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Evaluation of Antioxidant, ROS Scavenging Activity and Metal Chelation Activity of Herbal Extracts from *Calotropis gigantea*.

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Abstract

The imbalance between free radicals and antioxidants in the body causes oxidative stress, which plays a crucial role in the development of various diseases. Naturally occurring antioxidants derived from plants have emerged as a potentially interesting candidate for lowering oxidative stress due to their capacity to scavenge free radicals. As *Calotropis gigantea* widely acknowledged to have therapeutic properties, an extensive investigation of its antioxidant potential is required. Therefore, objective of this study is to systematically evaluate the antioxidant activity of different leaf extracts of *C. gigantea.* The phytochemical composition, antioxidant capacity, and free radical scavenging ability of the plant's methanol, chloroform, acetone, and aqueous leaf extracts were investigated. The presence of phenols, flavonoids, saponins, tannins, and alkaloids was demonstrated by phytochemical analysis. The high levels of total phenol and flavonoid content, along with substantial amount of reducing activity observed in the reducing assay, suggested that the leaves possessed antioxidant potential. A dose-dependent response by all extracts on DPPH assay confirmed the free radical activity of plant. The primary cause of oxidative stress in our bodies is reactive oxygen species. Our study demonstrates that all plant extracts have a neutralizing effect on superoxide, hydrogen peroxide, and hydroxyl radicals. The study was extended to evaluate the metal chelating capability of plant extracts, which was also found to be dose-dependent. Here, we report that *C. gigantea* has great promise as a multipurpose therapeutic agent due to its significant antioxidant activity and bioactive components.

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Key Words: *Calotropis gigantea*, Antioxidant activity, ROS, Medicinal plants

1. Introduction

Oxidative stress is the major driving force associated with the potential development of various diseases, including cancer, neurodegenerative disorders, diabetes, and heart disease [1]. Oxidative stress occurs due to the imbalance between the generation of reactive oxygen and nitrogen species (ROS) within cells and the ability

of cell to effectively eliminate them [2]. Every living system possesses an inherent antioxidant defense system designed to shield against oxidative harm and facilitate the repair of enzymes for the elimination of damaged molecules [3]. Nevertheless, in states of disease, this inherent antioxidant mechanism may operate inefficiently, resulting in the buildup of an excess of free radicals. This accumulation subsequently induces the damage to cellular machinery that associated with oxidative stress, contributing to the development of various diseases [4].

Conventionally, oxidative stress has been managed through the application of different synthetic antioxidants. However, the use of these synthetic antioxidant compounds has been associated with several adverse effects [5]. With growing safety concerns, there is a current interest in exploring cost-effective and safer sources for antioxidants, particularly those derived from natural sources such as plants. Polyphenols emerge as the primary plant compounds renowned for their antioxidant activity. Secondary metabolites such as polyphenols demonstrated a wide spectrum of pharmacologic activities which accredited to their redox properties [6]. Flavonoids represent the most prevalent and extensively distributed class of phenolic compounds found in plants. These compounds are found in the majority of plants and are believed to protect against damages caused by free radicals through various mechanisms, such as directly scavenging free radicals and inhibiting enzymes responsible for free radical production [7]. In the search of better alternatives to synthetic antioxidants, our study aims to investigate the bioactive elements and antioxidant capacity of the traditional medicinal plant *C. gigantean*.

The sweet akand, scientifically known as *Calotropis gigantea* (L.) Dryand belonging to the Asclepiadaceae family, is indigenous to India and grows at altitudes of 900 m in the lower hills [8]. Different components of *C. gigantea* have been recorded for their therapeutic applications in treating conditions such as sprains, restlessness, fatigue, epilepsy, mental disorders, diarrhea, pain relief, and exhibiting interceptive properties during pregnancy. Additionally, they have been traditionally used to address toothaches and earaches [9,10, 8]. In terms of secondary metabolites, various species belonging to the genus *Calotropis* have been shown to possess vital classes of phytochemicals, which include fatty acids, glycosides, flavonoids, and triterpenoids [11].

Taking in to account the plants previously mentioned therapeutic and dietary uses, the aim of the current study is to emphasize the antioxidant potential of the *C. gigantea* as a means to prevent oxidative stress. This was achieved by quantifying the total phenolic content and flavonoids, as well as by qualitatively examining diverse categories of phytochemicals present in plant extracts. The antioxidant capability was initially evaluated through reducing potential and DPPH assays. Subsequently, the impact on oxygen-borne free radicals and metal chelation was investigated to elucidate the precise means of action as an antioxidant. The findings of the present work offer a scientific foundation for the traditional claims regarding *C. gigantea*, validate the practical application of plant in phytomedicines and create opportunities for the formulation of antioxidant-rich nutraceuticals.

2. Materials and Methods

2.1 Plant material collection and chemicals

The leaves of the plant *C. gigantea* were collected from the area around Modinagar, India. The plant components were identified and authenticated by Professor D. K. Awasthi and Professor Arun Kumar Maurya of the Department of Botany at Multanimal Modi College, Modinagar. Plant sample has been preserved at the Department of Botany, Modinagar. All of the chemicals of analytical-grade used for the current study were acquired from Sisco, HIMEDIA, CDH, and Sigma in India.

2.2 Preparation of plant extracts

The double distilled water was used to wash the leaves to remove any kind of dust or debris and allowed to air-dried in the shade, and formed a coarse powder. 10 grams of the grounded powder was individually subjected to extraction using 250 ml of methanol, chloroform., water and acetone in a Soxhlet apparatus for 24 hours. After that, solvents were vacuum-evaporated at 40°C in a Buchi-type rotary evaporator. A desiccator was used to keep the concentrated extracts until they reached a steady weight. These extracts were stored for future use at 4ºC [12]. The % yield of the plant extracts was calculated according to the method outlined by Aquil *et al.* [13]. Specific concentrations (w/v) of the extracts for experiments were prepared using a mixture of Tween 80 and distilled water in a 1:9 ratio.

2.3. Qualitative and Quantitative Analysis of Phytochemicals

2.3.1 Qualitative assessment of phytochemicals: For the identification of various classes of active chemical constituents, a qualitative analysis of all four plant extracts was conducted using methods outlined by Chaurasia and Sharma [14], with slight modifications.

Test for phenols:

A few drops of 10% of aqueous $FeCl_3$ were added to all the extracts to perform the Ferric chloride test. The appearance of a blue or green color signifies the presence of phenols [14].

Test for Flavonoids

A bright yellow color developed after mixing the few drops of diluted NaOH solution with the 1 ml of the plant solvent extract. The addition of few drops of diluted acid caused the developed yellow color to turn colorless, showing the presence of flavonoids [14].

Test for Saponin

In a test tube 20 ml of distilled water was mixed with 1 mL of each plant extract and the resulting mixture was shaken manually for 15 minutes. Subsequently, a foam layer developed at the top of the test tube, indicating the presence of saponins [14].

Test for tannins

In a test tube, each plant extract is diluted with 3 ml of chloroform followed by addition of 1 ml of acetic anhydride. Subsequently, 1 ml of H_2SO_4 was carefully added along the side of the test tube to the solution. The formation of a green color revealed the presence of tannins [14].

Test for alkaloids

For a duration of 2 minutes the extracts were warmed with 2% H_2SO_4 followed by filtration. A few drops of Wagner reagent (prepared by dissolving 2g of potassium iodide and 1.27g of iodine in 5 ml of water, and adjusting the volume to 100ml with distilled water) were added. The presence of alkaloids was indicated by the formation of reddish-brown color precipitate [14].

2.4 Quantitative Analysis of Phytochemicals

2.4.1 Determination of total phenol content (TPC) in plant extract

The spectrophotometric technique was used to determine the total phenol content in various solvent extracts [14]. 0.1 ml of each plant extract of 1 mg/ml concentration was diluted to 1 ml by using Double distilled water (DDW). 1.5 ml of Na_2CO_3 (0.2 g/ml) and 0.5 ml of Folin ciocalteu reagent were added in the ratio of 1:1 after dilution to this solution. At 25°C mixture was incubated for 2 hrs. 7.0 ml of double distilled water (DDW) was added to each sample after incubation, and the mixtures' absorbance was determined at 765nm. A standard curve was formed by using Gallic acid (GA) that was used to quantify the phenol concentration in each extract. The total phenol content of the different plant extracts was then represented as mg GAE/g PM (Gallic Acid Equivalent per gram of Plant Material).

2.4.2 Determination of total flavonoid content in plant extract

A spectrophotometric technique was used to assess the total flavonoid concentration in various solvent extracts [14] with slight modifications. 1 ml of each extract was mixed with 0.3 ml of 10% $AlCl_3$, and 0.3 ml of 5% $NaNO_2$ were added to and allowed to incubate for 5 minutes. Following this, 1M NaOH solution (2ml) was added. The volume of mixture was raised up to 10 ml and vortexed rigorously. The absorbance of pink-colored solution was taken at 510 nm. Using a quercetin standard curve, the flavonoid concentration was calculated and expressed as mg QE/g PM (or Quercetin equivalent per gram of Plant Material).

2.5 Antioxidant activity

2.5.1 DPPH radical scavenging assay

The procedure outlined by Mensor *et al.* [15], was followed with minor modifications. Each sample solution (at concentrations specified in the protocol) was added to the 1 ml of DPPH (0.3 mM) in methanol and left to start the reaction at RT in the dark for 30 mins. The blank was created using a 2 ml sample solution and 1 ml

of methanol, while the DPPH solution (1 ml) and methanol (2 ml) was treated as negative control. The reduction in absorbance was recorded at 517 nm. % AA (antioxidant activity) was calculated using the formula specified below:
$\% AA = [(A_0 - A_1)/A_0 \times 100]$... (1)

where A_0 is the absorbance of control, and A_1 is the absorbance of extracts or standards

2.5.2 Reducing power assay
The reducing power of different plant extracts was determined by the method of Jing *et al*. [16]. In brief, 2.5 ml of 1% $K_3[Fe(CN)_6]$ and 2.5 ml of phosphate buffer (200 mM of pH 6.6) were mixed with 1 ml of each extract at variable concentrations. The resulting mixture was then allowed to incubate at 50°C for twenty minutes. After incubation, mixture was combined with 10% TCA (2.5ml), followed by the centrifugation for 10 minutes at 3000 rpm. Afterward supernatant (2.5 ml) was combined with 0.5 ml of $FeCl_3$ (0.1%) and DW (2.5 ml), and the absorbance at 700 nm was determined using spectrophotometry.

2.5.3 Superoxide Anion Scavenging Assay
The superoxide anion scavenging activity in all the plant extracts was assessed by using the method outlined by Nishimiki *et al.* [17], with slight modifications. 1 ml of each plant extract at different concentrations were specifically added to, 1 ml solution of NADH (468 µM in phosphate buffer of 100 mM and pH 7.4) and 1 ml solution of NBT (156 µM NBT in phosphate buffer of 100 mM and pH 7.4). To the resultant mixture 100 µl of PMS solution (60 µM PMS in 100 mM phosphate buffer and pH 7.4) was added and reaction starts. The solution was left to incubate for 5 minutes at 25°C. Subsequently, the absorbance of the solution was determined at a wavelength of 560 nm against blank samples. As the absorbance of the mixture decreases, it appears that there is an increase in the activity of scavenging superoxide anion. Formula (1) is utilized to determine the percentage reduction of superoxide anion production.

2.5.4 H_2O_2Scavenging activity
The hydrogen peroxide scavenging activity of leaf extracts from *C. gigantean* was assessed using the method described by Ruch *et al.* [18]. Plant extract (4 ml) prepared at different concentrations by using distilled water, were mixed with 0.6 ml of a 4mM H_2O_2 solution (0.1 M phosphate buffer, pH 7.4) and followed by the incubation of 10 minutes. At 230nm, the absorbance of the resulting mixture was measured, compared to a blank solution that contains plant extract without H_2O_2. The percentage decomposition of H_2O_2 was calculated using the formula (1).

2.5.5 Hydroxyl radical scavenging assay
Method given by Jing *et al.* [16] was used to assay hydroxyl radical scavenging activity of *C. gigantea* leaf extracts. 1ml of each plant extract at variable concentrations was mixed with 0.2 M phosphate buffer of pH 7.8 (2.4 mL), 1 mM $FeCl_2$ (60 µl), 1,10-phenanthroline (90 µl), followed by the addition of 0.17 M H_2O_2 (150 µl) for the initiation of the reaction, and the resulting mixture was allowed to incubate for five minutes at room temperature. After incubation, the absorbance of the mixture was determined spectrophotometrically at the wavelength of 560 nm. The % inhibition was calculated using the formula mentioned earlier (Formula 1).

2.5.6 Metal chelating activity
The chelation of ferrous ions by extracts was determined by the method of Dinis *et al.* [19]. In brief, different concentrations of plant extracts were added to a solution of 0.05ml of 2 mM ferric chloride. To initiate the reaction 5 mM ferrozine (0.2 ml) was added, and vigorously shaken the mixture. After that, the resulting mixture was left to rest for 10 minutes at room temperature. The absorbance of the resulting solution was then determined at 562 nm. The metal chelating activities were computed using the provided formula (1).

2.6 Statistical analysis
In this study results are reported as the mean ± standard deviation of three replicates. The data were analyzed by using one-way ANOVA, and p-values < 0.05 were considered statistically significant. MS office was used to create all the graphical presentations.

3. Results and Discussion

3.1. Qualitative and Quantitative assessment of Phytochemicals

3.1.1. Qualitative Analysis of Phytochemicals.

Phytochemical analysis of various leaf extracts from *C. gigantea* indicated (Table 1) the presence of all examined phytochemicals in methanol and acetone extracts. However, the aqueous extract lacked in alkaloids and tannins, while the chloroform extract did not contain saponins. The concentration of phytochemicals was visually assessed based on color intensity. The absence of phytochemicals is denoted by (−), low presence by (+), moderate by (++), and high presence by (+++). The presence of these bioactive phytoconstituents in *C. gigantea* supports the reported health-promoting and disease-prevention properties of the plant described in the literature [20].

Table 1: Qualitative phytochemical analysis of *Calotropis gigantea*

Phytochemicals	Solvent Extracts			
	Chloroform	Acetone	Methanol	Aqueous
Alkaloids	+++	+	+++	-
Phenols	++	++	+++	++
Flavonoids	+++	+	+++	++
Saponins	-	+++	++	+++
Tannins	++	+	++	-

Note: - absent; + low in abundance, ++ Moderate in abundance; +++ high in abundance

3.1.2. Total content of Phenolics and Flavonoids.

Phenolic and flavonoids compounds are widely known for their antioxidant properties. As indicated in Table 2, the leaf extracts of *C. gigantea* possess a significant quantity of phenolic and flavonoid content. Among all the extracts, the highest levels of phenolic and flavonoids were exhibited by the methanol extract of *C. gigantea*. Specifically, the total phenolic content in methanol was measured at 725±0.002 mg GAE/gm, followed by chloroform, acetone, and aqueous extracts. Similarly, methanolic extract showed the highest total flavonoid content (1866±0.014 mg QE/gm), followed by chloroform, acetone, and aqueous extracts of *C. gigantea*. These secondary metabolites by providing hydrogen, neutralize free radicals and interrupt the chain that leads to the creation of new radicals [21]. The presence of these phytochemicals in extracts of *C. gigantea* indicates the antioxidant capabilities of the plant.

Table 2: Total phenolic and flavonoid content of *Calotropis gigantea*

Solvent Extracts	Total Phenolic content (mg GAE/g PM)	Total Flavonoid content (mg QE/g PM)
Acetone	215±0.004	635±0.004
Chloroform	357±0.006	806±0.006
Methanol	725±0.002	1866±0.014
Aqueous	54±0.004	587±0.001

Data given are mean of three replicates ± standard deviation

3.2 Antioxidant activity of plant

3.2.1. DPPH radical scavenging assay

The capacity of *C. gigantea* to transfer hydrogen atoms for the neutralization of free radicals was examined through the DPPH assay. The stable free radical DPPH, of deep violet color, accepts hydrogen from antioxidants and reduces to 1,1-diphenyl-2-picryl hydrazine, resulting in decoloration. Remarkably, All the extracts of *C. gigantea* demonstrated substantial scavenging activity against DPPH (Table 3). The chloroform extract showed highest scavenging activity (IC50 0.011 mg/ml) followed by methanol (IC50 0.059 mg/ml), acetone (IC50 0.196 mg/ml), and aqueous (IC50 0.271mg/ml). It's worth highlighting that all these extracts exhibited superior activity compared to ascorbic acid (IC50 0.486 mg/ml), which was used as the standard in the analysis. Moreover, the identification of hydrogen atom transfer (HAT) as a pivotal mechanism for detoxifying free radicals [22] underscores the remarkable capacity of *C. gigantea* to actively participate in this process by donating hydrogen atoms, thus highlighting its potential as a potent natural antioxidant.

Table 3: DPPH radical scavenging ability of different extracts of *Calotropis gigantea* leaves extracts

Conc.(mg/ml)	% Inhibition of DPPH				
	Ascorbic acid	Aqueous	Methanol	Acetone	Chloroform
0.1	33.13±0.231	46.69±0.464	51.63±0.438	47.63±0.562	53.75±0.628
0.25	47.50±0.421	49.37±0.853	58.13±0.282	52.00±0.782	61.63±0.453
0.5	50.81±0.531	52.50±0.233	61.88±0.621	56.56±0.431	72.50±0.281
0.75	58.75±0.322	65.62±0.648	78.13±0.314	74.06±0.454	85.31±0.326
1.0	65.31±0.449	68.75±0.379	82.81±0.247	77.19±0.324	95.31±0.243
IC50 (mg/ml)	0.486	0.271	0.059	0.196	0.011

Data given are mean of three replicates ± standard deviation, $p < 0.005$

3.2.2. Reducing power assay

The reducing power assay is commonly used to assess the antioxidant's capability to donate an electron [23]. This method was used to evaluate the capacity of solvent extracts of *C. gigantea* to convert Fe^{3+} to Fe^{2+}. Notably, Fig. 1 demonstrates a clear trend of increasing absorbance with increasing concentration, indicative of enhanced reducing ability. Remarkably, among the extracts tested, chloroform demonstrated the highest reducing power, followed by methanol, acetone, and aqueous extracts, respectively. Remarkably, all extracts outperformed the standard ascorbic acid, underscoring the potent antioxidant potential of *C. gigantea*. These findings suggest that *C. gigantea* possesses the capability to contribute electrons, which could play a pivotal role in combating oxidative stress by neutralizing free radicals.

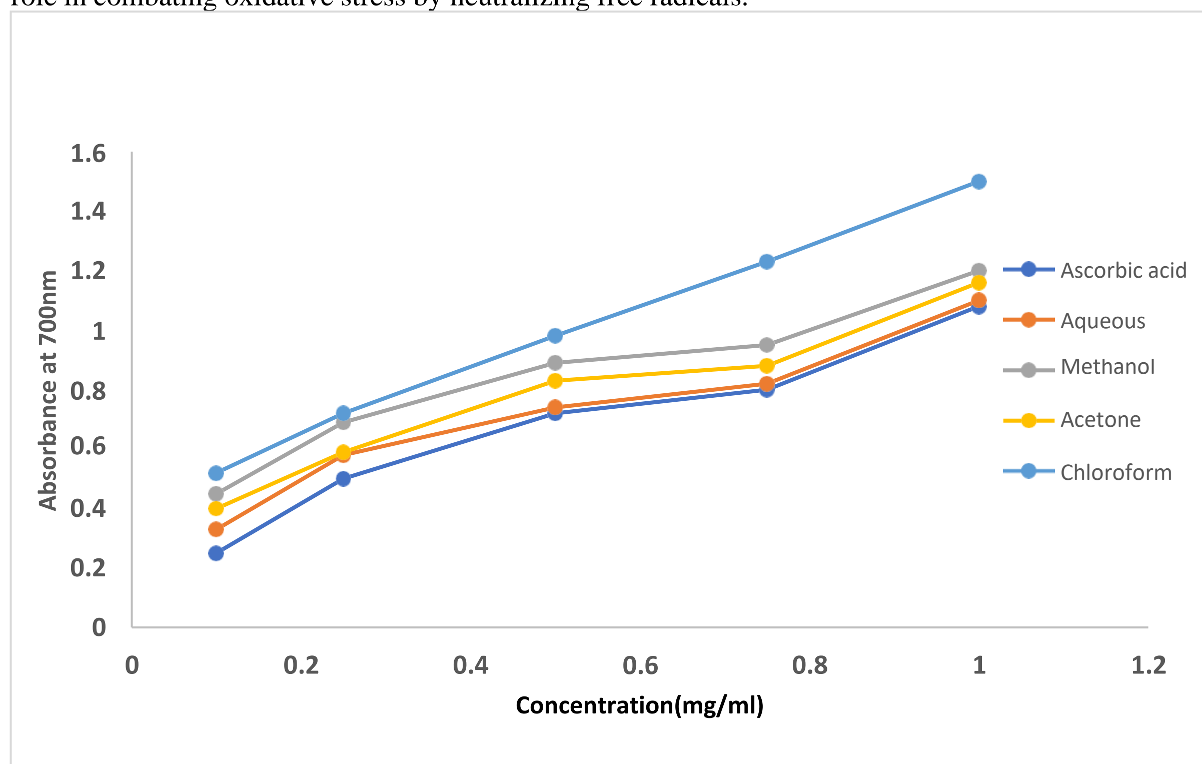

Fig.1: Reducing power ability of different extracts of *Calotropis gigantea* leaves.

3.2.3 Superoxide anion scavenging activity

A PMS/NADH-NBT system reduces NBT by generating superoxide anion through the PMS/NADH coupling reaction, whereas antioxidants inhibit this blue NBT formation [24]. Results obtained at various concentrations demonstrated that all the extracts of *C. gigantea* under investigation showed dose-dependent manner superoxide radical scavenging activity. Among these plant extracts, the highest inhibition was observed with the chloroform extract (IC50 0.256 mg/ml), followed by methanol (IC50 0.422 mg/ml), acetone(IC50 0.473 mg/ml), and aqueous (IC50 0.768 mg/ml) under similar experimental conditions. These findings were compared with the standard reducing agent quercetin (IC50 0.402 mg/ml). However, the outcomes obtained (Fig.2) at the investigated concentrations strongly suggest that *C. gigantea* possesses the capacity to neutralize superoxide radicals, demonstrating similar activity like superoxide dismutase, which can serve as a defense against reactive oxygen species (ROS).

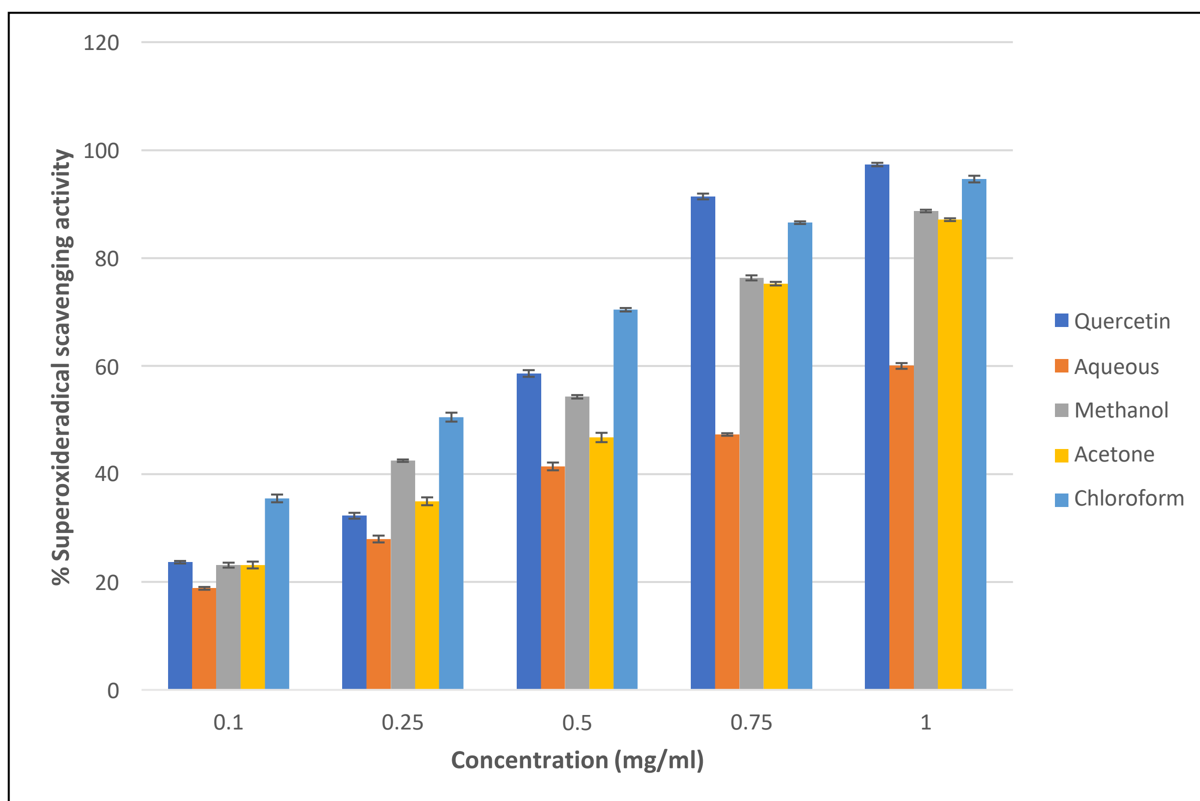

Fig.2: Superoxide radical scavenging activity of different extract of *Calotropis gigantea* leaves and Quercetin.

3.2.4. Hydrogen peroxide decomposition activity

Hydrogen peroxide is a moderately reactive ROS formed by the dismutation of superoxide radicals, either non-enzymatically under low pH conditions or mostly by inflammatory cells, which triggers oxidative stress [20]. The ability of leaf extracts of *C. gigantea* to decompose H_2O_2 was determined spectrophotometrically. The decomposition of H_2O_2 occurred in a concentration dependent manner and was comparable to that of the standard, ascorbic acid. All the extracts significantly decomposed H_2O_2 (Table 4). The methanol extract exhibited the highest decomposition activity (IC50 0.017 mg/ml), followed by chloroform (IC50 0.128 mg/ml), acetone (IC50 0.181 mg/ml), and aqueous (IC50 0.397mg/ml) extracts. The breakdown of H_2O_2 can be accredited to the presence of electron donating phenolic groups. These groups donate electrons to hydrogen peroxide, thereby reducing it into H_2O.

Table 4: Hydrogen peroxide scavenging ability of different extracts of *Calotropis gigantea* leaves extracts.

Conc.(mg/ml)	Percent decomposition of H_2O_2				
	Ascorbic Acid	Aqueous	Methanol	Acetone	Chloroform
0.1	45.41±0.241	40.98±0.231	50.73±0.431	47.41±0.731	48.29±0.674
0.25	51.85±0.671	42.41±0.541	60.61±0.563	52.85±0.282	57.16±0.521
0.5	60.29±0.483	53.50±0.369	75.05±0.282	61.17±0.914	63.63±0.423
0.75	73.17±0.265	65.85±0.884	85.37±0.534	78.05±0.324	78.05±0.242
1.0	82.93±0.712	68.29±0.218	90.24±0.262	85.37±0.562	87.80±0.531
IC50(mg/ml)	0.217	0.397	0.017	0.181	0.128

Data given are mean of three replicates ± standard deviation, $p < 0.005$

3.2.5. Hydroxyl radical scavenging activity

Hydroxyl radicals are recognized as major contributors to oxidative damage, given their extremely high reactivity and short half-life. Since our body lacks enzymes to neutralize this radical, its excessive accumulation can lead to cell death [25]. The results presented in Fig. 3 demonstrate significant hydroxyl radical scavenging properties in the studied plant extracts in a dose-dependent fashion. Methanol and chloroform extracts of *C. gigantea* exhibited approximately 92.62±0.264% and 92.62±0.474% inhibition at a dose of 1 mg/ml, followed by acetone (69.67±0.724) and aqueous (52.46±0.612) extracts. These results were compared with the standard, ascorbic acid (IC50 0.656 mg/ml). These findings suggest that *C. gigantea* serves as a

significant reservoir of natural antioxidants, potentially important for preventing the advancement of oxidative stress by effectively neutralizing all three reactive oxygen species (ROS).

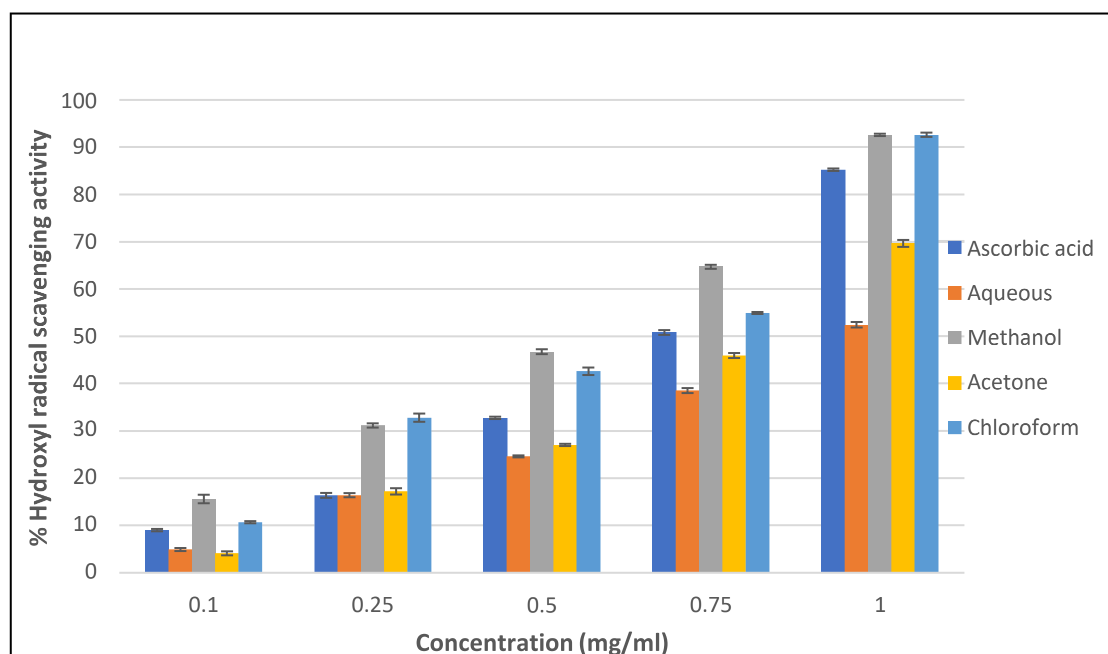

Fig.3: Hydroxyl radical scavenging activity of different extracts of *C. gigantea* leaves as compared to Ascorbic acid

3.2.6. Metal chelating assay

In the context of oxidative stress, iron plays a pivotal role as it can catalyze the generation of reactive oxygen species (ROS) via Fenton and Haber-Weiss reactions, exacerbating oxidative damage within [26, 27]. Given this significance, exploring natural compounds capable of chelating iron ions holds great relevance for potential therapeutic interventions against oxidative stress-related disease. In this study, it was observed (Table 5) that all the organic extracts examined displayed a dose-dependent potential for chelating Fe^{2+} ions. Among the various extracts, the highest metal chelation was observed with the chloroform extract of *C. gigantea* (IC50 0.083 mg/ml), subsequently followed by methanol (IC50 0.363 mg/ml), acetone (IC50 0.438 mg/ml), and aqueous extracts (IC50 0.571 mg/ml). The results were compared with the standard metal chelator EDTA (IC50 0.0436 mg/ml). The study underscores the potential of *C. gigantea* as a natural source of metal chelators, suggesting a potential protective role in preventing oxidative damage induced by metal-catalyzed decomposition reactions.

Table 5: Metal (Fe^{2+}) chelation ability of different extracts of *Calotropis gigantea* leaves extracts.

Conc.(mg/ml)	**Percent metal chelation**				
	EDTA	**Aqueous**	**Methanol**	**Acetone**	**Chloroform**
0.1	46.34±0.239	23.58±0.561	29.27±0.621	22.76±0.674	45.52±0.524
0.25	64.23±0.468	30.90±0.274	37.40±0.349	35.77±0.521	63.41±0.612
0.5	83.74±0.344	43.90±0.314	65.40±0.541	59.91±0.274	74.80±0.721
0.75	94.39±0.536	67.48±0.254	85.37±0.659	73.98±0.324	87.80±0.569
1.0	99.19±0.424	69.92±0.424	90.24±0.251	87.00±0.434	96.75±0.224
IC50(mg/ml)	0.0436	0.571	0.363	0.438	0.083

Data given are mean of three replicates ± standard deviation, $p < 0.005$

4. Conclusion

C. gigantea stands out as a promising plant with diverse therapeutic properties and significant economic importance. It has been used traditionally by tribal communities to treat variety of health problems, including as earaches, sprains, toothaches, pain, anxiety, diarrhea, and mental illnesses [28]. The present investigation on *C. gigantea* herbal leaf extracts revealed remarkable antioxidant and metal chelation activities, effectively

scavenging reactive oxygen species (ROS). The results indicate a multifaceted mechanism in providing antioxidant activity. The bioactive compounds found in leaves, enable them to neutralize free radicals by the mechanism of transferring single electron and hydrogen atom. Additionally, all leaf extracts from *C. gigantea* demonstrated scavenging activity against hydroxyl and superoxide radicals, along with the decomposition of hydrogen peroxide. The observed metal chelation activity further suggests a protective effect against oxidative damage induced by metal-catalyzed processes. These findings underscore the potential use of *C. gigantea* leaves as a natural source of antioxidant, offering promise in treating ailments associated with oxidative stress. Further investigation and study of the specific bioactive substances responsible for these activities could provide valuable insights for the development of innovative dietary or medicinal therapies.

5. Acknowledgements

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6. Conflicts of interest

There are no conflicts of interest between authors relevant to this article.

7. References

1. Arika W., Kibiti C. M., Njagi J. M., Ngugi M. P., 2019. In vitro antioxidant properties of dichloro methanolic leaf extract of Gnidiaglauca (Fresen) as a promising antiobesity drug. Journal of Evidence-Based Integrative Medicine, vol. 24: 2515690X19883258.
2. Gilgun-Sherki Y., Rosenbaum Z., Melamed E., Offen D., 2002. Antioxidant therapy in acute central nervous system injury: current state. Pharmacol Rev., 54: 271-284. 10.1124/pr.54.2.271.
3. Willett W. C., 2002. Balancing life-style and genomics research for disease prevention. Science., 296: 695-698. 10.1126/science.1071055.
4. Vertuani S., Angusti A., Manfredini S., 2014. The antioxidants and pro-antioxidants network: an overview. Current Pharmaceutical Design, vol. 10, no. 14, pp. 1677–1694.
5. Ndhlala A., Moyo M., Van Staden J., 2010. Natural antioxidants: fascinating or mythical biomolecules? Molecules, vol. 15, no. 10, pp. 6905–6930.
6. Zheng W., Wang S.Y., 2001. Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem, 49, pp. 5165-5170.
7. Sandhar H. K., Kumar B., Prasher S., Tiwari P., Salhan M., Sharma P., 2011. A review of phytochemistry and pharmacology of flavonoids. Int Pharmac Scien, 1, pp. 25-41.
8. Ghule S. D., Vidyasagar G., Bhandari A., Sharma P., Gunjal, A. P., 2014. CNS Activity of Leaves Extract of Calotropis Gigantea. Asian Pac. J. Trop. Dis. 4, S902–S905. doi:10.1016/s2222-1808(14)60755-6
9. Argal A., Pathak A. K., 2006. CNS Activity of Calotropis Gigantea Roots. J. Ethnopharmacology, 106, 142–145. doi:10.1016/j.jep.2005.12.024.
10. Pathak A. K., Argal A., 2007. Analgesic Activity of Calotropis Gigantea Flower. Fitoterapia 78, 40–42. doi:10.1016/j.fitote.2006.09.023.
11. Srivastava S., Singh A., and Rawat A., 2015. Comparative Botanical and Phytochemical Evaluation of Calotropis Procera Linn. And Calotropis Gigantea Linn. Root. J. App Pharm. Sci. 5, 041–047. doi:10.7324/japs.2015.50707
12. Kumar G., Karthik L., Rao K.V.B., 2010. In vitro anti-Canadida activity of Calotropis gigantean against clinical isolates of Candida. J Pharm Res; 3:539-542.
13. Aqil F, Maryam Z, Ahmad I., 2008. Antimutagenic activity of Methanolic extracts of four ayurvedic medicinal plants. Indian J Exp Biol; 46:668-672.
14. Chaurasia S, Sharma P., 2015. Evaluation of Total Phenolic, Flavonoid Contents and Antioxidant Activity of Acokanthera oppositifolia and Leucaena leucocephala. International Journal of Pharmacognosy and Phytochemical Research; 7(1); 175-180.
15. Mensor L. L., Menezes F.S., Leitao G. G., Reis A. S., Santos T. C., Coube C. S., Leitao S. G., 2001.Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res; 15: 127-130.

16. Jing M., Cai Y. X., Li J.R., Zhao H., 1996. 1, 10-Phenanthroline-Fe2+ oxidative assay of hydroxyl radical produced by H_2O_2/Fe_{2+}. Prog. Biochem. Biophys.; 22: 553–555
17. Nishikimi M, Appaji N, Yagi K., 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun.; 46:849–53.
18. Ruch R.J., Cheng S.J., Klaunig E., 1989. Prevention of cyto-toxicity and inhibition of intercellular communication by anti-oxidant catechins isolated from Chineese green tea. Carcinogenesis; 10:1003-8.
19. Dinis T.C., Madeira V.M., Almeida L.M., 1994. Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation as peroxyl radical scavenging effects. 199; 36:2090–97
20. Richards A., Chaurasia S., 2022. Antioxidant Activity and Reactive Oxygen Species (ROS) Scavenging Mechanism of Eriodictyon californium, an Edible Herb of North America Hindawi. Journal of Chemistry: Volume 2022, Article ID 6980121, 8 pages https://doi.org/10.1155/2022/6980121
21. Vo Q.V., Nam P.C., Thong N.M., Trung N.T., Phan D.C.T., Mechler A., 2019. Antioxidant motifs in flavonoids: O–H versus C–H bond dissociation. ACS Omega, vol. 4, no. 5, pp. 8935–8942.
22. Siddeeg A., AlKehayez N., M., Abu-Hiamed H. A., AlSanea E. A., and AL-Farga A. M., 2021. Mode of action and determination of antioxidant activity in the dietary sources: an overview. Saudi Journal of Biological Sciences, vol. 28, no. 3, pp. 1633–1644.
23. Yildirim A., Mavi A., Oktay M., Kara A. A., Algur O. F., Bilaloglu V., 2000 Comparison of antioxidant and antimicrobial activities of Tilia (Tilia argentea Desf ex DC), sage (Salvia triloba L.), and Black tea (Camellia sinensis) extracts. Journal of Agricultural and Food Chemistry;48(10):5030–5034.
24. Özen T., Türkekul I., 2010. Antioxidant activities of Sarcodon imbricatum wildly grown in the Black Sea Region of Turkey. Pharmacogn Mag.; 6(22): 89–97.
25. Das A., Roychoudhury A., 2014. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in Environmental Science, vol. 2 (53), 13 pages.
26. Kell D.B., 2009. Iron behaving badly: Inappropriate iron chelation as a major contributor to the etiology of vascular and other progressive inflammatory and degenerative diseases. BMC Medical Genomics, 10.1186/1755-8794-2.
27. Braughler J.M., Duncan L.A., Chase L.R., 1986. The involvement of iron in lipid peroxidation. Importance of ferric to ferrous ratios in initiation Journal of Biological Chemistry, 261, pp. 10282-10289
28. Kumar G., Karthik L., Rao K.V., 2010. Antibacterial activity of aqueous extract of Calotropis gigantea leaves – An in vitro study. Int J Pharm Sci Rev Res.; 4:141–144.