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ANTIOXIDANT ACTIVITY AND PHENOL CONTENT OF 50% ETHANOL AND ETHYL ACETATE EXTRACT OF *Polygonum nepalense* MEISSN

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Abstract: The current study was designed to evaluate antioxidant activity of the 50% ethanol extract and ethyl acetate of *Polygonum nepalense* Meissn. Nitric oxide generated from sodium Nitroprusside in aqueous solution at physiological pH, interact with oxygen to produce nitrite ions which were measured by using a modified Griess-Ilosvany method. The antioxidant activities of aqueous extract, 95% ethanol extract and ethyl acetate fraction was assessed on the basis of the radical scavenging effect of the stable DPPH free radical. 100 mg of the 50% ethanol extract and ethyl acetate fraction was taken and was dissolved separately in 10 volumes of ethanol, the resultant solutions were centrifuged at 10000 rpm for 20 min. The ethyl acetate fraction was prepared in order to enrich phenolic content in the fraction. In nitric oxide radical inhibition method ethyl acetate and 50% ethanol extract showed moderate antioxidant activity with IC₅₀ value 185.05±2.59 and 225.00±3.15 µg/mL respectively. 50% ethanol extract and ethyl acetate fraction exhibited good DPPH radical scavenging activity with IC₅₀ values ranging from 64.13±3.37 to 43.87±2.56µg/mL. Thus this study gives support for expanding future investigations of pharmacological activities associated with free radicals.

Keywords: Antioxidant; Centrifuged; Griess-Ilosvany; Radicals; Scavenging

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INTRODUCTION

Polygonum nepalense Meissn belonging to family Polygonaceae locally known as Sat balon is distributed throughout the range of East Asia-China, Japan, Korea, Indian Himalayas. The plant is an annual herb of about 2 m. long. The leaves are stiff, ovate acute, base truncate or round, sometimes cordate, glabrous, nerves conspicuous, 12.5 cm. long, 7.5 cm. wide; petioles 1 cm. long with round auricles at base; sheath 1.3 cm. long. Inflorescence of peduncles cymes 2.5-7.5 cm long with leafy bracts (Wealth of India., 1969). Regarding the phytochemical profile, plant contains new heptamethoxy-1, 3-diketo compound along with 3 known flavonoid glycosides, identified as quercetin-3-O-rhamnobioid, hyperoside, and luteolin-6-C-glucoside and β-sitosterol glycoside (Rathore *et al.*, 1986; 1987). Traditionally the leaves of *Polygonum nepalense* Meissn are employed as

a local application for swelling (Wealth of India., 1969). The reported medicinal uses of *Polygonum nepalense* species by indigenous people in different parts of the world show considerable similarities. Preparations of the plant were employed in colds, influenza, swelling, hemorrhoids, diarrhoea and rheumatism. The plant is also used as an abortive and diuretic and the entire plant have been reported to possess antihistaminic, anti-inflammatory, antiviral, hypotensive, antipyretic and fungitoxic activities (Maria *et al.*, 2000; Oliveiran *et al.*, 1989; Alves TMA *et al.*, 2000). An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. The current study was designed to evaluate antioxidant activity of the 50% ethanol extract and ethyl acetate.

EXPERIMENTAL

Plant Material: Aerial parts of *Polygonum nepalense* Meissn were collected from Doddabetta in Nilgiris in the month of July 2009. The plant material was identified, confirmed and authenticated by Dr. H.B. Singh, Scientist F, Head of Raw material Herbarium and Museum (RHMD), National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India (Ref. No. NISCAIR/RHMD/Consult/-2009-10/1358/160).

Preparation of Extract: The collected aerial part of plant was air dried and powdered. The powdered plant material was extracted with Petroleum ether (60°- 80 °C), chloroform, ethyl acetate and ethanol (99%) of C.R. grade were used for extraction of the dried powdered plant material. Solvents from extracts were recovered by vacuum distillation using Rotary vacuum evaporator and the dried extracts were preserved in vacuum desiccator containing anhydrous silica Gel blue. The 50% ethanol and ethyl acetate extracts was used for the investigation for antioxidant activity.

Antioxidant Studies

Sample solution for Nitric oxide radical inhibition activity: 21 mg of each of the 50% ethanol extract and ethyl acetate fraction was dissolved in 1 mL of distilled Dimethyl sulfoxide (DMSO) to obtain a solution of 21 mg/mL. Each of these solutions was serially diluted to obtain, concentrations of 1000 µg/mL to 0.015625 µg/mL (Dominic Sophia, Chinthamony (2012) and Ebrahimzadeh, Pourmorad (2008b). 10mg of ascorbic acid and rutin and dissolved in 1 mL of dimethyl sulfoxide separately. From this solution serial dilutions were made to obtain various concentrations using DMSO. Nitric oxide generated from sodium pentacyanonitrosylferrate in aqueous solution at physiological pH, interact with oxygen to produce nitrite ions which were measured by using a modified Griess-Ilosvany method. The reaction mixture (6 mL) containing sodium nitroprusside (10 mm, 4 mL), Phosphate buffer saline (PBS, 1 mL) and extract in DMSO were incubated at 25°C for 150 minutes. After incubation, 0.5 mL of the reaction mixture containing nitrate was removed, 1 mL of

sulphanilic acid reagent (0.33% in 20% glacial acetic acid) was added, mixed well and allowed to stand for 5 minutes for completion of diazotization, then 1 mL of Naphthyl ethylene di-aminedihydrochloride was added, mixed and allowed to stand for 30 minutes in diffused light. A pink coloured chromophore was formed. IC₅₀ value is the concentration of the sample required to inhibit 50% nitric oxide radical (Ebrahimzadeh *et al.*, 2009; Devasagayam *et al.*, 2004; Choudhary *et al.*, 2011; Manian *et al.*, 2008).

Preparation of test solutions for DPPH

Method: 21 mg of each of 50% ethanol extract and ethyl acetate fraction was dissolved in distilled Dimethyl sulfoxide (DMSO) to obtain a solution of 21 mg/mL concentration. Each of these solutions was serially diluted separately to obtain concentration of 1,000 µg/mL to 0.015625 µg/mL.

Preparation of standard solution: 10 mg of each of Ascorbic acid and Rutin were weighed separately and dissolved in 0.95 mL of DMSO to get 10.5 mg/mL concentrations. This solution was serially diluted with Dimethyl sulfoxide to get lower concentrations. The antioxidant activities of aqueous extract, 95% ethanol extract and ethyl acetate fraction was assessed on the basis of the radical scavenging effect of the stable DPPH free radical. The assay was carried out in a 96 well micro-well plate. To 200 µl of DPPH solution, 10 µL of each of the test sample or the standard solution was added separately in wells of the microwell plate. The final concentration of the test and standard solutions used are 1000 µg/mL, 500µg/mL, 250µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL and 15.625 µg/mL. The plates were incubated at 37°C for 30 minutes and the absorbance of each solution was measured at 490 nm, using ELISA reader against the corresponding test and standard blanks and the remaining DPPH was calculated (Kumar *et al.*, 2012; Sundarammal *et al.*, 2012; Lawrence *et al.*, 2012; Mbaebie *et al.*, 2012; Dolai *et al.*, 2012; Patel *et al.*, 2011; Kalita *et al.*, 2012).

Preparation of standard Gallic acid for Phenolic content:

Accurately weighted about 0.1 g of Gallic acid was dissolved in 100 mL of methanol which was known as stock solution I.

From the stock solution I further dilution was made to prepare a stock II (250 mg/mL) from which different aliquots were taken (2-10 mL), 0.5 mL of Folin's reagent was added and kept for 3 minutes and 2 mL of 20% sodium carbonate was added, boiled for 1 minute then volume was made to get the concentrations 20 to 100 µg/mL to obtain linearity curve. The absorbance was measured at the 765 nm against blank.

Sample Preparatio: 100 mg of the 50% ethanol extract and ethyl acetate fraction was taken and was dissolved separately in 10 volumes of ethanol, the resultant solutions were centrifuged at 10000 rpm for 20 min. The aliquots were separated and were diluted with water up to known volume. From the resultant solutions different aliquots (0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL) were taken and 0.5 mL of Follin's reagent was added, kept for 3 min., 2 mL of 20% sodium carbonate was added boiled for one minute and then the volume was made and the absorbance of the solutions of different extracts were obtained at 765 nm against blank.

RESULT AND DISCUSSION

The drugs derived from natural sources have a significant contribution in the modern system of medicines. The aerial parts of *Polygonum nepalense* Meissn (Polygonaceae) are being used traditionally for the treatment of several

diseases. No much work has been done to exploit the medicinal properties of this plant. Hence we were interested to submit this plant to antioxidant of 50% ethanol extract and ethyl acetate fraction. In phytochemical studies 50% ethanol extract was prepared by hot continuous percolation method using soxhlet apparatus for 12 hrs. The ethyl acetate fraction was prepared in order to enrich phenolic content in the fraction. The colour and percentage yield of the 50% ethanol extract and ethyl acetate fraction have been tabulated in Table 1. In 50% ethanol extract and ethyl acetate fraction of aerial parts of *Polygonum nepalense* Meissn were screened for their antioxidant property by DPPH and nitric oxide method. In nitric oxide radical inhibition method ethyl acetate and 50% ethanol extract showed moderate antioxidant activity with IC₅₀ value 185.05 ± 2.59 and 225.00 ± 3.15 µg/mL respectively. Ascorbic acid was used as standard antioxidant. 50% ethanol extract and ethyl acetate fraction exhibited good DPPH radical scavenging activity with IC₅₀ values ranging from 64.13 ± 3.37 to 43.87 ± 2.56 µg/mL. Results are given in Table 2. In the phenolic content estimation the absorbance obtained were plotted on the standard Gallic acid linearity curve to get respective concentrations and then the phenol content was calculated and results were presented in table 3.

Table 1. Colour, consistency and yield of 50% ethanol extract and ethyl acetate fraction of *Polygonum nepalense* Meissn

Name of the extract	Colour	Consistency	Yield (%w/w)
50% ethanol extract	Brown	Amorphous powder	14%
Ethyl acetate fraction	Shining amber-brown	Crystalline powder	4%

Table 2. Antioxidant activity [IC₅₀ value (µg/mL)]

S. No.	Test compound	Nitric oxide method	DPPH method
1.	50% ethanol extract	225.00 ± 3.15	64.13 ± 3.37
2.	Ethyl acetate fraction	185.05 ± 2.59	43.87 ± 2.56
3.	Rutin	180.50 ± 1.51	41.17 ± 1.27

Table 3. Phenol content of 50% ethanol extract and ethyl acetate fraction of aerial parts of *Polygonum nepalense* Meissn

S.No.	Extracts and fraction	Phenol content (% w/w)
1.	50% ethanol extract	18.33%
2.	Ethyl acetate fraction	73.66%

CONCLUSION

The aerial parts of *Polygonum nepalense* Meissn belonging to family

Polygonaceae were collected from Doddabetta in Nilgiris, authenticated and taken for present study. The 50% ethanol extract and ethyl acetate fraction were subjected to chemical

tests for the detection of phyto-constituents. The results revealed that the aerial parts contain flavonoids, tannins and phenol compounds and carbohydrates (50% ethanol extract only). The phenolic content of 50% ethanol extract and ethyl acetate fraction was determined. In ethyl acetate fraction higher phenolic content was found as compared to 50% ethanol extract. Both the extract and the fraction exhibited DPPH radical scavenging activity. The 50% ethanol extract and ethyl acetate fraction were also found to have antioxidant activity by nitric oxide method. *Polygonum nepalense* Meissn has potential phyto-constituents and demonstrated significant antioxidant. An attempt can be taken to investigate medicinal properties of different fractions of the extracts and can be subjected for detailed antimicrobial studies and other biological activities. Thus this study gives support for expanding future investigations of pharmacological activities associated with free radicals.

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