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Phytochemical Analysis and Antioxidant Assay of *Pseuderanthemum crenulatum* (Wall.Ex Lindl.) Radlk.

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ABSTRACT

India has a large diversity of plants. Plants have been used as a source of therapeutic processes. *Pseuderanthemum crenulatum* (Wallich Ex Lindley) Radlk belongs to Acanthaceae family. The objective of the study was Phytochemical analysis and antioxidant assay in methanolic extracts of *Pseuderanthemum crenulatum* (Wall.ex Lindl.) Radlk leaves. The antioxidant effect of methanolic extraction of plant leaves was studied by DPPH (1, 1diphenyl-2picryl hydrazyl) radical scavenging assay. The antioxidant studies done by DPPH assay revealed low antioxidant activity against ascorbic acid as reference standard. Absorbance was measured immediately at 515 nm by UV spectrophotometer. Lower absorbance of reaction mixture indicated higher free radical activity. As increasing concentration, the absorbance of sample was decreased. The methanolic extract of dried leaves of *Pseuderanthemum crenulatum* revealed the presence of secondary metabolites such as phenols, flavonoids and saponin. It indicates that the plant has a therapeutic activity.

Key words: *Pseuderanthemum crenulatum, Acanthaceae, DPPH*

Introduction

India is rich in biodiversity. Medicinal plants are potential source of development of new herbal drugs (Shakya, 2016). Many of the plant material used in traditional medicine are more efficient and cheaper than modern medicine (Mann *et al.*, 2008). Secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plant. The medicinal actions of plant are unique to a particular plant species or group.

Antioxidants play an important role in health protection factor; they reduce risks for chronic diseases including cancer and heart disease. *Pseuderanthemum crenulatum* (Wall.ex.Lindl.) Radlk. also called Blue twilight, Blue crossandra, Florida twilight is a herbaceous plant species belongs to the family of Acanthaceae, It has glossy green leaves with showy sprays of lavender flowers and blooms heavily in fall and winter. It is a very ramified semi shrubby species, with a height of 1-1.5m height, with brown pubescent stems. Leaves are simple, opposite elliptic-lanceolate with sharp apex, slightly crenate, bent upwards and has seven prominent veins on sides of central one. Inflorescence is racemose terminal with a crowd of bilabiate flowers, which is pale blue to lilac with a white spot at centre of lower lip. Externally there is a pubescent calyx.

Phenolics are large category of Phytochemicals and are widely distributed in plant kingdom. The three most important groups of phenolics are flavonoids, phenolic acids and polyphenols. These reduces blood cholesterol and has antimicrobial activ-

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ity against some strains of bacteria. They also possess anti-ulcer, antioxidant and antitumor activities (Saxena *et al.,* 2013). Flavonoids have antimicrobial, cytotoxicity, anti-inflammatory and antitumor activities. Mostly, they are powerful antioxidants, Antioxidants helps to protect the body from free radical and reactive oxygen species (Saxena *et al.,* 2013). Saponins are major part of defense system of plants. So, they are included in the group of protective molecules and named phytoprotectants or phytoanticipins (Saxena *et al.,* 2013). *Pseuderanthemum crenulatum* revealed the presence of secondary metabolites such as phenols, flavonoids and saponin

Materials and Methods

The study area

Fresh leaves of *Psuederanthemum crenulatum* were collected from Kollam district of Kerala. The district lies between 8° 48' 0.00" North latitude and between 76° 35' 60.00" East longitudes.

Studymethod

Fresh leaves of *Psuederanthemum crenulatum* were collected and shade dried for 20 days and powdered using mechanical grinder. 30g of leaf powder is subjected to Soxhlet extraction with 250 ml of methanol. The extraction was carried out 10 cycles until the solution becomes colourless and temperature was maintained 65 °C. The colour of extract was dark green colour. The concentrated extract was collected in a beaker and kept on room temperature till all solvent got evaporated. The extract obtained was dark green coloured and sticky and it was kept in small air tight container and this extract was used for further studies.

Sample preparation

Preliminary Phytochemical analysis

Phenol

2 ml of plant extract was taken in a test tube and add 1% lead acetate solution. Formation of white precipitate indicates presence of phenolic compounds (Raaman *et al*., 2006).

Flavonoids

The extract was treated with a few drops of sodium hydroxide. Formation of intense yellow colour, which becomes colour less on addition of few drops of dilute acid, indicates the presence of flavonoids (Gul *et al.,* 2017).

Alkaloids

To 2 ml of plant extract add 2 ml of Wagner's reagent. Test tubes were observed for appearance of reddish-brown precipitate for alkaloids (Wagner 1990).

Tannins

Take 1ml of extract and treat it with 1ml of 10% alcoholic ferric chloride solution. Observe for the formation of blue or greenish colour Appearance of brownish green coloration showed the presence of tannins (Ciulci, 1994).

Glycosides

In 5 ml plant extract, 2 ml glacial acetic acid, one drop of 5% ferric chloride and concentrated sulphuric acid were added. Brown ring appears indicating presence of glycosides.

Saponins

To about 1ml of extract, was added to 2ml of distilled water in a test tube and shaken vigorously with few drops of olive oil. Foam which persisted was taken as evidence for presence of saponins (Kokate, 1999).

Steroids

1ml of extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of test tube. Upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicates presence of steroids (Ciulci, 1994).

Terpenoids

2ml of extract of sample was mixed with 2 ml of chloroform. Then allow evaporating and adding 2ml of concentrated sulphuric acid, then heat for 2 minutes. Greyish colour indicates presence of terpenoids.

Quinones

2 ml of extract of plant was mixed with 3or 4 drops of concentrated HCl. A yellow colour precipitate indicates presence of quinones.

Fatty acids

0.5 ml of extract was added to 5ml of ether and allowed it to evaporate on filter paper. Then the filter paper was dried and appearance of transparency on filter paper is indication of presence of fatty acids.
DPPH radical scavenging assay

Principle

Radical scavenging activity of test sample against stable 2,2-diphenyl 2picrylhydrazyl hydrate (DPPH) was determined according to method of Brand William *et al., (*1995*)* with slight modification. DPPH reacts with an antioxidant compound, which can donate hydrogen and reduce DPPH. The change in colour (from deep violet to light yellow) was measured at optical density 515nm on a UV visible spectrophotometer.

Procedure

For DPPH assay the ascorbic acid was used as reference standard. The ascorbic acid stock solution was prepared in distilled water (1mg/ml; w/v). A 60 micromolar solution of DPPH in methanol was freshly prepared and a 200 µl of this solution was mixed with 50 µl of test sample at various concentrations (1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200, 400, 800 microgram/ml). The plates were kept in dark for 15minutes at room temperature and decrease in absorbance was measured at 515 nm. Control was prepared with DPPH solution only, without any extract or ascorbic acid 95% methanol was used as blank.

Radical scavenging activity was calculated by the formula,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test} \times 100}{\text{Absorbance of control}}$$

Results

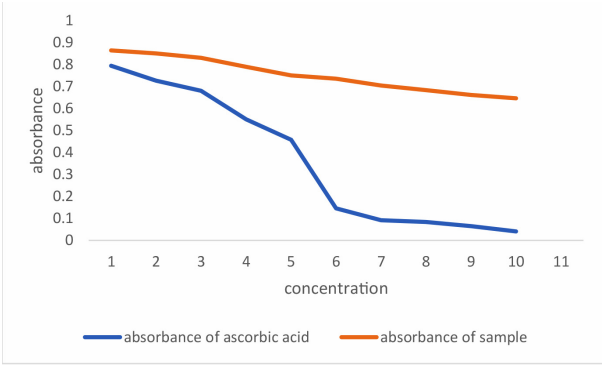
The leaves of plant *Pseuderanthemum crenulatum* were used for Phytochemical analysis and antioxidant assay. Dried leaf powder extraction of *Pseuderanthemum crenulatum* in 100% methanol was used for qualitative phytochemical analysis. The qualitative phytochemical analysis indicated the presence of phenols, flavonoids and saponin. Phytochemicals such as Alkaloids, Tannins, Glycosides, Steroids, Terpenoids, Quinones and fatty acids were absent.

Antioxidant Assay

DPPH radical assay was done for analysing antioxidant activity in *P. crenulatum*. For this assay, Ascorbic acid was used as reference standard. The optical density or the absorbance at 515nm was measured using UV-visible spectrophotometer at various concentrations 1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200, 400, 800 µg/ml. On assay, the absorbance obtained for the sample at 1.56 is 0.8655, 3.12 is 0.8517, 6.25 is 0.8318, 12 is 0.7904, 25 is 0.7512, 50 is 0.7364, 100 is 0.7056, 200 is 0.6844, 400 is 0.6624, and 800 is 0.6476 accordingly. The absorbance of control of sample obtained is 0.8785. The plant showed feeble activity as compared with the standard (Table 1 and Graph 1).

Table 1. Absorbance at 515 nm of DPPH assay

Concentration	Absorbance of ascorbic acid	Absorbance of sample
1.56	0.795	0.865
3.12	0.727	0.851
6.25	0.681	0.831
12.5	0.551	0.790
25	0.458	0.751
50	0.146	0.736
100	0.092	0.705
200	0.084	0.684
400	0.065	0.662
800	0.041	0.647

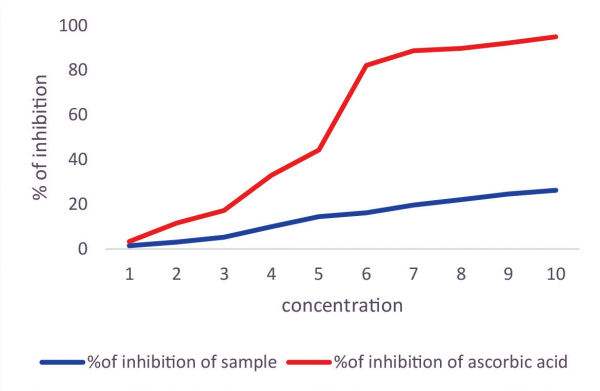


Graph 1. Concentration – Absorbance curve of DPPH assay

As compared with standard, the inhibition of sample is least hence the antioxidant activity of *Pseuderanthemum crenulatum* (Wall.ex.Lindl) Radlk is feeble (Table 2 and Graph 2).

Table % of inhibition at 515nm of DPPH assay

Concentration	%of inhibition of ascorbic acid	% of inhibition of sample
1.56	3.36	1.48
3.12	11.61	3.05
6.25	17.22	5.32
12.5	32.98	10.02
25	44.26	14.49
50	82.19	16.17
100	88.75	19.68
200	89.75	22.09
400	92.11	24.60
800	94.95	26.28



Graph 2. Concentration - %of inhibition curve of DPPH assay

Discussion

For curing bodily disorders, humans had been using plants since pre-historic times and that kept their health in perfect state of fitness. The consumption of synthetic drugs causes many health problems hence people are now increasingly approaching traditional medicines. The present study on the methanolic extract of dried leaves of plant *Pseuderanthemum crenulatum* revealed the presence of secondary metabolites such as phenols, flavonoids and saponin that are of therapeutic benefits. These metabolites are useful in medicinal field as phenols are used as oral analgesic, vaccine preservative etc. Flavonoids have anticancer, antiviral and anti-inflammatory properties. Saponins are used as an antidote against lead poisoning. Since these metabolites are present in the plant, it has medicinal properties and can be used for therapeutic purposes. The studies on the plant *Pseuderanthemum crenulatum* (Wall.ex. Lindl) Radlk. revealed that it has feeble antioxidant activity and has therapeutic benefits because of the presence of secondary metabolites such as phenols, flavonoids and saponin.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper

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