ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 15, Issue 1, 2022

Online - 2455-3891
Print - 0974-2441

Research Article

PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY OF *CLERODENDRUM PANICULATUM* (L.) LEAF AND FLOWER EXTRACTS

DEEPA R HEBBAR[1], DEVIKA M[2], RASHMI GN[3], NALINI MS[3]*

[1]Department of Botany, Maharani's Science College for Women, Mysuru, Karnataka, India. [2]Department of Botany, Saradavilas College, Mysuru, Karnataka, India. [3]Department of Studies in Botany, University of Mysore, Mysore, Karnataka, India.
Email: nmsomaiah@gmail.com

Received: 12 October 2021, Revised and Accepted: 09 December 2021

ABSTRACT

Objective: This study was designed to evaluate the phytochemicals present in the flower and leaf extracts of *Clerodendrum paniculatum* L., collected from Nelji village of Kodagu district, Karnataka.

Methods: The healthy leaves and flowers of *C. paniculatum* were collected and the plant extracts were prepared using ethanol, hexane, and distilled water separately. Phytochemical analysis was conducted using standard procedures for the flower and leaf extracts. The antioxidant activity in leaf and flower extracts of *C. paniculatum* was determined by three assays, estimation of total phenolic content, reducing power assay, and radical scavenging activity (1,1-diphenyl-2-picrylhydrazyl) using standard procedures.

Results: Phytochemical screening conducted for the flower and leaf extracts of *C. paniculatum* L. showed the presence of three phytochemicals, namely, saponins, alkaloids, and terpenoids. Terpenoids were commonly present in all the extracts of flower and leaf that are in both polar (aqueous and ethanol) solvents and in non-polar (hexane) solvents. The extracts tested for the antioxidant activity showed the presence of total phenolics in ethanol, aqueous, and hexane extracts. The aqueous extract showed high redox potential followed by ethanol and hexane extracts. The aqueous leaf extract showed high radical scavenging activity when compared to the flower extracts of *C. paniculatum.*

Conclusion: The present study shows *C. paniculatum* to be an important medicinal plant, since the flower and leaves showed good antioxidant activity. Thus, it may use in the treatment of diseases and may also use in the preparation of natural or herbal drugs due to the presence of antioxidants.

Keywords: *Clerodendrum paniculatum*, phytochemicals, antioxidant activity, phenolic content, reducing power assay, 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity.

 DOI: http://dx.doi.org/10.22159/ajpcr.2022v15i1.43361. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Medicinal plants are the richest bioresource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs [1]. Indian natural products, particularly from the traditional medicinal plants reported in the classic texts such as Ayurveda and Charaka Samhita, have contributed toward this boom in drug discovery. Some of the classical examples of drug discovery are morphine, quinine, and digoxin [2]. The study and identification of chemical constituents present in the plants is termed as phytochemistry [3]. Phytochemicals are naturally occurring in the medicinal plants that contain defense mechanism and protect from various diseases [4]. Antioxidants are the substances that significantly delay or inhibit oxidative damage to a target molecule. Antioxidants prevent cell and tissue damage hence they act as scavengers [5].

Clerodendrum is a very large and diverse genus with about 580 identified species which are distributed throughout the world [6]. It is the largest genus of the tribe Teucrieae. In India, 23 species were recorded [7], of which 16 are from Arunachal Pradesh [8]. A number of species from this genus have been used in traditional system of medicine by various tribes in many countries such as China, India, Japan, Korea, and Thailand [6].

Clerodendrum paniculatum L. is a shrub, 1 m tall, branchlets 4-angled, subglabrous to pubescent, nodes villous. Leaves palmately lobed, petiole 3–11 cm, yellow-brown pubescent, leaf blade broadly ovate to surrounded, 5–17×7.5–19 cm, abaxially sparsely pubescent and sandy glandular, adaxially sparsely pubescent to subglabrous, base cordate, margin entire or minutely denticulate, apex acute. Inflorescences conical to rounded thyrses, 15–26×16–22 cm; peduncle long; bracts ovate lanceolate to ovate, bractlets linear. Calyx 7 mm, deeply 5-lobed, dotted, lobes usually lanceolate 5 mm. Corolla red to orange, tube 1–1.5 cm, dotted, outside pubescent, inside subglabrous, lobes oblong to ovate, spreading, stamens, and style 4 as long as corolla tube. Fruit-drupes globose, 5–9 mm in diameter [9].

The presence of phenolic compounds, carbohydrates, glycosides sterols, tannins, sugars, etc., was noticed and the presence of these secondary metabolites confirms that the plant can be used for pharmaceutical manufacturing and drug discovery [10]. *C. paniculatum* is mainly found in Andaman and Nicobar Islands, Karnataka, Kerala, Tamil Nadu, Uttar Pradesh, and West Bengal of India (http://www.indianbiodiversity.org/species/slow/266140).

METHODS

The healthy leaves and flowers of *C. paniculatum* were collected from the Western Ghats region of Kodagu district during October 2014 (Fig. 1). The plant specimen was identified and authenticated by Dr. K.K. Sampathkumara, Taxonomist, Davanagere, Karnataka, India, during October 2014 and a herbarium specimen has been deposited in the Department of Studies in Botany Herbarium Collection of the University of Mysore with the accession number UOMCP92. The collected flowers and leaves were separated carefully and washed under running tap water and dried under shade. The shade dried plant materials were

ground into a fine powder using a mixer. The weight of the sample was recorded and preserved in a polythene zip lock cover for the further use. The plant extracts were prepared using ethanol, hexane, and distilled water separately.

Phytochemical screening

Qualitative phytochemical analysis of the crude powder and different solvent extracts was determined using standard procedures [11,12]. The extracts were tested qualitatively for the presence of phytochemical constituents such as tannins, saponins, terpenoids, flavonoids, alkaloids, steroids, cardiac glycosides, phlobatannins, anthraquinones, and reducing sugars.

Evaluation of antioxidant activity in leaf and flower extracts

The antioxidant activity in leaf and flower extracts of *C. paniculatum* L. was determined by three assays, estimation of total phenolic content, reducing power assay, and radical scavenging activity 1,1-diphenyl-2-picrylhydrazyl (DPPH) using standard procedure.

Estimation of total phenolic content

Total phenolic content in the extracts was determined with Folin–Ciocalteu reagent using gallic acid as a standard as per the procedure of Volluri *et al.* [13]. Various concentrations of the standard gallic acid (1.0 µg/ml) ranging from 5 to 25 µg/ml were prepared. One milligram of hexane and ethanol extracts is weighed and dissolved in respective solvents up to 1 ml. Solution of 1 mg/mL of different solvent and aqueous extracts was prepared in various concentrations ranging from 20 to 100 mg/ml. 0.5 ml of FC reagent was added and 1 ml of Na_2CO_3 (20 %, w/v) was added. The tubes were kept for 45 min in dark condition. The absorbance was measured by 765 nm using UV–Vis spectrophotometer. The absorbance of standard as well as the test samples was recorded and was plotted and expressed in terms of gallic acid equivalence (µg GAE/g of extract).

Evaluation of reducing power assay

The reducing power of the material extracts was determined by the procedure of Yen and Chen [14] with some modifications. Butylated hydroxyl toluene (BHT) was taken as standard. For preparing a sample for reducing power, BHT, leaf, and flower extracts were dissolved in 1 mL of methanol solvent. The BHT concentration ranging from 5 to 25 µg/ml and sample concentration ranging from 20 to 100 µg/mL, phosphate buffer (480–400 ml), and potassium ferricyanide (500 ml) were added to the sample. The mixture was kept it in water bath for 20 min incubation at 50°C. After incubation, trichloroacetic acid was added to the mixture, which was then centrifuged at 300 rpm for 10 min. Then, equal volume of distilled water was added to the mixture followed by 0.5 ml of ferric chloride solution. The absorbance was measured at 700 nm.

Evaluation of radical scavenging assay

The radical scavenging activities of the all extracts were determined by the procedure of Pannangpetch *et al.* [15] using DPPH. Different aliquots of standard ascorbic acid (5–25 µg/ml) and extracts of plant source (20–100 µg/ml) were taken. To this, 2 mL of DPPH were added and the tubes were kept in dark condition for incubation at room temperature for 20 min. The absorbance was measured at 517 nm and the percent radical scavenging was calculated based on the extent of reduction in the color.

The percent radical scavenging activity was calculated as follows:

$$\text{Percent radical scavenging activity} = \frac{Ac - As}{Ac} \times 100$$

Where, Ac = absorbance of control and As = absorbance of test sample.

RESULTS

Phytochemical analysis was conducted for the flower and leaf extracts of *C. paniculatum* L. The hexane extract of (non-polar solvent) flower showed the presence of terpenoids (Fig. 2). The ethanol extract of flower showed the presence of alkaloids and terpenoids. Alkaloids were present only in the ethanol extract of flower. The aqueous extract of flower and leaf showed (polar solvent) the presence of saponins and terpenoids. The flowers and leaves were positive for the presence of terpenoids. Aqueous extracts of both leaf and flower showed the presence of saponins (Fig. 2).

The flower and leaf extracts of *C. paniculatum* L. showed the presence of three phytochemicals, they are saponins, alkaloids, and terpenoids. Terpenoids were commonly present in all the extracts of flower and leaf that are in both polar solvents and in non-polar solvents. The presence and absence of phytochemical constituents are represented in Table 1.

Estimation of total phenolic content

The total phenolic content of flowers and leaf extracts of *C. paniculatum* was determined by Folin–Ciocalteu method with gallic acid as standard. The phenolic concentration of the extract was expressed in milligram of standard gallic acid equivalents per gram of extract (µg GAE/g). The aqueous extract of flower contained 104.7 µg GAE/g of total phenolics. The ethanolic extract of flower showed 100.6 µg/g GAE of total phenolics and hexane extract contained 142.6 µg GAE/g (Fig. 3a). The aqueous extract of leaf contained 110.0 µg GAE/g of total phenolics (Fig. 3b). A good correlation was observed in the ethanol (R^2 = 0.991) and aqueous extracts of flower (R^2=0.975).

Estimation of reducing power assay

The reducing power of leaf and flower extracts of *C. paniculatum* was determined [14] with some modifications. BHT was taken as the standard. In the aqueous extract of flower, high reducing power was found which was followed by ethanol and hexane extracts (Fig. 4a). In the leaf, reducing power assay was performed using aqueous extract only (Fig. 4b) and it showed good redox potential.

Estimation of radical scavenging activity using DPPH

The percentage of radical scavenging activity in flower extracts of *C. paniculatum* ranged from 0.13% to 58.53% µg/ml. The high percentage of radical scavenging activity was found in the ethanolic extract (58.53% µg/ml) and aqueous extract (51.83% µg/ml) of

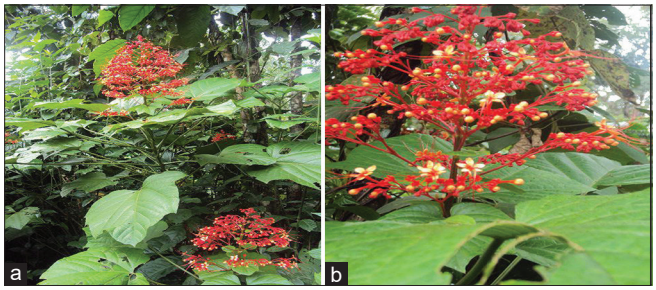

Fig. 1: (a) Habit of *Clerodendrum paniculatum* L. (b) Close-up of the inflorescence

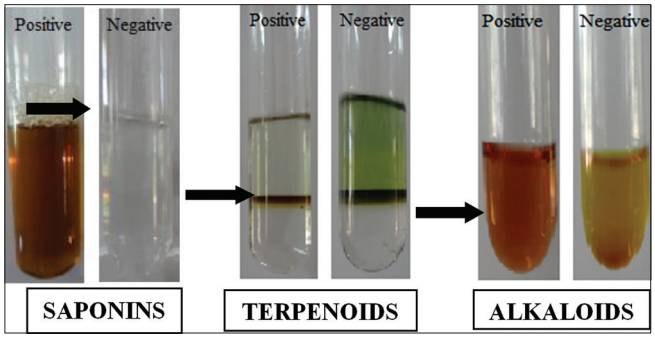

Fig. 2: Phytochemical tests for the plant extracts

flower which is followed by hexane extract (3.40% µg/ml). The IC_{50} values of ethanol, aqueous, and hexane extracts of flower were found to be 3.57, 61.98, and 400.15 µg/ml, respectively (Fig. 5a). In leaf, the radical scavenging assay was performed only using aqueous extract and the high percentage of radical scavenging activity was detected in leaf that is 92% µg/ml. The IC50 value of aqueous extract in leaf was 1.303 µg/ml (Fig. 5b).

DISCUSSION

An antioxidant is a substance and when it is present at low concentrations, it gradually delays or prevents oxidation of cell content such as proteins, lipids, carbohydrates, and DNA. In nature, we find many types of naturally occurring antioxidants which differ in their composition, physical and chemical properties, mechanisms, and site of action [16].

Reducing power assay was based on the principle that is substances which have reduction potential react with potassium ferricyanide (Fe 3+) to form potassium ferrocyanide (Fe2+), then it reacts with ferric chloride to form a ferric ferrous complex which has a maximum absorption at 700 nm [17]. Potassium ferricyanide + Ferric chloride → Potassium ferrocyanide + Ferrous chloride.

The reducing power assay was performed which revealed that the aqueous and ethanol extracts of *C. paniculatum* showed high reducing

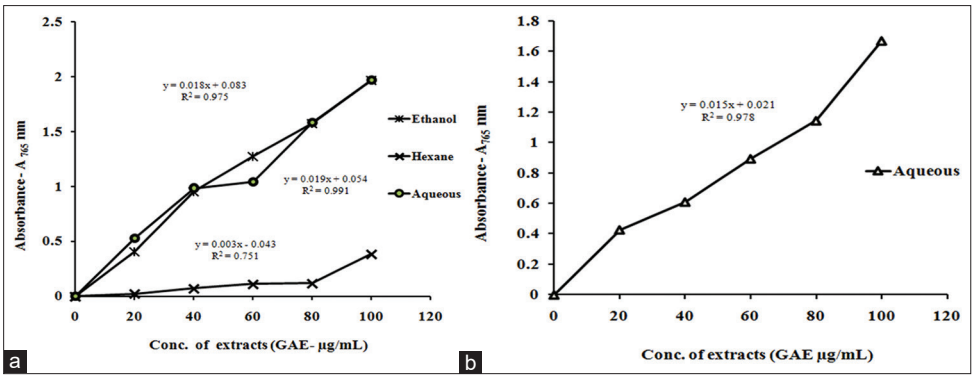

Fig. 3: (a and b) Total phenolic content of flower and leaf extracts of *Clerodendrum paniculatum*

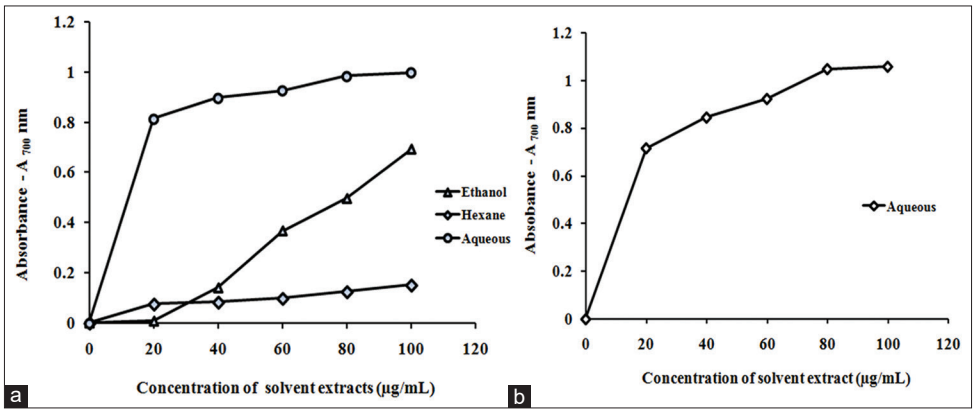

Fig. 4: (a and b) Reducing power of flower and leaf extracts of *Clerodendrum paniculatum*

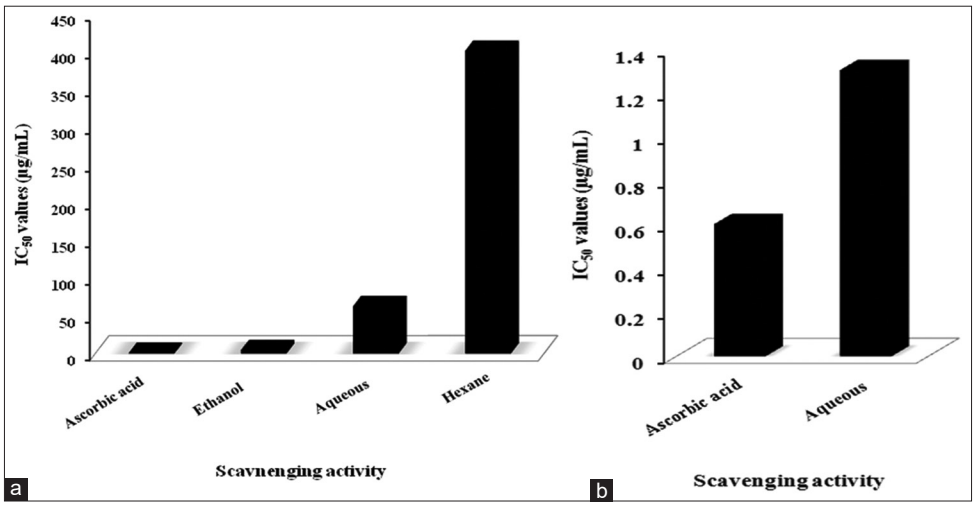

Fig. 5: (a and b) IC_{50} values of *Clerodendrum paniculatum* flower and leaf extracts for radical scavenging assay

Table 1: Phytochemical screening using the solvent extracts of leaves and flowers of *Clerodendrum paniculatum* L.

Phytochemical tests	Flower extracts			Leaf extract
	Aqueous	Ethanol	Hexane	Aqueous
Saponins	+	−	−	+
Tannins	−	−	−	−
Alkaloids	−	+	−	−
Cardiac glycosides	−	−	−	−
Terpenoids	+	+	+	+
Steroids	−	−	−	−
Flavonoids	−	−	−	−
Anthraquinones	−	−	−	−
Phlobatannins	−	−	−	−
Reducing sugars	−	−	−	−

"+": Indicates the presence and "−": Indicates the absence of phytochemicals

potential (0.693 µg/ml and 0.998 µg/ml). The aqueous leaf extract (1.058 µg/ml) also revealed good redox potential when compared to the standard BHT.

The DPPH free radical scavenging activity is one of the simple and rapid methods to determine the antioxidant activity which was determined by spectrophotometrically. 1,1-diphenyl-2-picrylhydrazyl is a stable free radical. It indicates deep violet color and turns to yellow color when the plant extract is scavenged. Thus, the DPPH assay uses this character to show free radical scavenging activity. Between an antioxidant (H-A) and DPPH, the scavenging reaction can be written as,

(DPPH)+(H-A)→DPPH-H+(A)

When an antioxidant reacts with DPPH, it reduces the DPPH to DPPH-H (diphenyl hydrazine), which leads to the decrease in absorbance [18]. Hence, the degree of discoloration indicates the scavenging potential of the antioxidant compounds or an extract is mentioned in terms of hydrogen donating ability.

CONCLUSION

The present study showed *C. paniculatum* to be an important medicinal plant, since the flower and leaves showed good antioxidant activity. Thus it may use in the treatment of diseases and may also use in the preparation of natural or herbal drugs due to the presence of antioxidants. Further studies have to be conducted, to determine the phytochemicals responsible for their antioxidant activity.

ACKNOWLEDGMENTS

The authors thank the Chairman, DOS in Botany for providing the necessary facilities.

AUTHORS' CONTRIBUTIONS

DRH collected the plant specimen and performed the phytochemical analysis, DM estimated the total phenolic content in the extracts, RGN assayed for the antioxidant assays, and MSN is responsible for the overall presentation of the manuscript in its final form.

FUNDING

There is no funding involved in this study.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

1. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. Afr J Biotechnol 2008;7:1797-806.
2. Bhutani KK, Gohil VM. Natural products drug discovery research in India: Status and appraisal. Indian J Exp Biol 2010;48:199-207.
3. Evans WC. Trease and Evans Pharmacognosy. 15th ed. London: W. B. Saunders; 2002. p. 137-45.
4. Wadood A, Ghufran M, Jamal SB, Naeem M, Khan A, Rukhsana G, *et al*. Phytochemical analysis of medicinal plants occurring in local area of Mardan. Biochem Anal Biochem 2013;2:144.
5. Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K. Methods for testing antioxidant activity. R Soc Chem 2001;127:183-98.
6. Shrivastava N, Patel T. *Clerodendrum* and healthcare: An overview-Part II phytochemistry and biotechnology. In: Medicinal and Aromatic Plant Science and Biotechnology. United Kingdom: Global Science Books; 2007. p. 209-10.
7. Rajendran A, Daniel P. The Indian Verbenceae: A Taxanomic Revision. Dehradun: Bishen Singh Mahendra Pal Singh; 2002. p. 1-2.
8. Shrivastava RC, Choudhary RK. Species diversity and economic importance of the family Verbenaceae in Arunachal Pradesh. Bull Arunachal For Res 2008;24:1-21.
9. Shou-liang C, Gilbert MG. Flora of China (Verbenaceae). Vol. 17. Beijing: Science Press; 1994. p. 1-49.
10. Praveen M, Radha K, Kumar RH, Padmaja V, Mathew A, Kumar AP. Preliminary phytochemical, antimicrobial and toxicity studies on *Clerodendrum paniculatum* L. leaves. Hygeia J Drug Med 2012;4:41-50.
11. Harborne J. Methods of plant analysis. In: Phytochemical Methods. Berlin: Springer; 1984. p. 1-36.
12. Trease GE, Evans WC. Phytochemicals. In: Pharmacognosy. London: Saunders Publishers; 2002.
13. Volluri SS, Bammidi RS, Chippada CS, Vangalpati M. *In vitro* antioxidant activity and estimation of total phenolic content in methanolic extract of *Bacopa monniera*. Indian J Pharmacol 2011;4:381-6.
14. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agric Food Chem 1995;43:27-32.
15. Pannangpetch P, Laupatarakasem P, Kukonviriyapan V, Kukongriyapa C, Kongyinayose B, Aromdee C. Antioxidant activity and protective effect against oxidative hemolysis of *Clinacanthus nutans* (Burm.f). Lindau. Songklanakarin J Sci Technol 2007;29:1-9.
16. Naik SR. Antioxidants and their role in biological functions: An overview. Indian Drugs 2003;40:501-8.
17. Bhalodia NR, Pankaj BN, Acharya RN, Shukla VJ. Evaluation of *in vitro* antioxidant activity of flowers of *Cassia fistula* L. Int J Pharma Tech Res 2011;3:589-99.
18. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid content of some selected Iranian medicinal plants. Afr J Biotechnol 2006;5:1142-5.