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A proanthocyanidin-rich extract from *Cassia abbreviata* exhibits antioxidant and hepatoprotective activities *in vivo*

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ABSTRACT

Ethnopharmacological relevance: *Cassia abbreviata* is a small to medium sized branched umbrella-shaped deciduous tree. It is widely spread in the tropics, especially in Africa, having a long history in traditional medicine for the treatment of numerous conditions such as headaches, diarrhea, constipation, some skin diseases, malaria, syphilis, pneumonia, stomach troubles, uterine pains, and against gonorrhea.

Aim of the study: We investigated the phytochemical constituents of a root extract from *Cassia abbreviata* using HPLC-PDA-ESI-MS/MS. We also determined the antioxidant activities *in vitro* and *in vivo* using the nematode *Caenorhabditis elegans* as a model organism. The hepatoprotective activities in case of D-galactosamine (D-GaIN)-induced hepatotoxicity were studied in a rat model.

Materials and methods: HPLC-PDA-ESI-MS/MS analysis allowed the identification of the secondary metabolites of the methanol extract. DPPH and FRAP assays were used to determine the antioxidant activities *in vitro*. Using the *C. elegans* model, survival rates under juglone induced oxidative stress, intracellular ROS content, quantification of *Phsp-16.2*: GFP expression and subcellular DAF-16: GFP localization were investigated to determine the antioxidant activities *in vivo*. The *in vivo* hepatoprotective potential of the root extract was evaluated for D-galactosamine (D-GaIN)-induced hepatotoxicity in rats. The activity of the liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT), in addition to liver peroxidation product malondialdehyde (MDA) and glutathione content (GSH), as well as albumin and total bilirubin concentration, were determined. A histopathological study was also performed.

Results and conclusions: *C. abbreviata* root extract is rich in polyphenolics, particularly proanthocyanidins. HPLC-PDA-ESI-MS/MS analysis resulted in the identification of 57 compounds on the bases of their mass spectra. (*epi*)-Catechin, (*epi*)-afzelechin, (*epi*)-guibourtinidol, and (*ent*)-cassiaflavan monomers as well as their dimers, trimers, and their diastereomers are the main components of the extract.

The total phenolic content amounted for 474 mg/g root extract expressed as gallic acid equivalent using the Folin-Ciocalteu method. The extract exhibited powerful antioxidant activity with EC_{50} of 6.3 μg/mL in DPPH and 19.15 mM $FeSO_4$ equivalent/mg sample in FRAP assay. In *C. elegans* model, the extract (200 μg/mL) was able to increase the survival rate by 44.56% and reduced the ROS level to 61.73%, compared to control group.

Pretreatment of rats with 100 mg extract/kg (b. wt.) reduced MDA by 47.36% and elevated GSH by 59.1%. The extract caused a significant reduction of ALT, AST and GGT activities by 11%, 35.7% and 65%, respectively.

The findings of this study suggest that the proanthocyanidin-rich extract from *C. abbreviata* may be an interesting candidate for hepatoprotective activity in case of hepatocellular injury.

Abbreviations: ANOVA, analysis of variance; ALT, alanine aminotransferase; AST, aspartate aminotransferase; *C. elegans*, *Caenorhabditis elegans*; D-GaIN, D-galactosamine; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; GGT, gamma-glutamyltransferase; GFP, green fluorescent protein; GSH, reduced glutathione content; HSP, heat shock protein; HPLC-PDA-ESI-MS/MS, high performance liquid chromatography-photodiode array detector- electrospray ionization mass spectrometry; MDA, malondialdehyde; ROS, reactive oxygen species

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1. Introduction

Medicinal plants have been used in folk medicine for ages against a wide range of ailments and health disorders in the form of herbal infusions and decoctions. Several health benefits of medicinal plants are attributed to certain chemical entities known as secondary metabolites among them tannins as a biologically highly active class (van Wyk and Wink, 2004, 2015).

Tannins have shown powerful potential as antioxidant, antimicrobial, antiviral, antitumor, antidiarrheal, and anti-inflammatory agents beside their ability to treat some cardiovascular diseases (Gali et al., 1992; Kashiwada et al., 1992; Souza et al., 2007; Diouf et al., 2009; Lee et al., 2010). Moreover, several epidemiological correlations have been established between the regular intake of tannins and the decreased incidence of chronic diseases. These broad biological properties have promoted the application of tannin-rich plants in various pharmacological and nutritional studies (Sakagami et al., 2000; Beninger and Hosfield, 2003; Koleckar et al., 2008; Abbas and Wink, 2009).

Cassia abbreviata is a small to medium sized branched umbrella-shaped deciduous tree with very distinctive cylindrically shaped fruits. It is widely spread in the tropics, especially in Africa in the whole region spanning from Somalia to South Africa (Mojeremane et al., 2005).

Phytochemical investigations of this plant have revealed many secondary metabolites belonging to different chemical classes such as anthraquinones, phenolics, triterpenoids, organic acids, and proanthocyanidins (Dehmlow et al., 1998; Erasto and Majinda, 2003; Mongalo and Mafoko, 2013). Several guibourtinidins (flavan-3-ol derivatives) e.g. (2R, 3S)-guibourtinidol and its diastereomers have been isolated from the bark and heartwood (Nel et al., 1999). Additionally, a 2,4-trans-7,4′-dihydroxy-4-methoxyflavan was isolated and characterized from polar extracts of shredded leaves and twigs of *C. abbreviata* (Dehmlow et al., 1998). Moreover, two novel trimeric proanthocyanidins, cassinidin A (3,7,4′-trihydroxyflavan-(4β→8)-3,5,7,4′-tetrahydroxyflavan-(3′→6)-3,5,7,2′,4′-pentahydroxyflavan) and cassinidin B (3,7,2′,4′-tetrahydroxyflavan-(4α→8)-3,5,7,4′-tetrahydroxyflavan-(4α→6)-3,5,7,2′,4′-pentahydroxyflavan) (Erasto and Majinda, 2003), in addition to 2,3-dihydro-5-hydroxy-8-methoxy-2-(4-methoxyphenyl) chromen-4-one and 3,4-dihydro-2-(4-hydroxyphenyl)-4-methoxy-2H-chromen-7-ol) (Kiplagat et al., 2012), were isolated from the root bark.

Traditionally, the fruits, leaves, bark, and roots of *C. abbreviata* are used to treat numerous conditions: The bark was employed to treat headaches, diarrhea, constipation, some skin diseases, and malaria. The roots are taken orally for the treatment of syphilis and their decoction is used for the treatment of pneumonia, stomach troubles, and uterine pains, and against gonorrhea. Extracts from the roots and bark exhibited anti-schistosomiasis (Bruschi et al., 2011; Leteane et al., 2012) and anti-malaria activities (Kiplagat et al., 2012). Furthermore, antimicrobial (Mulubwa and Prakash, 2015), anthelmintic, antiviral (Molgaard et al., 2001; Leteane et al., 2012), anti-diabetic, and antioxidant activities were reported (Shai et al., 2011). Recently, evidence was provided that a leaf extract can inhibit CYP450 in a concentration dependent manner (Thomford et al., 2016).

In this study, we comprehensively investigated the polyphenolic constituents of a methanol extract from roots of *C. abbreviata* using HPLC-MS/MS coupled with a PDA detector, the method of choice to analyze complex mixtures of non-volatile secondary metabolites (Clifford et al., 2003; Theodoridis et al., 2008; Link et al., 2015). Moreover, the antioxidant activities, as well as the hepatoprotective activities of the extract, were investigated *in vitro* and in *in vivo* models (*Caenorhabditis elegans* and D-galactosamine-induced hepatotoxicity in rats).

2. Material and methods

2.1. Plant material

Roots of *Cassia abbreviata* Oliv. (Fabaceae - Caesalpinioideae) were collected from Lupaga Site in Shinyanga, Tanzania by Dr. C. D. Rubanza (Tanzania Forestry Research Institute, Shinyanga). The trypanocidal activities have been investigated before for this plant in our lab (Nibret et al., 2010). The identity of the plant was confirmed by DNA barcoding carried out in our laboratory using *rbc*L as a marker gene. The voucher specimens (leaves and root) were deposited at the Department of Biology, Institute of Pharmacy and Molecular Biotechnology, Heidelberg University under the accession number P7291 and P7294 at IPMB Heidelberg (Germany), respectively.

The dried root (100 g) was ground and exhaustively extracted with 100% methanol at room temperature for an overall extraction period of 3 d. The combined extracts were reduced under vacuum at 40 °C until 500 mL, centrifuged, and then the soluble fraction was further evaporated till dryness. The residue was frozen at −70 °C, and then lyophilized yielding fine dried powder (27 g).

2.2. Drugs and chemicals

Chemicals were purchased from AppliChem (Darmstadt, Germany), Fluka (Buchs, Switzerland) and Sigma Aldrich GmbH (Sternheim, Germany). D-galactosamine was supplied by Sigma Chemicals (ST. Louis. Mo, USA). All solvents for extraction and separation were of analytical grade.

2.3. High performance liquid chromatography (HPLC-PDA-MS/MS)

The phytochemical analysis of polyphenolic compounds was carried out using high performance liquid chromatography–Mass spectrometry (HPLC-PDA-MS/MS). 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 over 60 min with flow rate 1 mL/min with a 1:1 split before the ESI source. The samples were injected automatically using autosampler surveyor ThermoQuest. The instrument was controlled by Xcalibur software (Xcalibur™ 2.0.7, Thermo Scientific). The MS operated in the negative mode with a capillary voltage of − 10 V, a source temperature of 200 °C, and high purity nitrogen as a sheath and auxiliary gas at a flow rate of 80 and 40 (arbitrary units), respectively. Collision energy of 35% was used in MS/MS fragmentation. The ions were detected in a full scan mode and mass range of 50–2000 *m/z*.

2.4. Biological activity experiments

2.4.1. Antioxidant activities in vitro

Determination of total phenolic content as well as antioxidant activities in *in vitro* assays (DPPH and FRAP) were performed as previously described by us (Sobeh et al., 2016).

2.4.2. Antioxidant activity in vivo

2.4.2.1. Caenorhabditis elegans strains and maintenance. Nematodes were maintained under standard conditions [20 °C, on nematode growth medium (NGM), fed with living *E. coli* OP50]. Age synchronized cultures were obtained by sodium hypochlorite treatment of gravid adults; the eggs were kept in M9 buffer for hatching and larvae obtained were subsequently transferred to S-media seeded with living *E. coli* OP50 ($D.O_{600}$ = 1.0) (Stiernagle, 2006). In the current work, the following strains of *C. elegans* were used: Wild type (N2), TJ375 [P*hsp-16.2: GFP(gpls1)*] and TJ356. All of them