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Short Communication

Screening of the Ethanolic Extract of Rosa Chinensis Jacq Leaves for Free Radical Scavenging Activity

Ranjit Singh and C. Sankar
KMCH College of Pharmacy, Department of Pharmaceutics, Kovai Estate, Kalapatti road, Coimbatore, Tamil Nadu, INDIA

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Abstract

The free radical scavenging activity of the ethanolic extract from fresh leaves of Rosa chinensis Jacq was assessed with the aid of the stable DPPH radical. This species had strong free radical scavenging capacities, with IC50 values lower than 0.6 mg leaves per milliliter. The activity of this species may be directly linked to the content in tannins and flavonoids and consequently to their free radical scavenging activities.

Keywords: Free radical scavenging activity, tannins, flavonoids, *Rosa chinensis Jacq*, DPPH radical.

Introduction

The DPPH free radical scavenging assay is a simple and widely used screen for bioactive compound discovery. In conjunction with a microtitre plate reader, the assay can be easily carried out as a high throughput test[1]. The leaves, flowers and roots of this plant have many uses such as stagnation menstruation, distending pain in the chest and abdomen, amenorrhea and swelling pain of scrofula with pus prior to rupture.

Material and Methods

Materials: 1, 1-Diphenyl-2-picryhydrazyl (DPPH) was bought from Aldrich (Shanghai). Analytical grade methanol was from Shree Hari Biotech Chemicals. UV-VISIBLE spectrophotometer 2202 (Schimadzu, Japan and Systronics) were used for finding absorbance.

Collection of plant material: The fresh leaves of *Rosa chinensis Jacq* were collected collected from South Garo Hills district of Meghalaya, India. The leaves were authentificated and checked for purity. The leaves were used for extraction and analysis for free radical scavenging activity.

Extraction Procedure: The fresh leaves of *Rosa chinensis Jacq* were crushed and extracted for 48 hrs at room temperature with ethanol. For every milligram of fresh leaves, 10 ml were added. Extraction was carried out under shaking with 80 rev/min. The suspensions were centrifuged, and the supernatant diluted at different concentrations, which were used as test samples[2].

Quantitative analysis: Free radical scavenging activity on α, α -diphenyl-β-picrylhydrazyl (DPPH$^{\bullet}$): The antioxidant activity of the ethanolic extract of the leaves of *Rosa chinensis Jacq* and BHA was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH$^{\bullet}$ method. A methanol solution (0.1 ml) of the sample extracts at various concentrations was added to 3.9 ml (0.025 $g1^{-}$) of DPPH$^{\bullet}$ solution. The decrease in absorbance at 517 nm was determined continuously at every minute with a Hitachi UV–Vis model U-2000 spectrophotometer until the reaction reached a plateau. The remaining concentration of DPPH$^{\bullet}$ in the reaction medium was calculated from a calibration curve obtained with DPPH$^{\bullet}$ at 517 nm. The percentage of remaining DPPH$^{\bullet}$ ($DPPH_R$) was calculated as follows:

$$\% \ DPPH_R^{\bullet} = [(DPPH^{\bullet})_T / (DPPH^{\bullet})_{T=0}] \ X100$$

Where $DPPH^{\bullet}_T$ was the concentration of DPPH$^{\bullet}$ at the time of steady-state and $DPPHT^{\bullet}_{T=0}$ was the concentration of DPPH. At time zero (initial concentration). Based on the parameter IC50, the result was expressed in terms of mg dry matter of sample/standard equivalent g^{-1} DPPH$^{\bullet}$ in the reaction medium.

Results and Discussion

Radical-scavenging activities on α, α -diphenyl-b-picrylhydrazyl (DPPH$^{\bullet}$): The free radical-scavenging activities of ethanolic extract of unprocessed and processed *Rosa chinensis Jacq* leaves along with reference standards, such as BHA, were determined by the DPPH$^{\bullet}$, and the results are shown in table 1. The decrease in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radial by hydrogen donation. It is visually noticeable as a colour change from purple to yellow. A lower value of IC50 indicates a higher antioxidant activity. Extracts obtained from *Rosa chinensis Jacq* leaves registered the highest DPPH radical scavenging activity are directly proportional to the concentration of total phenolic including tannins of respective samples[3,4]. In the present study, the order of scavenging activity of the leaves extracts was as follows: LBD > LBA > DB > LB > BHA. Such anti-radical scavenging activity of untreated and treated leaves extracts

would be related to substitution of hydroxyl groups in the aromatic rings of phenolic, particularly the presence of high concentration of protocatechuic acid, thus contributing to their hydrogen donating ability[5, 6].

Table-1
DPPH• Radical Scavenging Activity

Sample	DPPH(DM g⁻ DPPH•)	
	Mean	Deviation
LB	836	14
DB	749	13
LBD	577	10
LBA	678	11
BHA	967	16

Values are mean of three independent determinations, SD-standard deviation, LB- light brown, DB-dark brown, LBD-light brown, dry heated, LBA-light brown, Soaking followed by autoclaving.

On the other hand, the DPPH radical scavenging efficiency of extracts from both dry heated and hydrothermally processed seed samples might have also been partly contributed by the Maillard reaction products, other than the phenolic constituents, because they are also effectively participating as radical scavengers. However, when compared to standards BHA the tested extract showed significantly radical scavenging activity.

Conclusion

Ethanolic extract of *Rosa chinensis Jacq* leaves were screened for DPPH radical scavenging activity according to the method described above. The extract had shown strong free radical scavenging capacities, with IC50 values lower than 0.6 mg leaves per milliliter of ethanol. The active constituent present in the leaves responsible for free radical scavenger activity is rosamultin**.** This species contain tannins and other polyphenols such as flavonoids. Although the secondary metabolites of this species screened are only known in part or have not even been studied up to now, it is likely that tannins are, at least in part, responsible for the strong free radical scavenging activities [7]. When considering the traditional uses of this plant it has been used for several purposes such as stagnation menstruation, distending pain in the chest and abdomen, amenorrhea and swelling pain of scrofula with pus prior to rupture, antimicrobial or for treatment of dysentery. This uses may also relate to the presence of tannins. It is well known that tannins have anti-inflammatory, antidiarrheic and wound healing properties. Phenols such as methyl gallate possess antimicrobial properties, and physiological redox processes in the microbial cells may be disrupted by the strong reducing activity of tannins[8]. Furthermore, some tannin has been shown to act via inhibition of specific enzymatic functions. Elaeocarpusin, for example, inhibits AIDS virus replication, and casuarinin inhibits FAD oxidase and topoisomerase[9]. The anti-inflammatory properties of tannins are mainly due to their free radical scavenging activities, as free radical scavenders can stop some of the processes of inflammatory response[10]. Flavonoids are also widely encountered in the plants tested as the most active radical scavengers. Again, this is not surprising given that most flavonoids have strong free radical scavenging properties[10,11]. The multiple pharmacological properties of flavonoids, such as anti-inflammatory, antibiotic and cardiovascular activities[8] are, to a large extent, linked to their polyphenolic and hence radical scavenging nature[10]. Besides that, certain flavonoids such as hikino flavone possess specific properties as antiviral or antitumoral compounds[9]. There is no doubt that tannins and flavonoids play important roles in the scavenging activity and medical uses.

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