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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/11140
DOI URL: http://dx.doi.org/10.21474/IJAR01/11140

RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF METHANOLIC AND AQUEOUS LEAF EXTRACTS OF *Phlogacanthus thyrsiflorus*

K. Gitaranjan Singh, Sahana D., Dr. Rama T. and Kiran Kumar D.J
Department of Biotechnology, Government Science College, Bangalore.

Manuscript Info

Manuscript History
Received: 07 April 2020
Final Accepted: 10 May 2020
Published: June 2020

Key words:-
Phlogacanthus thyrsiflorus,
Phytoextraction, DPPH, ABTS

Abstract

Phlogancanthus thyrsiflorus is an underexploited medicinal plant of Acanthaceae family found in Himalayan range comprising of Bhutan, Assam, Arunachal Pradesh and Manipur. Various parts of the plant are used in traditional and folk medicine for fever, gastritis, pharyngitis, cough, bronchial asthma and other ailments. The present study was taken up to analyze the phytochemical contents of leaves of plant using aqeous and methanolic extracts of *Phlogancanthus thyrsiflorus* in order to check the feasibility of the plant extract to be used as a source for various phytopharmaceutical applications. Qualitative phytochemical tests confirmed the presence of alkaloids, glycosides, flavonoids, phenols, tannins, quinones, and terpenoids. Further, antioxidant activity was determined using DPPH and ABTS radical scavenging assay.

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Introduction:-

Phlogacanthus thyrsiflorus is an evergreen shrubby plant which belongs to Acanthaceae family. They grow in the northeast regions of India such as Assam, Manipur, Meghalaya, parts of Bihar, North Bengal, upper gangetic plain and neighbouring countries such as Bangladesh and Bhutan[1]. *Phlogacanthus thyrsiflorus*is used as medicinal plant by many local tribes belonging to northeast region of India. Kasbi tribes of Assam use the leaves for treatment of fever [2]. Aerial parts of *Phlogacanthus thyrsiflorus*plant is used for the treatment of allergies in folklore in regions of Lakhimpur of Assam distsrict[3]. Meetei community of Manipur use almost all organs of the plants for the treatment of many ailments. Leaves are the most frequently used organ and many a times combinations of two or more plants are also utilized for treating various ailments. Bacterial infections, stomach ache, diarrhea, wounds, cough, high blood pressure, diabetes, boils, irregular mensuration, viral influenza, constipation are just a few ailments treated with the help of *Phlogacanthus thyrsiflorus* plants[4]. Many researchers have tried to understand the logic behind utiliziation of *Phlogacanthus thyrsiflorus* plant in traditional medicine and folk medicine in a scientific way. In this regard after extensive research Ahmed R. and his group, have found that in diabetic Long-Evans rats, flowers of the plant exhibited hypoglycaemic and mildly hypolipidemic activity was exhibited when tested on[5]. A study by Sharmistha Chakravarthy and Jogen.Ch.Kalita showed that aqeous extracts of *Phlogocanthus thyrsiflorus* flowers have therapeutic effect in artificially induced diabetes in mice[6]. The plant also has been studied for its antibacterial and anticancerous activity by Anupam Kumar, *et al*.[7] Analgesic activity has also been reported by Mukherjee A and his team [8]. Inspite of so much study on the plant *Phlogacanthus thyrsiflorus*, a lot is left to be understood about this plant looking at its extensive use in ethnobotany and folklore.

Corresponding Author:- Kiran Kumar D.J
Address**:-** Department of Biotechnology, Government Science College, Bangalore.

Materials and Method

Collection of plant material and preparation of leaf powder;

Disease free plant material was collected from Jiribam district of Manipur state. The plant was identified and authenticated to be *Phlogacanthus thyrsiflorus* by Dr. A.N. Sringeswara, curator of botanical garden from University of Agricultural sciences, Bengaluru, after comparing it with available herbarium specimen KEW (K000950020). A voucher specimen was prepared and submitted to UAS, GKVK for future reference. Leaves were utilized for the preparation of phytoextracts. Leaves were first shade dried and pulverized into fine powder. The powder was sieved to get a uniform sized leaf powder for preparation of extracts.

Preparation of plant extract

50g of leaf powder was immersed in 500ml of methanol. 20gm of leaf powder was immersed in 200ml of water. The above suspensions were stored at 40°C and kept in continuous agitation for about 24 hours to assist phytoextraction. Soxhlet apparatus was utilized to prepare methanolic extracts of leaves. For preparation of aqueous extracts the suspension was first filtered and the solvents were taken out using simple technique of evaporation in hot air oven at 40°C. The obtained extracts were carefully stored under refrigeration and used for further analysis. Percentage yield in both methanolic and aqueous extracts was found to be around 4%.

Preliminary phytochemical screening

Preliminary phytochemical screening was performed for both the methanolic and aqueous extracts. The extracts were tested for the presence of phytochemicals such as alkaloids, flavonoids, cardiac glycosides, phenolic compounds, phlobatannin, amino acids, carbohydrate, saponins, sterols, tannins, terpenoids, Quinones and Oxalates. The results of these screening tests are tabulated in table 1.

Evaluation of Antioxidant Activity

Sample Preparation

The extracted methanolic and aqueous concentrates were diluted to a concentration of 1mg/ml using methanol solvent for estimation of antioxidant activity using DPPH radical scavenging assay as well as ABTS assay

Preparation of Gallic acid Standard

Gallic acid which was used in both the assays as standard antioxidant was prepared using methanol and the final concentration of gallicacid was 1mg/ml.

DPPH radical scavenging assay

In DPPH radical scavenging assay the antioxidant compound reacts with DPPH converting it into a yellow coloured compound 1,1 diphenyl-2-piercryl hydrazine. The intensity of colour indicates positive reaction.

Gallic acid was pipetted out into different test tubes(10µl, 20µl, 30µl, 40µl,50µl) and the volume was made upto 100µl using methanol. 3ml of DPPH was added to each test tube and they were incubated for 15minutes at room temperature for colour development[9]. After incubation period absorbance of the reaction mixture was read against blank (methanol) at 517nm.

Using the same method DPPH radical scavenging assay was performed for methanolic and aqueous extracts of *Phlogacanthus thyrsiflorus* and the results were tabulated. Percentage inhibition of the assay was calculated using the formula.

$$\%inhibition = \frac{o.d.of the control - o.d.of the sample}{o.d.of the control} \times 100$$

The IC50 of the reaction were obtained through extrapolation from regression analysis. The antioxidant was evaluated based on this IC50. The percentage inhibition to the concentration is represented in figure 2.

ABTS radical scavenging assay

ABTS assay works on the principle that the light gets scavenged by ABTS radicals. An antioxidant molecule such as gallic acid or the ones from phytoextract will be able to donate a hydrogen atom which in turn would neutralize the free radical.

Samples of methanolic and aqueous extracts were prepared using methanol as solvent and their concentration was 1mg/ml, Gallic acid was used as standard antioxidant molecule and it's concentration prepared using methanol was 1mg/ml. ABTS reagent was prepared by mixing 5ml of 7mM ABTS with 88µl of 140mM potassium per sulphate. The mixture was then kept in the dark at room temperature for 16h to allow free radical generation and was then diluted with water (1:44 v/v).

10µl, 20µl, 30µl, 40µl, 50µl of gallic acid was pipetted out into different test tubes and their volume was made up to 100µl using methanol. This gave us gallic acid concentrations of 100-500µg respectively. To each of the above test tube 3ml of ABTS reagent was added and the tubes were incubated in dark conditions at room temperature for a period of 30minutes[10]. After incubation period absorbance of the reaction mixture was measured against methanol blank at 734nm using UV Visible spectrophotometer. The percentage inhibition of the ABTS by sample was calculated using the same above mentioned formula.

The IC50 of the reaction were obtained through extrapolation from regression analysis. The antioxidant was evaluated based on this IC50. The percentage inhibition to the concentration is represented in figure 3.

Result:-

DPPH Assay

Total antioxidant capacity of the plant extracts was calculated using % inhibition against concentration of gallic acid compared to standard methanolic and aqueous extracts showed lesser percentage of inhibition. The results are tabulated in the table 1. Probably in our experimental conditions our extracts seems not very sensitive to DPPH radical scavenging assay since they showed very less amount of scavenging activity. Even at the concentration of 500µg/ml methanolic extract and aqueous extracts are showing little percentage of inhibition of DPPH radicals (9.2 and 24.6 respectively). The IC50 values of Gallic acid, aqueous extract and methanol extract were found to be 390.339, 1042.66, and 3656.38 respectively.

ABTS antioxidant Assay

The relative antioxidant ability of extracts to scavenge the radicals of ABTS was compared with that of standard Gallic acid. ABTS radical cation was produced in the stable form using potassium per sulphate. After getting the stable absorbance, the antioxidant plant extracts were added to the reaction medium and the antioxidant power was measured by studying decolourization. Compared to DPPH assay, ABTS assay was more sensitive with respect to our plant extracts and to our laboratory conditions. With respect to standard Gallic acid almost complete inhibition could be observed at a concentration of around 300µg/ml onwards itself. With respect to methanol and aqueous extracts nearly 50% inhibition was observed at around 500µg/ml concentrations. IC50 values of Gallic acid, aqueous extract and methanol extract were found to be 9.54, 447.00 and 705.09 respectively.

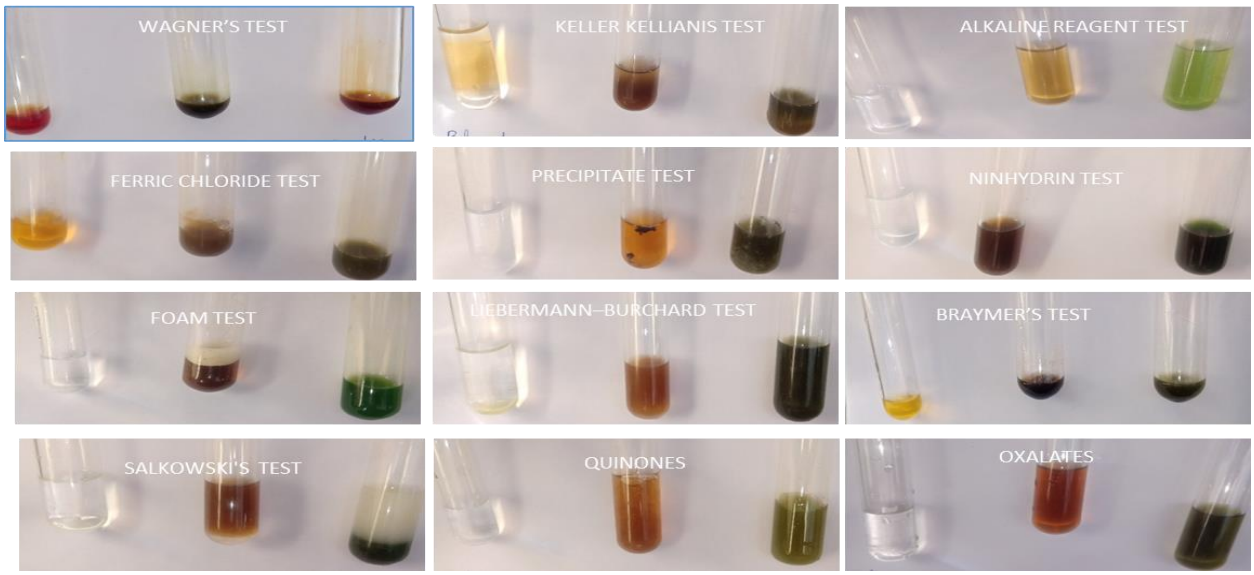

Figure 1:- Preliminary Phytochemical screening.

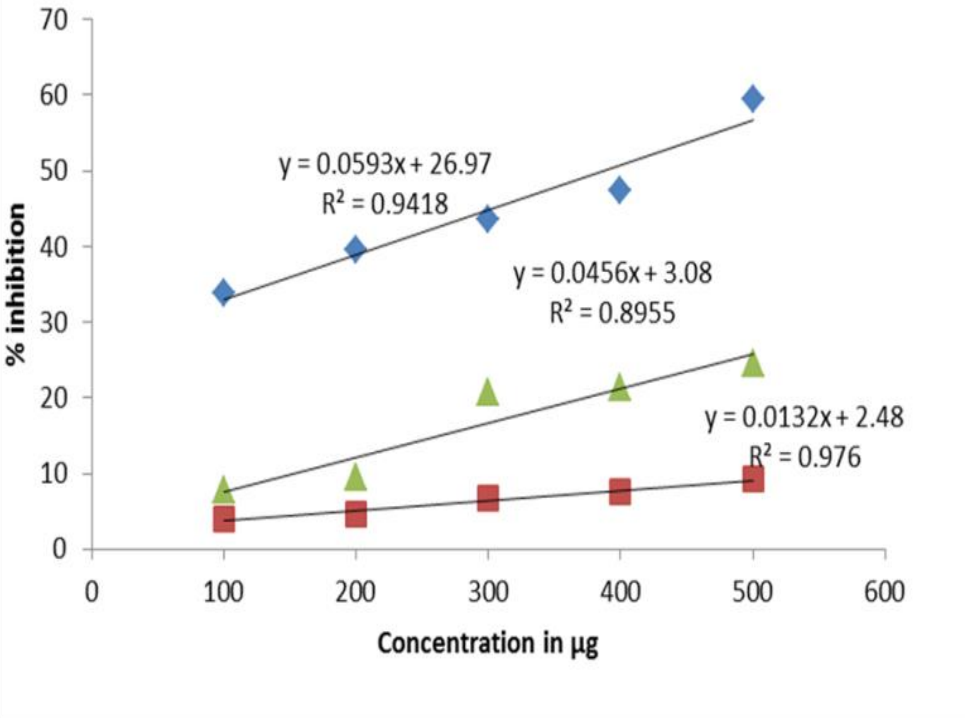

FIGURE 2:DPPH Assay

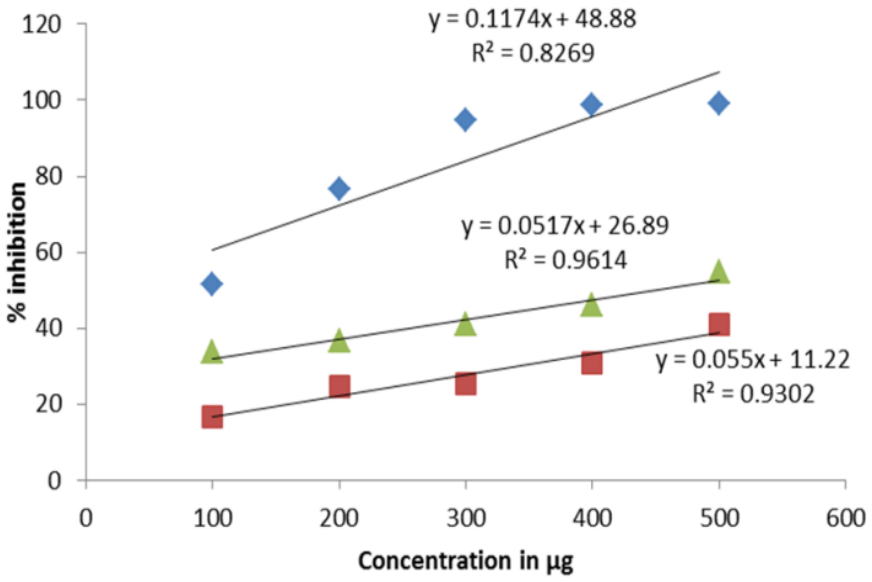

Figure 3:ABTS Assay

Table 1:- Phytochemical screening, tests performed and test results.

Sl.No.	Phytochemical tested	Test performed	Test Result	
			Aqueous extract	**Methanolic extract**
1	Alkaloids	Wagner's test	+	+
2	Flavonoids	Alkaline reagent test	+	+
3	Cardiac glycosides	Keller Kellianis test	+	+
4	Phenolic compounds	Ferric chloride test	+	+
5	Phlobatannin	Precipitate test	+	+
6	Amino acids	Ninhydrin test	-	-
7	Carbohydrate	Molisch's test	+	+
8	Saponins	Foam test	+	-
9	Sterols	Liebermann–Burchard test	-	-
10	Tannins	Braymer's test	+	+
11	Terpenoids	Salkowski's test	+	-
12	Quinones	Precipitation test with HCl	+	+
13	Oxalates	Glacial acetic acid test	-	+

Table 2:- DPPH Assay.

Concentration In µg/ml	% Inhibition		
	Gallic acid	Methanol Extract	Aqueous extract
100	33.8	4.0	7.8
200	39.5	4.7	9.4
300	43.6	6.8	20.6
400	47.4	7.5	21.4
500	59.5	9.2	24.6

Table 3:- ABTS Assay

Concentration In µg/ml	% Inhibition		
	Gallic acid	Methanol Extract	Aqueous extract
100	51.6	16.5	33.6
200	76.4	24.8	36.6
300	94.6	25.5	41.0
400	98.8	30.8	46.1
500	99.1	41.0	54.7

Discussion:-

From table 1 it is evident that a lot of active phytoacitves belonging to group alkaloids, flavonoids, glycosides, phenolics, tannins, terpenoids and quinones are present both in aqueous and methanolic extracts of *Phlogacanthus thyrsiflorus.* The presence of these phytochemicals itself might be one of the reasons why there is significant antioxidant activity as it is evident in results realted DPPH assay and ABTS assay. From the above observed data we conclude that *Phlogacanthus thyrsiflorus* has very good antioxidant property as well as contains diverse group of phytoactive compounds which makes it a very interesting plant in drug discovery.

References:-

1. B. Gogoi, B. BhusanKakoti, N. Sharma Bora, and A. K. Goswami, "Phytochemistry and pharmacology of Phlogacanthusthyrsiflorusnees: A review," Int. J. Pharm. Sci. Rev. Res., vol. 23, no. 2, pp. 175–179, 2013.
2. P. B., "In a: A glossary of medicinal plants of Assam and Meghalaya," Guwahati, India, M N Printers, vol. 1st editio. p. 98, 1992.
3. D. Kalita and R. L. Bora, "Some folk medicines from Lakhimpur district, Assam," Indian J. Tradit. Knowl., vol. 7, no. 3, pp. 414–416, 2008.

4. D. Singh Ningombam and P. Kumar Singh, "Ethnobotanical Study of PhologacanthusthyrsiformisNees: A Conserved Medicinal Plant of Manipur, Northeast India," ~ 10 ~ Int. J. Herb. Med., vol. 1, no. 5, pp. 10–14, 2014.
5. M. R. Ahmed, T. Sultana, and R. Routary, "Chemistry and Antidiabetic Effects of PhlogacanthusthyrsiflorusNees Flowers," Nat. Prod. Chem. Res., vol. 4, no. 5, 2016, doi: 10.4172/2329-6836.1000229.
6. S. Chakravarty and J. C. Kalita, "Antihyperglycaemic effect of flower of PhlogacanthusThyrsiflorusNees on streptozotocin induced diabetic mice," Asian Pac. J. Trop. Biomed., vol. 2, no. 3 SUPPL., pp. S1357–S1361, 2012, doi: 10.1016/S2221-1691(12)60416-X.
7. Kumar, T. Bidyapani, S. Digvijay, N. R. Sharma, and A. Mohan, "Study of phytochemical compositions of leaves extracts of Phlogacanthusthyrsiformis, its antibacterial and silver nanoparticle derived cell cytotoxicity on HeLa cell line," J. Pharm. Res., vol. 11, no. 12, pp. 1513–1517, 2017.
8. Mukherjee, M. Chaliha, and S. Das, "Study of analgesic activity of ethanol extract of Phlogacanthusthyrsiflorus on experimental animal models," Bangladesh J. Pharmacol., vol. 4, no. 2, pp. 147–149, 2009, doi: 10.3329/bjp.v4i2.3808.
9. Braca, C. Sortino, M. Politi, I. Morelli, and J. Mendez, "Antioxidant activity of flavonoids from Licanialicaniaeflora," J. Ethnopharmacol., vol. 79, no. 3, pp. 379–381, 2002, doi: 10.1016/S0378-8741(01)00413-5.
10. S. N. Formagio, C. R. F. Volobuff, M. Santiago, C. A. L. Cardoso, M. D. C. Vieira, and Z. V. Pereira, "Evaluation of antioxidant activity, total flavonoids, tannins and phenolic compounds in Psychotria leaf extracts," Antioxidants, vol. 3, no. 4, pp. 745–757, 2014, doi: 10.3390/antiox3040745.