

Original article

Antioxidant activity of proanthocyanidins from *adansonia digitata* fruitAbdelaaty A. Shahat¹, Hanaa H. Ahmed², Rasmeia A. Hassan¹, Ahmed A. Hussein¹¹Department of Phytochemistry, ²Department of Hormone, National Research Centre, Bohouth St. 12311 Dokki, Cairo, Egypt

Abstract

Besides (-)-epicatechin, epicatechin-(4 β -8)-epicatechin (procyanidin B2), epicatechin-(4 β -6)-epicatechin (procyanidin B5), epicatechin-(4 β -8, 2 β -O-7)-epicatechin (proanthocyanidin A2) and epicatechin-(4 β -8)-epicatechin-(4 β -8)-epicatechin (procyanidin C1), which were isolated before from *Adansonia digitata*, in this work an A-type proanthocyanidin trimer, i. e. epicatechin-(4 β -8)-epicatechin-(4 β -8, 2 β -O-7)-epicatechin, tetrameric procyanidin D1, i. e. epicatechin-(4 β -8)-epicatechin-(4 β -8)-epicatechin-(4 β -8)-epicatechin and a polymeric compound were isolated from the pericarp (fruit wall) of the fruits for the first time from this plant. The antioxidant activity of different fractions and pure compounds was experimentally evaluated in the DPPH⁻ assay. The ethyl acetate fraction, and most of the isolated compounds displayed a high activity (IC₅₀ 2.40-9.60 μ g/ml) compared with the reference antioxidant Trolox (IC₅₀ 12.18 μ g/ml) as a standard.

Keywords: *adansonia digitata*, antioxidant activity, bombacaceae, proanthocyanidins.

INTRODUCTION

The Baobab (*Adansonia digitata* L.) belongs to the family Bombacaceae. It is unmistakable in the field, given its strange appearance and barrel-like enormous trunk, which tapers into branches. It is a deciduous tree whose rounded crown is bare during the dry season. The generic name comes from the French botanist, M. Adanson (1727-1806)^[1]. The baobab is one of the longest-lived trees in the world. The French botanist Adanson contended that some specimens of the baobab were as much as 5000 years old. Baobabs are often-photographed trees with distinctive shapes. The genus *Adansonia* comprises eight species, one species is found in Africa, six in

Madagascar and one in northwestern Australia^[2]. In West Africa, the leaves (and bark) are used for treating urinary disorders and diarrhoea. Young roots are cooked and eaten^[3,4]. A variety of chemicals have been isolated and characterised from *A. digitata*, belonging to the classes of terpenoids, sterols, vitamins, amino acids, carbohydrates, lipids, flavonoids and proanthocyanidins^[2,5].

Proanthocyanidins are polyphenolic compounds are ubiquitous in the plant kingdom i. e. in pine bark^[6], grapes (seeds and skin)^[7], cocoa, cranberries, and apples^[8], hawthorn and *adansonia digitata*^[9-11]. Monomeric procyanidins are polyhydroxyflavan-3-ol monomers, and are generally called catechins, including catechin, epicatechin, galocatechin, epigallocatechin, and their gallic acid esters (catechin gallate and epicatechin gallate). A-type procyanidins are formed through a 4-8 C-C bond and an interflavonid C-O bond^[12]. Procyanidins formed through 4-8 or 4-6 C-C bonds of flavan-3-ol mono-

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mers are B-type procyanidins. Oligomeric procyanidins (OPCs) including dimers, trimers, and tetramers are also called proanthocyanidins. Procyanidins were reported to be potent antioxidants [11,13]. Problems of oxidation may arise when the electron flow becomes uncoupled (transfer of unpaired single electrons), generating free radicals. Examples of oxygen-centered free radicals, known as reactive oxygen species (ROS), include superoxide (O_2^-), peroxy (ROO^-), alkoxy (RO^-), hydroxyl (HO^-), and nitric oxide (NO^-). The hydroxyl and the alkoxy free radicals are very reactive and rapidly attack the molecules in nearby cells, and probably the damage caused by them is unavoidable and is dealt with by repair processes [14]. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids, or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols, and flavonoids scavenge free radicals such as peroxide, hydroperoxide, or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases [15].

Five procyanidins compounds were isolated before from this species [16]. In this work two oligomeric proanthocyanidins (A- and B-types) were isolated for the first time from this plant and identified, and the antioxidant activity of the isolated compounds, the total alcoholic extract and the ethyl acetate, n-butanol and water fractions was tested using the DPPH⁻ assay, which is one of the best-known, frequently employed, and accurate methods. DPPH⁻ (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical because of its spare electron delocalization over the whole molecule. The delocalization causes a deep violet color with λ_{max} around 520 nm. When a solution of DPPH⁻ is mixed with a substrate acting as a hydrogen atom donor, a stable nonradical form of DPPH is obtained with simultaneous change of the violet color to pale yellow.

MATERIALS AND METHODS

Plant Materials

Adansonia digitata was purchased at the Harraz Herbal Drugstore (Cairo, Egypt) in October, 2001 and identified by taxonomist Prof. Dr. Ibrahim El-Garf, Department of Botany, Faculty of Science, Cairo University, Cairo, Egypt. A voucher specimen is kept in the Phytochemistry Department, National Research Centre, Cairo, Egypt.

General

TLC was carried out on precoated silica gel F254 plates (0.2 mm, Merck) (Darmstadt, Germany) developed with the solvent system: EtOAc-HOAc-HCOOH-H₂O (30/0.8/1.2/8) v/v. Spots were detected using vanillin- H₂SO₄ (vanillin 1% in MeOH and 5% H₂SO₄ in EtOH) followed by heating the plates to 110 °C for 15-20 min. Column chromatography (CC) was performed on Sephadex LH-20 (Pharmacia), and Silica gel 60 (230-400 mesh ASTM) (Merck). ¹H and ¹³C NMR spectra were recorded in acetone-d₆ on a Bruker DRX-400 spectrometer operating at 400 MHz for ¹H and at 100 MHz for ¹³C. Chemical shifts are presented in ppm downfield of TMS. Electrospray Ionization Mass Spectra (ESI-MS) were obtained on a Bruker Esquire 3000 plus mass spectrometer.

DPPH⁻ scavenging activity:

The DPPH⁻ (1, 1-diphenyl-2-picrylhydrazyl free radical) assay is an excellent in vitro method to investigate the free radical scavenging activity of an antioxidant, i. e. the donation of a hydrogen atom to a free radical species. The stable free radical DPPH⁻ was dissolved in EtOH to give a 100 μ M solution; 0.5 ml of a test compound in EtOH (or EtOH itself as control) was added to 3.0 ml of the ethanol DPPH⁻ solution. For each test compound, different concentrations were tested. The mixtures were shaken vigorously and left to stand in the dark for 20 min. The decrease in DPPH⁻ absorption was measured at 517 nm (purple colour) and the actual decrease in absorption induced by the test compound was calculated by subtracting that of the control. The antioxidant activity of each test sample was tested in triplicate and was expressed as an IC₅₀ value, i. e. the concentration in μ g/ml that inhibits DPPH⁻ absorption by 50%, and was calculated by linear re-

gression analysis. Trolox (6-hydroxy- 2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) was used as control^[5,17].

Extraction and Isolation

The dried pericarp (200 g) was ground to powder and extracted exhaustively with 80% aqueous MeOH (4 x 500 ml). The combined extract was concentrated on a rotatory evaporator at 35 °C under reduced pressure and the residue was diluted with 20% aqueous MeOH and defatted by extracting 2 times with an equal volume of petroleum ether. The aqueous phase was reduced to a smaller volume and extracted 4 times with an equal volume of EtOAc. The EtOAc extract was combined and concentrated to afford 5.2 g of solid. 5 g of the solid was dissolved in a small amount of methanol and chromatographed on a Sephadex LH-20 column using EtOH as the eluting solvent. Fractions (12 mL) were collected with a fraction collector and monitored by TLC. On the basis of the TLC results, fractions were collected to give six sub fractions (I-VI) Compounds 1-5, i. e. epicatechin (1), epicatechin-(4 β -8)-epicatechin B2 (2), epicatechin-(4 β -8, 2 β -O-7)-epicatechin A2 (3) epicatechin-(4 β -6)-epicatechin B5 (4), and epicatechin-(4 β -8)-epicatechin-(4 β -8)-epicatechin C1 (5) were isolated before from fraction (1-IV) as published before (Shahat, 2006). Repeated column chromatography of fractions V and VI on Sephadex LH-20 with methanol or ethanol yielded a procyanidin trimer, i. e. epicatechin-(4 β -8)-epicatechin-(4 β -8, 2 β -O-7)-epicatechin (6), tetrameric procyanidin D1, i. e. epicatechin-(4 β -8)-epicatechin-(4 β -8)-epicatechin-(4 β -8)-epicatechin (7), and a polymeric compound, epicatechin-(4 β -8)-{epicatechin-(4 β -8)}*n*-epicatechin (8)

RESULTS AND DISCUSSION

Compounds 1-5 were isolated and identified before^[16]. Based on the [M + Na]⁺ peak at m/z 887, corresponding to a molecular weight of 864, com-

pound 6 was characterised as a procyanidin trimer, containing one B-type single linkage, and one A-type double linkage. The 13C NMR spectrum (table 1) was in good agreement with the upper unit of a procyanidin B2 dimer, combined with a procyanidin A2 dimer^[16]. Hence, compound 6 was identified as epicatechin-(4 -8)-epicatechin-(4 -8, 2 -O-7)-epicatechin. ESI-MS of compound 7 produced a [M + Na]⁺ peak at m/z 1177, indicating a molecular weight of 1154, which was in agreement with a tetrameric procyanidin containing only single (B-type) linkages, but no double (A-type) linkages. The 13C NMR data (table 2) were in good agreement with those reported for procyanidin D1, consisting of 4 epicatechin units with 4 β -8 linkages, i. e. epicatechin-(4 β -8)-epicatechin-(4 β -8)-epicatechin-(4 β -8)-epicatechin (Morimoto et al., 1986). For the upper and both middle units, C-2 resonances were found at 76.78/76.90 ppm, C-3 resonances at 72.05/72.21/73.02 ppm, and C-4 resonances at 36.82/36.95 ppm. For the terminal unit, C-2 occurred at 79.17 ppm, C-3 at 66.41 ppm, and C-4 at 28.99 ppm. Although for compound 8 no ESI-MS spectrum could be obtained, its 13C NMR was very similar to that of compound 7, suggesting that this oligomeric fraction consisted of epicatechin units connected by 4 β -8 linkages.

The antioxidant activity of different fractions and of these isolated compounds was evaluated in the DPPH⁻ assay (Table 3). The EtOAc fraction showed a high activity, and bioassay-guided fractionation resulted in the characterization of compounds 7, 5, 6, 1, 2, 4, and 6, which were stepwise the most active compounds. The n-BuOH fraction and the EtOH extract gave moderate activity while the chloroform and aqueous fractions were least active.

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Table 1: Characteristic ¹³C-NMR signals of compound 6 (epicatechin-(4β-8)-epicatechin)-(4-8, (2β-0-7)-epicatechin) (100 MHz, acetone-d₆)

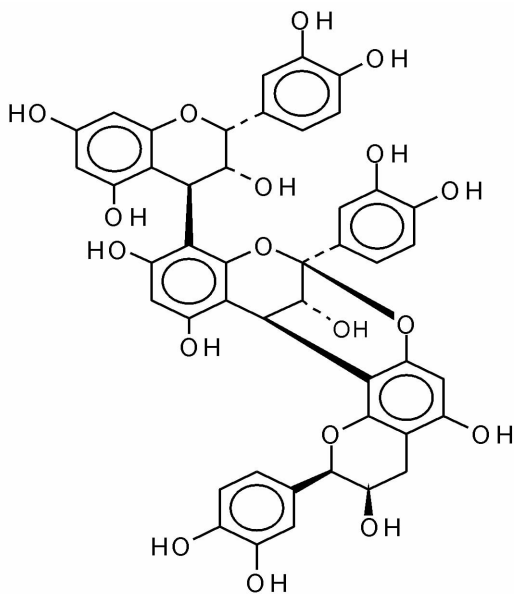
C No.	δC (ppm)
2u	77.01
2m	100.19
2t	81.77
3u	72.62
3m	67.09
3t	66.01
4u	36.93
4m	28.93
4t	30.00

Table 2: Characteristic ¹³C-NMR signals of compound 7 (epicatechin-(4β-8)-(epicatechin)₂-(4β-8)-epicatechin) (procyanidin D1) (100 MHz, acetone-d₆)

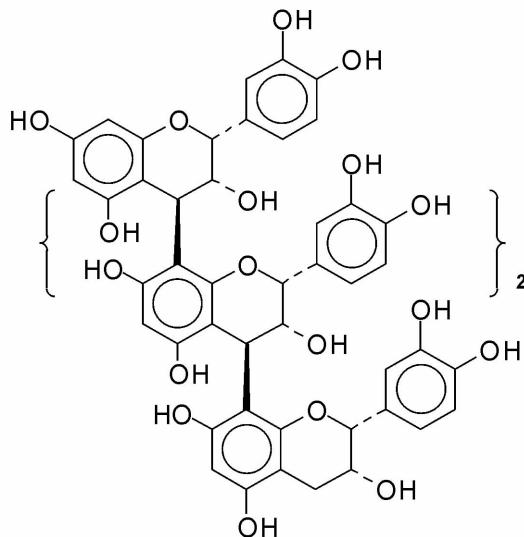
C No.	δC (ppm)
2u, 2m, 2m'	76.78 / 76.90
2t	79.17
3u, 3m, 3m'	72.05 / 72.21 / 73.02
3t	66.41
4u, 4m, 4m'	36.82 / 36.92
4t	28.99

Table 3: Antioxidant activity of the fractions and isolated compounds

Extract/Fractions /compounds	IC ₅₀ (μg/ml) ± SD
EtOH extract	17.30 ± 1.79
CHCl ₃ fraction	38 ± 1
EtOAc fraction	2.4 ± 0.2
BuOH fraction	12.20 ± 0.27
Aq fraction	52 ± 9
Epicatechine (1)	6.89 ± 0.77
B2 (2)	5.50 ± 0.26
A2 (3)	9.60 ± 0.93
B5 (4)	7.70 ± 0.14
C1 (5)	3.10 ± 0.78
A-type Trimmer (6)	4.90 ± 0.14
Tetramer (7)	2.55 ± 0.35
Trolox	12.18 ± 0.36



epicatechin-(4 β -8)-epicatechin)-(4-8, (2 β -O-7)-epicatechin (6)



epicatechin-(4 β -8)-(epicatechin)₂-(4 β -8)-epicatechin (procyanidin D1) (7)

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