Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 99 (2008) 7692–7698
BIORESOURCE TECHNOLOGY

Antioxidant activity of the phenol rich fractions of leaves of *Chukrasia tabularis* A. Juss.

Rajbir Kaur [a], Saroj Arora [a,*], Bikram Singh [b]

[a] *Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar 143005, Punjab, India*
[b] *Natural Plant Product Division, Institute of Himalayan Bioresource Technology (IHBT), Palampur, Post Box No. 6-176061, Himachal Pradesh, India*

Received 24 October 2007; received in revised form 25 January 2008; accepted 30 January 2008
Available online 18 March 2008

Abstract

The present study was designed to explore the antioxidant potential of chloroform, ethyl acetate, *n*-butanol and water fractions of 80% methanol extract of leaves *Chukrasia tabularis* by 2,2′-diphenyl-1-picrylhydrazyl (DPPH), deoxyribose degradation (non-site specific and site specific), reducing power and DNA nicking assays. The different fractions showed significant activities in all the free radical scavenging tests and these findings have also shown direct relationship between antioxidant activity and phenolic content. Among the fractions, ethyl acetate fraction exhibited highest inhibition of 93.14%, 89.99%, 87.04% in DPPH, non-site specific and site specific deoxyribose degradation assays, respectively and 91.20% reduction of ferricyanide to give Prussian blue coloured complex in reducing power assay at maximum concentration tested. This preliminary study indicates the antioxidant activity of the leaves of *Chukrasia tabularis*, and moreover the results showed correlation with the amount of phenolic content present in different fractions.

Keywords: *Chukrasia tabularis*; Phenolic compounds; Free radical scavenging activities; DNA nicking assay; Total phenol content

1. Introduction

The plants play a more comprehensive role in the human diet as they prevent diseases that are responsible for devastation of human population. Epidemiological studies have shown that a diet rich in fruits and vegetables is associated with a decreased risk of cardiovascular diseases and certain cancers (Bazzano et al., 2002; Block et al., 1992). These health effects have been attributed in part to the presence of phenolic compounds in dietry plants, which may exert their effects as a result of their antioxidant properties. The antioxidant activities of phenolics are mainly due to their redox properties that allow them to act as a reducing agents, hydrogen donors and singlet oxygen quenchers (Rice-Evans et al., 1996; Ramarathnam et al., 1997; Khokhar and Apenten, 2003). In addition, they have a metal chelation potential (Amarowicz et al., 2004; Zhao et al., 2005; Othmana et al., 2007).

Keeping this in view, coupled with the fact that *Chukrasia tabularis* has a high content of secondary metabolites including phenolic compounds, the present study was designed to estimate the antioxidant efficiency employing free radical scavenging tests viz. (2,2′-diphenyl-1-picrylhydrazyl (DPPH), non-site specific and site specific deoxyribose degradation, reducing power assays) together with inhibition of strand breaking of supercoiled deoxyribonucleic acid in DNA nicking assay. The total phenolic content was determined by Folin–Ciocalteu method.

C. tabularis A. Juss. (Meliaceae) commonly known as Chittgong wood or lal devdari etc. is a medicinal plant that accumulates a variety of secondary metabolites including phenolic compounds, terpenes, limonoids, steroids, chuktabularins and tabularisins (Rastogi and Mehrotra, 1993; Nakatani et al., 2004; Fan et al., 2007; Zhang et al.,

* Corresponding author. Tel.: +91 0183 2451048, +91 0941 7285485; fax: +91 0183 2258819/2258820.
E-mail address: dr.sarojarora@gmail.com (S. Arora).

0960-8524/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2008.01.070

2007). It has got reference in Ayurveda as astringent and antipyretic drug. Recent studies have also explored its antimalarial and antibacterial activities (Mackinnon et al., 1997; Nagalakshmi et al., 2001, 2003).

2. Methods

2.1. Plant material

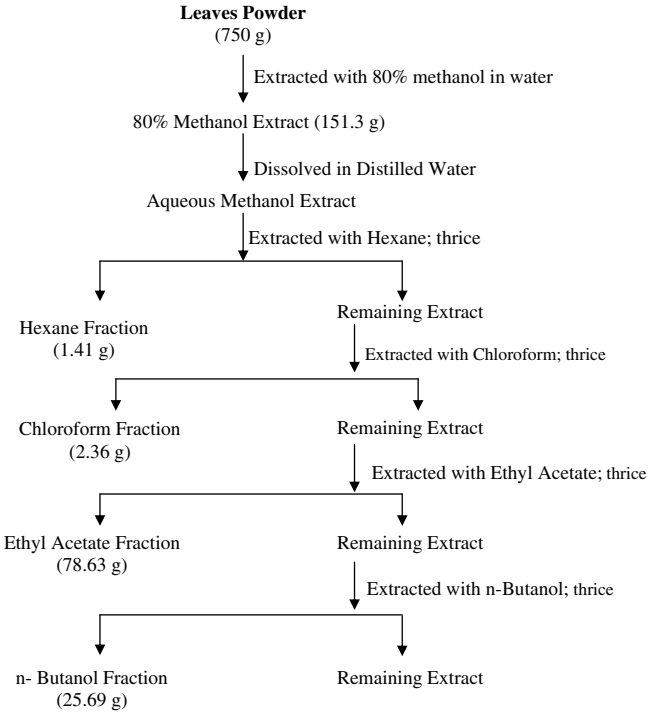
Leaves were collected from the tree growing in the Guru Nanak Dev University campus, Amritsar. Botanical identification was made from Herbarium of Department of Botanical and Environmental Sciences, GNDU, Amritsar and voucher specimen was submitted in the herbarium.

2.2. Plant extract

The leaves of plant were thoroughly washed with tap water, dried at room temperature and ground to fine powder. The powdered leaves were extracted with 80% methanol by employing maceration method. The 80% methanol extract was fractionated using different solvents viz. hexane, chloroform, ethyl acetate, *n*-butanol and water. The supernatant was filtered using Whatman No. 1 sheet, pooled and concentrated using vacuum rotary evaporator. The concentrated solutions were then lyophilized to get the dry form of respective fractions (Flow Chart 1).

2.3. Chemicals

2,2′-Diphenyl-1-picryl hydrazyl (DPPH), 2-thiobarbituric acid (TBA), ethidium bromide were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Ellagic acid and 2-deoxyribose were obtained from Lancaster Synthesis Inc. USA. Supercoiled plasmid pBR 322 DNA and agarose was obtained from Genei, Bangalore. Bromophenol blue, EDTA, L-ascorbic acid, Tris (hydroxymethyl) aminomethane, Folin–Ciocalteu reagent, trichloroacetic acid (TCA) and gallic acid were of analytical grade.



Flow Chart 1. Extraction procedure of leaves of *Chukrasia tabularis*.

2.4. Determination of total phenol content

Folin–Ciocalteu procedure given by Yu et al. (2002) was used to estimate the total phenol content in the different fractions of *C. tabularis*. Following this method, 0.1 ml aliquots of fractions were diluted to 1 ml with distilled water. To this solution 0.5 ml of Folin–Ciocalteu reagent (1:1) and 1.5 ml of 20% sodium carbonate solution was added. The mixture was incubated for 2 h at room temperature. The volume was raised to 10 ml with distilled water and the absorbance of blue coloured mixture was measured at 765 nm (Systronics 2202 UV–Vis Spectrophotometer). The amount of total phenol was calculated as mg/g (Gallic Acid Equivalents) from calibration curve of gallic acid standard solution. For the gallic acid, the curve absorbance versus concentration is described by the equation $y = 0.0012x - 0.0084$ ($R^2 = 0.9908$). Here, y = absorbance and x = concentration.

2.5. Free radical scavenging tests

2.5.1. DPPH radical scavenging activity

DPPH, a stable nitrogen centered radical was used to assess the hydrogen donating ability of different fractions of *C. tabularis* as it offers a convenient and accurate method because of the relatively short time required for analysis. For assessing the DPPH radical scavenging activity, method described by Blois (1958) was used with slight modifications. In this assay, 0.2 ml of extract solution was added to 3 ml of 0.1 mM methanolic DPPH solution in a cuvette and absorbance was read at 517 nm. The decrease in absorption at ambient temperature was recorded for 10 min and correlated with the scavenging action of the test compound. The radical scavenging activity was expressed as the inhibition percentage and monitored as per the equation: % DPPH radical scavenging $= (1 - A_S/A_C) \times 100$; A_C = absorbance of control and A_S = absorbance of sample solution.

The DPPH solution without sample solution was used as control. IC_{50} value is the concentration of sample (μg/ml) required to scavenge 50% DPPH free radical and was calculated from inhibition curve. Gallic acid being a phenolic compound was used as positive control.

2.5.2. Reducing power assay

The reducing power of different fractions was determined by the method of Oyaizu (1986). One milliliter of extract of different concentrations was mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 1%