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Research Article

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Relative evaluation of two euphorbiaceae plants for their hydrogen donating and hydroxyl radical scavenging activities

Arvind Kumar*, Rajbir Kaur, Tarunpreet Singh Thind, Rohit Arora, Pardeep Kaur and Saroj Arora

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

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ABSTRACT

Methanolic extract of leaves of two plants belonging to Euphorbiaceae family, Putranjiva roxburghii and Ricinus communis were screened for total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity and DNA protective ability. P. roxburghii extract bestowed highest phenolic (629 ±3.21) and flavonoid content (37.76 ± 0.42) than R. communis. In DPPH radical scavenging assay, P. roxburghii extract showed good radical scavenging activity (54.14 ± 0.21) at the highest test concentration (200 µg/ml) with IC_{50} value of 190 µg/ml whereas R. comminis exhibited poor (16.18 ±0.39) percent inhibition with IC_{50} value of 562.8190 µg/ml. P. roxburghii extract also protected the pBR322 plasmid DNA against hydroxyl radicals generated by Fenton's reagent and retained the native supercoiled form whereas on the other hand R. communis showed very poor results.

Keywords: Antioxidant activity; DPPH; plasmid nicking assay; Medicinal plants; *Putranjiva roxburghii*; *Ricinus communis*

INTRODUCTION

Reactive oxygen species (ROS) are chemically reactive compounds generated as a byproduct of cellular metabolism, primarily through mitochondria, endogenously and exogenously by ionizing radiations and also through environmental pollutants. In the normal state there is a balance between free radicals generated and antioxidants produced in the body; whenever this balance is disturbed it leads to chronic and degenerative diseases like cancer, diabetes, atherosclerosis and ageing related problems [1–5]. This condition related to the imbalance of proxidants and antioxidants level is referred to as oxidative stress. Aerobic organisms have developed both enzymatic and non-enzymatic defence systems for curbing the oxidative stress. The role of antioxidants is to neutralize the excess of free radicals, to protect the cells against their toxic effects, contributing to disease prevention. The major endogenous antioxidant enzymes directly involved in the neutralization of ROS are superoxide dismutase, catalase and glutathione reductase whereas non-enzymatic antioxidants produced by metabolism in the body are lipoic acid, glutathione, L–arginine, coenzyme Q10, melatonin, uric acid, bilirubin, metal chelating proteins, transferrin etc. [7]. In recent years, there has been a great interest in finding natural antioxidants from plants [8–10] as many Indian plants have been used as flavours, pigments and food [11–13]. Euphorbiaceae is a large family comprising about 300 genera and 5000 species. Most of the members of this family have been recognized and reported for their medicinal properties (anti-cancer and anti-hepatitis B) and the herbal plants from this family have been used for the treatment of health related problems from the very beginning [14–15]. A wide variety of *in vitro* chemical models have been developed to assess the ability of herbal plants and their products to prevent oxidative damage. The most

widely employed chemical test to measure the antioxidant potential of plants is phenolic and flavonoid contents and radical scavenging capacity (determined by DPPH and plasmid nicking method).

EXPERIMENTAL SECTION

2.1. Collection of plant material and preparation of extract

Putranjiva roxburghii and *Ricinus communis* plant leaves were collected from trees growing in the botanical garden of Guru Nanak Dev University, Amritsar. The leaves of plant were washed with tap water twice and then dried in the shade. Dried leaves were finally grounded and three successive extractions with 80% methanol were carried out at room temperature for 24 h. The extracts were filtered using Whatman no. 1 sheet. The filtrates obtained were concentrated under vacuum on a rotary evaporator at 40 ˚C and the concentrated solution was then lyophilized to get the dried form.

2.2. Phytochemical analysis

2.2.1 Determination of total phenolic content

Total phenolic content was determined by the Folin-Ciocalteu method [16]. In this procedure, 900 µl of double distilled water was added to 100 µl of extract fraction (100 µg/ml) for making the final volume 1000 µl. To this solution, 1.5 ml of 20% sodium carbonate solution was added. Following which, 0.5 ml of 1:1 Folin-Ciocalteu reagent was added to the reaction mixture. The volume of solution was raised to 5 ml and incubated for 2 h at room temperature. The absorbance of the mixture was measured at 765 nm using UV-VIS spectrophotometer.

2.2.2 Determination of total flavonoid content

Total flavonoid content was analysed by the method of Kim et al. [17]. In this procedure, 1 ml of extract (each of 100 µg/ml concentration) was added to 4 ml of double distilled water and then 300 µl of $NaNO_3$ and 300 µl of $AlCl_3$ was added. The mixture was then incubated at room temperature for 5 minutes. After incubation 2 ml of sodium hydroxide (1M) was added and the volume of the solution was raised to 10 ml by further addition of distilled water. The absorbance of samples and blank were taken at 510 nm by UV-VIS spectrophotometer. The total flavonoid content was then expressed as rutin equivalents (RE) in mg/g of dry sample.

2.3 Antioxidant assay

2.3.1. DPPH radical scavenging activity

Hydrogen atom donating activity of plant extracts was determined spectrophotometrically by the DPPH radical scavenging potential method given by Blois [18] with slight modifications. In this method, 200 µl of extract (concentrations ranging from 20-200 µg/ml) was used and to this solution freshly prepared 3 ml of 0.1 mM DPPH in methanol solution was added. The absorbance of the reaction mixture was taken at 517 nm. 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 percent 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.

2.3.2. Plasmid nicking assay

The ability of plant extracts to protect the plasmid DNA (pBR322) DNA from destructing effects of Fenton's reagent was assessed by the DNA nicking assay described by Lee *et al*. [19] with slight modifications.The reaction was initiated by mixing 0.5 µg of plasmid DNA (pBR 322) in a micro centrifuge tube with 10 µl of Fenton's reagent (30 mM H_2O_2 + 50 mM ascorbic acid and 80 mM $FeCl_3$). To this mixture, plant extract (200 µg/ml) was added and final volume of the mixture was brought up to 20 µl by using double distilled water. The mixture was then incubated for 30 minutes at 37 ˚C followed by the addition of 2-5 µl of loading buffer (0.25% bromophenol blue, 50% glycerol). Ellagic acid (100 µg/ml), a positive scavenger of hydroxyl radical, was used as a control. DNA was analysed using the Gel Doc XR system (Bio-Rad, USA) after agarose gel electrophoresis using 1% agarose gel in TBE buffer at 50 V (1.5-2 V/cm) for 4 h.

2.4 Statistical Analysis

Experiment was performed in triplicates and the results were expressed as mean ± SE. One way analysis of variance (ANOVA) and Tukey's HSD post hoc test were carried out to determine significant differences between the mean at $p \leq 0.05$.

RESULTS AND DISCUSSION

A number of studies deal with the antioxidant activity and health benefits from herbs, medicinal plants, spices, beans, vegetable sources, trees, berries and cherries [20–25]. Literature survey has revealed that there is a direct relationship between antioxidant activity and total phenolic content [26–28]. For a polyphenol to be defined as an antioxidant it should satisfy two basic conditions: first, when present in low concentrations relative to the substrate to be oxidized it can delay, retard, or prevent the autoxidation of free radical mediated oxidation [29], second, the resulting radicals formed after scavenging must be stable through intramolecular hydrogen bonding on further oxidation [30]. Basically, free radical scavenging and antioxidant ability of phenolics (e.g. flavonoids, phenolic acids) mainly depends upon the number and position of hydroxyl groups on aromatic rings of the phenolic molecules [31–32]. It is believed that thousands of phenolic compounds occur in medicinal herbs. For instance, more than 4000 different kinds of flavonoids and hundreds of coumarins and lignans have been reported as naturally occurring compounds [33–34]. Total phenolic content (gallic acid equivalent) assessed by Folin-Ciocalteu procedure and flavonoid content (rutin equivalent) assessed by aluminium chloride colorimetric procedure in methanolic plant extracts are shown in Table 1. *P. roxburghii* extracts exhibited the highest phenolic and flavonoid contents 629 ± 3.21 mg GAE/g dry weight of extract and 37.76 ± 0.42 mg RE/g dry weight of extract respectively whereas in *R. communis* it was found to be 19.66 ± 0.88 mg GAE/g and 10.23 ± 0.14 mg RE/g dry weight of extract respectively. In the present study high phenolic and flavonoid content in *P. roxburghii* imparts more antioxidative potential than *R. communis* which exhibited a lesser amount of polyphenolic compounds and therefore reduced activity [35–37].

Table 1: Total phenolic and flavonoid content in methanolic plant extracts

Plant	Hindi name	Total Phenolic content (mg GAE/g dry wt)	Total Flavonoid content (mg RE/g dry wt)
Putranjiva roxburghii	Putrajiv	629.0 ± 3.21	37.76 ± 0.42
Ricinus communis	Erandi	19.66 ± 0.88	10.23 ± 0.14

**The results are expressed as mean±SE (n=3) and significance of results was tested at $p \leq 0.05$*

Assessment of antioxidative activity was done by DPPH assay- a widely used methodology for screening antioxidant activities in plant extracts [38]. Plant extract donates hydrogen atom to DPPH (a stable organic radical) and converts it into reduced form with a change in colour indicating the free radical scavenging potential of plant extract. In case of DPPH radical scavenging activity (Table 2) *P. roxburghii* exhibited the highest activity of 54.14% at 200 µg/ml concentration with IC_{50} values of 190 µg/ml while *R. communis* showed 16.18% inhibition at 200 µg/ml with IC_{50} values of 562.8 µg/ml.

Table 2: DPPH radical scavenging activity in methanolic plant extracts

Concentration (µg/ml)	*Putranjiva roxburghii* (% activity)	*Ricinus communis* (% activity)
20	3.88 ± 0.09	0.56 ± 0.07
40	7.12 ± 0.18	1.76 ± 0.06
60	12.16 ± 0.15	3.40 ± 0.10
80	17.86 ± 0.27	6.50 ± 0.43
100	24.53 ± 0.22	6.92 ± 0.07
120	28.59 ± 0.23	9.46 ± 0.06
140	33.78 ± 0.15	10.93 ± 0.10
160	38.99 ± 0.62	13.85 ± 0.48
180	53.90 ± 0.94	15.17 ± 0.12
200	54.14 ± 0.21	16.18 ± 0.39
IC_{50} (µg/ml)	190	562.8

**The results are expressed as mean ±SE (n=3) and significance of results was tested at $p \leq 0.05$*

The protective effect of plant extracts against damage induced by hydroxyl radicals on pBR322 plasmid DNA in plasmid nicking assay is presented in Figure 1. Findings from this study showed that extract from *P. roxburghii* is a potent inhibitor of DNA damage and maintain the native supercoiled form of DNA by reducing nicked form of

DNA, whereas *R.communis* showed poor activity against DNA damage. Reduction in plasmid DNA (pBR 322) damage in *P. roxburghii* extract is more than *R. communis* from hydroxyl radicals generated due to Fenton's reagent, which may be due to high phenolic and flavonoid content [39].

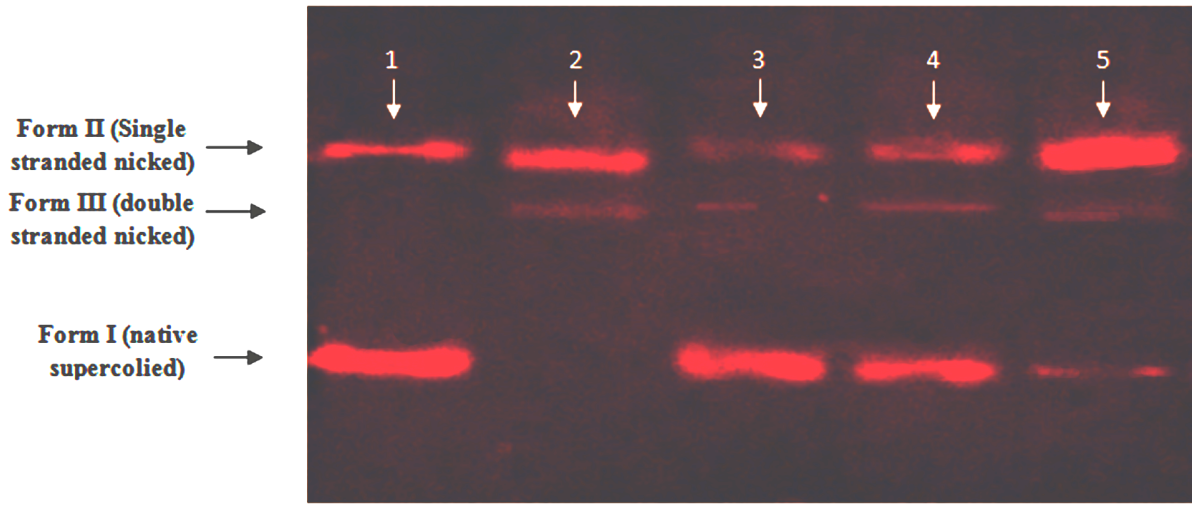

Figure 1: The inhibitory effects of two plant extracts on DNA nicking caused by hydroxyl radical. Here, Lane 1: pBR322 DNA + Distilled water; Lane 2: DNA + Fenton's reagent; Lane 3: DNA + Fenton's reagent + Ellagic acid; Lane 4: DNA + Fenton's reagent + *P. roxburghii* extract; Lane 5: DNA + Fenton's reagent + *R. communis* extract

CONCLUSION

Findings from the current *in vitro* research showed that the methanolic extract from *Putranjiva roxburghii* exhibited strong antioxidant activities and therefore, might assist in scavenging the free radicals generated through oxidative stress. However, it should be kept in mind that free radical scavenging activity measured by *in vitro* methods may not exhibit similar *in vivo* effects of antioxidative activity [40].

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