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COMPARATIVE *IN VITRO* FREE RADICAL SCAVENGING ACTIVITY OF *POLYGALA* JAVANA DC., *POLYGALA CHINENSIS* L. AND *POLYGALA ROSMARINIFOLIA* WIGHT & ARN (POLYGALACEAE)

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ABSTRACT

The aim of this research work was to investigate the antioxidant potential of whole plant extracts of *Polygala javana, Polygala chinensis* and *Polygala rosmarinifolia*. The results of the present study revealed that the ethanol extract was found to be the highest DPPH radical scavenging activity when compared with standard ascorbic acid. The DPPH radical scavenging activities were 83.57%, 89.21%, 73.15% and 48.28%, respectively for *Polygala javana, Polygala chinensis, Polygala rosmarinifolia* and standard ascorbic acid. These results showed that whole plant extracts of *Polygala* species can be used as a significant source of natural antioxidant.

Key words: *Polygala* species, Antioxidant, DPPH, Ascorbic acid.

INTRODCUTION

There is extensive evidence to implicate free radicals in the development of degenerative diseases. Oxygen free radicals are formed in tissue cells by various endogenous and exogenous causes such as metabolism, chemicals and ionizing radiation[1]. Approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. All these radicals are known as reactive oxygen species (ROS) exert oxidative stress to the cells. When the generation of ROS overtakes the antioxidant defense of the cells, the free radicals start attacking cellular proteins, lipids and carbohydrates leading to the pathogenesis of many disorders including arthritis and connective tissue disorders, liver disorders, neurodegenerative disorders, cardiovascular disorders, diabetes, chronic inflammation, mutagenesis, carcinogenesis and in the processing of ageing[2].

Antioxidant supplements or food containing antioxidants may be used to help the human body reduce oxidative damage. The most commonly used antioxidants are BHA, BHT, propyl gallate and tert-butyl-hydroquinone[3]. However, they have been suspected of being responsible for liver damage and carcinogenesis in laboratory animals[4]. Therefore, the development and use of more effective antioxidants is derived.

Medicinal plants are a source for a wide variety of natural products, such as the phenolic acids and flavonoids, which are very interesting for their antioxidant properties[5]. In addition to their ability to act as an

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efficient free radical scavengers[6]. Their natural origin represents an advantage to consumer in contrast to synthetic antioxidants, which their use is being restricted due to their carcinogenicity[7].

Polygala was traditionally used by native Americans to treat snake bites[8] and as an expectorant to treat cough and bronchitis. In traditional Chinese medicine, *Polygala* is used for a variety of purposes including the promotion to sleep and calming the spirit. *Polygala* considered as a powerful tonic herb[9] that can help to develop the mind and aid in creative thinking.

Taking into consideration of the medicinal importance of *Polygala* species, the ethanol extract of whole plants of *Polygala javana, Polygala chinensis* and *Polygala rosmarinifolia* were analyzed for their *in vitro* antioxidant activity.

EXPERIMENTAL

Materials and methods

Collection of plant sample

Whole plant of *Polygala javana* was collected from Courtallam, Tirunelveli District, Tamil Nadu and whole plant of *Polygala chinensis* and *Polygala rosmarinifolia* were collected from Vadavalli, Coimbatore, Tamil Nadu. With the help of local flora, voucher specimens were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin, Tamil Nadu for further references.

Plant sample extraction

Whole plants were cleaned, shade dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to Stoppard flask and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the first six hours and then it was kept aside and again shaken after 24 hours. This process was repeated for three days and then the extract was filtered. The extract was collected and evaporated to dryness by using vacuum distillation unit. The final extract thus obtained was used for *in vitro* antioxidants activity.

Estimation of total phenolic content

Total phenolic content was estimated using the Folin-Ciocalteu method[10]. Samples (100 µL) were mixed thoroughly with 2 mL of 2% Na_2CO_3. After 2 min. 100 µL of Folin-Ciocalteu reagent was added to the mixture. The resulting mixture was allowed to stand at room temperature for 30 min and the absorbance was measured at 743 nm against a blank. Total phenolic content was expressed as gram of gallic equivalents per 100 g of dry weight (g 100 g^{-1} DW) of the plant samples.

Estimation of Flavonoids

The flavonoids content was determined according to Eom *et al.*[11] An aliquot of 0.5 mL of sample (1 mg/mL) was mixed with 0.1 mL of 10% aluminium chloride and 0.1 mL of potassium acetate (1 M). In this mixture, 4.3 mL of 80% methanol was added to make 5 mL volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415 nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

DPPH radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence

of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H[12].

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method[12]. Briefly, an 0.1 mm solution of DPPH in methanol was prepared, and 1 mL of this solution was added to 3 mL of the solution of all extracts in methanol at different concentration (125, 250, 500 & 1000 µg/mL). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula.

$$\text{DPPH scavenging effect (\% inhibition)} = \{(A_0 - A_1)/A_0) \times 100\}$$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

RESULTS AND DISCUSSION

The total phenolic and flavonoid content of whole plant of *Polygala javana, Polygala chinensis* and *Polygala rosmarinifolia* are presented in Table 1. Among the studied plants, *Polygala chinensis* was found to be more amounts of total phenolics and flavonoids. Phenolics are presented in a variety of plants utilized as important components of both human and animal diets. The health benefits associated with the consumption of fruits and vegetables have been partly attributed to the flavonoid content.

Table 1: Total phenolic and flavonoid content of *Polygala javana, Polygala chinensis* and *Polygala rosmarinifolia*[a]

Plant materials	Total phenolics g 100 g $^{-1}$	Total flavonoids g 100 g $^{-1}$
Polygala javana	0.54 ± 0.03	1.01 ± 0.04
Polygala chinensis	0.68 ± 0.02	1.24 ± 0.05
Polygala rosmarinifolia	0.48 ± 0.01	0.96 ± 0.06

[a]Data given are the means of three replicates; ± Standard error

Unlike other free radicals, such as the hydroxyl radical and superoxide anion. DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition[13]. A freshly prepared DPPH solution exhibits a deep purple colour with absorption maximum at 517 nm. The purple colour generally fades or disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably via a free radical attack on the DPPH molecule) and convert them to a colourless (i.e., 2, 2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm. Hence, the more rapidly the absorbance decreases, the more potent the antioxidant activity of the extract. This test is a commonly employed assay in antioxidant studies of specific compounds or extracts across a short time scale. The principle advantage of DPPH is that its reduction can be measured directly in the reaction medium by a continuous spectrophotometric assay. DPPH assay is known to give reliable information concerning the antioxidant ability of the tested compounds.

The ethanol extract of whole plant of *Polygala javana, Polygala chinensis* and *Polygala rosmarinifolia* show the free radical scavenging property at all the six concentrations studied. DPPH can be used in determining radical scavenging activity as it forms a stable molecule on accepting an electron or hydrogen atom[14]. The results obtained are shown in Table 2. Plant ethanolic extracts show satisfactory effect in inhibiting DPPH. At a concentration of 400 µg/mL, the scavenging effects of the whole plant extracts on the DPPH radical increased when compared with standard ascorbic acid. The results show that among the studied *Polygala* species, *Polygala chinensis* whole plant exhibit higher DPPH radical scavenging activity. The maximum inhibitory concentrations (IC_{50}) in DPPH were found to be 48.26, 59.19, 41.58 and 22.51 µg/mL, respectively for *Polygala javana, Polygala chinensis* and *Polygala rosmarinifolia* and standard ascorbic acid.

Table 2: DPPH scavenging activity on whole plant extract extracts of *Polygala javana, Polygala chinensis* and *Polygala rosmarinifolia*

Concentration (µg/mL)	% of activity (± SEM)			
	Polygala javana	***Polygala chinensis***	***Polygala rosmarinifolia***	**Standard (Ascorbic acid)**
10	15.24 ± 1.45	18.37 ± 1.82	16.36 ± 1.38	14.56 ± 1.32
20	21.34 ± 1.92	27.25 ± 1.28	24.59 ± 1.22	21.46 ± 1.67
50	37.99 ± 1.27	43.19 ± 1.41	35.27 ± 1.52	32.55 ± 1.94
100	56.36 ± 1.84	63.78 ± 2.14	53.28 ± 1.98	39.25 ± 1.32
200	71.33 ± 2.03	82.67 ± 2.32	67.65 ± 2.04	48.28 ± 1.46
400	83.57 ± 2.75	89.21 ± 2.92	73.15 ± 1.39	48.28 ± 1.46
IC 50	48.26	59.19	41.58	22.51

CONCLUSION

It is reported that total phenolics and flavonoids are natural products, which have been shown to possess various biological properties related to antioxidant mechanisms. Thus in the present study, the antioxidant potential of *Polygala javana, Polygala chinensis, Polygala rosmarinifolia* may be attributed to the presence of total phenolics and flavonoids and other constituents present therein.

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