



## Research Article

# Exploring Natural Medicinal Resources: Comparative Study on Antioxidant and Cytotoxicity Potential

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**Citation:** Yadav N, Saini N, Saini N, Saini M, Kumari S, Sangwan NS (2024) Exploring Natural Medicinal Resources: Comparative Study on Antioxidant and Cytotoxicity Potential. Curr Res Cmpl Alt Med 8: 228. DOI: 10.29011/2577-2201.100228

**Received Date:** 21 January 2024; **Accepted Date:** 14 March 2024; **Published Date:** 16 March 2024

## Abstract

**Background:** Plants have served as the foundation of traditional medicines. The myriad of compounds belonging to chemical classes found in plants have been associated with various therapeutic potential. Recent research reports showed that plant-derived phenolic compounds possess great antioxidant properties and exhibit cytotoxic potential as well. Southern Haryana has a rich diversity of plants many of which remained under-explored for their therapeutic properties. **Objective:** In the present study, selected widely growing plants in the region were evaluated for their antioxidant and cytotoxicity potential. **Methods:** The antioxidant activity was evaluated using (2,2-dyphenyl-1-picrylhydrazyl (DPPH) scavenging, ferric reducing antioxidant power (FRAP) assay, and cytotoxic potential was evaluated using (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (MTT) assay. **Results:** *Glycyrrhiza glabra* showed maximum radical scavenging activity whereas *Cinnamomum verum* was found to have maximum antioxidant potential as estimated by FRAP assay i.e., range from 0.8 to 1 mg FSE/g DW. *Tectona grandis* has maximum phenolic content with a range of 30 to 35 ± 3.01 mg GAE/g DW). The maximum flavonoid content was found in the *Saraca asoca*. The highest antioxidant potential was shown by *Fraxinus excelsior* and *Cinnamon verum*. **Conclusion:** This study demonstrated that the widely growing plants could be a potential source of natural antioxidants and selected plants may further prove beneficial in targeting diseases associated with oxidative stress. Plants have the potential to be a long-term sustainable resource of bioactive chemicals. The present study also exhibited that the therapeutic potential of plants is highly reliant on the type of plant material used.

**Keywords:** Polyphenols; Antioxidant; Medicinal plants, Oxidative stress; Cytotoxicity

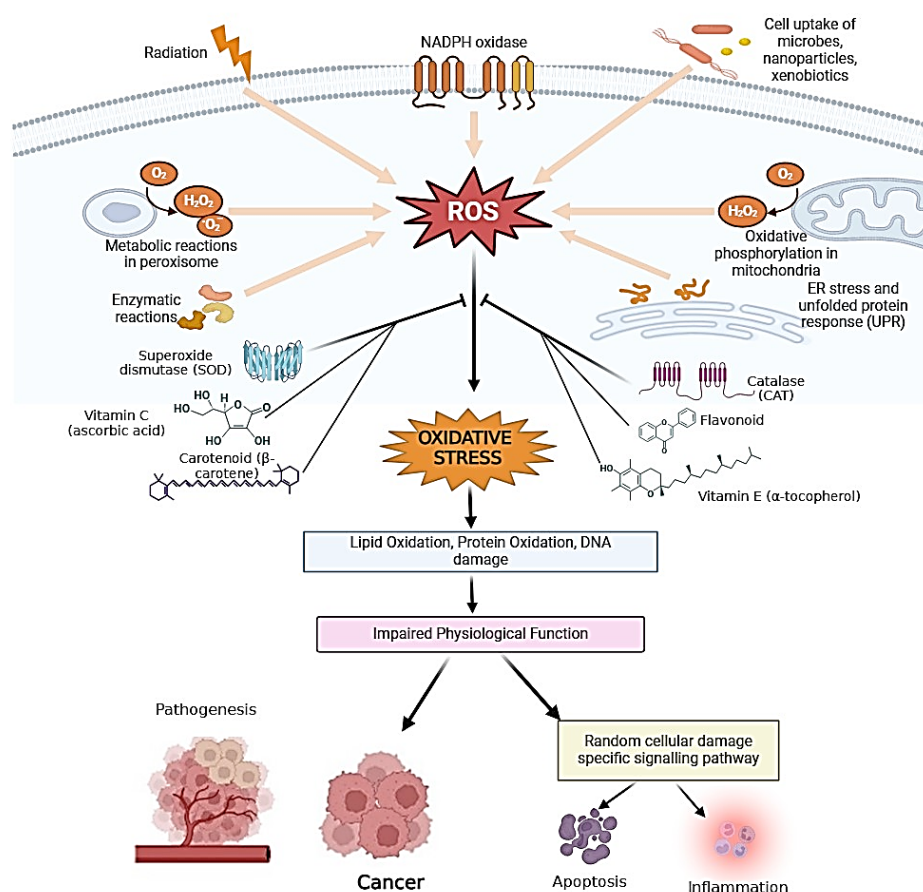
## Introduction

Humans rely heavily on plants as a source of food, feed, traditional medicine, and medication. According to current statistics, at least 35 - 40 thousand different plant species have been utilized for medical purposes [1]. As there are safety concerns with the adverse effects of synthetic chemicals and their medications, the demand for herbal therapies is steadily expanding. According to the World Health Organization (WHO) traditional herbal plants, have a significant role in effectively achieving health goals. India's ancient system of plant-based treatment and executive food preparation has a long history. As reported by Samhita and Samhita [2] ayurvedic science explained the use of over 700 herbal plants and elaborated it for further medicinal use. Many herbal plants possess antioxidants, antibacterial, antifungal, and other therapeutic uses [3-5]. Recent studies have further elucidated the role of plants in managing newer healthcare usages [6].

Herbal plant medications are a prominent therapy in the traditional system, and these can be employed in modern therapeutics owing to their biological properties. There is a potential threat due to the harmful effects of chemical drugs on the human body. Therefore, various plants and their parts have been utilized for the management of a variety of diseases in Ayurvedic and folk medicine. Plants synthesize primary metabolites for both necessary and specialized tasks, such as growth and development [7-9].

Phenolic compounds are primarily used as a cellular support material and are a key component made up of polymeric phenolics. The bioactivities of phenolic compounds (flavonoids and phenolic acids) may be connected to their ability to chelate metals, inhibit lipoxygenase, and scavenge free radicals [10]. Bioactive polyphenols have gained a lot of interest as they protect the human body from cellular damage resulting from enhanced free radicals, and cause oxidative stress [11,12]. The term "oxidative stress" refers to a disruption in the balance between the production of free radicals, and the status of *in vivo* antioxidant defense mechanisms [13].

This biological circumstance has a significant impact on the emergence of several illnesses, like aging, cancer, coronary heart disease, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataract formation, and inflammation [11]. Enzymatic antioxidant systems, such as peroxidases, polyphenol oxidase, superoxide dismutase, catalase, etc. lower the effect of free radicals generated reactive oxygen species (ROS), which are responsible for proteins damage, DNA damage, genomic instability, the function of macromolecules, the addition of mutation and ultimately results in cellular functional alteration (Figure 1) [14-15]. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butyl hydroxyhydroquinone (tBHQ), propyl gallate (PG), and others are limited for use since some of them are reported for their harmful effects [16,17].



**Figure 1:** Scavenging effect of free radicals and its associated diseases with antioxidants and polyphenols.

Antioxidants that can be found in medicinal plants in abundance are prominent therapeutic agents that scavenge free radicals from the body. Natural antioxidants boost the antioxidant capacity. Daily consumption of bioactive compounds such as polyphenols or flavonoids leads to reducing of various life-threatening diseases including heart disease, stroke, pulmonary disease, and different types of cancer [18-20]. The natural antioxidants found in the leaves, fruits, seeds, roots, and bark of plants show anti-inflammatory, antiviral, antimicrobial, and antioxidant properties [2, 21-23]. Plant-based antioxidant agents such as flavonoids, alkaloids, and polyphenols may downregulate the uncontrolled cell division caused by oxidative stress imposed by free radicals [24]. Many plant components regulate the genes or signaling pathways involved in antioxidant activities that internally regulate the inflammatory response [25]. Antioxidants protect cells from ROS-induced DNA damage which is associated with disease prevention [22]. Given the foregoing; the current study was conducted to investigate the antioxidant potential of various category plants, and their cytotoxic potential was also evaluated.

## Material & Methods

### Reagents & Chemicals

DPPH 2,2, Diphenyl picryl hydrazyl, Gallic acid, L Ascorbic acid, MTT reagent, DMEM high glucose media, FBS, PenStrep, DMSO, trypsin-EDTA were purchased from sigma. Aluminum chloride, Sodium carbonate, Ferrous sulfate, and 2,4,6-tripyridyl-S-triazine (TPTZ) were purchased from Himedia. All the other chemicals used were of analytical grade.

### Plant material collection and preparation of extracts

Plant material was collected from their natural habitat or procured. The freshly harvested material was properly cleaned, dried and powdered. Each sample was incubated in absolute ethanol for 72 hours. Following that, each extract was centrifuged and resultant ethanolic was filtered with Whatman's filter paper and the filtrate was evaporated using a Rotatory evaporator under low pressure. A known weight of the obtained extract was dissolved with a known volume of solvent to have an extract with a known concentration. The obtained extract was stored at 4°C until analysis [26].

### Determination of total phenolic content (TPC)

The total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent method with some modifications [12]. A suitable amount of aliquot of extract was added into the reaction mixture. The absorbance of the extract was measured at 760 nm against a blank. The total phenolic content of the plant extracts was expressed as milligram Gallic acid equivalents per gram of dry weight (mg GAE/g DW) using the calibration curve of Gallic acid.

### Determination of total flavonoid content (TFC)

The total flavonoid content (TFC) was determined using a modified aluminum chloride colorimetric method [22]. An aliquot of 0.04 ml & 0.1ml (1mg/ml) of extract was added in 2% aluminum chloride (0.1ml). To the mixture, 0.8 ml methanol was added. After spinning the mixture, the reaction was incubated for 60 minutes at room temperature in the dark. The absorbance of the solution was expressed as milligram Quercetin equivalent per gram of dry weight (mg QE/g DW) using the calibration curve of quercetin.

### Ferric reducing antioxidant power assay

The reducing power was determined according to the method of [27]. An aliquot of 0.03 ml (1mg/ml) of plant extract was added with FRAP reagent in a 1:30 ratio. The reagent included acetate buffer (300mM, pH 3.6), 2,4,6-tri[2-pyridyl]-s-triazine (10mM in 40mM HCl) solution, FeCl<sub>3</sub>.6H<sub>2</sub>O (20mmol/liter) in 10:1:1 ration respectively. The absorbance was measured at 593 nm against blank. The absorbance of the extract was expressed as milligram Ferrous sulfate equivalent per gram of dry weight (mg FSE/g DW) using the calibration curve of Ferrous sulfate.

### DPPH• radical scavenging activity

DPPH radical scavenging activity was determined with some modifications [28]. The hydrogen atom-donating ability of the plants was analyzed by decolorization of the methanol solution of DPPH. DPPH produces a violet/purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants. DPPH stock solution was prepared with a concentration of 0.002% in methanol. 1mg/ml extract was prepared using methanol as

solvent. 0.1ml of extract was added with 0.7ml of DPPH and volume was made up to 1ml with methanol. A control was prepared by adding 0.7 ml of DPPH and 0.3 ml of methanol. The reaction mixture was incubated and absorbance was measured against the blank. Free radical inhibition percentage was calculated using the following equation.

$$\text{Scavenging, DPPH (\%)} = (\text{Abs}_{\text{control, DPPH}} - \text{Abs}_{\text{sample, DPPH}}) / \text{Abs}_{\text{control, DPPH}} \times 100$$

### Cytotoxic activity against Human Embryonic Kidney cells

Human embryonic kidney cells (HEK-293) were procured from the National Centre for Cell Sciences (NCCS), Pune, India, and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% of inactivated Fetal Bovine Serum (FBS), 1% antibiotic/antimycin cocktail at 37°C in humidifier containing 5% CO<sub>2</sub>. The cells were allowed to develop to 80-90% confluence in 25cm<sup>2</sup> culture flasks before being harvested with 0.25% trypsin/EDTA solution. The viability of the cells was determined by the trypan blue dye exclusion technique. The cells were sub-cultured in 96-well plates cells, the cell count was adjusted to 9000 cells/ml using DMEM containing 10%FBS. After 24 hours, when a partial monolayer was formed, the supernatant was removed and 0.1ml of different concentrations of plant samples were added to 96 well plates. The plate was incubated at 37°C for 24h in a 5% CO<sub>2</sub> atmosphere. After 24h, the sample was removed from the wells and 0.5mg/ml MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) working solution was added in each well for 4hrs. After the incubation period, the medium was withdrawn, and each experimental well was filled with 100µl of DMSO, which was further incubated for 20min at room temperature. Finally, MTT activity was measured using a microplate reader at a wavelength of 570nm [29]. The percentage viability of the cell was determined using the equation:

$$\% \text{Cell viability} = [\text{OD}_{(\text{Control cells})} - \text{OD}_{(\text{treated cells})} / \text{OD}_{(\text{control wells})}] \times 100$$

### Statistical Analysis

All tests were carried out in triplicates and results were expressed as mean ± standard deviations. Both, the mean and standard deviation were determined using GraphPad Prism. The biochemical parameters were subjected to statistical analysis following One-way ANOVA. Significance difference was adapted to all the parameters under study to test the level of statistical significance  $p < 0.05$ .

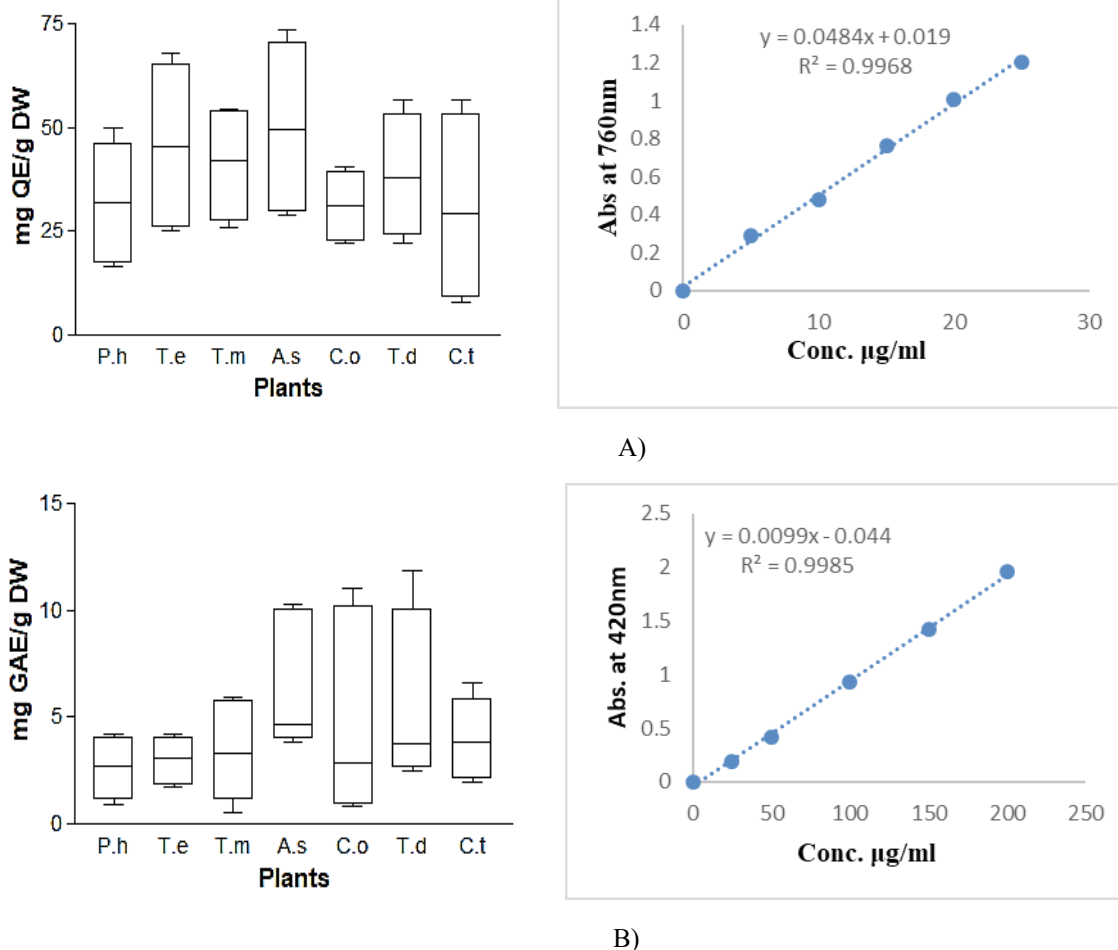
### Results

#### Determination of Total Phenolic and Flavonoid Content:

The total phenolic content (TPC) of standard gallic acid was estimated using the calibration curve ( $Y = 0.0484x + 0.019$ ,  $R^2 = 0.9968$ ) and represented as mg GAE/g DW. The total flavonoid

content (TFC) of standard quercetin was estimated using the calibration curve ( $Y = 0.0099x - 0.044$ ,  $R^2 = 0.9985$ ) and represented as mg QE/g DW. The result of total phenolic and flavonoid contents is illustrated in Figures (2-4).

The result of the study showed that the total phenolic and flavonoid content in the flowering group was reported highest in *Alstonia scholaris* and *Tagetes erecta* respectively followed by others as shown in Figure 2.

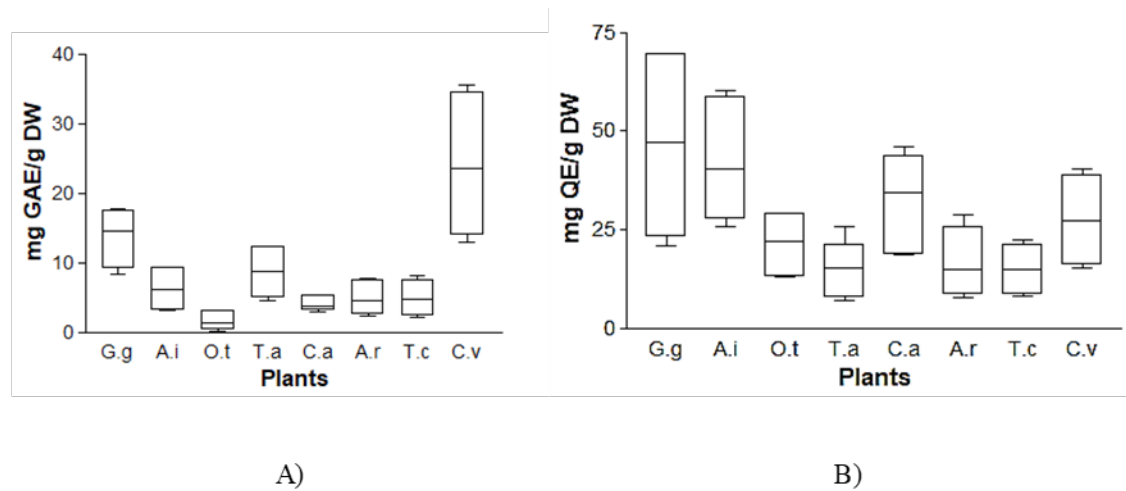


**Figure 2:** A) Total phenolic content (TPC) and a standard curve with gallic acid concentration ranging from 0.005 to 0.025mg/ml in the reaction mixture.

B) Total flavonoid content (TFC) and a standard curve with quercetin concentration ranging from 0.025 to 0.25mg/ml in the reaction mixture.

(P.h; *Physalis hybrida*; T.e; *Tagetes erecta*; T.m; *Tropaeolum majus*; A.s; *Alstonia scholaris*; C.o; *Calendula officinalis*; T.d; *Tabernaemontana divaricate*; C.t; *Clitoria ternatea*)

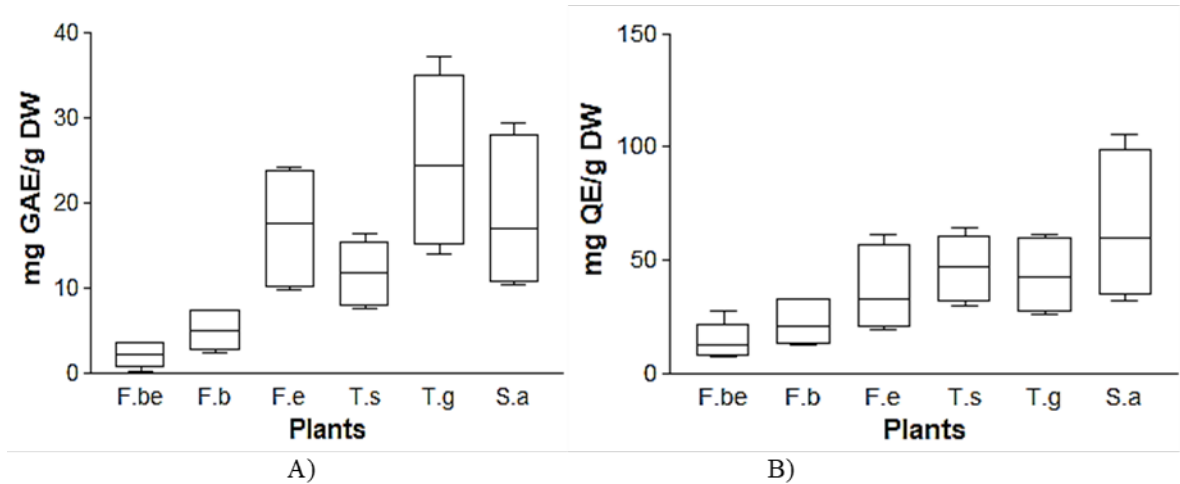
Likewise, for medicinal plant clusters, *Cinnamon verum* showed the highest phenolic content and *Glycyrrhiza glabra* showed the highest flavonoid content among others as shown in Figure 3.



**Figure 3:** A) Total phenolic content (TPC) B) Total flavonoid content (TFC)

(G.g; *Glycyrrhiza glabra*; A.i; *Azadirachta indica*; O.t; *Ocimum tenuiflorum*; T.a; *Terminalia arjuna*; C.a; *Crinum asiaticum*; A.r; *Asparagus racemosus*; T.c; *Tinospora cordifolia*; C.v; *Cinnamomum verum*).

Whereas in the case of trees, the highest phenolic content was found in *Tectona grandis* and the highest flavonoid content was shown by *Saraca asoca*. Total phenolics and flavonoid content among tree samples used in this study were reported in Figure 4.



**Figure 4:** A) Total phenolic content (TPC) B) Total flavonoid content (TFC)

(F.be; *Ficus benghalensis*; F.b; *Ficus benjamin*; F.e; *Fraxinus excelsior*; T.s; *Tecoma stans*; T.g; *Tectona grandis*; S.a; *Saraca asoca*)

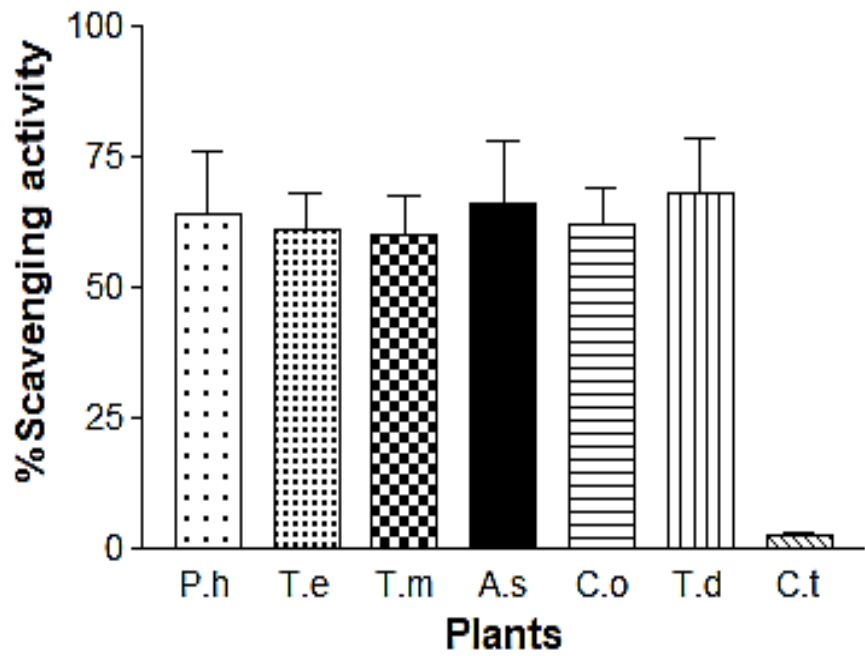
**Determination of Antioxidant Activity**

**Determination of Free Radical Scavenging Activity:**

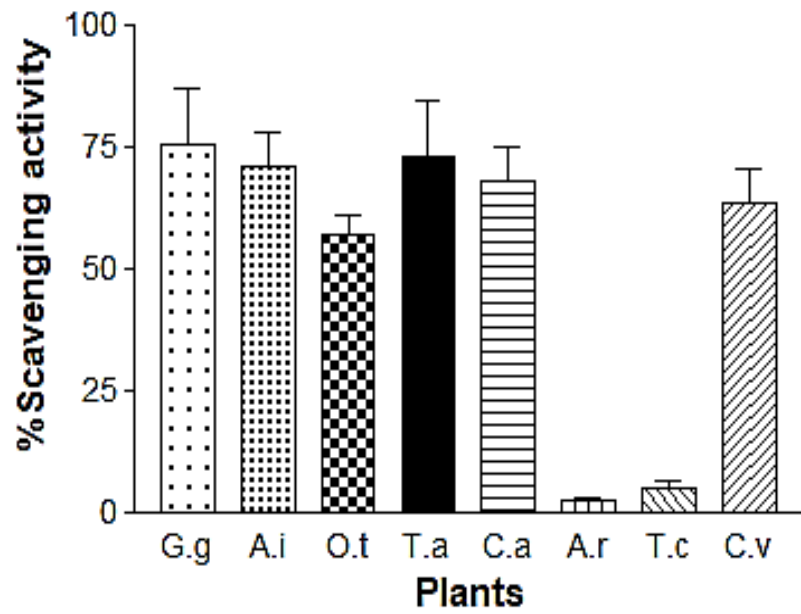
Plant extracts were evaluated by DPPH assay, to investigate their radical scavenging activity and the results are shown in Figure (5). DPPH is a stable free radical widely used to evaluate the free radical scavenging activity from many plant extracts. DPPH assay undergoes a reduction in the presence of antioxidants. The disappearance of DPPH in the presence of antioxidants is measured spectrophotometrically at 517nm. In the case of flowering plants, the maximum percentage of scavenging activity was shown by



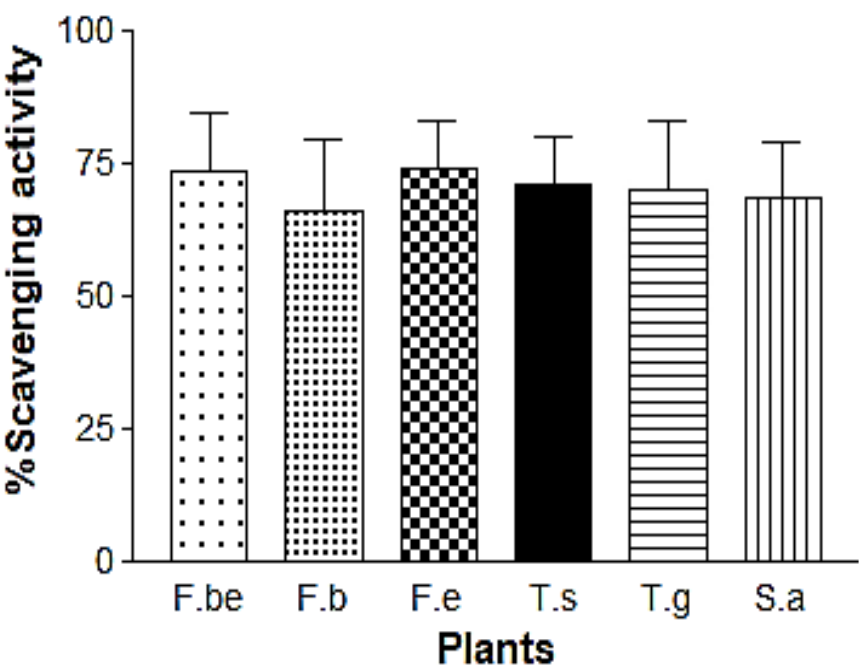
*Tabernaemontana divaricata* 65 to 70%. Whereas in the case of medicinal plants, maximum inhibition was shown by *Glycyrrhiza glabra* 70 to 80 %. In the case of trees, maximum inhibition was shown by *Fraxinus excelsior* 70 to 80%, and the results revealed that all the plants showed promising antioxidant activities.



(A)



(B)



(C)

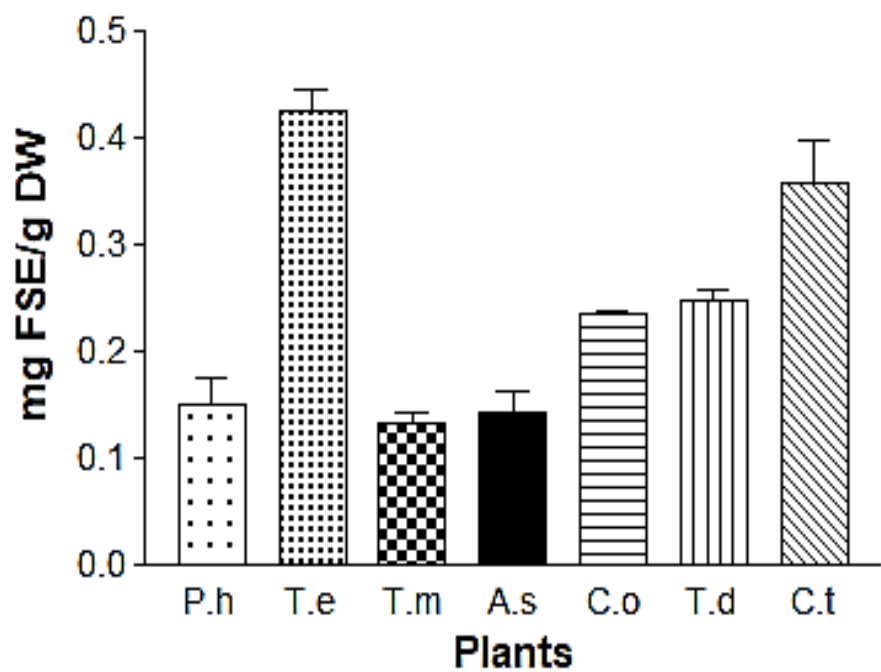
**Figure 5:** Percent scavenging activity of DPPH free radical in different plants.

**Determination of Total Antioxidant Capacity**

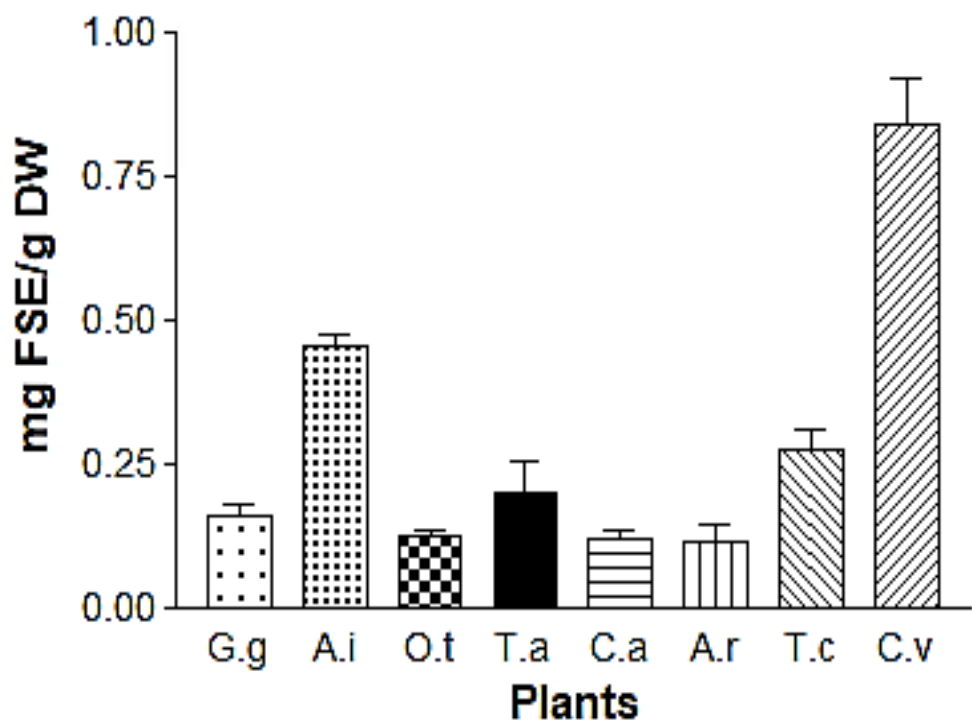
Plants were evaluated by using FRAP assay, to investigate the reducing antioxidant power. The ferric-reducing antioxidant power was expressed as mg FSE/g DW, and calculated using the calibration curve ( $Y= 1.36x + 0.000005$ ,  $R^2 = 0.999$ ) shown in Figure (6).

In the case of flowering plants, the maximum antioxidant power was shown by *Tagetus erecta*, in medicinal plants *Cinnamomum verum*, and trees *Ficus benjamin* and *Ficus excelsior* showed considerable activity.

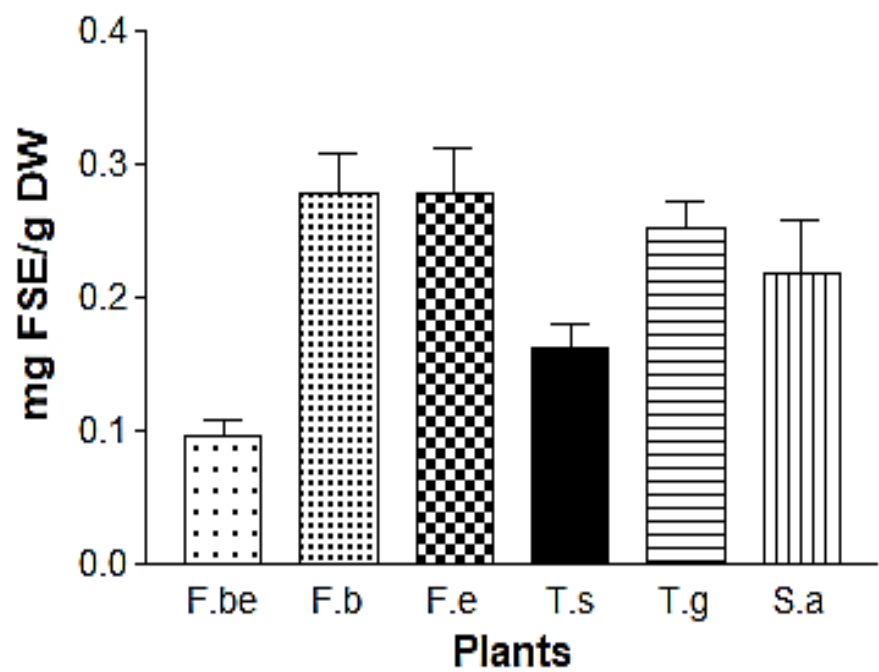




A)



B)

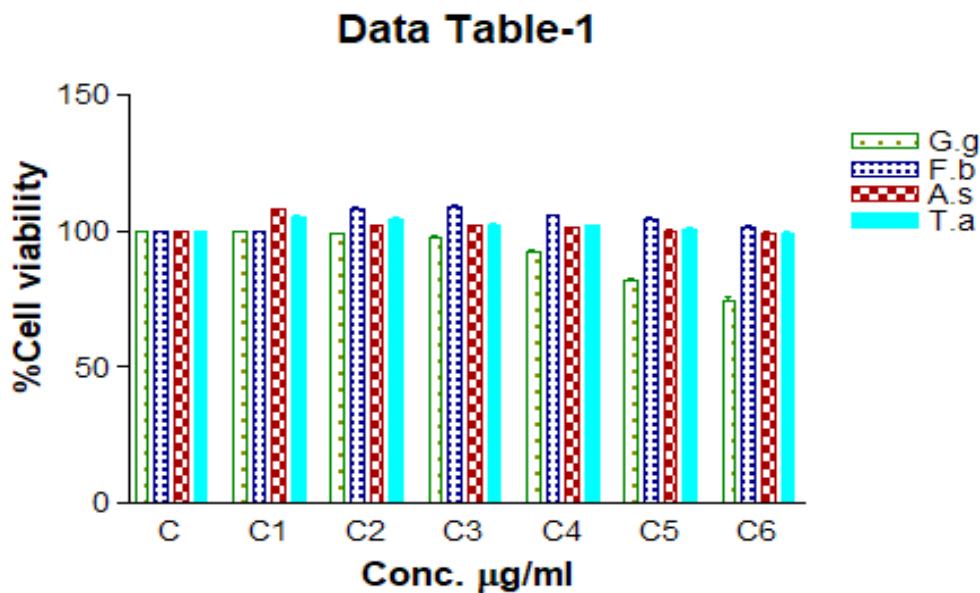


(C)

**Figure 6:** Antioxidant potential of different plant groups expressed as mg Ferrous sulfate equivalent per gram of dry weight. The findings of the studies agreed with earlier reports; nevertheless, variation in value could be caused by differences in the origin of the raw materials under different geographical variations and genotype-environment interaction [14].

**Cytotoxic Activity**

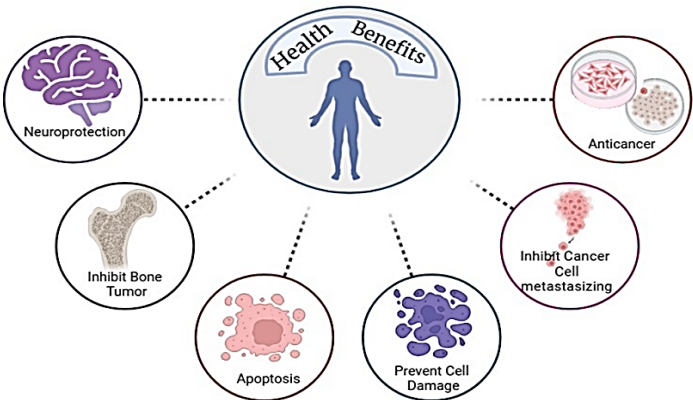
In the present study, the MTT assay was used to evaluate the cytotoxic potential of four selected plants exhibiting good antioxidant potential as per the parameter’s studies such as TPC, TFC, FRAP, and DPPH. These plants belongs to different clusters of medicinal, and tree plant groups *Alstonia scholaris*; *Glycyrrhiza glabra*; *Terminalia arjuna*; *Ficus benghalensis*. The result of the cytotoxicity assay conducted against HEK-293 is shown in Figure 7. The cytotoxicity activity was found to be dose-dependent, as the higher the concentration higher the activity was observed. *Glycyrrhiza glabra* showed 70 to 80% cell viability at 150µg/ml in the case of HEK-293 cell lines. *F. benghalensis*, *A. scholaris*, and *T. arjuna* show no cytotoxicity up to the dose of 150µg/ml in the case of HEK-293 cells. A one-way ANOVA test was done to evaluate the statistical difference and significance.



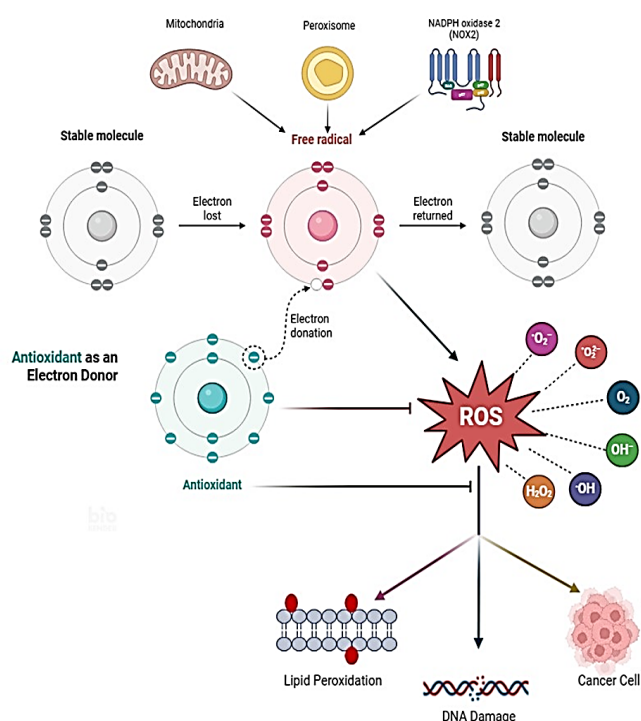
**Figure 7:** Growth inhibition of HEK-293 (Human embryonic kidney cells) by (G.g *Glycyrrhiza glabra*; F.b *Ficus benghalensis*; A.s *Alstonia scholaris*; T.a *Terminalia arjuna*). Cells were seeded at a density of  $10 \times 10^3$  cells/well and treated in a dose-dependent manner for 24 hours, Here C stands for Control, C1 concentration 25µg/ml, C2 50µg/ml, C3 75µg/ml, C4 100µg/ml, C5 125µg/ml, C6 150µg/ml. Cell viability was measured by MTT assay. The data is shown as the average of three separate studies, with standard errors represented by mean  $\pm$  standard error (p-value <0.01).

**Discussion**

Plants are a potent source of antioxidants. In epidemiological studies, fruits and vegetables in the diet have proven to protect against various chronic illnesses including cancer, cardiovascular disease, cataracts, and immunological dysfunction (Figure 8) [6, 30]. Various phenolic and flavonoid constituents have been linked with natural protective benefits [31-33]. The biological actions of phenolics, which are powerful antioxidants and free radical scavengers, have been subject to several earlier investigations [34, 35, 28]. Phenolic compounds are secondary metabolites having an aromatic ring with one or more hydroxyl substituents. The redox characteristics of phenolics, which allow them to operate as reducing agents, hydrogen donors, and singlet oxygen quenchers, are primarily responsible for their antioxidant action. As a result, their nutritional roles are expanding and promising. The antioxidant properties of plants can be tested with several methods [22].



**Figure 8:** Health benefits associated with natural antioxidants from plants.



**Figure 9:** Role of antioxidants as free radical scavengers and in neutralizing oxidative stress

The radical scavenging activity and polyphenolic profile reported were higher in the case of medicinal plants. Medicinal plants are potentially rich sources of natural antioxidants. *G. glabra* shows maximum antioxidant potential i.e., 75.12% at 100µg/ml. Similar studies which were done earlier on the same plant showed less activity at 80 µg/ml, and there is 50% hydroxyl radical scavenging activity [36]. According to [29,37] *G. glabra* showed low cytotoxicity to HEK293 cells as compared with cancerous cells. *Asparagus racemosus* shows the lowest antioxidant potential because of its low phenolic and flavonoid content. *Cinnamomum verum* shows the highest while *F. benghalensis* shows the lowest ferric-reducing antioxidant potential. Previous studies showed high phenolics as well as flavonoid content in cinnamon [38], implying it has potential uses in the mitigation of the disease associated with oxidative stress. This antioxidant activity might be attributed to the presence of flavonoids, as revealed by the phytochemical analysis of plants [23]. *Petunia hybrida* shows the lowest total flavonoid content. *Tectona grandis* shows maximum phenolic content i.e. a range of 30 to 35 mg GAE/g DW with good antioxidant potential 70% at 100µg/ml while *Ocimum tenuiflorum* shows the lowest total phenolic content. Phenolics and flavonoids are the key class of chemical constituents that operate as principal antioxidants and may be used as a substitute for many artificial antioxidants. Natural antioxidants are safer to

use compared to artificial antioxidants [39]. Previous reports have established that these polyphenols are a broad and diverse group of chemicals that may be found in a variety of plants, many of these polyphenols are now under active research. As a result of intense research, phenolics, and flavonoids are potent ingredients that minimize the risk of causing cancer has been attributed to their antioxidant potential [13, 40]. Hence, polyphenols protect against human diseases associated with oxidative stress, like coronary heart disease and cancer [28, 31]. After studying the literature, it was found that major polyphenolic classes which have prominent effects as antioxidants, and other health benefits present in *Alstonia scholaris*, *Tabernaemontana divaricata*, *Tagetes erecta* [8, 13, 18, 33]. Antioxidant activity evaluation revealed that *Saraca asoca* showed great antioxidant activity in eliminating hydroxyl radicals (Figure 9). These can lead to the development of new potential antioxidants. *Ficus benjamin* leaves are a good source of health-promoting polyphenols and flavonoids which are beneficial in showing antioxidant activity [35]. According to a previous study the genus *Ficus* has shown the promising potential of possessing antioxidant activity. Similarly, the study done on *F. benghalensis* shows 73% radical scavenging activity and shows no effects on normal human cell lines, owing to the phenolics and flavonoids content found in the leaves [41]. *Alstonia scholaris* and *Terminalia arjuna* show a high antioxidant potential of 69% & 73% respectively showing no cytotoxicity against HEK293 cells and till now no study has been available on HEK293 cells of these plant extracts. A previous study also supported these results due to the high antioxidant potential of *A. scholaris* which has recently been used in the formulation specifically for COVID-19 [25]. According to previous reports, dietary polyphenols act as strong antioxidants, prevent oxidative damage as well as decrease inflammation [42]. Plant enriched with phenolic decreases the chances of occurrence of many health ailments such as cancer, cardiovascular disease, chronic inflammation as well as metabolic disorders that interact with several cellular signaling pathways as well as their associated metabolic reactions [17, 18, 31, 41]. Polyphenols can neutralize free radicals by donating an electron or hydrogen atom to a wide range of reactive oxygen, nitrogen, and chlorine species, and gain antioxidant enzyme activity as well as balancing cytokine-induced inflammation [35, 43]. In most of the plant species, scavenging potential exhibited a correlation between total phenolic compounds and the antioxidant potential of the respective plant/tree samples. This further explores the potential of tree species, flowering plants, and medicinal plants under natural wild conditions to possess antioxidant properties owing to the naturally occurring antioxidant phenolic compounds which may have a role in lipid peroxidation. Thus, the present study revealed that there is a great scope for finding good sources of antioxidant molecules in the plants presented, and shows minimum or no toxicity against normal human cell lines. So, these plants give a

scope of study on different cell lines w.r.t. diseased conditions. The study has screened and evaluated the potential of selected plants for their use as natural antioxidants.

## Conclusions

Our study examined the potential of several plants for their antioxidant potential parameter as the overall capacity of each sample is the result of the combined activity of phenolic and nonphenolic compounds. Total phenolic content and total flavonoid content which were responsible for the antioxidant potential were evaluated and showed a wide range of occurrences were further used for their cytotoxic potential and proved nontoxic to normal human cell lines in a dose-dependent manner. The information generated may be useful in further development of plant types, food and pharmaceutical industry preparation for antioxidant properties. Foods that are rich in phenolic compounds may be encouraged for safe ingestion, as they can help in the prevention of long-term diseases.

**Ethics Approval and Consent of Participate:** There is no need for ethical approval and informed consent in this study.

**Human and Animal Rights:** This study involved no human or animal participation.

**Conflict of interest:** The authors declare no conflict of interest.

**Acknowledgments:** NSS thanks University Grants Commission (UGC) and collaborators for grants and assistance. NY thanks UGC, New Delhi for the research fellowship. The agency had no role in the interpretation or writing the manuscript.

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