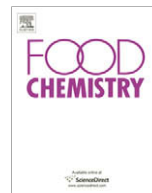
Food Chemistry 114 (2009) 1271–1277

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Total phenolic contents and free radical scavenging activity of certain Egyptian *Ficus* species leaf samples

El-Sayed Saleh Abdel-Hameed *

Laboratory of Medicinal Chemistry, Theodor Bilharz Research Institute, Warrak El-Hader, Giza, Egypt

ARTICLE INFO

Article history:
Received 31 March 2008
Received in revised form 23 September 2008
Accepted 3 November 2008

Keywords:
Antioxidant
DPPH
Ficus
Phenolic
Flavonoid

ABSTRACT

The antioxidative phytochemicals in grains, vegetables, fruits and medicinal plants have received increasing attention for their potential role in prevention of human diseases. The methanol extracts of the leaves of eleven *Ficus* species growing in Egypt were subjected to free radical scavenging activity using 1,1-diphenyl picrylhydrazyl (DPPH·) method. Six methanol extracts of six species showed high activity in order: *Ficus lyrata* Warb. > *Ficus afzelli* G. > *Ficus nitida* L. > *Ficus virens* Ait. > *Ficus sycomorus* L. > *Ficus decora* Hort. with SC_{50} 38.37, 60.22, 61.67, 74.00, 79.50 and 81.62 µg/ml, respectively. The free radical scavenging activity of different fractions obtained from successive fractionation of the six methanol extracts with organic solvents of different polarities; petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH; showed that, the EtOAc and *n*-BuOH fractions have the high activity with $SC_{50} < 50$ µg/ml whereas petroleum ether and $CHCl_3$ fractions have weak activity at $SC_{50} > 200$ and 100 µg/ml, respectively. The EtOAc and *n*-BuOH fractions of *F. lyrata* showed the strongest free radical scavenging activity on DPPH· with $SC_{50} = 8.27$ and 12.14 µg/ml, respectively and also the highest antioxidant capacity monitored by phosphomolybdenum method (928.48 and 728.53 mg equivalent to ascorbic acid/g extract). Preliminary phytochemical investigation of the six active EtOAc and *n*-BuOH fractions guided by review of literatures of this genus showed that phenolic compounds constitute the major components of it. The total phenolic, tannin, flavonoid and flavonol contents of the six active EtOAc and *n*-BuOH fractions were measured using the well established methods. Linear correlation between the total antioxidant capacity of the six active EtOAc and *n*-BuOH fractions and the total phenolic and flavonoid contents were observed. More *in vivo* and *in vitro* studies along with detailed photochemical investigation are needed in the hope to can use these species (crude extract, fractions, sub-fractions or pure compounds) in the prevention and therapies of diseases in which oxidants or free radicals are involved.

© 2008 Published by Elsevier Ltd.

1. Introduction

Free radicals are highly reactive oxygen species; superoxide ($O_2^{\cdot-}$), hydroxyl (OH·), peroxyl (ROO·), peroxinitrite (·ONOO⁻), and nitric oxide (NO·) radicals; produced through oxidative process within the mammalian body, biologically important materials (e.g., lipids, foods, and oils) and industrially important ones (e.g., rubber and lubricant) (Atta-ur-Rahman & Choudhary, 2001). The human body possesses many defence mechanisms against oxidative stress, including antioxidant enzymes and non-enzymatic compounds. Under some circumstances including exposure to some environmental pollutants, e.g., cigarette smoke, pesticides, smog, UV radiation, etc. the natural antioxidant mammalian mechanism become insufficient and then the excess of free radicals can damage both the structure and function of a cell membrane in a chain reaction leading to degenerative diseases and a conditions such as Alzheimer, ageing process, cataracts, acute liver toxicity, cardiovascular disease, arteriosclerosis, nephritis, diabetes mellitus, rheumatism, inflammation process and DNA damage that can lead to carcinogenesis. Many antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer (Mosquera, Correa, Buitrago, & Niö, 2007; Wong, Li, Cheng, & Chen, 2006).

Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods, cosmetics or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity (Sasaki et al., 2002). The antioxidative phytochemicals especially phenolic compounds found in vegetables, fruits and medicinal plants have received increasing attention for their potential role in prevention of human diseases (Cai, Luo, Sun, & Corke, 2004). The human can use antioxidants either as dietary, food supplement or as a drug.

Ficus is a genus of about 800 species of woody trees, shrubs and vines in the family Moraceae. Collectively known as fig trees and the most well known species in the genus is the common Fig

* Fax: +20 235408125.
E-mail address: Shzssayed@yahoo.com

0308-8146/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2008.11.005

(*Ficus carica* L.), which produces the commercial fruit called fig (http://www.figweb.org/Ficus/index.htm). Several members of the genus *Ficus* are being used traditionally in a wide variety of ethnomedical remedies all over the world (Hansson, Zelada, & Noriega, 2005; Koné et al., 2004). Phytochemical investigations of some *Ficus* species revealed that phenolic compounds constitute the major components of them (Li, Bu, Yue, & Sun, 2006; Sandabe, Onyeyili, & Chibuzo, 2006; Tuyen et al., 1999). Also, some studies reported the presence of antioxidant activity of some *Ficus* species which attributed the antioxidant activity to the phenolic content of them (Al-Fatimi, Wurster, Schröder, & Lindequist, 2007; Daniel, Mathew, Devi, & Augusti, 1998; Manian, Anusuya, Siddhuraju, & Manian, 2008; Shukla, Gupta, Gambhir, Prabhu, & Murthy, 2004).

Research on *Ficus* has focused on its edible part (fruits) followed by aerial roots and barks while the leaves are rarely studied on comparing with other parts. In Egypt, many *Ficus* species found in streets, gardens, parks and outside the canal banks. The fruits of *F. carica* and *Ficus sycomorus* L. are two of the most favourable fruits eaten by Egyptian peoples. Mousa et al. (1994); approved and supported the traditional uses of certain Egyptian *Ficus* species in folk medicine for respiratory disorders and certain skin diseases.

In this study, the leaves of eleven *Ficus* species were collected and its methanolic extracts were subjected to antioxidant evaluation due to the lake of data on the antioxidant properties of these plants part using 1,1-diphenyl picrylhydrazyl (DPPH·) method. The free radical scavenging activity of the fractions obtained from successive fractionation of methanolic extract with organic solvents with different polarities were measured. The most active fractions were estimated for its total antioxidant capacity using phosphomolybdenum methods. The total phenolic, tannin, flavonoid and flavonol contents were measured using the well known methods.

2. Materials and methods

2.1. Materials and chemicals

The solvents petroleum ether, chloroform, ethyl acetate, *n*-butanol, acetic acid and sulphuric acid were purchases from Egyptian chemical company. DPPH free radical from Fluka Chemicals, aluminium chloride, sodium carbonate, sodium phosphate, ammonium molybedate, rutin, ascorbic acid and gallic acid from Aldrich chemicals. Paper chromatography (PC) was done on Whatmann No. 1 (57×46 cm) while thin layer chromatography (TLC) was performed over pre-coated silica plates (GF_{254}, Merck). The Folin–Ciocalteu's reagent (FCR) for determination total phenolic compounds was freshly prepared according to the described method by Huang, Ou, and Prior (2005). The FCR is typically made by boiling (for 10 h) the mixture of sodium tungstate ($Na_2WO_4 \cdot 2H_2O$, 10 g), sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$, 2.5 g), concentrated hydrochloric acid (10 ml), 85% phosphoric acid (5 ml), and water (70 ml). After boiling, lithium sulphate ($Li_2SO_4 \cdot 4H_2O$, 1.5 g), 5 ml water and one drop of bromine were added followed by reflux for 15 min. Cool to room temperature and bring to 100 ml with water. About 1 hexavalent phosphomolybdic/phosphotungestic acid complex is formed. The absorbance measurements were recorded using the UV–vis spectrophotometer Milton Roy, Spectronic 601.

2.2. Plant materials

The leaves of eleven *Ficus* species under investigation; *Ficus afzelli* G., *Ficus benjamina* L., *Ficus carica* L., *Ficus decora* Hort., *Ficus glomerata* Roxb., *Ficus lyrata* Warb., *Ficus microcarpa* L., *Ficus nitida* L., *Ficus platyphylla* Del., *Ficus virens* Ait. and *Ficus sycomorus* L.; were collected between June and July 2007 from El-Orman botanical garden and the Giza Zoo, Giza, Egypt. The plants were kindly identified by Mrs. Traes Labib, general manager and head of specialists of Plant Taxonomy in El-Orman botanical garden, Giza, Egypt. Voucher specimens were deposited at the laboratory of Medicinal Chemistry, Theodor Bilharz Research Institute. The plants were dried in shade, finely powdered with an electric mill and become ready for extraction process.

2.3. Preparation of methanol extracts and its successive fractions

One hundred gram of each fine powdered leaves of the eleven *Ficus* species under investigation were soaked in 500 ml methanol for one week at room temperature with shaking day by day followed by filtration and again extraction for four times. The organic solvents were removed in vacuo using rotatory evaporator affording known weight of each methanol extract which became ready for primary radical scavenging estimation using DPPH methods.

Five grams of the most active methanol extracts were dissolved in 20 ml distiled water and then successively partitioned with petroleum ether, chloroform, ethyl acetate and finally with *n*-butanol (4×25 ml solvent) affording known weight of each respective fractions.

2.4. Antioxidant estimation

It is important to select and employ a stable and rapid method to assay antioxidant activity, because the determination of many samples is time-consuming. Several methods have been developed to assay free radical scavenging capacity and total antioxidant activity of plant extracts. The most common and reliable method involves the determination of the disappearance of free radicals using a spectrophotometer. In this work, two methods were used, 1,1-diphenyl picrylhydrazyl scavenging activity for estimation the free radical scavenging properties and phosphomolybdenum method used for measurement the total antioxidant capacity.

2.4.1. Scavenging ability towards DPPH radical (DPPH· assay)

The DPPH· assay was performed as described by Shirwaikar, Rajendran, and Punithaa (2006). This method depends on the reduction of purple DPPH· to a yellow coloured diphenyl picrylhydrazine and the remaining DPPH· which showed maximum absorption at 517 nm was measured. About 2 ml of various concentrations of each extract were added to 2 ml solution of 0.1 mM DPPH·. An equal amount of methanol and DPPH· served as control. After 20 min of incubation at 37 °C in the dark, the absorbance was recorded at 517 nm. The experiment was performed in duplicates. The DPPH radical scavenging activity was calculated according to the following equation:

$$\% \text{ DPPH radical scavenging activity} = 1 - [A_{sample}/A_{control}] \times 100$$

where A_{sample} and $A_{control}$ are absorbance of sample and control. The concentration of sample required to scavenge 50% of DPPH· (SC_{50}) were determined. Decreasing of the DPPH· solution absorbance indicates an increase of the DPPH radical scavenging activity.

2.4.2. Determination of total antioxidant capacity by phosphomolybdenum method

The total antioxidant capacities of the extracts were evaluated by the phosphomolybdenum method as described by Prieto, Pineda, and Aguilar (1999). The assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. 0.3 ml of each sample solution and ascorbic acid (100 μg/ml) were combined with 3 ml of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). A typical blank solution contained 3 ml of reagent solution and the appropriate volume of the same solvent used for the sample. All tubes were capped and incubated in a boiling water bath at 95 °C for 90 min. After the samples had been cooled to room temperature, the absorbance of the solution of each

Download English Version:

https://daneshyari.com/en/article/1186228

Download Persian Version:

https://daneshyari.com/article/1186228

Daneshyari.com