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Research Article

Role of Phenolic and Flavonoid Compounds from Selected Plants in Inhibiting Pro-Oxidants Elicited Strand Breaks in Plasmid pBR322 DNA

Kumar A*, Arora S

Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar-143005, Punjab, India

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ABSTRACT

Free radical scavenging properties of methanolic extract from *Pongamia glabra* and *Pterospermum acerifolium* leaves were assessed dy DPPH and pBR322 plasmid DNA nicking assay and were correlated with their phenolic and flavonoid content. The extracts from *P. acerifolium* exhibited good DPPH radical scavenging potential (59.02±0.30 %) with IC_{50} value of 163.50 then *P. glabra* which showed poor activity (8.28±0.10 %) with IC_{50} values of 1243.45 at highest test concentration of 200µg/ml. The high antioxidant potential of *P. acerifolium* extract could be attributed to its High phenolic (mg GAE/g dry wt) and flavonoid (mg RE/g dry wt) content 296.66±1.76 and 430.23±1.18 respectively.

Keywords: Antioxidant potential; DPPH; pBR322 DNA damage; Medicinal plants.

INTRODUCTION

The body has several mechanisms to counteract oxidative stress by producing antioxidants, either naturally generated *in situ* (endogenous antioxidants) or externally supplied through foods (exogenous antioxidants). Aerobic organisms have developed both enzymatic and non-enzymatic defense system. The function of antioxidants is to counterbalance the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention[1]. Medicinal plants have been employed in the treatment of diabetes, cancer, hypertension, arthritis and in various other health related problems since time immemorial[2]. There have been surveyed about 2,500,000 species of higher plants, however the majority of them is not studied for their pharmacological activities[3]. Polyphenolic compounds in plants play the role as chemopreventive and antifungal, antibacterial agents[4,5]. The role of plants as DNA damage protective activity and anti-quorum sensing potential has been studied by many researchers[6,7].

Pongamia glabra (Karanj) is a medium sized tree with a large canopy from family Fabaceae and have been widely used as folk medicine. In Ayurvedic system different parts of plants have been used in the treatment of various diseases leprosy, piles, chronic fever, liver pain[7] and extract form plants also possess antioxidant, anti-inflammatory, antiplasmodial and antihyperammonic activities[8-10]. Similarly flowers and bark of *Pterospermum acerifolium* a medium sized tree included in the family Sterculiaceae is of great medicinal importance and various studies have reported their role as antimicrobial, anticancer, antiulcer, anti-inflammatory agents and also in the treatment of smallpox[11-13].

The present study was aimed to evaluate the protective effect of phenolic and flavonoid content in the methanolic extract of leaves of *P. glabra* and *P. acerifolium* against Fenton' s reagent induced oxidative damage.

MATERIALS AND METHODS

Collection of Plant material and Preparation of Extract

Leaves of the *P. glabra* and *P. acerifolium* were collected from the trees growing in Botanical Garden Guru Nanak Dev University, Amritsar and were identified at the herbarium, department of Botanical and Environmental sciences (Fig. 1). The leaves were washed thoroughly with water and were air dried at room temperature and were then ground into fine powder. Fine leaf powder was extracted by soaking in 80% methanol for 24 hrs at room temperature. After filtration through Whatman No.1 filter paper the residue was processed similarly for two more times. The filtrate was dried each time with a rotary evaporator and stored at 4˚C for further usage.

Determination of total flavonoid contents

The radical scavenging activity of flavonoids has been reported to be dependent upon the number and position of hydroxyl groups on their ringed structure. The method given by Kim *et al.*[14] was used for analyzing total flavonoid content (TFC) employing rutin as a standard. Briefly to 1 ml extract (each of 100µg/ml concentration) 4ml of ddH_2O was added, and then 300µl of $NaNO_3$ and 300µl of $AlCl_3$ were added. The mixture was then incubated at room temperature for 5 minutes. After incubation, 2 ml of sodium hydroxide (1M) was added. Then the final volume of the solution was raised to 10ml by further addition of distilled water. The absorbance of samples and blank were taken at 510 nm by UV-VIS spectrophotometer. The total

*Author for Correspondence

Fig. 1: Plant specimens a) *Pongamia glabra* and b) *Pterospermum acerifolium*

Table 1: Total phenolic and flavonoid content in the leaves of two plants used in the study

Sr. No.	Plant Name	Phenolic content (mg GAE/g dry wt)	Flavonoid content (mg RE/g dry wt)
1.	*Pongamia glabra*	161.0 ± 2.08	Nil
2.	*Pterospermum acerifolium*	296.66± 1.76	430.23± 1.18

The results are expressed as Mean ± SE (n=3)

Table 2: Percent Inhibition and IC_{50} values in the leaves of two plants used in the study

Sr. No.	Plant Name	Highest % Inhibition (200µg/ml)	IC_{50} value
1.	*Pongamia glabra*	8.28±0.10	1223.45
2.	*Pterospermum acerifolium*	59.02±0.30	163.50

The results are expressed as Mean ± SE (n=3)

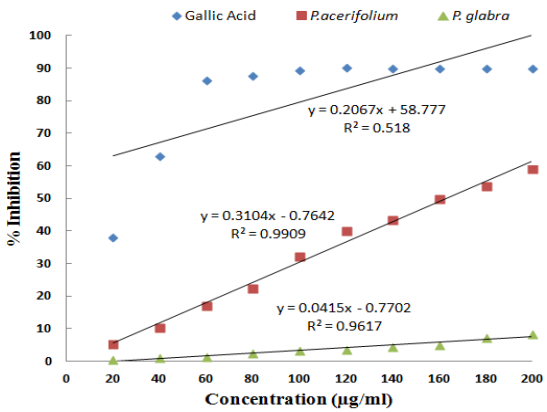

Fig. 2: Antioxidant activity in terms of percent inhibition of P. acerifolium and *P. glabra* methanolic extracts in comparison to the natural antioxidant (gallic acid).

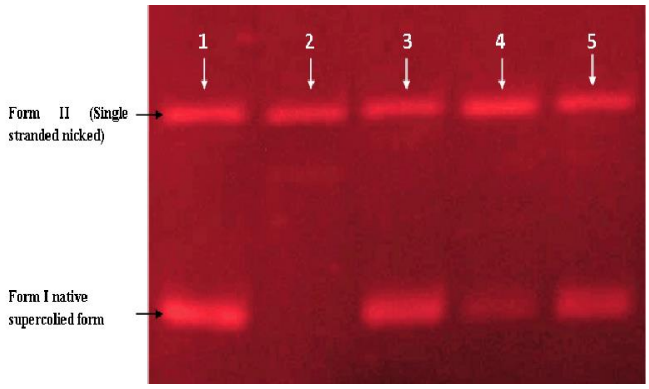

Fig. 3: The inhibitory effects of two plant extracts on DNA nicking caused by hydroxyl radical. Here, Lane 1: pBR322 DNA + H_2O; Lane 2: pBR322 DNA + Fenton reagent + H_2O; Lane 3: pBR322 DNA + Fenton's reagent + Ellagic acid; Lane 4: pBR322 DNA + Fenton reagent + *Pongamia glabra* extract; Lane 5: pBR322 DNA + Fenton reagent + *Pterospermum acerifolium* extract.

flavonoid content was quantified as rutin equivalents (RE) in mg/g of dry sample.

Determination of Total Phenolic content

The concentration of total phenols in plant extract was assessed by the method given by Yu *et al.*[15]. In this procedure to the 100µl of extract fraction (100µg/ml) add 900µl of double distilled water to make final volume 1000 µl. To this solution 1.5 ml of 20% sodium carbonate solution was added. Then 0.5 ml of 1:1 Folin- Ciocalteu reagent was used. The volume was raised to 5 ml. The mixture was then incubated for 2h at room temperature. The absorbance of the mixture was measured at 765 nm using UV-VIS spectrophotometer. Total phenolic content of samples were expressed as gallic acid equivalents (GAE) which reflected the phenolic content as the amount of gallic acid (mg) in 1 g of dry material.

DPPH free radical scavenging assay

The hydrogen atom donating ability of the different plant extracts was determined from the decolorization of a purple coloured methanol solution of DPPH. It is stable nitrogen centred radical. The odd electrons in the DPPH free radical give a strong absorption maximum at 517nm. The antioxidant activity was determined according to the method reported by Blois[16] with minor modifications. In this assay 200µl of extract solution (concentrations ranging from 20-200µg/ml) was used and to this solution freshly prepared 3ml of 0.1mM DPPH in methanol solution was added. The reaction mixture was then placed in the cuvette holder of spectrophotometer, against blank, which did not contain the plant extract. All determinations were performed in triplicate. The absorbance of the reaction mixture at 517 nm was taken. The decrease in absorption was correlated with the scavenging action of the test compound. Gallic acid being a phenolic compound was used as a positive control. The radical scavenging activities were expressed as percentage of inhibition and calculated according to the following equation.

Percentage of DPPH inhibition= [(Ac-As) /Ac] ×100; where Ac = absorbance of control and As= absorbance of the sample. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and expressed as IC_{50} value.

Plasmid nicking assay

The capacity of plant extracts to preserve supercioled pBR322 DNA from detrimental effects of hydroxyl radicals generated by Fenton reagent was assessed by the DNA nicking assay described by Lee *et al*.[17] with slight alterations. The reaction mixture was initiated by 0.5 µg of plasmid DNA (pBR 322) in a micro centrifuge tube with 10 µl of Fenton reagent (30mM H_2O_2 + 50 mM ascorbic acid and 80 mM $FeCl_3$). To this mixture plant extract solution (200µg/ml) was added and final volume of the mixture was brought up to 20µl by using ddH_2O. The mixture was then incubated for 30 min. at 37˚C followed by the addition of 2-5µl of loading dye (0.25% bromophenol blue, 50% glycerol) and centrifugation for 10 seconds. Ellagic acid (100µg/ml) a positive scavenger of hydroxyl radical was used as a control. DNA was analysed using Gel Doc XR system (Bio-Rad, USA) after agarose gel electrophoresis.

Statistical Analysis

Results are depicted as the average and standard error. The data was analysed for statistical significance using one way ANOVA (Analysis of Variance) and the difference among means was compared by high range statistical domain (HSD) using Tukey's test.

RESULTS AND DISCUSSION

Total Phenolic & Flavonoid content

Total Phenolic & Flavonoid contents in methanolic extracts of *P. glabra* and *P. acerifolium* leaves are presented in Table 1. *P. acerifolium* and *P. glabra* exhibited total phenolic content 296.66 ± 2.08 and 161 ± 1.76 mg GAE/g dry weight, respectively at highest test concentration 200µg/ml. In case of flavonoid content *P. glabra* leaf extract depicted nil flavonoid content than *P. acerifolium* which showed good flavonoid amount of 430.23 ± 1.18. Phenolic compounds present in the plants are of much importance because of their role as a radical scavenger and metal chelator[18,19] however antioxidative potential does not depend only on phenolics compounds, but also on other secondary metabolites present in the plants[20].

DPPH free radical scavenging activity

The *in vitro* radical scavenging property and IC_{50} values of methanolic plant extracts is presented in Table 2. The results showed that free radical scavenging property increases with increasing the concentration of extracts (Fig. 2). *Pongamia glabra* extracts exhibited poor % inhibition (8.28 ± 0.10µg/ml) whereas *P. acerifolium* showed good radical scavenging activity (59.02 ± 0.30µg/ml). IC_{50} values are 1223.45 and 163.50 respectively in *P. glabra* and *P. acerifolium* respectively, with standard Gallic acid (24.01µg/ml). The antioxidant activity of methanolic extracts could be because of the hydrogen atom transfer and is measured by colour change from purple to yellow as DPPH free radical is scavenged [21,22].

Plasmid Nicking Assay

Methanolic extracts from *P. glabra* and *P. acerifolium* were evaluated for DNA damage protective activity against the Fenton's reaction generated hydroxyl radicals in plasmid DNA pBR322 and results are presented in Fig. 3. Extracts from *P. acerifolium* depicted good scavenging and helped in maintaining native supercoiled form (lane 5), on the other hand *P. glabra* extracts showed poor DNA protection activity (lane 4) which could be ascribed to its inability to scavenge hydroxyl radicals. The inhibition of pBR 322DNA damage against hydroxyl radicals by *P. acerifolium* extract is in accordance with its ability to scavenge DPPH radicals, which could be attributed to its high phenolic and flavonoids contents as these compounds have potential natural antioxidant activity in terms of their ability to act as a radical scavenger and metal chelator [23-25].

CONCLUSION

Findings from current study suggested that extracts from *P. acerifolium* leaves possessed high phenolic and flavonoid content than *P. glabra* and thus good antioxidant and DNA damage protective potential against reactive oxygen species. However the extracts should be further assessed for their nutritional and therapeutic use worldwide by conducting *in vitro* trials.

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REFERENCES

1. Vinha AF, Barreiral, SVP, Castro A, Machado M.Comparison Between the Phytochemical and Antioxidant Properties of Plants Used in Plant

Infusions for Medicinal Purposes. Journal of Agricultural Science 2013; 5 (11) : 11-19.
2. Salem MZM, Ali HM, El-Shanhorey NA, Abdel-Megeed A. Evaluation of extracts and essential oil from *Callistemon viminalis* leaves: Antibacterial and antioxidant activities, total phenolic and flavonoid contents. Asian Pacific Journal of Tropical Medicine 2013; 785: 791-785.
3. Ram AJ, Bhakshu LM, Raju RRV. *In vitro* antimicrobial activity of certain medicinal plants from Eastern Ghats, India, used for skin diseases. Journal of Ethnopharmacology 2004; 90 : 353–357.
4. Kavitha KS, Satish S. Antibacterial activity of *Callistemon lanceolatus* DC. Against human and phytopathogenic bacteria. Journal of Pharmacy Research 2013; 7: 235-240.
5. Jaberian H, Piri K, Nazari J. Phytochemical composition and *in vitro* antimicrobial and antioxidant activities of some medicinal plants. Food Chemistry *2013*; 136: 237–244.
6. Sinha S, Biswas D, Mukherjee A. Antigenotoxic and antioxidant activities of *Palmarosa* and *Citronella* essential oils. Journal of Ethnopharmacology 2011; 137: 1521– 1527.
7. Hazra B, Sarkar R, Biswas S, Mandal N. Antioxidant and iron chelating potential of *Pongammia pinnata* and its role in preventing free radical induced oxidative damage in plasmid DNA. International Journal of Phytomedicine 2011; 3:240-253.
8. Saiprasanna B, Babu SM, Ramani YR. Phytochemical Investigation and Study on Antioxidant Properties of *Pongamia pinnata* Hydro-Alcoholic Leaf Extract. Plant Sciences Feed 2012; 2 (5): 74-78.
9. Porwal P, Shukla K, Mishra DK, Mahajan SC, Tiwari A.Comparative *In vitro* Antioxidant Activity of *Pongamia pinnata* Linn. Leaves Extracts and Isolated Compound. International Journal of Pharmaceutical & Biological Archives 2010; 1(1): 69 – 75.
10. Anuradha R, Krishnamoorthy P. Antioxidant activity of methanolic extract of *Pongamia pinnata* on lead acetate induced hepatic damage in rats. African Journal of Biochemistry Research 2011; 5(12): 348-351.
11. Sannigrahi S, Parida S, Patro VJ, Mishra US, Pathak A. Antioxidant and Anti-Inflammatory Potential Of *Pterospermum acerifolium*. International Journal of Pharmaceutical Sciences Review and Research 2010; 2 (1): 1-5.
12. Chatterjee P, Chakraborty B, Nandy S, Dwivedi A, Datta R. *Pterospermum acerifolium* Linn. : A comprehensive review with significant pharmacological activities. International Journal Of Pharmacy and Life Sciences 2012; 3 (2): 1453-1458.
13. Malik SK, Khan Z, Ajaib M. (2012). Investigation of *in-vitro* Antioxidant potential of Ethnobotanically important tree, *Pterospermum acerifolium* l. Pakistan Journal of Botany 2012; 44: 105-109.
14. Kim D, Jeong S, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chemistry 2003; 81, 321-326.
15. Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M. Free radical scavenging properties of wheat extracts. Journal of Agricultural and Food Chemistry 2002; 50, 1619-1624.
16. Blois, MS. Antioxidant determinations by the use of a stable free radical. Nature 1958; 181, 1199-1200.
17. Lee KW, Lee HJ, Lee, CY. Antioxidant activity of black tea vs. green tea. Journal of Nutrition 2002; 132: 785.
18. Wojdylo A, Oszmian´ski J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemistry 2007; 105 :940–949.
19. Atanassova M, Georgieva S, Ivancheva K.Total phenolic and total flavonoid contents, Antioxidant capacity and biological contaminants in medicinal herbs. Journal of the University of Chemical Technology and Metallurgy 2011; 46(1):81-88.
20. Awah FM, Uzoegwu PN, Ifeonu P, Oyugi JO, Rutherford J, Yao XJ, Fehrmann F, Fowke KR, Eze MO. Free radical scavenging activity, phenolic contents and cytotoxicity of selected Nigerian medicinal plants. Food Chemistry 2012; 131 :1279–1286.
21. Garg D, Shaikh A, Muley A, Marar T. *In-vitro* antioxidant activity and phytochemical analysis in extracts of *Hibiscus rosa-sinensis* stem and leaves. Free Radicals and Antioxidants 2012; 2 (3): 41-46.
22. Conforti F, Sosa S, Marrelli M, Menichini F, Statti GA, Uzunov D, Tubaro A, Menichini F. The protective ability of Mediterranean dietary plants against the oxidative damage: The role of radical oxygen species in inflammation and the polyphenol, flavonoid and sterol contents. Food Chemistry 2009; 112: 587–594.
23. Verma AR, Vijayakumar M, Mathela CS, Rao CV. *In vitro* and *in vivo* antioxidant properties of different fractions of *Moringa oleifera* leaves. Food and Chemical Toxicology 2009; 47: 2196–2201.
24. Walia H, Kumar S, Arora S. Attenuation of Protective Effect on DNA and Antioxidant Efficacy of Extracts from *Terminalia chebula* Prepared by Sequential Method. Advances in Biological Research 2012; 6 (6): 231-239.
25. Paramaguru R, Mazumder PM, Sasmal D, Kumar D, Mukhopadhyay. Evaluation of antioxidant and DNA nicking potential along with HPTLC fingerprint analysis of different parts of *Pterospermum acerifolium* (L.) Willd. Free Radicals and Antioxidants 2013; 3: 100-106