1958) and Benzie and Strain (1996), respectively, and adapted to a 96 well microplate as previously described (Sobeh et al., 2016).

Hepatoprotective experiments

Animals and study design

Male Wistar rats (200–220 g; Zagazig University, Zagazig, Egypt) were used in the current study. The protocol was approved by the institutional Animal Care and Use Committee (approval number P2-6-2016) of the Faculty of Pharmacy, Zagazig University. All rats were kept on a 12-h light/12-h dark regime, with free access to food and water.

Rats were randomly assigned into four groups. Extract, silymarin and/or vehicle were either administrated intraperitoneally (i.p.) or orally after one week adaptation period. Control group received equal volume of vehicle. D-galactosamine (D-GalN) group received 800 mg/kg D-GalN dissolved in normal saline to induce acute liver failure similar to human acute hepatitis. Root extract and silymarin (100 mg/kg b.w