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Original Article

Identification of Bioactive Compounds by GC-MS Analysis and *in vitro* Antioxidant Activities of *Debregeasia longifolia* (Burm.f.) Wedd.: Medicinal Plant

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ABSTRACT

Objectives: The study was aimed to investigate the presences of bioactive components by *Debregeasia longifolia* through Gas Chromatography-Mass Spectrometry (GC-MS) analysis and evaluate its *in vitro* free radical scavenging activity. *Debregeasia longifolia* is a member of the Urticaceae family that is used medicinally. **Materials and Methods:** The methanolic extract was prepared by shade-drying the gathered leaves, grinding them into a fine powder, and then extracting them with methanol. The *Debregeasia longfolia* methanolic leaf extracts was analyzed for potential bioactive components using GC-MS (Agilent 8890) and evaluated for its *in vitro* free radical scavenging activity. **Results:** A total of around fourteen bioactive components were identified during the GC-MS investigation, the current study focused on compounds with highest peak value and pharmacologic importance which include Phytol, Cyclobarbital, Hexadecanoic acid, methyl ester, Ipriflavone, Pinolenic acid, 2-Myristynoylglycinamide and some other components. The leaves were subjected to *in vitro* free radicles scavenging activity viz., DPPH, Nitric oxide, Hydroxyl, Total antioxidant activity, reducing power assay and all the assays shown strong antioxidant activity. **Conclusion:** *Debregeasia longifolia* found to contain potent therapeutic components and related curative properties, hence suggested as a plant of phytopharmaceutical interest.

Keywords: *Debregeasia longifolia* leaves, Medicinal plant, GC-MS analysis, Phytocomponents, Free radical Scavenging assays.

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INTRODUCTION

Medicinal plants are a valuable gift from nature, serving as a source of various phytoconstituents widely used by people across the globe. For generations, plant-derived medicines have been utilized to treat illnesses, with this knowledge passed down over time. These plants can synthesize a diverse range of organic compounds, known as secondary metabolites, which often have unique and complex structures (Briskin, 2010). They are used in traditional medicine to maintain health, prevent diseases, and treat physical and mental ailments in both humans and animals (Thomas *et al.*, 2013).

In recent decades, extensive research has focused on evaluating the therapeutic effects of phytoconstituents and how they work as pharmacological agents' significance (WHO, 2002; Kaushik *et al.*, 2014). Various modern techniques have been employed to identify and quantify bioactive components in plants. Among these, Gas Chromatography-Mass Spectrometry (GC-MS) has emerged as a reliable and essential tool for profiling of secondary metabolites in plants and animals with low molecular weight species (Robertson, 2005; Kell *et al.*, 2005).

Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are chemically reactive molecules that are naturally produced as byproducts of normal cellular metabolic processes. These molecules play significant roles in various physiological functions, such as cell signaling and immune defense. Superoxide anion, hydrogen peroxide, peroxyl radicals, and reactive hydroxyl radicals are the most prevalent Reactive Oxygen Species (ROS). The main components of Reactive Nitrogen Species (RNS) are Nitric Oxide (NO) and Peroxynitrite Anion (ONOO-) (Joyce, 1987).

In the pathogenesis of various oxidative stress-related illnesses, reactive species play a crucial role, including neurological degenerative disorders, rheumatoid arthritis, ulcerative colitis, cardiovascular diseases, and carcinogenesis (Halliwell and Gutteridge, 1990). Through scavenging free radicals, lipid peroxidation inhibition, and a variety of other mechanisms,

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antioxidants give protection against oxidative stress and prevent the development of diseases (Braugghler, 1987).

The formation of free radicals is naturally controlled by various compounds called antioxidants, which play a vital role in preventing diseases associated with oxidative stress. The antioxidant plays a significant function in preserving our body from diseases by decreasing the oxidative damage. Plants have natural antioxidants originate inside their leaves, fruits, roots, stems, and seeds. Phytocompounds such flavonoids, alkaloids, tannins, and phenolic compounds generate these kinds of antioxidants.

Herbs have been known for their medicinal properties and wealth of natural chemicals since ancient times. In recent years, advanced natural antioxidants derived from plants have been extensively studied for their antioxidant and radical scavenging properties (Sangameswaran *et al.*, 2008). *Debregeasia longifolia* (Burm.f.) Wedd., an evergreen shrub commonly known as "Kattunoochi" in Tamil, is native to regions from China to tropical Asia. *Debregeasia longifolia* belongs to the Urticaceae long history of usage as a remedy for a variety of gastrointestinal issues, skin conditions, and arthritic pains within this plant's family. This research set out to use Gas Chromatography-Mass Spectrometry (GC-MS) to identify bioactive components and assess the free radical scavenging ability of methanolic leaf extracts of *Debregeasia longifolia* (Figure 1).

MATERIALS AND METHODS

Study area

The Western Ghats of Nilgiris are native to the region over 5,000 unique vascular plants species that belongs to almost 2,200 genera, approximately 1,700 species (34%) are indigenous. It is altitude of 2240 m above sea level. The plants were collected in the geographical range of Latitude 11.360823º Longitude 76.784422º.

Plant collection and Authentication

Debregeasia longifolia, a medicinal plant, was collected from the Shola Forest near the Western Ghats of Nilgiris, Tamil Nadu. The plant specimen was authenticated by the South Regional Centre of the Botanical Survey of India, Coimbatore (ID: BSI/SRC/5/23/2023/Tech-211). The fresh leaves of *Debregeasia longifolia* were collected and washed under running water to remove dirt. The leaves were then cut into small pieces and air-dry in a well-ventilated area. Once dried, the leaves were ground into a fine powder. The dry plant powder was labeled properly and stored in plastic container.

Preparation of plant extract

20 g of the dry powdered plant material was extracted with 200 mL of methanol using a Soxhlet apparatus. The extraction was conveyed out at 60ºC until the material was decolorized. The resulting extract was concentrated, evaporated, and dried under reduced pressure to obtain a solid crude extract, which was then stored for further analysis.

Examination of Gas Chromatography and Mass Spectrometry (GC-MS) for bioactive compounds

GC-MS analysis was conducted using a mass spectrometer and gas chromatograph (Agilent 8890). The system had a triple-axis detector and an HP-5ms ultra-Inert MSD, as well as a fused silica column with dimensions of 30.0 m×250 µm and a film thickness of 0.25 µm, which contained 5% phenyl methyl siloxane. The column flow rate was set at 1.0 mL/min, and the carrier gas was helium gas. The following parameters were used in the GC-MS experiment: an injection temperature of 350ºC, an ion source of 250ºC, an interface of 300ºC, a pressure of 11.367 psi, an out time of 1.2 mL/min, and an injector operating in split mode with a 15:1 split ratio. The temperature of the column was raised to 150ºC at a rate of 4ºC per minute after being maintained at 36ºC for 5 min. The temperature was maintained at 280ºC for 5 min after being raised to that level at a pace of 20ºC per minute. A total of 53.5 min were required for elution. By comparing the peak area to the entire area, we were able to calculate the relative percentage of each component. The system was operated using the MS solution provided by the source, and data were collected accordingly.

Identification of Compounds

The components of the methanol leaf extracts of *Debregeasia longifolia* were identified by comparing their retention indices, and their mass spectra were determined using the National Institute of Standards and Technology (NIST) database. This database contains over 62,000 recognized chemical patterns, providing a comprehensive reference for analysis. The spectra of the unknown components in the *Debregeasia longifolia* methanolic leaf extracts were associated to the standard mass spectra of recognized compounds recorded in the NIST library (NISTII), allowing for accurate identification of the components present.

In vitro free radical scavenging activity of methanolic leaf extract of *Debregeasia longifolia*

The DPPH Radical Scavenging Assay for the Evaluation of Antioxidant Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is a widely used method for evaluating the antioxidant potential of natural substances. It specifically measures the ability of antioxidant compounds to neutralize the stable DPPH radical, making it an effective approach for assessing free radical scavenging activity.

The *in vitro* antioxidant activity of *Debregeasia longifolia* methanolic leaf extracts was determined using the DPPH radical scavenging method, following the procedure with slight modifications (Shimada *et al.*, 1992). A reaction

mixture was prepared by combining 0.8 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution with 1 mL of the methanolic leaf extracts at varying concentrations (200-1000 µg/mL). The mixture was thoroughly mixed and left to incubate at room temperature for 30 min. After incubation, a blank sample was used to measure the solution's absorbance at 517 nm.

For the comparison, ascorbic acid was utilized as the standard antioxidant. We used the following formula to determine the percentage of DPPH radicals that were inhibited:

$$\text{Inhibition } \% = [(\text{Control - Test}) / \text{Control}] \times 100$$

This formula provided a quantitative measure of how effective the extract is in scavenging DPPH radicals, indicating its potential as a natural antioxidant.

Examination of antioxidant activity using the Nitric oxide radical scavenging assay

The Griess reaction is a widely used method to inhibit the formation of nitrite ions, which occur when sodium nitroprusside reacts with oxygen to produce nitric oxide. This experiment was performed using the method to evaluate the capacity to reduce NO (Nitric Oxide) from the air by utilizing *Debregeasia longifolia* methanolic leaf extracts (Green *et al.*, 1982).

The reaction mixture contained different quantities of the reactants, as well as 3 mL of 10 mM sodium nitroprusside in pH 7.4 Phosphate-Buffered Saline (PBS). Methanolic leaf extracts *Debregeasia longifolia* in various concentrations (200-1000 µg/mL). The mixture was incubated at 25°C for 60 min to allow the reaction to progress. After incubation, 5 mL of Griess reagent, which consists of 0.1% NEDD (N-1-naphthylethylenediamine dihydrochloride) in 2% Phosphoric acid (H_3PO_4) and 1% sulphanilamide, was added to the sample. The solution was thoroughly mixed, and the absorbance was measured at 546 nm against a blank sample to determine the extent of nitrite ion production.

The inhibitory effect of the extracts on nitrite ion formation was calculated using the following formula:

$$\text{Inhibition } \% = [(\text{Control - Test}) / \text{Control}] \times 100$$

This calculation helped quantify the nitric oxide scavenging activity, highlighting the potential antioxidant properties of the *Debregeasia longifolia* methanolic leaf extracts.

Examination of antioxidant activity using the Hydroxyl radical scavenging assay

Hydroxyl radical scavenging assay, which was employed to evaluate the ability of the methanolic extracts of the leaves to moderate the effects of free radicals *Debregeasia longifolia* (Klein *et al.*, 1981). The reaction mixture consisted of 1 mL of the methanolic leaf extracts (200-1000 µg/mL), 1 mL of iron-EDTA (Ethylene Diamine Tetra Acetic Acid) solution (comprising 0.13% ferrous ammonium sulfate and 0.26% EDTA), 1 mL of DMSO (Dimethyl sulfoxide) (0.85% in 0.1 M phosphate buffer, pH 7.4), 0.5 mL of 0.018% EDTA, and 0.5 mL of 0.22% ascorbic acid.

A water bath was used to heat the reaction tubes to 80-90°C for 15 min after they were securely sealed. Following the heating process, 1 mL of trichloroacetic acid (17.5% concentration) was drizzled in followed by 3 mL of Nash reagent. The Nash reagent was prepared by mixing 2 mL of acetylacetone, 3 mL of glacial acetic acid, and 75 g of ammonium acetate with distilled water to make a total volume of 1 L. After 15 min of incubation at room temperature, the reaction mixture was colored.

At 412 nm, in comparison to a blank for the reagent, the strength of the resulting yellow manner was determined. The assay's standard reference was ascorbic acid. In order to determine the % inhibition of hydroxyl radicals, the formula was used:

$$\text{Inhibition } \% = [(\text{Control - Test}) / \text{Control}] \times 100$$

This method provides a quantitative measure of the extract's ability to scavenge hydroxyl radicals, highlighting its antioxidant potential.

Examination of Total antioxidant activity

Following the protocol described, the phosphomolybdenum technique was used to assess the total antioxidant activity of the methanolic leaf extracts of *Debregeasia longifolia* (Prieto *et al.*, 1999). To perform the assay, 1 mL of the methanolic leaf extracts, at concentrations ranging from 200 to 1000 µg/mL, combined with 1 mL of a reagent solution that included 28 mM of sodium phosphate, 4 mM of ammonium molybdate, and 0.6 m of sulfuric acid. After sealing the tubes, the reaction mixture was heated in a thermal block to 95°C for half an hour.

Following incubation, the tubes were allowed to cool to ambient temperature. Then, the solution's absorbance was measured at 695 nm compared to a blank for the reagent. Milligrams (Mg) of Ascorbic Acid Equivalent (AAE) per gram of extract were used to represent the overall antioxidant activity. The percentage inhibition of antioxidant activity was calculated using the formula:

$$\text{Inhibition } \% = [(\text{Control - Test}) / \text{Control}] \times 100$$

This method provides a quantitative measure of the extract's antioxidant potential, highlighting its ability to neutralize oxidative agents effectively.

Examination of antioxidant activity using the Reducing power assay

The reducing power of the methanolic leaf extracts of *Debregeasia longifolia* was assessed using the method (Oyaizu, 1986). This technique evaluates the ability of a substance to reduce oxidants, an indicator of antioxidant potential.

A 1 mL extract solution ranging from 200 to 1000 µg/mL, 2.5 mL of 1% potassium ferricyanide, and 2.5 mL of 0.2 M sodium phosphate buffer were all part of the reaction mixture used for this test. After 30 min of incubation at 50°C, the reaction may activate. Next, 2.5 mL of 10% Trichloroacetic Acid (TCA) was added to stop the reaction. The mixture was then centrifuged at 3000 rpm for 10 min to separate the layers.

Next, 2.5 mL of the supernatant was combined with 0.5 mL of 0.1% ferric chloride solution and 2.5 mL of deionized water. The absorbance of the resulting solution was measured at 700 nm against a blank sample. As the absorbance increased, the reducing power also increased, reflecting the antioxidant capacity of the extract.

RESULTS

Gas chromatography-mass spectroscopy reporting of methanolic leaf extracts of *Debregeasia longifolia*

Over the Gas Chromatography-Mass Spectroscopy (GC-MS) study of the methanol extract of *Debregeasia longifolia* leaves revealing several phytochemical activities, a total of 14 bioactive compounds were discovered. The chromatogram is represented in Figure 2 and while the 14 bioactive compounds were presented in Table 1 with their Retention time, name of the phytocomponents, molecular formula, molecular weight, compound peak area percentage, structure of the compounds.

In vitro free radical scavenging activity

Debregeasia longifolia leaf extract made from methanol were subjected to *in vitro* free radical scavenging activities.

DISCUSSION

GC-MS of Bioactive components and their biological activities

The bioactive phytocomponents identified in methanolic leaf extracts of *Debregeasia longifolia* through GC MS analysis were mentioned in Table 1. Among the reported bioactive components, 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R- [R*, R is known as phytol, a diterpene compound of chlorophyll which acts as an antimicrobial, anti-inflammatory, anticancer (Gnanavel and Mary, 2013), diuretic, antifungal, resistant gonorrhea, Stimulant and antimalarial compound (Dandekar *et al.*, 2015) and pioneer molecule for the artificial forms of vitamin E and vitamin K1 (Devakumar *et al.*, 2017).

Hexadecanoic acid, methyl ester is found to possess an antifungal and antibacterial properties (Devakumar *et al.*, 2017). Pentadecanoic acid, 14-methyl-methyl ester is a fatty acid ester and act as an anti-inflammatory, antiandrogenic, antioxidant, hypercholesterolemic, and antimicrobial compound (Imtiaz *et al.*, 2022). Tetradecanoic acid, 12-methyl-, methyl ester, (S)-is a fatty acid methyl ester that was not reported with any pharmacological activity. Ipriflavone, is a synthetic isoflavone effective in treating intervention for Postmenopausal Osteoporosis (PMO) (John *et al.*, 2023) and other estrogen-related disorders (Kuiper *et al.*, 1998).

Disopyramide is an anti-arrhythmic medication used to manage life-threatening ventricular arrhythmias, including sustained ventricular tachycardia. It is also effective in treating documented cases of ventricular pre-excitation, ventricular tachycardia, and other cardiac dysrhythmias (NCBI). Cyclobarbital is a barbiturate molecule that is synthesized similarly to phenobarbital, but with the use of cyclohexyl urea rather than methyl urea. It is mostly used as a restful to treat tension, anxiety, and epileptic attacks (Alexander *et al.*, 2005) and used as an antimicrobial and anticancerous agents (Rajendran *et al.*, 2017).

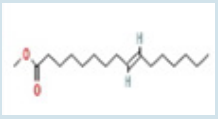
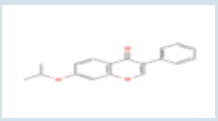
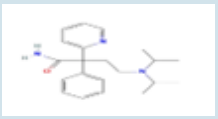
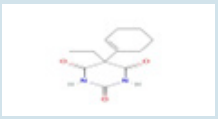
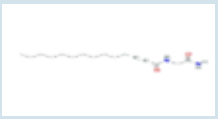
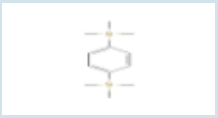
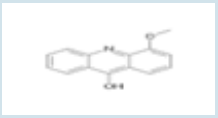
3,7,11,15-Tetramethyl hexadec-2-en-1-yl acetate which has been reported to have an antimicrobial activity (Somashekar *et al.*, 2023). 2-Myristynoyl-glycinamide is an amino compound, it is therapeutically used as an antimicrobial (Saikarthik *et al.*, 2017), antiprotozoal, antioxidant, antitumor drug (Leon Stephan Raj *et al.*, 2023). 1,4- Bis(trimethylsilyl)benzene is found to have antibacterial, antioxidant, and anti-fungal activities (Leon Stephan Raj *et al.*, 2023). Pinolenic acid is found to possess anti-inflammatory and anti-atherogenic effects (Takala *et al.*, 2022). Androstane-11,17-dione, 3-[(trimethylsilyl) oxy]-, 17-[O-(phenylmethyl) oxime], (3 alpha, 5 alpha)- is an antimicrobial, anti-inflammatory compound (Susheela *et al.*, 2018). Until now, the separation and characterization of individual phytochemical compounds continue to play a crucial role in discovering new drugs and exploring their pharmacological properties.

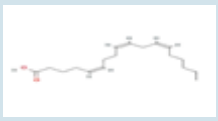
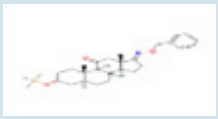
Free radical scavenging activities of leaf extracts

The methanolic leaf extracts of *Debregeasia longifolia* were thoroughly investigated for their antioxidant properties through various assays, including DPPH radical scavenging activity, hydroxyl radical scavenging activity, nitric oxide radical scavenging activity, total antioxidant capacity, and reducing power

Figure 1: *Debregeasia longifolia.*

Table 1: GC-MS phytochemical components of Methanol extract of *Debregeasia longifolia* leaves.

Sl. No.	RT (min)	Name of the compounds	Molecular formula	Component area (Peck %)	Molecular weight (g/mol)	Structure of compounds
1	24.929	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	15.66	270.45	
2	24.929	Pentadecanoic acid, 14-methyl-methyl ester	$C_{17}H_{34}O_2$	15.66	270.45	
3	24.929	Tetradecanoic acid, 12-methyl-, methyl ester, (S)-	$C_{16}H_{32}O_2$	15.66	256.42	
4	26.792	Ipriflavone	$C_{18}H_{16}O_3$	5.51	280.3	
5	26.792	Disopyramide	$C_{21}H_{29}N_3O$	5.51	387.4	
6	29.331	Cyclobarbital	$C_{12}H_{16}N_2O_3$	4.74	236.27	
7	29.331	Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)-	$C_{13}H_{22}N_2O$	4.74	222.33	
8	29.605	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R- [R*, R	$C_{20}H_{40}O$	60.25	296.5310	
9	29.605	3,7,11,15-Tetramethyl hexadec-2-en-1-yl acetate	$C_{22}H_{42}O_2$	4.74	338.56	
10	30.674	2-Myristynoylglycinamide	$C_{16}H_{28}N_2O_2$	4.82	280.412	
11	30.674	1,4-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$	4.82	222.478	
12	30.674	4-Methylacridin-9-ol	$C_{14}H_{11}NO$	4.82	225.24	

Sl. No.	RT (min)	Name of the compounds	Molecular formula	Component area (Peck %)	Molecular weight (g/mol)	Structure of compounds
13	30.811	Pinolenic acid	$C_{18}H_{30}O_2$	9.01	278.42	
14	30.811	Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl) oxime], (3alpha,5alpha)-	$C_{29}H_{43}NO_3Si$	9.01	481.7	

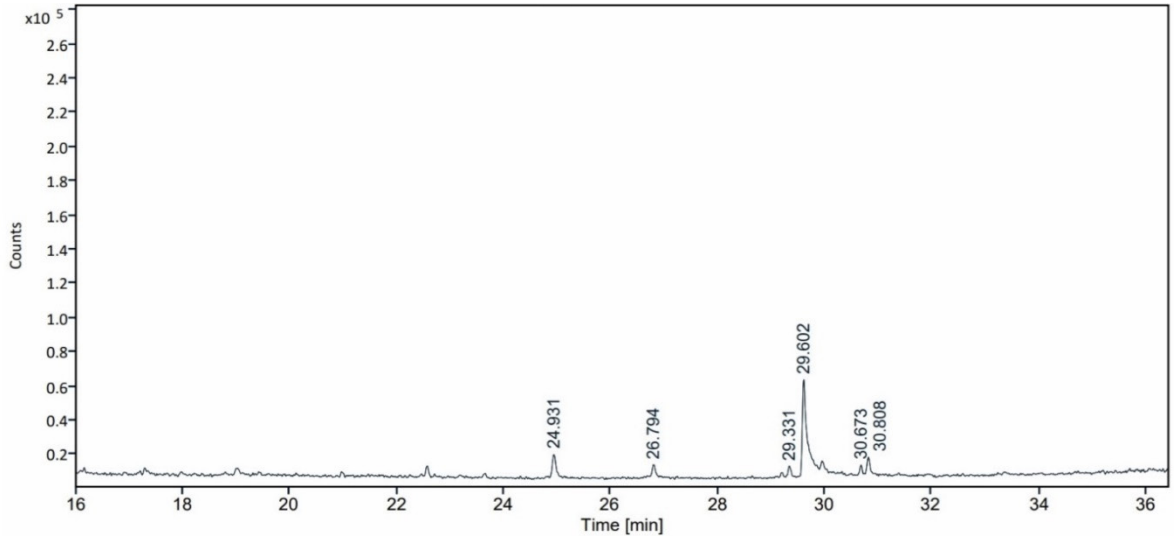


Figure 2: GC-MS chromatogram of Methanolic leaf extract of *Debregeasia longifolia*.

assays. These activities were tested at different concentrations ranging from 200 to 1000 µg/mL. The findings indicated that the extract exhibited remarkable antioxidant potential, which increased in a dose-dependent manner across all the evaluated assays.

DPPH, a constant free radical, is widely used to evaluate the antioxidant potential of various compounds due to its ability to accept an electron or hydrogen atom, transforming it into a stable diamagnetic molecule (Bijaya and Bikash, 2013). The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of the methanolic leaf extracts of *Debregeasia longifolia* is presented in Figure 3, with an IC_{50} value calculated to be 502.95 µg/mL. Remarkably, its ability to neutralize free radicals in methanolic leaf extracts was observed to be relatively higher compared to the standard ascorbic acid, indicating its strong free radical scavenging ability.

Nitric oxide plays a crucial role in various inflammatory processes; however, its elevated levels can be directly toxic to tissues, leading to vascular damage and contributing to several health disorders. This toxicity is further exacerbated when nitric oxide reacts with superoxide radicals to form Peroxynitrite Anion (ONOO-), a highly reactive and damaging compound (Balavoine and Geletti, 1999). The methanolic leaf's radical scavenging activity extract of *Debregeasia longifolia* is depicted in Figure 3, with the IC_{50} value calculated as 869.15 µg/mL. Remarkably, the nitric oxide scavenging ability of the methanol extract was significantly higher compared to the standard ascorbic acid, highlighting its potential efficacy in justifying oxidative stress.

Hydroxyl radicals are highly reactive and have an extremely short life, making them one of the most dangerous reactive oxygens species (Hayyan *et al.*, 2016). Due to their high reactivity, they have the potential to inflict severe damage on essential macromolecules such as proteins, lipids, and nucleic acids, thereby disrupting vital cellular functions (Floyd and Lewis, 1983). Because of their extreme reactivity, hydroxyl radicals seriously damage cells and their components, which in turn affects the organisms as a whole (Dizdaroglu and Jaruga, 2012). The hydroxyl radical scavenging activity of the methanolic leaf extracts of *Debregeasia longifolia* is shown in Figure 3, with an IC_{50} value calculated at 560 µg/mL. Remarkably, the hydroxyl radical scavenging potential of the methanolic leaf extract was observed to be significantly higher than that of the standard ascorbic acid, indicating the extract has a strong ability to neutralize these harmful radicals and protect biological systems from oxidative damage.

The phosphomolybdate method is a widely used technique for evaluating the total antioxidant capacity of plant extracts (Prieto *et al.*, 1999; Prasad *et al.*, 2009). It is particularly effective in assessing antioxidants such as carotenoids, α-tocopherol, ascorbic acid, and various phenolic compounds (Prieto *et al.*, 1999). In this method, molybdenum (VI) is reduced to molybdenum (V) in the presence of the methanolic leaf extract, resulting in the formation of a green-colored phosphomolybdenum (V) complex, which serves as an indicator of antioxidant activity. The total antioxidant activity of the methanolic leaf extracts of *Debregeasia longifolia* is illustrated in Figure 3, with an IC_{50} value calculated to be 622.36 µg/mL. Remarkably, the total antioxidant capacity of the methanolic leaf extracts was observed to be significantly higher compared to the standard ascorbic acid, highlighting its strong ability to neutralize free radicals and combat oxidative stress.

The reducing power of a compound is directly linked to its ability to donate electrons, which is an important mechanism of antioxidant activity. This assay is widely used to assess the antioxidant properties of polyphenolic compounds, which owe their reducing power to the presence of reductones. Reductones act by interrupting free radical chain reactions through the donation of hydrogen atoms, thereby neutralizing reactive species and preventing oxidative damage (Duan *et al.*, 2007).

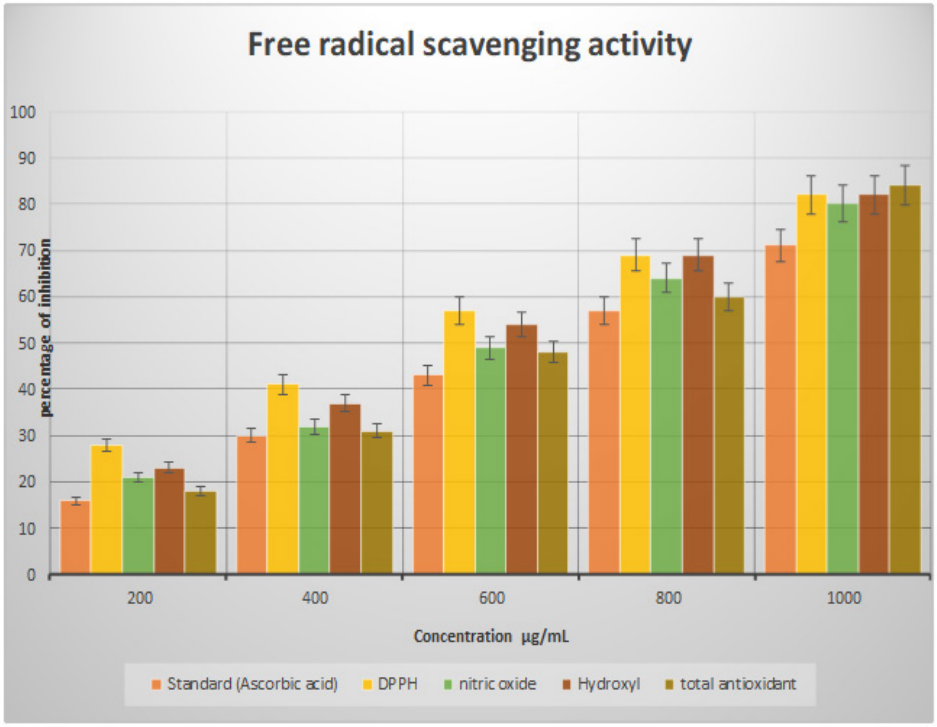


Figure 3: Free radical scavenging activity of *Debregeasia longifolia* methanolic leaf extract.

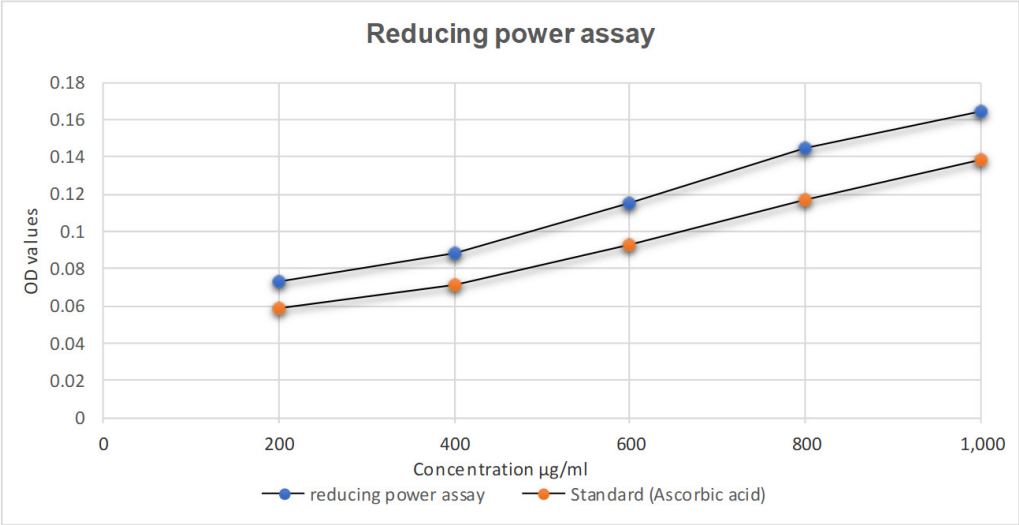


Figure 4: Reducing power activity of *Debregeasia longifolia* methanolic leaf extract.

The reducing power assay of the methanolic leaf extracts of *Debregeasia longifolia* is depicted in Figure 4, with the IC_{50} value calculated to be 499.55 µg/mL. Remarkably, the reducing power exhibited by the methanolic leaf extracts was significantly higher when compared to the standard ascorbic acid. This finding highlights the extract's strong electron-donating capacity, further emphasizing its potential as a powerful natural antioxidant capable of mitigating oxidative stress and protecting biological systems from free radical-induced damage.

CONCLUSION

Currently, the antioxidant qualities of plants have developed increasingly important outstanding because of their possible application as all-natural substitutes for artificial antioxidants. The current investigation's finding suggested that methanolic leaf extracts of *Debregeasia longifolia* exhibits strong antioxidant activity, with a notable ability to scavenge free radicals. The scavenging activity observed in the leaf extracts closely correlates with the total phenolic content, suggesting that the process of scavenging free radicals' ability is largely attributed to the hydroxyl groups present in the phenolic compounds.

The methanolic extract of *Debregeasia longifolia* possessed significant molecules like Hexadecanoic acid, methyl ester, Pentadecanoic acid, 14-methyl- methyl ester, 3,7,11,15-Tetramethyl hexadec-2-en-1-yl acetate, Pinolenic acid offering potential health benefits antifungal, anti-inflammatory, antimicrobial, antioxidant and anti-atherogenic effects. These results will lead to the further development of drug molecules in pharmaceutical industry.

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ABBREVIATIONS

GC-MS: Gas Chromatography-Mass Spectrometry; **DPPH:** 2,2-Diphenyl-1-Picrylhydrazyl; **ROS:** Reactive Oxygen Species; **RNS:** Reactive Nitrogen Species; **NO:** Nitric Oxide; **µg:** Micro gram; **nm:** Nanometer; **Mg:** Milligrams; **RT:** Retention time; **BSI:** Botanical Survey of India; **m:** Mole; **mL:** Milliliter; **mM:** Millimoles; **TCA:** Trichloroacetic Acid; **AAE:** Ascorbic Acid Equivalent; **EDTA:** Ethylene Diamine Tetra Acetic Acid; **DMSO:** Dimethyl sulfoxide; **IC_{50}:** Half-Maximal Inhibitory Concentration; **PBS:** Phosphate Buffered Saline; **%:** Percentage.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

AUTHORS CONTRIBUTIONS

Suresh Kumar Thirumoorthi- collection of plant sample, analysis and writing the original manuscript. Rama prabha mani -planning of work, editing, supervision and draft the manuscript. All authors have read and agreed to the manuscript.

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