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ORIGINAL ARTICLE

Evaluation of antioxidant and antibacterial activity of methanolic extracts of *Gentiana kurroo* royle

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Abstract In this study our objective was to evaluate the antioxidant and antimicrobial activity of methanolic extracts of leaves and roots of *Gentiana kurroo*. The antioxidant activities of the extracts were examined using different biochemical assays namely diphenylpicrylhydrazyl (DPPH), nitro-blue tetrazolium (NBT) and ferric reducing power (FRAP). In all the assays, root extract exhibited stronger antioxidant activity than that of leaves. The antibacterial activity of the extracts was also evaluated and MIC values were calculated by broth dilution method. Although, the extracts prevented the growth of both Gram positive and Gram negative bacteria, the MIC values of methanolic extract of the leaves were higher than those of the root extract. The antibacterial and antioxidant activity of the extracts was found to be positively associated with the total phenolic and flavonoid content of the extracts.

1. Introduction

Plants have to adapt to the changing environmental conditions for their sustenance. The oxidative environment presents a range of free radicals including superoxide, hydroxyl radical, nitric oxide and peroxynitrite, for living organisms to deal with. There are a number of concrete evidences about the role of free radicals in the development of various diseases including Cancer, neurodegeneration and some inflammatory diseases (Halliwell, 2006, 2007; Ferguson, 2010). Antioxidants have therefore gained importance for their capacity to neutralize free radicals. In this context, the antibacterial and antioxidant properties of various medicinal plants are being investigated throughout the world because of the toxicological concerns associated with the synthetic antioxidants and preservatives (Peschel et al., 2006).*Gentiana kurroo* is one of the critically endangered and endemic medicinal plants of the northwestern Himalayas. It belongs to family Gentianaceae, a cosmopolitan family comprising of more than 1600 species (Struwe and Albert, 2002; Daniel and Sabins, 2002). *G. kurroo* is a perennial herb with stem as modified rhizome (Behera and Raina, 2012). The dried roots and rhizomes of the plant contain some important bitter glycosides (gentiopicrin, gentianin)

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and alkaloids (gentiomarin) that have a wide range of pharmaceutical and medicinal utilities. The root stock and other parts of the plant are used as bitter tonic, expectorant, anthelmintic, stomachic and carminative (Kirtikar and Basu, 1935). In folklore medicine, the leaf powder of the plant is mixed with oil and applied on ulcers and fungal infections (Unial and Shiva, 2005). In the Amchi system of medicine the plant is used to treat fever, cough and hepatic ailments (Sharma et al., 2006). Although a number pharmacological activities have been attributed to different parts of *G. kurroo*, only anti-inflammatory and analgesic properties have been scientifically validated (Behera and Raina, 2012). Therefore, the aim of the present study was to determine the total phenolic and flavonoid content and to evaluate the antioxidant and antibacterial activity of methanolic extracts of leaves and roots of *G. kurroo*.

2. Materials and methods

2.1. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, trichloroacetic acid, gallic acid (GA), rutin (RU), nitroblue tetrazolium (NBT), and Folin–Ciocalteu's reagent, were purchased from Sigma–Aldrich (St. Louis, MO, USA). Aluminium chloride, Sodium carbonate, Mueller Hinton media were purchased from Himedia (Mumbai, India). All other chemical reagents used were of analytical grade.

2.2. Collection of plant material

The fresh leaves and roots of *G. kurroo* were collected from lower reaches of the Pir-Panjal range of Kashmir Himalaya. The taxonomic identification of plant was confirmed by Akhtar H. Malik at the Centre of Plant Taxonomy and Biodiversity, University of Kashmir (India).

2.3. Preparation of extracts

Leaves and roots of the plant were collected and dried under shade at room temperature. The plant material was then chopped and ground to fine powder using a mechanical blender. Dried root powder was packed in a Soxhlet apparatus and extracted with methanol at 60–65 °C for 3–4 h. The extract was filtered through a Whatman filter paper No. 1 and the filtrate collected was concentrated under reduced pressure at 40 °C. The extract was dried and stored at 4 °C in storage vials for experimental use.

2.4. Determination of total phenolic content

Total phenolic content of methanolic extracts root and leaf of *G. kurroo* was measured using the Folin–Ciocalteu reagent method as described earlier (Kaur and Kapoor, 2002). Briefly, from the stock solution of (1 mg/ml methanol) 200 µl of both of the crude extracts were made up to 3 ml with distilled water then mixed thoroughly with 0.5 ml of Folin–Ciocalteu reagent for 3 min, followed by the addition of 2 ml of 20% (w/v) sodium carbonate. The mixture was allowed to stand for a further 60 min in the dark and absorbance of the reaction mixtures was measured at 650 nm. Quantification was done on the basis of the standard curve of Gallic acid concentration range from 50 to 500 mg/ml (r^2 = 0.998). Total phenolic content calculated from the calibration curve was expressed as mg of gallic acid equivalent (GAE)/g″dry weight.

2.5. Determination of total flavonoid content

Total flavonoid content of both crude extracts was determined using the aluminium chloride colorimetric method as described earlier (Chang et al., 2002). In brief, from the stock solution of 1 mg/ml crude extracts, 50 µl of each extract was made up to 1 ml with methanol, mixed with 4 ml of distilled water and subsequently with 0.3 ml of 5% $NaNO_2$ solution. 0.3 ml of 10% $AlCl_3$ solution was added after 5 min of incubation and then allowed to stand for 6 min. This was followed by the addition of 2 ml of 1 M NaOH solution to the mixture and final volume of the mixture was brought to 10 ml by the addition of double distilled water. The mixture was allowed to stand for 15 min and absorbance was measured at 510 nm. Quantification was done on the basis of the standard curve of rutin concentration ranging from 50 to 500 mg/ml (r^2 = 0.999). Total flavonoid content calculated from a calibration curve was expressed as mg of rutin equivalent (RU)/g of dry weight.

2.6. DPPH assay

The antioxidant activity was determined by DPPH assay as described earlier with some modifications (Villano et al., 2007). From the stock solution different concentrations of extract (100 µg–600 µg/ml) were prepared. 200 µl of each concentration was mixed with 3.8 ml DPPH solution and incubated in the dark at room temperature for 60 min. Absorbance of the mixture was then measured at 517 nm control and Vitamin E was used as a positive. Scavenging ability of the sample to DPPH radical was determined according to the following equation:

$$\text{DPPH scavenging effect}(\%) = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

2.7. NBT assay

Superoxide anion scavenging activity was performed as described earlier (Vyas and Kumar, 2005). From the stock solution (1 mg/ml) different concentrations of extract (100 µg–500 µg/ml) were prepared. The reaction was performed in 50 mM phosphate buffer (pH 7.8) containing extracts of various concentrations (100–600 µg/ml), 1.5 mM riboflavin, 50 mM NBT, 10 mM DL-methionine, and 0.025% v/v Triton X-100. The reaction was initiated by illuminating the reaction mixture and absorbance of formazan was recorded at 560 nm and percentage scavenging activity was described as inverse of the produced formazan.

2.8. Ferric reducing power assay

Ferric reducing/antioxidant power (FRAP) was determined following the method as described earlier (Zhao et al., 2008). Briefly, 100 µl of each concentration of the extracts (100–500 µg/ml) was mixed with 2.5 ml of 200 mM phosphate