Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating: 3.53
JMPS 2018; 6(6): 242-244

Received: 06-09-2018
Accepted: 07-10-2018

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Evaluation of antioxidant activity of *Phlogacanthus thyrsiflorus* Nees: A medicinal plant

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Abstract
The Ethnobotanical and pharmacological evaluations of plant based chemicals have shown rapid strides in the last few decades. The present study was carried out to investigate the anti oxidant potential of methanolic extracts of flowers and leaves of *Phlogacanthus thyrsiflorus* by DPPH assay. Edible plant parts *viz*., fresh young and matured leaf, fresh flower, dry flower and dry matured leaf showed antioxidant activity and there was significant difference in the IC_{50} values of the plant parts studied. The highest IC_{50} was observed in fresh young leaf (57.20±0.96 mg) indicating the lowest antioxidant activity in fresh young leaf and the lowest IC_{50} was observed in dry matured leaf (7.86±0.35 mg) indicating the highest antioxidant activity in dry matured leaf. Dried flower sample had higher antioxidant activity than fresh sample of flower. The results suggest that *Phlogacanthus thyrsiflorus* is a natural source of antioxidants.

Keywords: *Phlogacanthus thyrsiflorus*, antioxidant potential, DPPH, Free radical, IC_{50}.

Introduction
Plants have been used for medicine from time immemorial because they are easily accessible and inexpensive. They are a rich source of important therapeutic agents and form the basis of herbal systems of medicine, like ayurveda, resulting in the revival of ancient traditions of medicine. Some of the most important compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. Presence of flavonoids and other polyphenolic compounds in the plant parts have been reported to show their remarkable biological effects like antioxidant property and anti cancer activity [1]. Tannins are considered antioxidants and prevent the onset of degenerative diseases such as cancer and cardiovascular disease. These are also used in photography and refining beer and wine. The anti-inflammatory effect of tannins help control all indication of gastritis, esophagitis, enteritis and irritating bowel disorders [2]. They not only heal burns and stop bleeding, but they also stop infection while they continue to heal the wound internally. The ability of tannins to form a protective layer over the exposed tissue keeps the wound from being further infected [3].

Phlogacanthus thyrsiflorus – Nees belonging to the Acanthaceae family is an important group of medicinal plant found in the sub tropical Himalayas spreading up to Bhutan, upper gangetic plains, Bihar, North Bengal, Assam, Arunachal Pradesh and Manipur. It is an evergreen shrub growing up to 2.4 m high with long oblanceolate leaves and terminal elongated, thyrsoid panicles up to 30 cm long. The plant is used in various ailments by the people. Flowers are antidote to pox; prevent skin disease like sore, scabies etc. It has the antimicrobial properties, been known to possess antibacterial, antifungal, anti-diabetic, anti-inflammatory, anticancerous, hypolipidaemic and hepatoprotective [4].

Antioxidants are diverse group of chemicals which protect the body from oxidative damage induced by free radicals and reactive oxygen by suppressing their formation [5] and acting as scavengers [6]. Natural antioxidants from plant sources are potent and safe due to their harmless nature. Among the various medicinal and culinary herbs, some endemic species may be used for the production of raw materials or preparations containing phytochemicals with significant antioxidant capacities and health benefits [7]. Therefore, many wild herbs have been investigated for their antioxidant properties. The present study was undertaken to explore the anti oxidant property of the leaves and flowers of *Phlogacanthus thyrsiflorus* using DPPH assay.

Materials and Methods
Antioxidant activity was determined by method given by Molyneux [8]. Free radical scavenging ability of DPPH (1, 1-diphenyl-2-picrylhydrazyl) was determined on methanolic

extracts of dried sample (young and matured leaves and flower) of *Phlogacanthus thyrsiflorus.* One g of each of dried leaves and flowers of *Phlogacanthus thyrsiflorus* and 1 g of each of fresh young, fresh matured leaves and fresh flower were extracted in 10 ml methanol, centrifuged at 10000 rpm for 20 mins and the supernatant was used for assay, after making up volume to 10 ml by methanol. To 100-1400 µl of methanolic sample extract, methanol was added to make up the volume to 1500 µl. To it 1500 µl of DPPH reagent (1 mM in methanol) was added and the mixture was incubated at room temperature at dark for 30 mins. The absorbance was measured at 517 nm taking methanol as blank. A mixture of equal volume of methanol and DPPH reagent served as control. A decreasing intensity of the purple colouration was taken as increasing scavenging activity.

The inhibition of DPPH radicals by the sample was calculated using the following formula

$$\text{DPPH inhibition (\%)} = \frac{\text{(Absorbance of control - absorbance of sample)}}{\text{Absorbance of control}} \times 100$$

Antioxidant activity of various edible plant parts were tested by analysis of variance (ANOVA). The levels of probability used for 'F' test was at 0.05%. CD values given in the table are at 5% level of significance, where 'F' test was significant.

Results

Free radical scavenging activity of the crude methanolic sample extracts were measured by colorimetric assay using DPPH as a source of free radical. The results are presented in Table 1. With increase in the concentration of the samples, the DPPH inhibition percentage increased, which shows the antioxidant activity of the samples. Maximum DPPH inhibition was seen in dry leaf where 10 mg of sample gave 59.1±2.4 DPPH inhibition percentage which got increased to 85.6±1.5 DPPH inhibition percent in 100 mg followed by fresh matured leaf where 10 mg of the sample gave 48.3±2.1 and 100 mg gave 85.5±2.3 DPPH inhibition percent. Lowest DPPH inhibition percent was 9.8±2.4 observed in 10 mg of the fresh young leaf.

The IC_{50} value, a measure of the extract concentration which is required for the 50% inhibition of the free radical DPPH was determined. There was significant difference in the IC_{50} values of the different samples studied (Table 1). Dry leaf samples showed the highest DPPH scavenging activity as the IC_{50} value (7.86±0.35mg) was recorded to be the lowest (Table 1). The fresh young leaf showed the lowest DPPH scavenging activity as the IC_{50} value for it was observed to be the highest (57.20±0.96 mg). Fresh and dried flower samples recorded 34.8±2.86 and 20.23±3.70 IC_{50} value respectively. The leaf samples were found to have more DPPH scavenging activity than the flower samples.

Discussion

DPPH, a relatively stable organic radical has been used widely to test the potential of compounds as free radical scavengers and to investigate the antioxidant activity of plant extracts. The antioxidants react with DPPH, a purple coloured stable free radical and convert it into a colourless α-α, diphenyl-β-picryl hydrazine. Antioxidants, on interaction with DPPH, either transfer an electron or hydrogen atom to DPPH, neutralizing its free radical character [9]. Total antioxidant necessary to decrease the initial DPPH radical concentration by 50% is referred as IC_{50}. Thus a lower IC_{50} would reflect greater antioxidant activity of the sample.

It is reported that composition of antioxidants varied widely with several factors like the stage of maturity, variety, climatic condition, part of the plant analyzed, post harvest handling, processing and storage [10]. In the present study, antioxidant activity of *Phlogacanthus thyrsiflorus* also varied. Dry matured leaves exhibited the highest antioxidant activity than the fresh matured leaves, fresh young leaves, fresh flower and dry flower samples. Similarly, Ali *et al.* [11] observed that methanolic extract of matured of *Scurrula parasite* L. showed the higher DPPH scavenging activity than tender leaves.

However, Sreelatha and Padma [12] observed that there were minor differences in the antioxidant activity in the two maturity stages, matured and tender leaves of *Moringa oleifera*. Though, the flower samples contained different phenolic compounds (phenols and flavonoids) in amount higher than those found in leaf samples, the antioxidant activity was observed to be higher in leaf samples. So, the other phenol compounds like tannins may contribute to the higher antioxidant activity of leaf samples of *Phlogacanthus thyrsiflorus*.

Siddhuraju *et al.* [13] in their studies correlated the antioxidant activity with the total polyphenolic content of the flower and leaf extracts of Indiana laburnum (*Cassia fistula* L.) and found that leaf extracts contained higher antioxidant activity than the flower extracts. Similar results were observed by Shabir *et al.* [14] where the DPPH scavenging capacity was higher in leaves than flowers of Gold mohor (*Delonix regia*) whereas contrasting results were obtained by Barreira *et al.* [15] where flowers of chestnut were having higher flavonoid content than leaves contributing to higher antioxidant activity of flower than leaves.

Table 1: Percentage DPPH inhibition and IC_{50} values of different plant parts of *Phlogacanthus thyrsiflorus*

Sample	DPPH inhibition (%)						$IC_{50\ mg}$
	10mg	20mg	40mg	60mg	80mg	100mg	
Fresh flower	33.6±3.1	43.3±0.3	51.6±2.2	60.8±2.9	70.5±1.8	78.4±1.9	34.80±2.86
Dry flower	42.2±3.6	49.4±5.9	56.4±3.5	64.0±1.8	74.8±2.6	83.2±3.4	20.23±3.70
Fresh young leaf	9.8±2.4	23.6±2.5	36.9±2.8	52.1±0.8	65.5±0.4	78.8±1.1	57.20±0.96
Fresh matured leaf	48.3±2.1	55.0±2.7	62.7±2.6	71.0±3.0	80.4±2.4	85.5±2.3	12.90±3.79
Dry leaf	59.1±2.4	63.5±3.5	68.7±3.1	74.7±2.5	80.0±2.2	85.6±1.5	7.86±0.35
CD 0.05							1.90

Conclusion

P. thyrsiflorus is a common vegetable of north eastern states of India. The present study demonstrated that methanolic flower and leaf extracts of *Phlogacanthus thyrsiflorus* has promising antioxidant and radical scavenging activities. Therefore, it can be concluded that the leaves and flowers of this plant are good sources of natural antioxidants and might be useful in treating the diseases associated with oxidative stress.

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