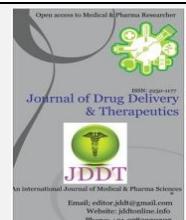


Available online on 15.09.2018 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

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Research Article

Antioxidant and antifungal activity of *Geophila repens* (L.) I. M. Johnst. (Rubiaceae)

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ABSTRACT

Objectives: The present study was conducted to investigate the potential of leaf extract of *Geophila repens* (L.) I.M. Johnst. (Rubiaceae) to exhibit antioxidant and antifungal potential.

Methods: Extraction of powdered leaf material was carried out by maceration process using methanol. Antioxidant activity of leaf extract was assessed by DPPH free radical scavenging assay and ferric reducing assay. Antifungal activity of leaf extract was determined by Poisoned food technique.

Results: Leaf extract was shown to scavenge DPPH radicals dose dependently with IC_{50} value of 51.39 μ g/ml. An increase in the absorbance on increasing concentration of leaf extract indicated ferric reducing activity. The extract showed more or less similar inhibitory activity against *Aspergillus niger* and *Bipolaris* sp. (>50% inhibition).

Conclusion: The results of the study indicate that leaf extract possesses bioactive principles with activity against oxidative damage and seed-borne fungi which are to be purified and screened for antioxidant and antifungal activity.

Keywords: *Geophila repens*, Maceration, DPPH, Ferric reducing, Seed-borne fungi, Poisoned food technique

Article Info: Received 29 June, 2018; Review Completed 09 Aug 2018; Accepted 18 Aug 2018; Available online 15 Sep 2018



Cite this article as:

Sahana B.K, Akhilesha S, Priyanka GS, Prashith Kekuda TR, Antioxidant and antifungal activity of *Geophila repens* (L.) I. M. Johnst. (Rubiaceae), Journal of Drug Delivery and Therapeutics. 2018; 8(5):268-272

DOI: <http://dx.doi.org/10.22270/jddt.v8i5.1837>

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INTRODUCTION

Free radicals such as superoxide radical, hydroxyl radical and non-radical chemical species such as hydrogen peroxide are formed in the body during normal metabolism. Excess levels of these reactive oxygen species affect biological macromolecules such as carbohydrates, nucleic acids, proteins and lipids resulting in oxidative damage which is implicated in several pathophysiological conditions such as cancer, ageing, cardiovascular diseases and neurodegenerative disorders. The onset of oxidative stress gets triggered when an imbalance between free radical generation and antioxidant defense occurs. Antioxidants are compounds which delay or inhibit the process of oxidation of other

molecules by means of inhibition of initiation as well as propagation of oxidizing chain reactions. Immense interest in plant based antioxidants is triggered due to suspected negative effects that are associated with the use of synthetic antioxidants. Crude extracts and purified compounds from higher plants are shown to exhibit marked antioxidant activity.¹⁻⁸

Seeds are known to harbor many fungi. Fungal genera such as *Cladosporium*, *Alternaria*, *Curvularia*, *Aspergillus*, *Penicillium*, *Cercospora*, *Colletotrichum*, *Chaetomium*, *Fusarium*, *Drechslera*, *Macrophomina*, *Bipolaris* and *Rhizopus* are commonly associated with seeds of many crops. These fungi are known to reduce nutritive content of seeds and result in deterioration in

seed quality. Fungal infestation of seeds drastically lowers the market value to seeds. Seed-borne fungi are known to affect germination and seedling vigor drastically. Controlling phytopathogenic fungi including seed-borne fungi usually involves the use of synthetic fungicides. However, the indiscriminate use of chemical agents results in several drawbacks which triggered immense interest in exploitation of botanicals as biopesticides. Botanicals are shown to be promising alternatives for synthetic fungicides.⁹⁻¹⁷

Geophila repens (L.) I. M. Johnst. (synonym *Geophila reniformis* D. Don) is a prostrate, creeping herb with orbicular-cordate leaves (Figure 1). It often forms dense and close colonies in shaded places. The leaves are 1-

4cm across and the apex of leaves is rounded. Petiole is up to 5cm long. Flowers are white, terminal and solitary or in peduncled 2-3 flowered umbels. Fruit is a fleshy drupe with 2 plano-convex pyrenes, up to 1cm across, globose and red when ripe. The plant is occasional in Western Ghats.¹⁸⁻¹⁹ The leaves as well as whole plants are boiled and the decoction obtained is used as remedy for cough. Fruits are crushed and rubbed on facial dermatitis. Seeds are reputedly edible.²⁰ *G. repens* is used traditionally in Paraguay for treating fungal infection of skin.²¹ The plant is reported to exhibit antifungal²¹, antioxidant²², antibacterial²³, anticholinesterase²² activity. The present study was carried out to evaluate antioxidant and antifungal activity of methanolic extract of *G. repens* leaves.



Figure 1: *G. repens* (Photograph by Prashith Kekuda)

MATERIALS AND METHODS

Collection and identification of plant

The plant *G. repens* was collected at Haniya, Hosanagara Taluk, Shivamogga district, Karnataka in the month of December 2017. The plant was identified by Dr. Vinayaka K.S, Principal, KFGC, Shikarpura, Karnataka.

Extraction

Leaves were separated, cleaned, dried under shade and powdered. Maceration technique was performed for extraction of powdered leaf material of *G. repens*. Methanol was used as solvent. Filtrate was evaporated to dryness to obtain crude leaf extract.⁸

Antioxidant activity of leaf extract

DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity

Various concentrations viz. 12.5 μ g/ml - 200 μ g/ml of leaf extract and ascorbic acid (reference standard) were prepared in methanol. Antiradical activity was assessed by scavenging potential of leaf extract and ascorbic acid against DPPH free radicals.⁸ Scavenging activity was determined using the formula:

Scavenging of DPPH radicals (%) = [Ac - At / Ac] x 100, Where 'Ac' and 'At' refers to absorbance of DPPH control and absorbance of DPPH in the presence of leaf extract/ascorbic acid, respectively.

Ferric reducing activity

Various concentrations viz. 12.5 μ g/ml - 200 μ g/ml of leaf extract were prepared in methanol. The potential of leaf extract of *G. repens* to exhibit reducing property was investigated by ferric reducing assay.⁸ An increase in the absorbance of reaction mixture on increasing the concentration of extract/ascorbic acid indicated reducing activity. Ascorbic acid was used as reference standard.

Antifungal activity of leaf extract

The efficacy of leaf extract to exhibit antifungal activity was assessed against two seed-borne fungi viz. *Aspergillus niger* and *Bipolaris* sp. by Poisoned food technique.⁸ Test fungi were inoculated on control (without leaf extract) and poisoned potato dextrose agar (1mg extract/ml of medium) plates aseptically followed by incubation of plates at room temperature for 96 hours. Antifungal activity, in terms of inhibition of mycelial growth of test fungi (%) was calculated using the formula:

Inhibition of growth (%) = (Dc - Dt / Dc) x 100, where 'Dc' and 'Dt' denotes colony diameter (in cm) of test fungi in control and poisoned plates, respectively.

Statistical analysis

Biological activities conducted were carried out in triplicates (n=3). Results are presented as Mean \pm Standard Deviation (S.D). IC₅₀ values were obtained by linear regression analysis using Origin (Data Analysis and Graphing) Software version 7.0.

RESULTS AND DISCUSSION

DPPH radical scavenging activity of leaf extract

Among various in vitro antiradical assays, the DPPH free radical scavenging assay is one the most extensively used methods. The method is simple, rapid and the results obtained are reproducible. The method of scavenging of DPPH radicals is widely used to investigate antiradical potential of extracts from higher plants.^{8,24-27} In this study, we investigated the potential of leaf extract of *G. repens* to scavenge DPPH radicals and the result is shown in Figure 2. Bleaching of color of DPPH radicals (purple to yellow) by leaf extract/ascorbic acid was monitored by measuring absorbance of reaction mixture at 520nm. Leaf extract was shown to scavenge DPPH radicals dose dependently with a scavenging activity of 22.68% and 68.66% at extract concentration 12.50 μ g/ml and 200 μ g/ml respectively. Ascorbic acid scavenged radicals more efficiently than leaf extract. The IC₅₀ value for leaf extract and ascorbic acid was found to be 51.39 μ g/ml and 6.12 μ g/ml respectively. In an earlier study, Dash and Sahoo et al.²² showed the efficacy of methanol extract of *G. repens* leaves to scavenge DPPH radicals with an IC₅₀ value of 69.01 μ g/ml. It is evident from the result of this study that the leaf extract possess bioactive principles capable of scavenging free radicals and hence, the leaf of *G. repens* can act as free radical scavenger.

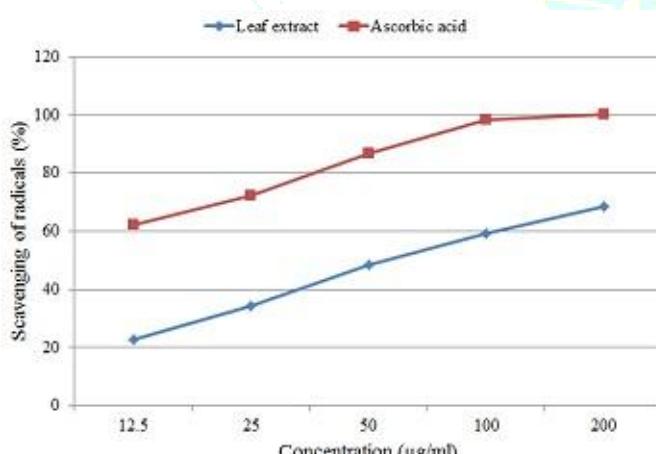


Figure 2: Scavenging of DPPH radicals by leaf extract and ascorbic acid

Ferric reducing activity of leaf extract

Reducing power of a substance is considered as a significant indicator of antioxidant capacity. The reducing potential of a compound is due to the presence of reductones which are involved in causing the reduction of Fe³⁺/ferricyanide complex to the ferrous form that result in the formation of Perl's Prussian blue complex. The intensity of color complex formed can be measured at 700 nm as the Fe²⁺ concentration. Ferric reducing assay is one of the widely used assays to evaluate antioxidant activity of plant extracts.^{8,24,25,26,28,29} In the present study, we investigated reducing capacity of leaf extract of *G. repens* by ferric reducing assay. An increase in the absorbance with increase in the concentration of leaf extract/ascorbic acid was observed and indicated the ferric reducing ability of extract (Figure 3). It is evident from the result of this study that

the leaf extract of *G. repens* can serve as electron donor and hence can terminate the radical chain reactions.

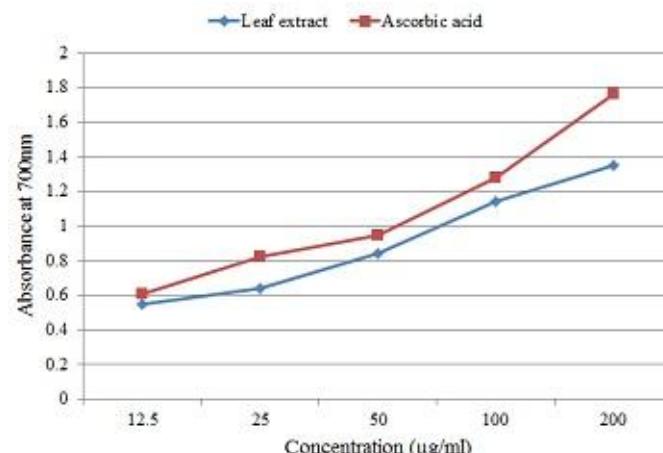


Figure 3: Ferric reducing activity of leaf extract and ascorbic acid

Antifungal activity of leaf extract

Fungi play a predominant role as pathogens causing several diseases in plants resulting in drastic reduction in crop yield and thereby cause huge economic loss in severe cases. Control of phytopathogenic fungi including seed-borne fungi is greatly accomplished by the use of synthetic fungicides. However, their indiscriminate use is associated with many drawbacks viz. emergence of resistant strains of fungal pathogens, residual effect in environment leading to pollution, deleterious effect on non-target organisms and the high cost of fungicides which is not usually afforded by poor farmers. These adverse and alarming effects of synthetic chemicals triggered an immense search for alternatives for pathogen control. Higher plants have been considered as one of the potential alternatives for fungicides from synthetic origin. Extracts and metabolites obtained from several plant species showed promising antifungal activity against a range of phytopathogenic fungi including seed mycoflora.^{8,30-35} In the present study, we evaluated antifungal potential of leaf extract of *G. repens* against two seed-borne fungi viz. *A. niger* and *Bipolaris* sp. by poisoned food technique which is one of the most commonly used assays for determining antifungal activity of plant extracts. Poisoning of medium with the leaf extract resulted in considerable reduction in the mycelial growth of test fungi (Table 1; Figure 4). At concentration of 1mg extract/ml of medium, an inhibition of >50% inhibition of both fungi was observed. Both the test fungi were inhibited by extract to more or less similar extent with slightly higher inhibition of *Bipolaris* sp. (60.41%) when compared to *A. niger* (59.61%). In a previous study, Portillo et al.²¹ revealed marked antifungal potential of dichloromethane extract of whole plant of *G. repens* against yeasts and molds.

Table 1: Colony diameter of test fungi in control and poisoned plates

Treatment	Colony diameter in cm	
	<i>A. niger</i>	<i>Bipolaris</i> sp.
Control	5.70 \pm 0.00	4.80 \pm 0.00
Leaf extract	2.33 \pm 0.05	1.90 \pm 0.00

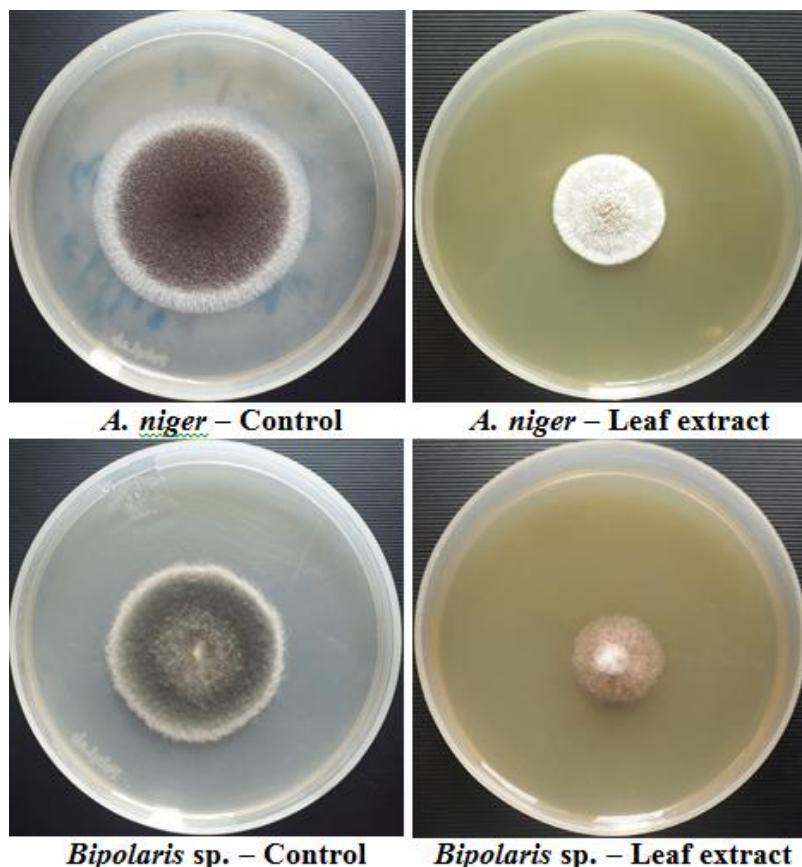


Figure 4: Growth of test fungi in control and poisoned plates

CONCLUSIONS

The plant can be used for developing therapeutic agents which can be used against oxidative damage induced by free radicals. In suitable formulation, the leaf of *G. repens* can be used to manage seed-borne fungal infections. Further investigations are to be conducted in order to recover active principles from leaves of *G. repens* and to evaluate their biological activities.

ACKNOWLEDGEMENTS

Authors are thankful to H.O.D, Department of Microbiology, and Principal, S.R.N.M.N College of

Applied Sciences, Shivamogga and N.E.S, Shivamogga for the immense support rendered to carryout work. Authors thank Dr. Vinayaka K.S, Principal, KFGC, Shikaripura for helping in collection and identification of plant material and Dr. Raghavendra H.L for helping us through statistical analysis of results.

SOURCES OF FUNDING

None

CONFLICTS OF INTEREST

None declared

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