Plant Sci. Arc.
Plant Science Archives
An International Journal of Plant Biology

Original Research Article
ISSN 2518-6965
Journal Homepage: www.plantarc.com

Evaluation of in vitro antioxidant activity and DNA damage inhibition potential of *Caralluma procumbens* (Gravely & Mayur.)

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Citation: Rajani Vemula, Gayathri Pachipala, Vijaya Bhaskar Reddy Pichili, and Kiranmai Chadipiralla (2026). Evaluation of in vitro antioxidant activity and DNA damage inhibition potential of *Caralluma procumbens* (Gravely & Mayur.). *Plant Science Archives.* **DOI: https://doi.org/10.51470/PSA.2026.11.2.07**

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Received 12 January 2026 | Revised 15 February 2026 | Accepted 09 March 2026 | Available Online 14 April 2026

ABSTRACT

Caralluma procumbens is a member of genus Caralluma and family Apocynaceae. C. procumbens is a perennial succulent herb that is edible in different forms and widely used in traditional and folk medicine for the treatment of various diseases and also used significantly in nutraceuticals. Although C. procumbens is known to possess high economic value, it is under explored for its pharmacological and therapeutic potential. The objective of the study to evaluate and assess the medicinal importance of one of the underexplored tropical plant species C. procumbens. Determination of antioxidant activity in aqueous and methanolic extracts of C. procumbens was performed by adapting the 2,2-diphenyl-1-picrylhydrazyl scavenging assay. DNA damage inhibition ability was evaluated by UV irradiated photolysis of H_2O_2in the presence of pET-28a plasmid with and without plant extracts. Aqueous and methanolic extracts of C. procumbens showed significant antioxidant activity as evidenced by DPPH radical scavenging activity. The IC50 values of aqueous and methanolic extracts were found to be 139.94 ug/ml and 86.91 ug/ml, respectively. Both the extracts significantly prevented the damage of pET-28a DNA. Our results suggest that C. Procumbens is endowed with high antioxidant potential and DNA damage inhibition ability. Hence this plant species could be a potential candidate for the development of phytochemical-based drugs and development of novel therapeutic strategies.

Keywords: *Caralluma procumbens, DPPH, Free radical, antioxidant activity, phytochemicals.*

1. Introduction

Oxidative stress is a condition associated with increased free radical generation along with a concomitant decrease in the counteracting antioxidant mechanisms. Free radicals usually contain an unpaired electron in the outer valence shell. Due to the presence of the unpaired electron, these radicals are highly reactive in nature and hence attack the neighbouring molecules to gain the paired electron status and to achieve stability there by stimulating a cascade of chain reactions which further result in increased production of free radicals. Reactive oxygen species include hydroxyl radicals (OH^-), superoxide anions (O_2^-), and peroxyl radicals (ROO^-) while reactive nitrogen species include radicals such as nitric oxide (NO^-), nitrogen dioxide (NO_2^-) and peroxynitrite ($ONOO^-$). In healthy conditions increased free radical generation is corrected by the cellular endogenous antioxidant defensive mechanisms. However, when cellular defensive mechanisms succumb to the increased free radical generation, it can lead to a condition called as oxidative stress that can result in lethal damage of membranes, lipids, and several other biomolecules. Oxidative stress has been implicated in various diseases such as cancers, hepatic diseases, neurological disorders, diabetes, kidney disorders, cardiovascular diseases, osteoporosis, inflammatory disorders and DNA damage etc [1-2].

Antioxidants play an important role in prevention of cellular damage, usually by acting as reducing agents. Normally the antioxidants from the diet such as vitamins, natural polyphenols, carotenoic acids, essential metals etc., try to help in suppression of the free radicals. However, when the dietary antioxidants fail to re-establish the red-ox homeostasis within the cell, extracellular exogenous antioxidant supplements could help in prevention of the oxidative damage. Earlier studies have shown that supplementation of good amounts of exogenous antioxidants significantly reduced the free radical mediated diseases [3]. Such supplementation has also shown to attenuate the degree of free radical induced oxidative damage in various chronic diseases [4].

In the recent years there has been a great deal of focus on natural sources of antioxidants due to their growing importance in the field of medicine. Plants have been a good source of a variety biologically active compounds that possess medicinal, therapeutic and pharmacological applications. Plant derived natural products such as phenols, tannins, flavonoids, anthocyanins, alkaloids, phytoestrogens etc have been identified to exhibit significant protection against various free radical-mediated diseases [5-6].

Caralluma procumbens is a member of genus *Caralluma* classified under the family Apocynaceae. *C. procumbens* is a perennial succulent herb that is edible in different forms such as preparation of curries and pickles. The genus *Caralluma* consists of 13 species and seven varieties within India among which 11 species are reported to be endemic to Southern parts of India [7].

Species of *Caralluma* have been widely used in traditional and folk medicine for treatment of various diseases such as cancer, diabetis, obesity, skin damage, skin infections, ulcers, inflammatory diseases, antidote [8-9]. Further *Carallumas* have also been significantly used in nutraceuticals [10]. Although *C. procumbens* is known to possess high economic value, it has been underexplored for its pharmacological and therapeutic potential.

DPPH is a crystalline, dark powder that consists of stable free radical and is used for evaluating free radical scavenging activity of antioxidants. DPPH upon gaining a proton from its neighbouring antioxidant molecule gets reduced and forms yellow colour. Intensity of the colour is determined by measuring the absorbance at 517nm. The reduction potential of DPPH radical is determined by the decrease in absorption at 517nm [11]. Ascorbic acid was used as the reference standard. Upon addition of the plant extracts the stable DPPH radical was reduced to yellow colour Diphenyl Picryl Hydrazine.

Ultraviolet radiation is a part of the electromagnetic spectrum which is typically categorised into UVC (200-280 nm), UVB (290-320 nm), and UVA (320-400 nm). Only UVA reaches Earth's surface completely, UVB is partially filtered and UVC is absorbed in the stratosphere. Exposing with these radiations can damage the cellular DNA. DNA bases directly absorb UVB rays as a result photoproducts like as cyclobutene pyrimidine dimers (CPDs) were induced. Therefore, leading to mutagenesis and carcinogenesis [12]. By irradiating cells or DNA under controlled doses of UV rays as well as treating with the plant extract can reveal the DNA damage inhibition potential of the plant.

The objective of the current study was to investigate *Caralluma procumbens* stem extracts, antioxidant potential by DPPH radical scavenging assay and the DNA damage inhibition activity against UV ray treatment.

2. Materials and Methods

2.1. Plant material: *C. procumbens* plant was collected from the natural growing habitat in the rocky hills of Maruthuvamalai, Kanyakumari district, Tamil Nadu. The plant was identified and authenticated by a plant taxonomist. The plant was acclimatized, grown and maintained in the shade net house of VS University, Kakutur, Nellore.

2.2. Processing of plant: Healthy mature stems of *C. procumbens* were collected fresh and washed thoroughly with distilled water and were chopped into small pieces, shade dried at room temperature. Dried stems were powdered uniformly using a mechanical grinder. The powder (1 gram) was extracted in 80ml of methanol using Soxhlet apparatus and distilled water using boiling water bath separately. These extracts were concentrated at 40˚C under reduced pressure (72 mbar) using rotary evaporator and dried and lyophilized. Dried extracts were collected in airtight container and stored at 4°C until further use.

2.3. DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay: Aqueous and methanolic extracts of *C. procumbens* were diluted to obtain concentrations of 10, 20, 40, 60, 80, 100, 200, 400, 800 µg/ml dilutions. Two ml of each dilution was mixed with 1 ml of DPPH solution (0.2mM/ml in methanol) and mixed thoroughly. The mixture was incubated in dark at 20˚C for 40 minutes.

Absorbance was measured at 517nm using a UV–Visible spectrophotometer with methanol as blank [13]. Each experiment was performed in triplicates at each concentration. The percentage scavenging of DPPH was calculated according to the following formula.

% DPPH Radical scavenging= [(Ac-At)/Ac] × 100

Where, Ac is the absorbance of the control. At is the absorbance of test.

The IC50 (concentration providing 50% inhibition) was calculated graphically using a calibration curve in the linear range by plotting the extract concentration vs. the corresponding free radical scavenging effect.

2.4. DNA damage inhibition efficiency: The methanolic and aqueous extracts (10 µg/ml, 20 µg/ml and 50 µg/ml) of *Caralluma procumbens* were evaluated to detect the DNA damage inhibition activity. DNA damage inhibition potential was determined by photolyzing H_2O_2 and exposing to UV radiation in presence of pET-28a plasmid DNA in the presence and absence of the plant extracts. An aliquot of 1 µl of pET-28a was taken from the stock solution (200 µg/ml) and distributed into five separate microfuge tubes. Among them, 4 µl of 3% Hydrogen peroxide was added to three microcentrifuges. Following this the irradiated DNA sample was loaded and run on agarose gel electrophoresis. A total of 5 wells were loaded in the following pattern. 1. UV-treated plasmid DNA (1.5 µl); 2. Untreated plasmid DNA; 3. UV treated plasmid DNA with H_2O_2; 4. UV treated plasmid DNA with H_2O_2 and methanol extract, 5. UV treated plasmid DNA with H_2O_2 and aqueous extract, respectively. All the samples except untreated Plasmid DNA were exposed to UV light (300nm) for 10 minutes after addition of H_2O_2 [12]. Tracking dye (6 µl containing 0.25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol) was added in all the wells to track the resolution and were analyzed by gel electrophoresis on a 1% agarose gel (containing ethidium bromide) in TBE buffer (pH 8).

3. Results and Discussion

In the current study both methanolic and aqueous extracts of *C. procumbens* exhibited a dose dependent and steady free radical scavenging ability (Fig.1).

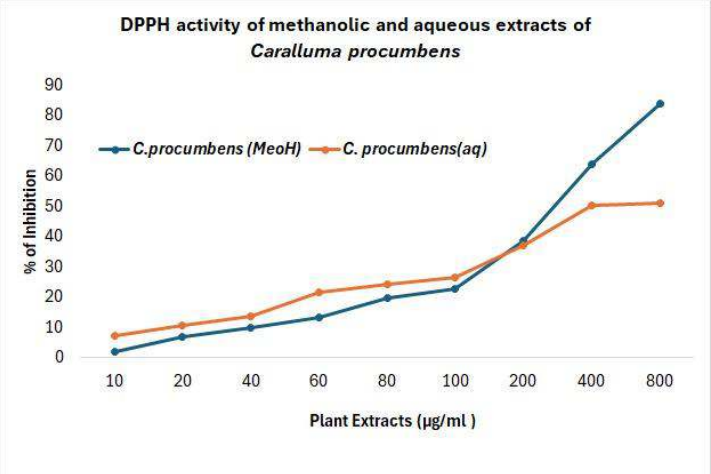

Fig. 1: DPPH activity of methanolic and aqueous extracts of Caralluma procumbens

It was observed that the methanolic extract exhibited its highest inhibition (83.81%) at 800µg/ml, while aqueous extract showed its highest inhibition (50.9 %) at 400µg/ml and remained the same at 800 µg/ml also. The IC_{50} values of methanolic and aqueous extracts were found to be 86.91 µg/ml and 139.94 µg/ml respectively. The experimental data showed that the methanolic extract of *C. procumbens* with low IC_{50} value indicating higher antioxidant potential compared to the aqueous extract. It also revealed that the extracts at all concentrations had a positive effect. Such inhibitory effect could be attributed to the presence of various flavonoids, tannins, glutathione, ascorbic acid, tocopherols in the plant extracts [14]. In consistent with these studies *Carallumas* have been reported to contain several important phytochemicals and secondary metabolites such as tannins, flavonoids and quinines, phenols, phytosteroids, and saponins [15]. Several bioactive compounds associated with antioxidant activity of *C. procumbens* were identified from our previous studies [15]. Presence of phenols and flavonoids in the herbal extracts have been reported to offer increased protection against oxidative stress induced cascades and further aid in the betterment of the health conditions [16]. Recent findings of Yada *et al*. reported an IC_{50} value of, 214.76 µg/ml in the DPPH assay for the ethanolic extracts of *C. adscendens* determining the phenolic and flavonoid concentrations [17]. In addition to this, other vital secondary metabolites like pregnane glycosides including carambelloside I and II were also reported in certain *Caralluma* species [18]. Similarly, the findings of Rehman *et al*. and Devi *et al*. demonstrating antioxidant activity in other *Caralluma* species namely *C. tuberculata* and *C. fimbriata* are in support of our findings [19-20].

DNA is quite susceptible to free radical mediated damage. In normal, healthy physiological conditions DNA is intact. However, when there is an enhanced production of free radicals, DNA gets damaged due to the interaction of free radicals with the sugar or nucleotide base, resulting in the formation of DNA adducts. It has been reported that damage caused to the deoxyribose sugars results in DNA strand breaks [21]. Such free radical induced DNA damage has been implicated in various types of diseases such as cancers, diabetes, neurological dysfunctions, mitochondrial diseases, cardiac disorders, kidney diseases, infectious and inflammatory disorders [22]. In order to study the effects of the extracts of *C. procumbens* on DNA damage, DNA inhibition assay was performed in the presence and absence of plant extracts. Methanolic and aqueous extracts of *C. procumbens* on UV induced-H_2O_2 photolysis of DNA on pET-28a plasmid was examined (Fig. 2).

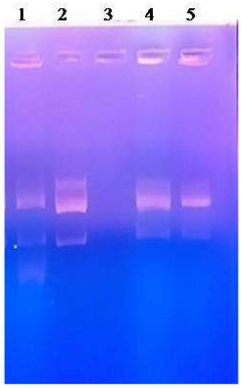

Fig.2: *Protection of plasmid DNA (pET-28a) against oxidative damage caused by UV-photolyzed H_2O_2 by methanol and aqueous extract of C procumbens plant.* 1. UV treated plasmid DNA; 2. Untreated plasmid DNA; 3. UV treated plasmid DNA with H_2O_2; 4. UV treated plasmid DNA with H_2O_2 and methanol (50 µg/ml) extract, 5. UV treated plasmid DNA with H_2O_2 and aqueous (50 µg/ml) extract respectively.

The results of the study show partial damage of pET-28a DNA upon UV exposure alone. Lane 2 shows standard intact DNA (Control), Lane 3 shows complete damage of Plasmid DNA upon UV-induced photolysis with H_2O_2. Addition of methanolic extract of *C. procumbens* exhibited complete and strong protection against UV induced photolysis of H_2O_2 (Lane 4). Similarly, the aqueous extract of *C. procumbens* also significantly attenuated the DNA damage caused by UV-induced photolysis of H_2O_2 (Lane 5). While the protective effect exhibited by aqueous extract is quite significant, its protective effect was found to be lesser than that of the methanolic extract. Although a dose dependent study was performed 50µg/ml of aqueous and methanolic extract showed the optimal protective activity compared to (10 µg/ml and 20 µg/ml). These results are in correlation with the results obtained in antioxidant activity. In both cases, the methanolic extract showed profound protective effect as compared to the aqueous extract.

Presence of high phenols as published in our earlier results [15] in *C. procumbens* could be a possible reason for the DNA damage inhibitory activity. In support of these findings earlier studies have reported a strong positive correlation between the phenol content and DNA damage inhibition [23]. Studies of Kalita *et al*. revealed that aqueous extract of *Lantana camera L*. showed significant DNA damage inhibition activity against UV induced DNA damage employing pBR322 plasmid DNA [24]. Further they have also shown presence of high amounts of phenols in the above plant extract. In another study, phenols, flavonoids and anthocyanins were shown to play a significant role in the prevention of peroxy radical induced DNA damage [25]. Similarly, studies have revealed that a ferric ion induced oxidative damage was effectively suppressed in the presence of methanolic extract of *Pothomorphe peltata* L [26]. Further oral supplementation of mice with leaf extracts of Jamun significantly reduced the radiation-mediated DNA damage in association with reduction of free radical production [27]. *Caralluma* species such as *C. fimbriata* has been shown to reduce cell proliferation by stimulating cytotoxicity in *in vitro* conditions in colon cell lines [28]. Similarly, *C. tuberculata* has been reported to induce caspase dependent apoptosis in cancer cells [29]. In another similar study, *C. tuberculata* (50-800µg/ml) exhibited 37.26% of hydrogen peroxide inhibition suggest the antioxidant potential of *Caralluma* species [30].

Recent studies on other Apocynaceae members, *Plumeria alba*, its flower essential oil [31] along with, extracts of *Allamanda cathartica* [32] and *Carissa carandas* [33] has reported better scavenging activity. Nearly 400 compounds have been isolated from the genus *Alstonia* R.Br including alkaloids (monoterpenoid indole alkaloids), triterpenes, flavonoids and phenolic acids which are responsible for anti-oxidant activity of the plants [34]. Since DNA damage is the central cause for cancers and aging, based on the results obtained from this study in conjunction with our earlier published results, the various phytochemicals present in the plant extracts of *C. procumbens* may be playing a role in mitigating two of the most lethal pathways namely oxidative stress and DNA damage.

4. Conclusion

Overall, the current study on the plant extracts proved to be valuable and advantageous and may help design phytotherapeutic medicinal drugs that can be validated for human health and welfare. However, a single isolated bioactive product from the plants might not be accounting for the exhibited activities.

There could be an interplay between several network of mechanisms playing a role in the manifestation of oxidative and DNA damage pathways.

5. Acknowledgments

We acknowledge the facilities provided by DST SERB and DST FIST, Department of Biotechnology, VSU, SPSR Nellore, Andhra Pradesh to do this work.

6. AUTHORS' CONTRIBUTIONS: All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. **FUNDING**: There is no funding to report.

8. **CONFLICT OF INTEREST:** The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS: This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY: All data generated and analyzed are included within this research article.

11. PUBLISHER'S NOTE: The views and statements presented in this article are entirely those of the authors and do not necessarily reflect the positions of the publisher, editors, or reviewers. The journal maintains a neutral stance on any jurisdictional claims related to institutional affiliations.

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