



Antioxidant activity, total phenolic and flavonoid content of selected medicinal plants from Gorkha district, Nepal

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Abstract

Objective: To determine the antioxidant activity, total phenolic and flavonoid content of the methanolic extracts of *Fritillaria cirrhosa*, *Rosa sericea*, *Neopicrorhiza scrophulariflora* and *Juniperus squamata*.

Methods: Antioxidant ability of the extracts was evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as the source of free radical and butylated hydroxyanisole and vitamin C as reference compounds. Total phenolic content of the sample was estimated by the Folin-Ciocalteu method and total flavonoid content by the aluminium chloride method.

Results: The extracts of *J. squamata* contained highest phenolic content whereas *R. sericea* contained highest amount of flavonoids. *F. cirrhosa* has negligible amount of total phenolic and flavonoids. Antioxidant activity, as determined by DPPH assay revealed that *J. squamata* and *R. sericea* had stronger activity.

Conclusion: *J. squamata* and *R. sericea* possess higher phenolic and flavonoid content and strong antioxidant activity and could be useful as natural antioxidants.

Keywords: DPPH, Phenolic Content, Flavonoid Content, Medicinal Plants

Introduction

Nepal is rich in diversity of medicinal plants and thousands of plants have been used in traditional and folk medicine for the treatment of various diseases. Flowers and the fruits of *Rosa sericea* Lindl. (Family: Rosaceae) are used in headache, jaundice, menstrual disorders and to treat liver, bile, lung, diseases [1, 2]. *Juniperus squamata* Buch Ham Ex D Don (Family: Cupressaceae) is used in wind, blood, kidney disorders; wound healing, fever, paralysis, skin diseases and an insecticide [1, 2]. Rhizomes of *Neopicrorhiza scrophulariiflora* (Penna) Hong (Family: Scrophulariaceae) are used in bile disorders, intestinal pain, fever, high blood pressure, sore throat, eye problems, gastritis, cough and cold [3]. Rhizomes promote secretion of bile, improve appetite, stimulate gastric secretion and are useful in edema, anemia and jaundice [4]. Bulb of *Fritillaria cirrhosa* D. Don (Family: Liliaceae) is used in asthma, bronchitis and prevents bleeding from wounds [2]. Bulbs are sweet, refrigerant, galactagogue, expectorant, aphrodisiac, diuretic, antipyretic [5] and are useful to enhance memory and lessen mental disorders [6].

Among the various groups of plant secondary metabolites, there has been increasing attraction towards phenolics. Phenolic compounds have great potential as natural antioxidants because of their high redox potentials, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [7]. They have ideal structural

chemistry for free radical scavenging activities [8]. Antioxidants can protect, prevent or reduce the extent of oxidative damage of biomolecules [9]. Medicinal plants contain high levels of antioxidants that can delay or inhibit the oxidation of lipids or other important biomolecules [10] and could contribute as remedial or preventive agents against free radical related health problems. Medicinal plants are widely used in food industry and cosmetics as preservatives and are regarded to have beneficial effects in decreasing blood pressure, preventing cardiovascular diseases, or reducing the risk of cancer [10].

Despite huge diversity of medicinal plants and rich tradition of their ethnomedical practice throughout the country, only few studies have been carried out in antioxidant properties of Nepalese medicinal plants and little is known about the antioxidant property of medicinal plants particularly from Manasalu conservation area (MCA). This study aims to examine the antioxidant activity, total phenolic and flavonoid content of selected plants from MCA and correlate of total phenolic and flavonoid content with the antioxidant activity of plant extracts.

Materials and methods

Plant materials

The samples were collected from Chekambar village development committee of MCA, Gorkha district in June 2012. *Rosa sericea* (Voucher No. L-10) was collected from

Rachen-gompa, *Juniperous squamata* (Voucher No. L-24) above Mo-gompa, *Fritillaria cirrhosa* (Voucher No. L-29) and *Neopicrorhiza scrophulariiflora* (Voucher No. L-34) from Cho-Seong. The plants were identified by one of us (LR Bhatt) and confirmed by comparing with the available literature [1, 11, 12].

Extraction

All the plant materials collected from the MCA were dried in room and finally in an oven below 40 °C for 4-8 h. The samples were then grinded with a laboratory blender to make fine powder. 50 gm of powder from each plant were extracted with methanol using soxhlet extractor. The samples were extracted until complete disappearance of the colour. The obtained organic solution was filtered with Whatman filter paper and completely dried by evaporating under reduced pressure at 40 °C and was kept at refrigerator until use.

Antioxidant activity of the sample

Antioxidant activity of the sample was determined using DPPH radical scavenging method [13] with slight modifications. Briefly, different concentrations of sample (1.25-80 µg/mL) prepared by dissolving the dried extract in methanol was mixed with methanolic DPPH solution (Final concentration; 0.15 mM). After 30 min incubation at room temperature, the absorbance was measured at 517 nm against blank. Butylated hydroxyanisole (BHA) and vitamin C were used as positive controls. The percentage DPPH radical scavenging activity was determined using following equation.

$$\text{DPPH radical scavenging (\%)} = \frac{[\text{Control absorbance} - \text{Sample absorbance}]}{[\text{Control absorbance}]} \times 100$$

Determination of total phenolic content

Total phenolic content (TPC) of the sample was estimated by the Folin-Ciocalteu method as described elsewhere [14]. In brief, 200 µL of sample and standard was added to 750 µL of ten time diluted Folin-Ciocalteu reagent. Then, after adding 2 mL of 7.5% sodium carbonate, the final mixture was diluted to 7 mL with distilled water. The samples prepared above were kept in the dark at room temperature with continuous shaking for 2 h. Thereafter, the absorbance of each sample was read at 765 nm against the blank. Gallic acid (0-500 mg/L) was used for calibration of a standard curve. TPC was determined as gallic acid equivalents (GAE) and values were expressed as mg of acid/g of plant material.

Determination of total flavonoid content

Total flavonoid content (TFC) of the sample was determined as described earlier [15]. In brief, 100 µL of sample was added to 96 well plates. Then 100 µL of 2% aluminium chloride solution was added to it and the mixture was incubated at room temperature for 1 h. Then the absorbance of above mixture was measured at 450 nm. Quercetin was used to plot calibration curve. TFC was expressed as mg

quercetin equivalents per gram of the extract.

Statistical analysis

All the experiments were performed in triplicates. Data analysis was carried out using GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego, CA) and Microsoft excel.

Results and discussion

Methanolic extracts of *F. cirrhosa*, *R. sericea*, *N. scrophulariiflora*, *J. squamata* were analyzed for their antioxidant activity, total phenolic and flavonoid contents. Antioxidant ability of the extracts was evaluated using DPPH as the source of free radical and BHA and vitamin C as reference standards. The concentration dependent percentage radical scavenging activity graph was plotted to compare the relative scavenging activity of the plants and standards (Figure 1). Among four plants, *R. sericea*, *N. scrophulariiflora* and *J. squamata* showed dose dependent increase in percentage radical scavenging activity while *F. cirrhosa* was indifferent to percentage radical scavenging activity with the range of concentration from 1.5-20 µg/mL and decreased instead when concentration was further increased upto 80 µg/mL. *R. sericea* and *J. squamata* extracts effectively exceed EC₅₀ with the concentration used below 41 µg/mL while *N. scrophulariiflora* could only scavenge 21% of free radical at highest (80 µg/mL) concentration used in this experiment. When a comparison was made between the percentage radical scavenging activity of *R. sericea* and *J. squamata* with BHA and vitamin C at their EC₅₀ values, they were not significantly different indicating the methanolic extracts of the plants were as effective as the standards in scavenging free radicals.

TPC of the extracts were calculated by using a calibration curve generated taking gallic acid as standard phenolic compound (0-500 µg/mL) and expressed as gallic acid equivalents per gm of plant material. A linear calibration curve of gallic acid, in the range of 0-500 µg/mL with r² value of 0.9934, was constructed. Among four plants, except bulb of *F. cirrhosa* all three plants showed remarkably high TPC (GAE >100 mg/g dry weight) (Figure 2). *J. squamata* showed the highest TPC of 321.5±0.7 mg GAE/g dry weight followed by *R. sericea* (181.6±0.4 mg GAE/g dry weight) and *N. scrophulariiflora* (124.0±0.3 mg GAE/g dry weight).

Quercetin (0-100 µg/mL) was used as standard flavonoid to obtain a calibration curve with r² value of 0.9923. The flavonoids content in the extracts were estimated and expressed as mg Quercetin/g dry weight of the plant material. Among four plants *R. sericea* had the highest TFC of 34.41±0.47 mg quercetin/g dry weight followed by *J. squamata*, *F. cirrhosa* and *N. scrophulariiflora* (Figure 2). Present study reveals positive linear correlation of antioxidant activity with TPC and TFC. It exhibited good correlation with r = 0.8996 and 0.8092 of antioxidant activity with TPC and TFC

respectively. Extracting methods and solvents play important role in determination of antioxidant ability and TPC of plant extracts. Soxhlet extraction employed in present study might

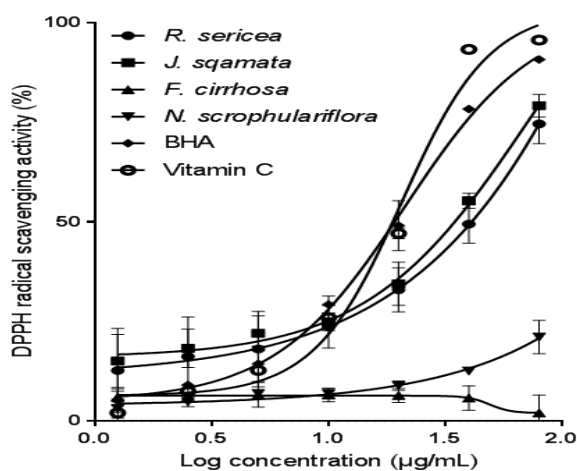


Figure 1: Percentage of DPPH radical-scavenging activity of plant extracts and reference standards at different concentration; Values represent the mean \pm SD of triplicate experiments.

The results revealed that phenolic compounds as the major groups contributing towards the antioxidant activities, particularly in case of *R. sericea*, *J. squamata* and *N. scrophulariflora* extracts. The synergic effects of various phenolics, including phenolic acids and flavonoids could be responsible towards the free radical scavenging activities of plant extracts. Similar to our study *P. scrophulariflora* had strong antioxidant activity and high amount of total phenolics but *F. cirrhosa* showed poor antioxidant capacity and contained negligible amount of phenolics [17]. The leaf extract of Junipers were richer in phenolic and flavonoid content and exhibited strong antioxidant ability in all tested model systems, particularly in DPPH assay [18-20]. Interestingly, *J. foetidissima* from Republic of Macedonia showed stronger activity than those from Turkey, indicating geographical variation could affect the antioxidant ability of same cultivars [18]. However, in spite of low phenolic content, the root bark extract of same plant showed higher antioxidant activity than needle extract indicating that high phenolic content always doesn't show strong antioxidant activity [20]. Our results are in agreement with several previous reports, which showed a strong positive correlation of antioxidant activity with total polyphenols [17-19, 21]. However, in case of crude extracts it is difficult to compare the results obtained by various research groups and laboratories as the differences may arise due to variation in sample collection, processing, extraction method and solvent employed [23, 24]. Several medicinal plants are reported to possess stronger antioxidant

have contributed towards higher phenolic content and strong antioxidant activities of some extracts [16].

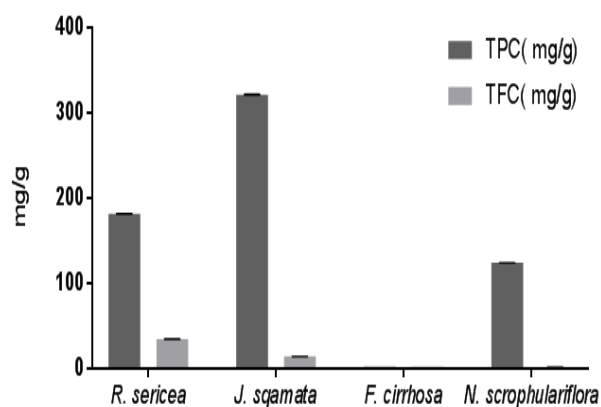


Figure 2: Total phenolic and flavonoid content of plant extracts; values represent the mean \pm SD of triplicate experiments.

activity than common fruits, vegetables and dietary plants and phenolic compounds are responsible for their stronger activity [22]. Their strong antioxidant activities and popular use in traditional medicine make them promising sources of natural antioxidants [17].

Conclusion

The strong antioxidant ability of *J. squamata* and *R. sericea* along with their traditional use in the treatment of various ailments suggest their potential as natural antioxidants.

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Conflict of Interest: None declared

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