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ScienceDirect
Journal of Ethnopharmacology 113 (2007) 284–291
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Antioxidant activity of *Caesalpinia digyna* root

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Received 11 August 2006; received in revised form 6 June 2007; accepted 18 June 2007
Available online 26 June 2007

Abstract

The antioxidant properties of three successive extracts of *Caesalpinia digyna* Rottler root and the isolated compound, bergenin, were tested using standard *in vitro* and *in vivo* models. The amount of the total phenolic compounds present was also determined. The successive methanol extract of *Caesalpinia digyna* root (CDM) exhibited strong scavenging effect on 2,2-diphenyl-2-picryl hydrazyl (DPPH) free radical, 2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt (ABTS) radical cation, hydrogen peroxide, nitric oxide, hydroxyl radical and inhibition of lipid peroxidation. The free radical scavenging effect of CDM was comparable with that of reference antioxidants. The CDM having the highest content of phenolic compounds and strong free radical scavenging effect when administered orally to male albino rats at 100, 200 and 400 mg/kg body weight for 7 days, prior to carbontetrachloride (CCl_4) treatment, caused a significant increase in the levels of catalase (CAT) and superoxide dismutase (SOD) and significant decrease in the levels of lipidperoxidation (LPO) in serum, liver and kidney in a dose dependent manner, when compared to CCl_4 treated control. These results clearly indicate the strong antioxidant property of *Caesalpinia digyna* root. The study provides a proof for the ethnomedical claims and reported biological activities. The plant has, therefore, very good therapeutic potential.

Keywords: *Caesalpinia digyna*; Free radicals; Bergenin; *In vivo*; CCl_4

1. Introduction

Free radicals and reactive oxygen species (ROS) are well known inducers of cellular and tissue pathogenesis leading to several human diseases such as cancer, inflammatory disorders, as well as in aging processes (Halliwell, 1994; Aviram, 2000). Antioxidants provide protection to living organisms from damage caused by uncontrolled production of ROS and the concomitant lipid peroxidation, protein damage and DNA strand breaking (Ghosal et al., 1996). Several anti-inflammatory, digestive, antinecrotic, neuroprotective and hepatoprotective drugs have recently been shown to have antioxidant and/or radical scavenging mechanism as part of their activity (Lin and Huang, 2000; Repetto and Llesuy, 2002).

The use of traditional medicine is widespread, and plants still present a large source of natural antioxidants that might serve as leads for the development of novel drugs (Perry et al., 1999). Several members of the species of genus *Caesalpinia* like *Caesalpinia sappan* and *Caesalpinia bonducella*, etc., are used traditionally for a wide variety of ethnomedical properties such as anti-inflammatory, antidiabetic, antioxidant and hepatoprotective (Badami et al., 2003), etc. Among them, *Caesalpinia digyna* Rottler (Family: Leguminosae) is a large, scandent, prickly shrub or climber, upto 10 m in height, growing wild in the scrub forests of the eastern Himalayas in Assam and West Bengal, the Eastern Ghats in Andhra Pradesh, Madhya Pradesh and also in Ceylon and Malay Islands. The plant is one of the ingredients of an indigenous drug preparation "Geriforte", which has been used for curing senile prurites with excellent results. The drug also exhibits antifatigue effect in rats (Anon., 1992). The root has marked astringent properties. It is given internally in pthisis and scrofulous affections; when sores exist, it is applied externally as well. It is also used in diabetes. In some parts of the Burma the root, pounded and mixed with water, is drunk as a febrifuge. It is said to have intoxicating effect (Kiritikar and Basu, 1999). The ethanol water extract of roots inhibits the growth of *Mycobacterium tuberculosis* (Patel et al., 1966). Chemical investigations of the plant have shown the presence of caesalpinine A, cellallocinnine, ellagic acid, gallic acid, bergenin, bonducellin, intricatinol and tannins (Biswas,

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doi:10.1016/j.jep.2007.06.006

1944; Chaudhry et al., 1954; Evans and Bell, 1978; Mahato et al., 1983, 1985; Boonsri et al., 2005; Chantrapromma et al., 2006). In view of the several ethnobotanical uses of *Caesalpinia digyna* described above, it was proposed to screen its successive extracts and isolated compound(s) for the *in vitro* and *in vivo* antioxidant activity using standard procedures.

2. Materials and methods

2.1. Plant material

The root of *Caesalpinia digyna* was purchased from Abirami Botanicals, Tuticorin, Tamilnadu, India, and authenticated by Dr. D. Suresh Baburaj, Survey of Medicinal Plants and Collection Unit, Ootacamund, India. A voucher specimen (TIFAC 01) has been deposited for further reference at J.S.S College of Pharmacy herbarium, Ootacamund, India.

2.2. Chemicals

2,2-Diphenyl-2-picryl hydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt (ABTS) were obtained from Sigma–Aldrich Co., St. Louis, USA. Rutin and *p*-nitroso dimethyl aniline (*p*-NDA) were obtained from Acros Organics, NJ, USA. Naphthyl ethylene diamine dihydrochloride (NEDD) was from Roch-Light Ltd., Suffolk, UK, ascorbic acid, nitro blue tetrazolium (NBT) and butylated hydroxy anisole (BHA) were from SD Fine Chemicals Ltd., Mumbai, India and 2-deoxy-D-ribose was from Hi-Media Laboratories Pvt. Ltd., Mumbai, India. Sodium nitroprusside and Silymarin were from Ranbaxy Laboratories Ltd., Mohali, India. Sulphanilic acid used was from E-Merck (India) Ltd., Mumbai, India. All chemicals used were of analytical grade.

2.3. Animals

Healthy male albino rats of wistar strain (180–220 g) were obtained from the animal house, J.S.S. College of Pharmacy, Ootacamund, India, and were maintained under standard environment conditions (22–28 °C, 60–70% relative humidity, 12-h dark:12-h light cycle) and were fed with standard rat feed (M/S Hindustan Lever Ltd., Bangalore, India) and water *ad libitum*. The experiments were conducted as per the guidelines of CPCSEA, Chennai, India (approval no. JSSCP/IAEC/Ph.D/PH.Chemistry/01/2005–2006).

2.4. Extraction procedure

The root was chopped to small pieces and dried in shade. The dried root was powdered and passed through sieve no. 20 and extracted (100 g) successively with 600 mL each of petroleum ether (60–80 °C), methanol and water in a Soxhlet extractor for 18–20 h. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40–50 °C). The petroleum ether extract yielded a yellowish green sticky semisolid, weighing 0.3 g (0.30%). The methanol and water extracts yielded brown and dark brown semi-solid residues, weighing 7.0 g (7.0%) and 0.9 g (0.9%), respectively. All the extracts were preserved in a refrigerator till further use.

2.5. Isolation of bergenin

The powdered root was extracted (100 g) with 600 mL of methanol in a Soxhlet extractor for 18–20 h. The extract was concentrated and dissolved in a minimum amount of methanol and kept in room temperature for 24 h. A crystalline solid settled down to the bottom of the container. The crystalline solid was separated and washed with acetone. Repeated recrystallation of combined crystalline solids with methanol yielded a colourless crystalline compound. This was characterized by comparing its melting point, IR, NMR and mass spectrum with a pure specimen of bergenin (Taneyama et al., 1983).

2.6. Preparation of test and standard solutions

All the three extracts of *Caesalpinia digyna* Rottler root, the isolated compound and the standard antioxidants (ascorbic acid, rutin, butylated hydroxy anisole and alpha tocopherol) were dissolved in distilled dimethyl sulphoxide (DMSO) separately and used for the *in vitro* antioxidant assays using seven different methods except the hydrogen peroxide method. For the hydrogen peroxide method (where DMSO interferes with the method), the extracts and the standards were dissolved in distilled methanol and used. The stock solutions were serially diluted with the respective solvents to obtain lower dilutions.

A suspension of CDM and standard drug silymarin were prepared in sodium CMC (0.5%, w/v) using distilled water and used for *in vivo* experiments.

2.7. Total phenolic compounds estimation

Antioxidant compounds generally contain phenolic group(s) and hence, the amount of phenolic compounds in all the three extracts of the root was estimated by using Folin–Ciocalteu reagent (Sadasivam and Manikam, 1992). In a series of test tubes, 0.4 mL of the extract in methanol was taken, mixed with 2 mL of Folin–Ciocalteu reagent and 1.6 mL of sodium carbonate. After shaking, it was kept for 2 h and the absorbance was measured at 750 nm using a Shimadzu-UV-160 spectrophotometer. Using gallic acid monohydrate, a standard curve was prepared. The linearity obtained was in the range of 1–10 μg/mL. Using the standard curve, the total phenolic compounds content was calculated and expressed as gallic acid equivalent in mg/g of extracts.

2.8. In vitro antioxidant activity

The three extracts and the isolated compound were tested for their *in vitro* antioxidant activity using the standard methods. In all these methods, a particular concentration of the extract or standard solution was used which gave a final concentration of 1000–0.45 μg/mL after all the reagents were added. Absorbance was measured against a blank solution containing the extract or standard, but without the reagents. A control