



E-ISSN: 2278-4136

P-ISSN: 2349-8234

www.phytojournal.com

JPP 2021; 10(4): 390-394

Received: 14-05-2021

Accepted: 18-06-2021

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Antioxidant properties of *Clerodendrum* species found in north east India: A review

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DOI: <https://doi.org/10.22271/phyto.2021.v10.i4e.14186>

Abstract

The popularity of herbal medicine is increasing very rapidly among the people due to awareness of harmful side effects and adverse reactions caused by synthetic compounds. There are about 580 species of *Clerodendrum* which belongs to the family Verbenaceae. Its widely distributed in the tropical regions of the world mostly in the south east Asia. *Clerodendrum* is an important locally used medicinal plant. It is one of the essential herbs. The leaves *Clerodendrum* species found in North eastern part of India forms an important source of diet. Various plant parts such as root and leaf extracts of *C. phlomidis*, *C. petasites*, *C. serratum*, *C. trichotomum*, *C. chinense* and *C. indicum* have been used for the treatment of hypertension, diabetes, rheumatism, asthma, coughs, skin diseases, vermifuge, febrifuge, malaria, inflammatory diseases etc. *Clerodendrum* genus has also been widely explored for various biological activities to know its potential effect towards pharmaceuticals one of which is anti-oxidant property. In the present review the antioxidant property of *Clerodendrum* at different concentrations in the DPPH radical scavenging assay, is studied.

Keywords: *Clerodendrum*, north east India, antioxidant

Introduction

Human beings have depended on plants for survival since ever. Plants are not only the major source of food and shelter but also for the medicine to cure illness [1]. Chemicals extracted from different flora have demonstrated antiseptic, anti-oxidant, anti-fungal, anti-carcinogenic, analgesic, and insecticidal properties [3]. Chemical compounds responsible for therapeutic characteristics are generally called secondary metabolites [2]. Oxidative stress is believed to be a primary factor in various diseases as well as in the normal process of aging [4-5]. Free-radicals and reactive oxygen species are well known for initiating cellular and tissue pathogenesis leading to various human diseases such as cancer, atherosclerosis, inflammatory disorders and cardiovascular diseases. Most common cause of death in the industrialized countries is due to cardiovascular diseases. The beneficial effects of phytochemicals are associated with a multitude of biological activities, which includes antioxidant and free radical scavenging properties [6].

Clerodendrum philippinum which also known as Chinese glory flower is a perennial shrub (50-120 cm) tall. Branchlets are nearly four -angled to round, velvet-hairy when young, becoming hairless. Leaf-stalks are 3-17 cm long, velvet-hairy to woolly; leaf blade broadly relate to somewhat-heart-shaped (9-22 cm x 8-21 cm), below velvet-hairy on veins and with several large glands near base; above bristly; base flat, broadly flat, or somewhat heart-shaped, margin sparsely irregularly toothed and tip tapering. Flowers are born at branch-ends, in dense corymb-like cymes. Flower-cluster-stalk is woolly; bracts lance shaped (1.5-3 cm); velvet-hairy, with several large glands. Flowers are single or double and fragrant. Sepal-cup is (1.5-2.5 cm) deeply five-lobed, velvet-hairy, with several large glands; sepals linear-lance shaped or lance shaped. Flowers are white, pinkish, or red, lobes elliptic or ovate. Chinese glory bower is native to Assam, China, East Himalaya, Jawa, Myanmar, Nepal, Philippines, Sulawesi, Sumatera, Thailand, Vietnam [7].

Various different species from this genus have been used in almost every traditional systems of medicine by different tribes in many countries like China, India, Japan, Korea and Thailand [8]. Different plant parts such as leaf and root extracts of *C. phlomidis*, *C. petasites*, *C. serratum*, *C. trichotomum*, *C. chinense* and *C. indicum* have been used for the treatment of hypertension, diabetes, rheumatism, asthma, coughs, skin diseases, vermifuge, febrifuge, malaria, inflammatory diseases etc. [9, 10-14].

Plant species such as *C. indicum* is used for the treatment of coughs, vermifuge, febrifuge, malaria, venereal infections, skin diseases, elephantiasis, rheumatism, tropical burns etc. [15] *C. phlomidis*, *C. colebrookianum*, *C. calamitosum* and *C. trichotomum* have been used for anti-diabetic, anti-hypertensive and sedative properties [11, 13, 15].

Anti-oxidant Activity

Oxygen Species which are reactive (ROS) and many of the free radicals are natural by-products, which are constantly generated *in vivo*, both by “accidents of chemistry” and for specific metabolic purposes [16]. ROS has a high reactive potential and is responsible for many of the human diseases like diabetes, cancer, viral infections, cardiovascular diseases, and inflammations, and also damages the biological molecules like DNA, lipids, and proteins [17]. The antioxidants which are present in the medicinal plants minimise the formation of ROS [18]. The ethanolic extract of aerial parts of *Clerodendrum serratum* showed good antioxidant properties against 1,1-diphenyl, 2-picryl hydrazyl (DPPH) and nitric oxide radical whereas *Clerodendrum serratum* roots extract showed considerable antioxidant properties against DPPH [19]. The ethanolic extract of the leaves of *Clerodendrum infortunatum* Linn showed significant antioxidant activity against DPPH-free radical scavenging activity, reducing power assay and scavenging of hydrogen peroxide [20]. The methanolic extract of leaves of *Clerodendrum inerme* showed higher free radical and antioxidant activity [23]. The ethanolic extract of roots of *Clerodendrum viscosum* showed notable scavenging of the cation radical, nitric oxide radical, ferric-ion radical and DPPH. However, the aqueous extract showed moderate antioxidant activity [21]. The ethanolic extract of roots of *Clerodendrum phlomidis* showed best free radical scavenging activity compared to other three extracts *viz.* petroleum ether, chloroform, and ethyl acetate [22].

Chahal and Sarin [24] *et al.*, (2014) described that *Clerodendrum* genus is a valuable source of natural products such as bioactive compounds and antioxidants which are very advantageous for human health. Two conventionally valuable plants of spp. *Clerodendrum* (*C. inerme* and *C. phlomidis*) were tested for their antioxidant potential. *Clerodendrum* is found in various parts of Asia. Three solvents (Ethanol, Water, and Ethyl acetate) were used separately for extraction of dehydrated powder. The extract was separated for its prospective antioxidant potential using DPPH Radical inhibiting potential. The outcomes determined that the inhibiting potential of *C. inerme* shoot is greater than *C. phlomidis* shoot. Ethyl acetate and Ethanol both predicted the better potential for radical inhibiting activity and could be used as original parts of active antioxidant managers.

Khan [25] *et al.*, (2013) stated that *Clerodendrum inerme* also has antioxidant properties. The antioxidant activity of *C. inerme* shoot was measured by calculating whole phenolic, whole flavonoid, DPPH free radical, decreasing control and proportion of linoleic acid peroxidation. Whole phenolic insides and whole flavonoid insides amount were calculated as (0.89-1.89 mg/100 g GAE) and (3.18-5.68 mg/100 g CE) correspondingly. The IC₅₀ and average self-consciousness of linoleic acid peroxidation amount of *C. inerme* remove vary from (24.1-81 µg/mL) and (41-72 %) correspondingly. Peroxide significance, free fatty acid, and *para*-anisidine amounts were also realized by using canola oil as oxidation substrate. Results showed that methanol extract of *C. inerme* shoot gives improved antioxidant activity. The cytotoxicity values were concluded from hemolytic inactivity of plant

mines and checked on the human erythrocytes (RBCs) *in vitro*.

Devi [26] *et al.*, (2012) have ascribed the comprehensive study of pharmacognostic on the foliage of *C. inerme* and have revealed its role in Ayurveda and Siddha medication systems. This study was based on its physicochemical, macroscopic and organoleptic characteristics. Polyphenols like phenolic acids, flavonoids etc., triterpenoids, steroids, and sugars are present in *C. inerme*. Isolation of phenolic acid had been done and marker this compound as well as also recognized as Ursolic acid. By estimating free radical scavenging potential, anti-oxidant potential of Ursolic acid, as well as alcoholic extract, had been determined by following the assay of DPPH scavenging assay. Extract of plant presented better antioxidant potential which is analogous to that of Butylated Hydroxy Toluene that had been employed as a standard.

Gokani [27] *et al.*, (2011) had been studied the essence of *Clerodendrum phlomidis* (Verbenaceae) and revealed that Arni/Agnimantha, is its vital constituent. Methanolic extract of the roots was evaluated for its *in-vitro* antioxidant potential with the anti-radical, superoxide scavenging, anti-lipid peroxidation, hydroxyl radical scavenging and nitric oxide scavenging, assesses. The active part of the plant was consistently found to have anti-oxidant potential. *C. phlomidis* is recognised as a prospective origin of ordinary antioxidant.

Amirtharaj and Saravanan [28] (2010) analyzed the antioxidant potential of aerial extracts of *Clerodendrum inerme* which belongs to the family verbenace. Anti-oxidant potential of chloroform mixture of *Clerodendrum inerme* (CI) aerial portions was examined for its free radical inhibiting possessions in different *in vitro* activities as 1, 1 diphenyl-2-picryl hydrazyl, nitric oxide, decreasing command to examine and hydrogen peroxide radical treatment activity. Various amounts of the chloroform extract of CI were extracted and examined. The IC₅₀ values of the chloroform extract of CI contended with ascorbic acid (Standard) and it was noted that the extract gives important meditation used free radical inhibiting possessions in all the methods. Examined values have chloroform extract of CI hold *in vitro* free radical inhibiting potential. The answers rationalize the presence of this plant in the controlling of antioxidant potential.

Gurudeeban [29] *et al.*, (2010) revealed that *Clerodendrum inerme* is a medicinal plant conventionally used as an abortifacient to cure constipation, odema, bacterial infections, cancer, and diabetes. First phytochemical separation of the plant examined that the occurrence of huge quantity of phenolic compounds and flavonoids. Following quantification showed the existence of 0.74% (m/m) phenolic (calculated as gallic acid) and 0.13% (m/m) flavonoids considered as catechin per each 100 grams of new mass. The existence of phenolic compounds encouraged to lead this work to assess its antioxidant potential. Methanolic shrubberies cutting of *Clerodendrum inerme* was separated to examine its maximum antioxidant and free radical inhibiting potential of shrubberies extract. 2500 mg ml⁻¹ of antioxidants was detected.

Chourasiya [30] *et al.*, (2010) determined the antioxidant levels of methanolic and petroleum ether extract of *Clerodendron inerme*.

The methods used were DPPH (1, 1-diphenyl-2- picryl hydrazyl) examine, hydroxyl inhibiting analysis and the lipid peroxidation analysis. The *Clerodendron inerme* methanol extract (CIME) was most abundant in the DPPH test with an IC₅₀ value of 19.20 ± 0.27 µg/mL. The hydroxyl radical inhibiting potential of the petroleum ether extract disclosed the extreme potential 69.28±2.1% at 100µg/ml. In the lipid

peroxidation analysis, the highest amount was that of methanolic extract *i.e.* 36.38±1.3%.

Gouthamchandra^[31] *et al.*, (2010) analyzed three consecutive extracts of *Clerodendrum infortunatum* L shrubberries for their activity as antioxidant 1, 1-diphenyl 2 picrylhydrazyl (DPPH) model. The inhibiting potential of ethanol extract was examined to be maximum when analyzed with petroleum ether and chloroform extracts. So, it was chosen as a standard to test the potential candidates using *in vivo* and *in vitro* methods. The antioxidant properties encouraged oxidative pressure in rats considerably high. Further, to authenticate the conventionally therapeutic claim wound healing potential of plant extract also tested. Between the three extracts used, the petroleum ether and ethanol extract showed the significant results.

Bhujbal^[32] *et al.*, (2009) studied the antioxidant activity of ethanolic extract obtained from roots of *Clerodendrum serratum* at different concentrations in the DPPH radical inhibiting methods; FRAP method (Ferric Reducing Antioxidant Power) and the Hydrogen peroxide radical inhibiting method. In the last decade, herbal and Ayurvedic medicines were very popular due to their medicinal and economic advantages. Due to their worldwide acceptance and effectiveness, the use of plant extract throughout the world increased. Over the time, appropriate scientific methods have been developed to become the standards for recognition of herbal health rights.

Sannigrahi^[33] *et al.*, (2009) evaluated the antioxidant activity of methanolic extract *Clerodendrum infortunatum* Linn. (MECI), used in the Indian native medicine for wide uses, was examined. The antioxidant activity was calculated using various organized *in vitro* antioxidant tests. MECI was found to have a lot of polyphenolics and it holds important free radical inhibiting potential. The greater potential was probably due to the highest quantity of polyphenolics and flavonoids in it.

Chae^[34] *et al.*, (2007) studied the antioxidant activity of isoacteoside, separated from *Clerodendron trichotomum* (Verbenaceae), This compound inhibited intracellular sensitive oxygen species (ROS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, and prohibited lipid peroxidation. This radical inhibiting potential of isoacteoside sheltered cell viability of Chinese hamster lung fibroblast (V79-4) cells exposed to hydrogen peroxide (H₂O₂). Furthermore, isoacteoside decreased the cell apoptosis caused by H₂O₂. However, isoacteoside amplified the cellular antioxidant enzyme action including superoxide dismutase (SOD) and catalase (CAT). Altogether these results examined that isoacteoside, separated from *C. trichotomum*, have antioxidant potentials.

Methods

Antioxidant activity

The antioxidant activity of samples were determined using standard methods. VC and BHT were used as positive standards in the radical-scavenging assays. Gallic acid was considered as a standard in the ferric reducing power assay. Ethylene diamine-tetra acetic acid (EDTA) was used as a standard for the ferrous ion-chelating activity assay.

DPPH radical-scavenging activity: The DPPH radical scavenging activity was estimated by mixing 0.1 ml of the extract with 3.9 ml of 60 µM solution of DPPH in ethanol. After 30 min of reaction in the dark condition the absorbance

was measured at 517 nm. The inhibition percent and 50% inhibition (IC₅₀) values of DPPH radicals were calculated^[34].

ABTS radical-scavenging activity:^[34] This method was used to determine the ABTS radical-scavenging capacity. A small portion of extract (0.1 ml) was added to 3.9 ml of ABTS radical solution. The mixture was reacted for 30 min in dark condition and absorbance at 734 nm was measured. The inhibition percent and IC₅₀ values of the extracts for ABTS radical were calculated.

Superoxide radical-scavenging activity: The superoxide radical scavenging effects were examined^[35]. About, 1 ml of the extract was taken and added to 1 ml of 50 µM NBT solution, 1 ml of 468 µM NADH, and a 1 ml of the portion of 60 µM PMS reaction mixture. After 5 min, the absorbance was read at 560 nm. The inhibition percent and IC₅₀ values were calculated.

Hydroxyl radical-scavenging activity: The scavenging of hydroxyl radicals was determined following the method of Guo *et al.*^[35]. The reactions were performed with 0.3 ml of 20 mM sodium salicylate, 2.0 ml of 1.5 nM FeSO₄, 1.0 ml of sample, and 1.0 ml of 6 mM H₂O₂. The reaction mixture was incubated for 1 hour at 37°C. Then absorbance was measured at 510 nm. The inhibition percent and 50% of absorbance (EC₅₀) were calculated.

Reducing power: The reducing power of the samples were assayed using the method of Guo *et al.*^[36]. Briefly, 1 ml of extract solution was added to 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 1%, 2.5 ml potassium ferri-cyanide. After 20 min, 2.5 ml of 10% tri-chloro acetic acid (TCA) was added, and after that the mixture was centrifuged at 2800-3000 rpm for about 10 min. The upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride, and after 10 min, the absorbance was measured at 700nm. The EC₅₀ values were calculated from the graph of inhibition percentage against extract concentration.

Ferrous ion-chelating activity: About 1 ml portion of extract was added to a solution of 100 µL of FeCl₃ (2.0 mM), 3.7 ml of distilled water and 200 µL of ferrozine (5.0 mM). After 20 min, the absorbance was checked and recorded at 562 nm. The inhibition percent and IC₅₀ values were calculated.

Total phenolic content (TPC): The Total Phenolic Contents in the samples was determined by colorimetric method, Zhou *et al.*^[34]. Folin-Ciocalteu reagent (2 ml) was added to 2 ml of diluted extract. After 3 min, 750 µL of sodium carbonate anhydrous solution (7.5%, w/v) was added, and the mixture was adjusted to 10 ml with distilled water. After 2 h, the absorbance was checked at 765 nm. Calibration curves were constructed with gallic acid as the standard at concentrations ranging from 0–100 µg/ml.

Total flavonoid content (TFC): The reaction mixture consisted of 1.0 ml of extract, 0.3 ml of 5% sodium nitrite and 4 ml of 60% ethanol. About 6 min later, 0.3 ml of 10% aluminium nitrite was added. 6 mins later, 4 ml of 1M sodium hydroxide solution was added. Then, the volume was brought to 10 ml using distilled water, and the absorbance was measured at 510 nm. The TFC was calculated and is expressed as rutin equivalents (RE). A calibration curve was

constructed with different concentrations of rutin (15– 75 µg/ml) as a standard [35].

Conclusion

Clerodendrum forms an precious part of traditional/folk medicine in many countries like India, Japan, Korea, China and Thailand. A few works conducted on the anti-oxidant potential of *Clerodendrum* by various researchers reflects its huge potential as ethnomedicine, although deep investigation through scientific validation and experimentation is still scarce. *Clerodendrum* spp. Possesses multiple pharmacological and therapeutical activities such as anti-microbial, anti-inflammatory, anti-malarial, anti-diabetic, anti-cancer, analgesic. However, there are limited numbers of studies describing the bioactivities of *Clerodendrum* and this opens up new horizon for researchers working in this filed to explore this raw material as potent nutraceutical and pharmaceutical product.

DPPH (1,1-diphenyl-2-picryl-hydrazil) is a free radical which is stable and that accepts an electron or hydrogen radical to form a stable diamagnetic molecule. The model of scavenging the stable DPPH radical is widely used for relatively rapid evaluation of antioxidant activities compared to different other methods [32]. The reduction capability of the DPPH radical is calculated by its absorbance decrease at 517 nm, as induced by natural antioxidants [29].

Scavenging of H₂O₂ by antioxidants may be due to donation of electrons to H₂O₂, thus neutralizing it to water [30]. According to phytochemical investigation it is observed that the ethanolic extract of CSR contains phenolic compounds such as flavonoids, tannins. It has been recently shown that quercetin and its glycosides exert inhibitory activity against lipid peroxidation [28]. Since luteolin and its derivate, along with quercetin and rutin, belong to the same group of compounds – flavonoids, the following antioxidative mechanism can be proposed: conjugation of the double bound in position 2,3 with C4- -carbonyl group, and also the existence of a free OH group on C5 and C7, enable the formation of chelate complexes with d-elements (Fe²⁺, Cu²⁺, Zn²⁺). The formation of a complex with Fe²⁺ prevents the production of OH₂ radicals (Fenton's reaction), which was used to evaluate the inhibitory effects. The antioxidant activity of this extract can be linked up to the high polyphenols and flavonoids content. Divers studies mentioned an implication of the polyphenols and flavonoids in the antioxidant occupation of different plants extracts [31-32]. Phenols have been shown to possess an important antioxidant job toward these radicals, which is mainly based on the redox properties of their phenolic hydroxyl groups and the structural relationships between different parts of their chemical structure.

It have been established a highly good relationship between total phenols and antioxidant activity in many plant species. antioxidant activity through the DPPH free radical scavenging action, reducing power assay and scavenging of hydrogen peroxide. The initial phytochemical study indicates the presence of flavonoids in the plant. Polyphenols such as flavonoids and tannins are the well known natural antioxidants [32]. So, the antioxidant potential of the plant may be attributed to the presence of flavonoids. The separation and identification of flavonoids present in the roots can help scholars to find new molecules which can be used as natural antioxidants. Further studies are currently infact underway to isolate and characterize the active constituents responsible for its antioxidant activity.

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