

# ***Desmodium gangeticum* (L.) DC. (Fabaceae) extract protects cell by preventing oxidative stress and its possible Iron binding ability.**

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## ABSTRACT

Reactive oxygen species (ROS) play a pivotal role in the development of oxidative stress-related health disorders, driving the ongoing pursuit of natural antioxidants as potential remedies. *Desmodium gangeticum* (DG), a medicinal plant recognized for its antioxidant properties, The plant's roots, shoots, leaves, and bark have all exhibited medicinal attributes, notably its capacity to counteract oxidative stress which is an intricate process often initiated by iron-induced Fenton reactions that generate harmful free radicals. Our study delves into the multifaceted role of DG, encompassing its effects on cell viability, ROS reduction, and its potential as an iron chelator Given DG's rich content of flavonoids and polyphenols, we hypothesized that these compounds could interact with iron, potentially preventing its participation in free radical generation.

To test this hypothesis, we prepared a whole plant extract by grinding powdered DG parts in water and conducted an MTT assay and ROS estimation in untreated cultured cell and cells treated with it and carried out iron chelation assay. Our results revealed that the presence of DG extract reduced the level of cellular ROS and protected cell in H<sub>2</sub>O<sub>2</sub> induced oxidative stress. DG extract also reduced formation of the iron-ferrozine complex

by 50-70% compared to the complex formed in the absence of the extract. This outcome strongly suggests that DG extract indeed chelates or interacts with iron, impeding its ability to bind and form the characteristic-colored complex. These findings not only provide crucial insights into DG's iron-binding capabilities but also hint at its potential role in preventing oxidative stress.

In conclusion, our study underscores the significance of DG's iron-binding ability as a protective mechanism against oxidative stress. These findings underscore the therapeutic potential of DG, shedding light on its promising role in combating ROS-induced health disorders and encouraging further exploration of its multifaceted benefits.

**Keywords:** Oxidative stress, Natural antioxidants, HepG2 cells, Herbal medicine, Antioxidant properties, Cellular health

## INTRODUCTION

*Desmodium gangeticum* (L.) DC. (Fabaceae) has been used in the tradition of tribal people as medication to treat ailments. One among plants being used extensively in ayurvedic decoction is *Desmodium gangeticum* (DG), its lethal dose are

more than 7g/kg in mice when fed orally (1,2), excessive consumption may results in moderate anti- implantation, antifertility as observed in albino rats (3–5). The aqueous decoction of its roots, and aerial parts have been reported to possess anti-inflammatory activity in-vivo which increased in dose-dependent manner 5, 10 and 20mg/kg(6). The extracts of plant are chemotherapeutic and prophylactic against helminths, and microbes and has been well reported (7). The healing natures and therapeutic uses of DG extract are presumed to be due to its one of the antioxidative potential The beneficial properties of *Desmodium gangeticum* seem to be due to its richness in antioxidant constituents.

Oxidative balance is an important tendency that natural phenomenon and life maintains for sustainability. It is equally applicable for cellular homeostasis. In case of irregularity cells face oxidative imbalance, it needs a counter mechanism to be executed. Naturally occurring antioxidants and antimicrobials are in growing need. The plant contains important secondary metabolites, such as alkaloids, steroid, terpenoids, phenols etc. These compounds and derivatives have been shown to possess antioxidant activity etc. Antioxidants delay or prevent the oxidation of substrates in cellular milieu.

Phytochemicals from DG have been demonstrated to reduce the reactive oxygen species. Methanolic extract of the plant reduce oxidative stress through the mitochondria mediate pathway (8)). The phytochemicals present in extract of the plant are helpful biological antioxidant for defence system, and regulates superoxide dismutase, glutathione and catalase enzymes responsible for maintain cellular redox balance. There is significant reduction in the lipid peroxide content upon administration of DG extract (9). DG is rich in compounds containing catechols, alkaloids, flavonoids which can work as chelator or iron ion and prevent well known Fenton Reaction. The Fenton reaction is one among important contributors of free radical generation in the biological system. A substance preventing iron to participate in Fenton reaction enhances the antioxidant system in case of compromise due to injury or infections. Among several herbs, DG is known for its antioxidant property. In a series of studies various roles in preventing oxidative stress have been postulated by checking the effect on superoxide dismutase activity, Free radical scavenging, catalase etc. Therefore, in this study we have tried to identify the possible role of DG extract in interacting with iron and in turn its possible effects.

## MATERIALS AND METHODS

### *Sample preparation:*

Plant extracts are prepared, Fresh leaves and shoots of identified herb were collected and dried. The dried leaves and stem were grinded and powdered, 5g of dried powder was dissolved in 5ml of water, or ethanol by frequent and vigorous shaking. Finally, the mix was centrifuged and supernatant was taken for further study.

### *Cell Viability Assay:*

In this study, the impact of *Desmodium gangeticum* (DG) extract on cell viability was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. HepG2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) under standard conditions. These cells were then subjected to various treatment groups, each exposed to different concentrations ranging from 0-500mg/ml of DG extract, including root, leaf, and stem extracts. After a specified incubation period, MTT reagent was added to the treated cells, and they were incubated to allow viable cells to convert MTT into purple formazan crystals. Following incubation, formazan crystals were solubilized, and the resulting solution's absorbance was measured at 570 nm using a microplate reader. The concentration-dependent effects of DG extract on cell viability were evaluated, and the results demonstrated the safety of DG extracts, as they did not exhibit significant cytotoxicity even at higher concentrations.

### *Estimation of Reactive oxygen species levels*

To estimate reactive oxygen species (ROS) in *Desmodium gangeticum* (DG) root, shoot, and leaf extracts treated with HepG2 cells, a common method involves using 2',7'-dichlorofluorescein diacetate (DCF-DA) assay. First, DG extracts are prepared and filtered. HepG2 cells are cultured and divided into various treatment groups, including a control group, extract-treated groups, and a positive control group treated with a known ROS scavenger. After incubation, the cells are loaded with DCF-DA and incubated in the dark. The fluorescence intensity is measured, and the data is analyzed to determine relative ROS levels compared to the control group. This experiment helps assess the impact of DG extracts on ROS levels in HepG2 cells and their potential antioxidant or pro-oxidant effects. Careful controls and statistical analysis are essential for robust results.

### *Assay Procedures:*

Briefly the crude extract was serial diluted and treated with iron salt solution. Then ferrozine was added and incubated for 30 min. Thereafter Absorbance at 570 nm was measured using a microplate reader. The absorbance of untreated blank containing only iron and ferrozine solution were compared with solution treated with the plant extract. The reduction in absorbance in presence of the extract was calculated using inhibition percentage which indicates the ability of extract to limit iron to participate in reactive oxygen species(ROS) generation.

The decrease in absorbance is measured and proportionally converted into antioxidant potential index

$$\text{Inhibition percentage} = \{A_0 - A(\text{mix}) / A_0\} * 100$$

**Statistical Analysis**

Data were collected and arranged in MS excel sheets, descriptive statistics has been carried out. Mean +SEM has been compared and differences tested for significance using ANOVA, were considered significant for p values <0.001.

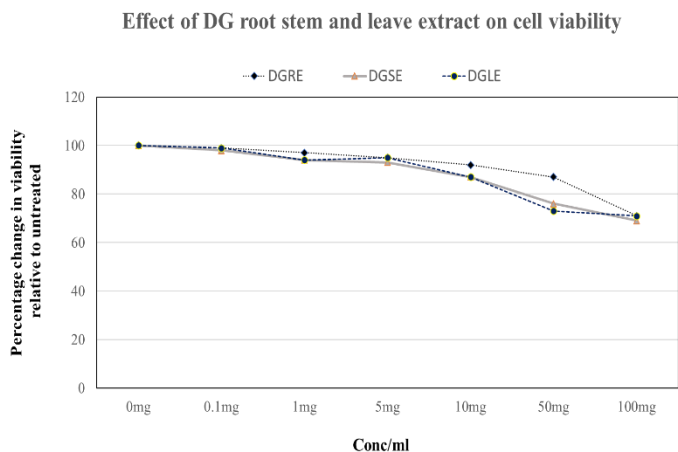
**RESULTS**

**DG extract protects cells viability in H2O2 induced oxidative stress**

The evaluation of cell viability in response to different Desmodium gangeticum extracts, including DGRE (Desmodium gangeticum root extract), DGLE (Desmodium gangeticum leaf extract), and DGSE (Desmodium gangeticum stem extract), The results of the MTT assay revealed a concentration-dependent effect of DG extract on cell viability as illustrated in Figure 1,

Conditions	Mean	SDEV
Untreated	2.55	0.02
DGRE	2.56	0.07
DGSE	2.57	0.07
DGLE	2.6	0.06
H2O2	4.79	0.16
DGRE +H2O2	3.28	0.16
DGSE +H2O2	3.78	0.24
DGLE +H2O2	3.51	0.17

concentration-dependent response on cell viability. Notably, even at higher concentrations, DGRE, DGLE, and DGSE did not exert substantial cytotoxic effects on the HepG2 cells. The calculated IC50 (half-maximal inhibitory concentration) for each of these extracts is expected to exceed 500 mg, underscoring their remarkable safety profiles.



**Figure 1** Illustrates the cell viability curve, depicting the representative percentage change in cell viability relative to untreated cells. Three different Desmodium gangeticum extracts were evaluated, including DGRE (Desmodium gangeticum root extract), DGLE (Desmodium gangeticum leaf extract), and DGSE (Desmodium gangeticum stem extract), across a range of concentrations from 0.0 to 500mg/ml. The cell viability curve demonstrates that DGRE, DGLE, and DGSE did not exhibit significant cytotoxic effects even at higher concentrations.

The cell viability curve demonstrates that DGRE, DGLE, and DGSE did not exhibit significant cytotoxic effects even at higher concentrations. The calculated IC50 (half-maximal inhibitory concentration) for these extracts is expected to exceed 500 mg, indicating a lack of substantial impact on cellular viability.

**DG extract reduces oxidative stress by reducing ROS levels in H2O2 treated cells**

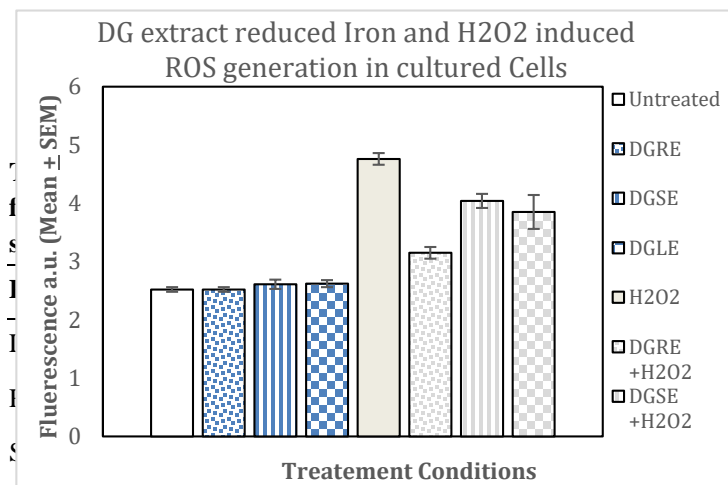
The results of the Cellular DCFDA fluorescence assay, measured in arbitrary units (AU), for both untreated and treated conditions are presented in the **Table 1** below. DCFDA fluorescence is indicative of reactive oxygen species (ROS) levels within the cells.

*Table 1 :Cellular DCFDA fluorescence (AU) in untreated and treated conditions*

**Table Legend:** Three different Desmodium gangeticum extracts were evaluated, including DGRE (Desmodium gangeticum root extract), DGLE (Desmodium gangeticum leaf extract), and DGSE (Desmodium gangeticum stem extract). The table displays the mean cellular DCFDA fluorescence levels in Arbitrary Units (AU) along with the corresponding standard deviations (SDEV) for each treatment condition. These conditions include untreated cells, individual DG extracts, exposure to hydrogen peroxide (H2O2), and co-treatment of DG extracts with H2O2. The data provides insights into the impact of these treatments on cellular oxidative stress levels.

The presence of hydrogen peroxide (H2O2) and iron significantly increased oxidative stress, as indicated by markedly elevated DCFDA fluorescence levels compared to the untreated control cells. This increase in fluorescence reflected the heightened production of reactive oxygen species (ROS) in the presence of H2O2 and iron.

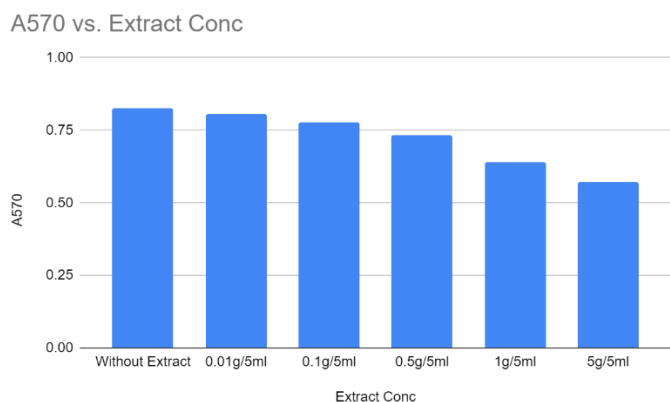
However, when *Desmodium gangeticum* (DG) extract was co-treated with the H<sub>2</sub>O<sub>2</sub> and iron mixture, a notable change occurred. The ROS levels, as indicated by fluorescence, were observed to return to normal or comparable levels to those seen in the untreated control cells. This intriguing finding suggests that DG extract has a potential protective effect against oxidative stress induced by H<sub>2</sub>O<sub>2</sub> and iron. These results highlight the ability of DG extract to mitigate the oxidative stress caused by the presence of H<sub>2</sub>O<sub>2</sub> and iron.



**Figure 2 :** Bar graph shows that DGRE, DGSE or DGLE treated cell have cellular ROS comparable to untreated cells, and H<sub>2</sub>O<sub>2</sub> Treated cells the level are significantly increase which are further reduced by DGRE DGSE or DGLE

**DG extract reduced the formation of FeFz Complex**

We carried out a dose response study to assess the effect of DG aqueous extract in formation of FeFz complex. DG extract decreased absorbance of the FeFz complex significantly and did at more than 0.5g/5ml. Fig shown a dose dependent response. Whole plant extract reduced ~40-50% or iron to participate complex formation with Ferrozine.



**Figure 3: Bar diagram of change in absorbance of FeFz complex in presences of *D. gagenticum* extracts. Increasing extract concentration reduced the complexation.**

**Comparison of iron binding properties of part of DG plant**

The above observation indicated the presence of active component in the DG extract we further explored which part of the plant is actively involved. The root shoot and leaves powder were prepared separately and their aqueous extract was used at 5mg/ml conc. Among the three root extract showed maximum percentage inhibition i.e. 30-40% in FeFz formation. However, the shoot and leaves were relatively less active upto 20%, when compared to whole plant extract all of these were found to be synergistic.

**DISCUSSION**

Free radicals and reactive oxygen species (ROS) are highly reactive molecules that are generated as byproducts of normal cellular processes, environmental stresses, and exposure to factors such as UV irradiation. These ROS can react with various cellular components, including DNA, carbohydrates, proteins, and lipids, leading to damage and causing cellular and tissue injury. Excessive production of ROS can also result in inflammation, premature aging disorders, and several disease states, including cancer, diabetes, and atherosclerosis. To counteract the harmful effects of ROS, organisms have evolved complex antioxidant systems. However, when ROS production exceeds the capacity of these antioxidant systems, it can lead to severe cellular damage.

One of the mechanisms by which ROS can be generated is through the presence of excess transition metal ions, such as Fe(II), which can participate in Fenton-like reactions. These reactions can lead to the production of highly reactive hydroxyl radicals [OH•], contributing to oxidative stress. In this context, any substance capable of preventing these reactions can potentially reduce oxidative stress and protect cells from the damaging effects of free radicals.

The Ferrous Ion Chelating Assay is a valuable tool for measuring the ability of test samples to chelate free ferrous ions in a solution, thereby inhibiting the binding of Fe(II) to ferrozine, which generates a highly colored complex. This assay provides insights into the potential of substances to act as iron chelators, which can prevent the formation of hydroxyl radicals and oxidative damage. In the present study, the FeFz assay(10) was used to assess the iron-chelating capacity of *Desmodium gangeticum* (DG) extract.

The results of the study revealed that DG extract demonstrated the ability to reduce the formation of the FeFz complex in a dose-dependent manner. This reduction in absorbance indicates that DG extract effectively chelates or

interacts with iron (II) ions, preventing them from participating in the formation of the colored complex. This suggests that DG extract contains compounds, possibly flavonoids and proteins, capable of binding to free iron and inhibiting its reactivity in Fenton-like reactions.

Furthermore, when different parts of the DG plant, including the root, shoot, and leaves, were separately evaluated, it was observed that the root extract exhibited the highest percentage inhibition in FeFz complex formation (30-40%), followed by the shoot and leaves extracts with relatively lower inhibitory effects (up to 20%). This variation suggests that the distribution of active iron-chelating components is not uniform throughout the plant. However, it is possible that more than one compound is contributing to this iron-chelating activity.

The results of the Cellular DCFDA fluorescence assay reveal noteworthy insights into the cellular oxidative state under various conditions. Notably, untreated cells exhibited a relatively low DCFDA fluorescence level, indicating baseline levels of reactive oxygen species (ROS). In contrast, the introduction of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), a known inducer of oxidative stress, resulted in a substantial increase in DCFDA fluorescence, signifying elevated ROS levels within the cells.

When DG extracts, specifically DGRE, DGSE, and DGLE, were applied to the cells independently, their respective DCFDA fluorescence levels remained similar to the untreated cells, suggesting that these extracts did not significantly affect ROS production under normal conditions.

Interestingly, when DG extracts were administered in combination with H<sub>2</sub>O<sub>2</sub>, the DCFDA fluorescence levels were notably lower compared to cells treated with H<sub>2</sub>O<sub>2</sub> and iron mixture. This observation implies that DG extracts may possess the ability to mitigate the oxidative stress induced by H<sub>2</sub>O<sub>2</sub>, as evidenced by the reduction in ROS levels.

In summary, these results suggest that *Desmodium gangeticum* extracts, under normal conditions, do not significantly alter cellular ROS levels. However, in the presence of an oxidative stressor like H<sub>2</sub>O<sub>2</sub>, these extracts demonstrate a potential protective effect by reducing ROS levels, which warrants further investigation into their antioxidant properties and mechanisms of action.

The calculated IC<sub>50</sub> (half-maximal inhibitory concentration) for these extracts is expected to exceed 500 mg, indicating a lack of substantial impact on cellular viability. This figure underscores the safety profile of *Desmodium gangeticum* extracts, suggesting that they do not adversely affect cell viability within the tested concentration range.

The findings of this study are consistent with earlier research(8,9) and add to the body of evidence supporting DG extract's potential antioxidant properties, particularly its ability to chelate iron and reduce oxidative stress. These results suggest that

DG extracts, derived from various parts of the plant, are well-tolerated by cells in vitro and do not significantly affect cellular viability within the tested concentration range. This safety profile makes DG extracts promising candidates for further investigation in antioxidant and cytoprotective therapies.

In conclusion, this study highlights the potential of DG extracts, particularly the root extract, to act as iron chelators and reduce oxidative stress by inhibiting the formation of hydroxyl radicals. These findings contribute to our understanding of DG's antioxidant properties and its potential applications in mitigating oxidative stress-related health disorders. Further research is warranted to identify and isolate the specific bioactive compounds responsible for these effects and to explore their therapeutic potential in various cellular and biomedical contexts.

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