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# The Bioactive Spectrum: Unlocking the Antidiabetic and Radical Scavenging potential of *Wedelia chinensis* leaf Extract

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**ABSTRACT:**
The present study was designed to pharmacologically evaluate the therapeutic potential of *Wedelia chinensis* Less. non-Rich. (Family: Asteraceae). Antioxidant and antidiabetic potentials were investigated. The phytochemical screening of the methanolic extract of *Wedelia chinensis* (WCME-L) revealed a diverse array of bioactive compounds, including high concentrations of flavonoids and phenols, and significant amounts of saponins, alkaloids, phytosterols, tannins, terpenoids, and glycosides. Despite the measurable phenolic content of 8.53 mg GAE/g extract, the study found no direct correlation with antioxidant activity, suggesting other factors at play. The thiocyanate method demonstrated potent antioxidant properties at 250 µg/ml, with the extract showing higher activity than α- Tocopherol but lower than BHA. Reducing power assays confirmed the extract's strong antioxidant capacity, surpassing α-Tocopherol and being second to BHA. In DPPH radical scavenging and superoxide anion scavenging activities, the extract exhibited significant efficacy, though slightly less potent than BHA and ascorbic acid, respectively. The extract also displayed dose-dependent alpha-amylase and alpha-glucosidase inhibition, indicating potential for managing blood sugar levels in diabetes. These findings suggest that WCME-L is a potent natural antioxidant with promising therapeutic applications, particularly in managing oxidative stress and metabolic disorders.
**Keywords:** *Wedelia chinensis,* Antioxidant, Free radical scavenging, Antidiabetic, blood sugar, alpha- amylase, alpha-glucosidase.

**INTRODUCTION**

Oxidative stress arises when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them using antioxidants. ROS, which include free radicals like superoxide anion (O2-), hydroxyl radical (OH), and non-radical species like hydrogen peroxide (H2O2), are by-products of normal cellular metabolism, primarily occurring in mitochondria during ATP production. While ROS play essential roles in cell signaling and homeostasis, excessive ROS can lead to cellular damage, affecting lipids, proteins, and DNA, thereby disrupting cellular function and integrity (Ellnain-Wojtaszek et al., 2003). Antioxidants are molecules that can donate an electron to neutralize ROS without becoming reactive themselves. The body has both enzymatic antioxidants (such as superoxide dismutase, catalase, and glutathione peroxidase) and non-enzymatic antioxidants (such as vitamin C, vitamin E, and glutathione) to combat oxidative stress (Ito and Hirose, 1989). When ROS production overwhelms these antioxidant defenses, oxidative stress occurs, leading to oxidative damage and contributing to the pathogenesis of numerous diseases, including diabetes (Gerber et al., 1996, *Kris-etherton et al*., 2002, Serafini *et al.*, 2002, Kodithala S *et al*. 2013).

Diabetes mellitus is a chronic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Type 1 diabetes (T1D) is an autoimmune condition leading to the destruction of pancreatic beta cells, resulting in insulin deficiency. Type 2 diabetes (T2D) is more common and is characterized by insulin resistance and eventual beta-cell dysfunction. Oxidative stress plays a significant role in the development and progression of both T1D and T2D. Hyperglycemia induces ROS production through several mechanisms, including glucose autoxidation, protein glycation, and activation of the polyol pathway. This increased ROS generation overwhelms the antioxidant defense system, leading to oxidative damage (Clement et al., 1998, Uniyal et al., 2006, Newman *et al*., 2000). In T1D, oxidative stress exacerbates the autoimmune destruction of beta cells, while in T2D, it contributes to insulin resistance and beta-cell dysfunction. Furthermore, oxidative stress is implicated in the development of diabetic complications, such as neuropathy, nephropathy, and retinopathy (Halliwell and Gutleridge, 1984). Understanding the relationship between oxidative stress and diabetes highlights the importance of antioxidant therapies in managing diabetes and preventing its complications. Addressing oxidative stress through lifestyle modifications, dietary interventions, and pharmacological approaches could offer significant benefits in the management of diabetes (Gülçin et al., 2002, Dhuley *et al.*, 1993).

*Wedelia chinensis* Less. non-rich. (Family: Asteraceae), known as pitabhringa-raaja in Hindi, pitabhringi in Sanskrit, gargari in Kannada, and manjal karisaalai in Tamil, is a traditional Ayurvedic herb (Khare, 2007, Chopra et al., 1956). This perennial plant, characterized by its bright yellow flowers and light camphor-like odor, is found in Assam, Arunachal Pradesh, Himachal Pradesh, Uttar Pradesh, and Tamil Nadu. Traditionally, it has been used as a cholagogue and deobstruent for hepatic enlargement and jaundice, as well as for treating uterine hemorrhage and menorrhagia (Khare, 2007, Chopra *et al*., 1956). Previous studies have identified the presence of isoflavonoids and wedelolactone in this plant (Chopra et al., 1956, Khare, 2007). Notably, the herb shares similar properties and key active constituents with *Eclipta alba* Hassk., a plant whose leaves are used as a tonic and alternative remedy (Khare, 2007). Therefore, this study aims to evaluate the antioxidant and antidiabetic profile of *Wedelia chinensis* using various *in vitro* models to assess its pharmacological activities.

**MATERIAL AND METHODS**

***Drugs, Chemicals and reagents***

Chemicals including Folin-Ciocalteau reagent, trichloroacetic acid (TCA), methanol, ethanol, ammonium thiocyanate, gallic acid, dimethylsulphoxide (DMSO), α-Tocopherol, butylated hydroxyanisole (BHA), and Tween 20 were sourced from E. Merck (India) Limited. Linoleic acids, N-(1-naphthyl) ethylenediamine dihydrochloride, sulfanilamide, riboflavin, ascorbic acid, NBT (Nitroblue tetrazolium), DPPH, and ferrozine were obtained from Sigma, USA.

Alpha-amylase, alpha-glucosidase, sodium carbonate, p-Nitrophenyl glucopyranoside (pNPG), iodine, and phosphate buffer were procured from either Sigma Aldrich or Himedia Laboratories, India. Additionally, Tween 80, petroleum ether, and all other commercially available analytical grade chemicals and solvents were acquired from SRL Mumbai, Himedia, and E. Merck India.

***Plant material***

*Wedelia chinensis* Less. non-rich leaves were gathered from Kullu district of Himachal Pradesh, India. Dr. A. K. Sharma, a botanist at the Research Institute in Indian System of Medicine (ISM), Himachal Pradesh, verified the authenticity of the plant. For future use, a voucher specimen (MK/AOL/09/2022) was placed in the pharmacy department of the College of Pharmacy in Himachal Pradesh.

***Preparation of extracts***

*Wedelia chinensis* Less. non-rich leaves were dried before being ground into a powder. Using a Soxhlet device, the powdered plant leaves were extracted with methanol and concentrated in-vacuo. From 100 g of dried leaf material, approximately 7.7 gm of dried methanolic extract of *Wedelia chinensis* Less. non-rich. leaves (WCME-L) were produced (Yield, 7.7%).

***Preliminary phytochemical screening***

Chemical techniques were used to identify the phytochemical components of the drug's powder and methanolic extract in accordance with previously proposed methodology (Harborne, 1973)

***Determination of total phenolic content***

To determine the total phenolic content of *Wedelia chinensis* leaves, a systematic procedure was followed (Slinkard and Singleton, 1977). Initially, 1 gram of dried leaves was extracted with 10 mL of methanol, left to stand for 24 hours, and then filtered through Whatman No. 1 filter paper. The Folin-Ciocalteu reagent was diluted with distilled water in a 1:10 ratio, and a 7.5% w/v sodium carbonate solution was prepared by dissolving 7.5 grams of sodium carbonate in 100 mL of distilled water. For the reaction mixture, 0.5 mL of the leaf extract was combined with 2.5 mL of the diluted Folin-Ciocalteu reagent and mixed thoroughly. This mixture was allowed to stand at room temperature for 5 minutes before adding 2 mL of the sodium carbonate solution and mixing again. The reaction mixture was then incubated at room temperature for 30 minutes, protected from light. The absorbance of the final mixture was measured at 765 nm using a UV-visible spectrophotometer. To create a calibration curve, standard solutions of gallic acid at various concentrations (10, 20, 40, 60, 80, and 100 µg/mL) were prepared, and their absorbance values were plotted against their concentrations. The total phenolic content of the leaf extract was calculated by comparing its absorbance with the standard curve and was expressed in milligrams of gallic acid equivalents (GAE) per gram of dry plant material. This method provided an accurate measurement of the total phenolic content in the Wedelia chinensis leaves. A formula derived from the standard gallic acid graph was used to calculate the concentration of total phenolic compounds in the extract as measured in grammes of gallic acid equivalent (GAE):

$Y= 0.0027x + 0.1361, R^2 = 0.8881$

Where Y was the absorbance and x were the concentration.

***Total antioxidant activity***

The total antioxidant activity of the extract was determined according to the thiocyanate method. (Mitsuda *et al*., 1996, Kodithala S *et al*.,2021) Ten milligrams of extract was dissolved in 10 ml water. Different concentration of extract (50-250 µg/mL) or standard samples in 2.5 ml of potassium phosphate buffer (0.04 M, pH 7.0) was added to linoleic acid emulsion (2.5 ml) in potassium phosphate buffer (0.04 M, pH 7.0). Five millilitres linoleic acid emulsion consists of 17.5g Tween-20,

15.5µl linoleic acid, and 0.04M potassium phosphate buffer (pH 7.0). On the other hand, 5.0 ml control consists of 2.5 ml linoleic acid emulsion and 2.5 ml potassium phosphate buffer (0.04 M, pH 7.0). The mixed solution was incubated at 37°C in a glass flask and in the dark. After the mixture was stirred for 3 min, the peroxide value was determined by reading the absorbance at 500 nm in a spectrophotometer (UV -1601 Shimadzu, Japan), after reaction with $FeCl_2$ and thiocyanate at intervals during incubation. During the linoleic acid oxidation,

peroxides formed. These compounds oxidize $Fe^{2+}$ to $Fe^{3+}$. The latter $Fe^{3+}$ ions form complex with $SCN^-$, which had maximum absorbance at 500 nm. Therefore, high absorbance indicates high linoleic acid oxidation. The solutions without extracts or standards were used as blank samples. All data about total antioxidant activity is the average of triplicate analyses. The inhibition of lipid peroxidation in percentage was calculated by following equation:

Inhibition (%) = $(A_0 - A_t / A_0) \times 100$

Where $A_0$ was the absorbance of the control reaction and $A_t$ was the absorbance in the presence of the sample. All the tests were performed in triplicate and graph was plotted with the mean ± SD values. BHA and α-Tocopherol were used as standard antioxidant compounds.

***Reducing power***

The reducing power of extract was determined according to the method described previously.(Oyaizu, 1986) The different concentrations of extract (50-250µg/mL) in 1ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer (UV -1601 Shimadzu, Japan). Higher absorbance of the reaction mixture indicated greater reducing power. BHA and α-Tocopherol were used as standard antioxidant compounds.

***Assay of superoxide radical ($O_2^{\bullet -}$) scavenging activity***

The assay was based on the capacity of the methanolic extract to inhibit blue formazon formation. Superoxide radical were generated in riboflavin-light-NBT (Nitroblue tetrazolium) system. (Beauchamp and Fridovich, 1971) The total volume of the reactant mixture was 3 ml. Each 3 ml of this reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 µg riboflavin, and 12 mM EDTA, and 0.1 mg NBT and 1 ml sample solution. Reaction was started by illuminating the reaction mixture with different concentrations of the methanolic plant extract (50-250 µg/ml) for 90 seconds. Immediately after illumination, the absorbance was measured at 590 nm. The reaction assembly was enclosed in a aluminium foil lined box. Unilluminated identical tubes containing reaction mixture served as blank. The percentage inhibition of superoxide anion generation was calculated using the following formula:

% Inhibition = $(A_0 - A_t / A_0) \times 100$

Where, $A_0$ was the absorbance of the control (without extract) and $A_t$ was the absorbance in the presence of the extract or standard. All the tests were performed in triplicate and graph was plotted with the mean ± SD values. Ascorbic acid was used as standard compound (et alKodithala S *et al* 2021-2022).

***Metal chelating activity***

The chelating of ferrous ions by the methanolic plant extract was measured by the method described previously.(Dinis et al., 1994, Kumaran and Joel Karunakaran, 2006) Different concentrations of the extract (50-250µg/ml) were added to a solution of $FeCl_2$ (0.05 ml, 2 mM). Then the reaction was initiated by addition of 5 mM ferrozine (0.2 ml). The reaction mixture was then shaken vigorously and allowed to stand at room temperature for 10 minutes. The absorbance of the solution was then measured at 562 nm. The percentage inhibition of ferrozine –ferrous complex formation was calculated by using the following formula:

% Inhibition = $(A_0 - A_t / A_0) \times 100$

Where, $A_0$ was the absorbance of the control (without extract) and at was the absorbance in the presence of the extract or standard. All the tests were performed in triplicate and graph was plotted with the mean ± SD values. EDTA (Ethylenediaminetetraacetic acid) was used as standard chelating compound (Kodithala S *et al* 2012-2014)

**Antidiabetic activity**

***Alpha-amylase inhibition assay***

The alpha-amylase inhibition assay is a widely used method for evaluating the in vitro antidiabetic activity of compounds, focusing on their ability to manage diabetes by inhibiting alpha-amylase, an enzyme crucial for the breakdown of starch into glucose. To perform this

assay, alpha amylase is first dissolved in a phosphate buffer. The test compound is then added to this enzyme solution and pre-incubated for a specified duration. Following the pre-incubation period, a starch solution is introduced to the mixture, and the reaction is allowed to proceed for a further incubation period. After this, the reaction is halted by adding a colour reagent, such as iodine, which reacts with any remaining starch to form a coloured complex. The intensity of this colour, which decreases with increasing enzyme inhibition, is measured using a spectrophotometer. This spectrophotometric measurement allows for the quantification of the enzyme activity and the assessment of the inhibitory potential of the test compound (Nair et al., 2013, Bhutkar and Bhise, 2012, Kodithala S *et al.* 2021*).*

***Alpha-glucosidase inhibition assay***

The alpha-glucosidase inhibition assay is an important method for assessing the antidiabetic potential of compounds by measuring their ability to inhibit alpha-glucosidase. This enzyme plays a critical role in the digestive tract by breaking down disaccharides into glucose. To conduct this assay, alpha-glucosidase is mixed with a chromogenic substrate, such as p-nitrophenyl glucopyranoside, in a buffer solution. The test compound is first pre-incubated with the enzyme. After pre-incubation, the substrate is added to initiate the reaction. The reaction is then stopped by adding sodium carbonate, which also helps develop the color if the substrate is chromogenic. The absorbance of the resulting solution is measured spectrophotometrically to determine the level of enzyme inhibition. The extent of color change correlates with the degree of alpha-glucosidase inhibition by the test compound, allowing for the evaluation of its antidiabetic activity (Nair et al., 2013, *Chougale et al*., 2009).

**Statistical analysis**

In this work, several experimental groups were compared against a control group using an ANOVA followed by a Dunnett's test in GraphPad Prism software. The findings were displayed as mean ± standard deviation (SD), with $p < 0.05$ designated as the significance threshold.

## RESULTS AND DISCUSSION

***Preliminary phytochemical screening***

The phytochemical screening of the extract (WCME-L) reveals a diverse range of bioactive compounds, each with varying degrees of presence indicated by "+" for moderate and "++" for high concentration. Notably, the extract contains a high concentration of flavonoids and phenols, both of which are known for their potent antioxidant properties. This suggests that WCME-L may have strong capabilities in neutralizing free radicals and reducing oxidative stress, which can be beneficial in preventing or managing diseases associated with oxidative damage, such as cardiovascular diseases and certain cancers. In addition to these, the extract also has a significant presence of saponins. Saponins are renowned for their cholesterol-lowering effects, immune system enhancement, and anti-cancer activities, indicating that the extract could play a role in managing cholesterol levels, boosting immune responses, and potentially preventing cancer. The presence of moderate amounts of alkaloids further enhances the extract's therapeutic potential. Alkaloids exhibit a wide range of pharmacological activities, including analgesic, anti-inflammatory, and antimalarial effects, suggesting that WCME-L could be beneficial in pain management and as part of antimalarial treatments. Phytosterols, present in moderate amounts, are known for their ability to lower cholesterol levels by inhibiting its absorption in the intestines. This indicates that the extract could contribute to cardiovascular health by helping reduce cholesterol levels. Additionally, tannins, which are also moderately present, possess astringent and antimicrobial properties. These characteristics could make the extract useful in wound healing and reducing inflammation, as well as in providing antimicrobial benefits (Table 1). The extract also contains terpenoids and glycosides in moderate amounts, which are known for their anti-inflammatory and cardioprotective

properties, respectively. However, the absence of coumarins indicates that the extract does not contain this class of phytochemicals, which are known for their anticoagulant properties. Overall, the diverse and abundant presence of these phytochemicals suggests that the extract (WCME-L) has considerable potential for therapeutic applications, particularly in areas such as antioxidant support, cardiovascular health, immune enhancement, and anti- inflammatory and antimicrobial treatments.

**Table 1.** Results of Preliminary Phytochemical Screening

| **Phytochemical Constituent** | **Extract (WCME-L)** |
|---|---|
| Flavonoids | ++ |
| Phenols | ++ |
| Alkaloids | + |
| Saponins | ++ |
| Phytosterols | + |
| Tannins | + |
| Borntrager Test | + |
| Terpenoids | + |
| Glycosides | + |
| Coumarins | - |

**+:** Presence of moderate active constituents, **++:** Presence of maximum active constituents

***Determination of total phenolic content***

Most antioxidant activities of plant sources are derived from phenolic-type compounds, which are known for their scavenging abilities due to their hydroxyl groups. Phenols are significant plant constituents contributing to antioxidant effects. However, the presence of large quantities of phenolics does not always correlate directly with antioxidant activity (Slinkard and Singleton, 1977). In this study, the methanolic extract of *Wedelia chinensis* (WCME-L) was evaluated for its total phenolic content. The analysis revealed that the total phenolic content of WCME-L was 8.53 mg of gallic acid equivalents (GAE) per gram of extract. Despite this measurable quantity of phenolics, the study did not find a correlation between the phenolic content and the antioxidant activity of the extract. This suggests that other factors or compounds within the extract may also play significant roles in its overall antioxidant capacity.

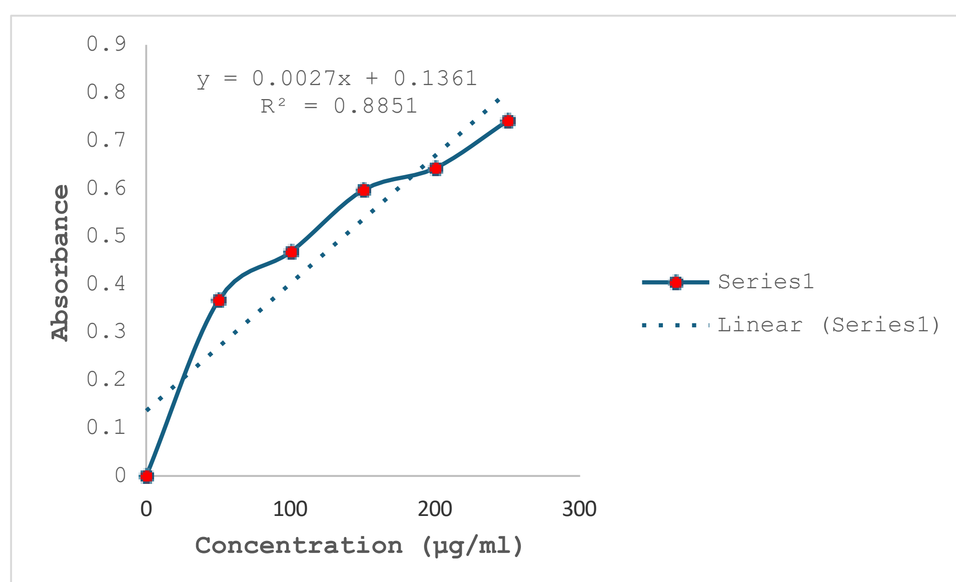


**Figure 1.** Estimating the total phenolic content

***Total antioxidant activity determination in linoleic acid system***

The thiocyanate method was employed to assess the total antioxidant activity of the plant extract. At a concentration of 250 µg/ml, the extract demonstrated effective and potent antioxidant properties. Figure 1 illustrates the impact of this concentration on the peroxidation of linoleic acid emulsion. Initially, the antioxidant activity of the extract increased with longer incubation times, but it subsequently decreased with further incubation. The extract at the studied concentration exhibited higher antioxidant activity compared to 250 µg/ml of α-Tocopherol but lower activity than the same concentration of BHA (Butylated hydroxyanisole). The percentage inhibition of peroxidation in the linoleic acid system by the extract was found to be 62.43%. In comparison, the percentage inhibition at 250 µg/ml concentration of BHA and α-Tocopherol was 98.34% and 32.76%, respectively.

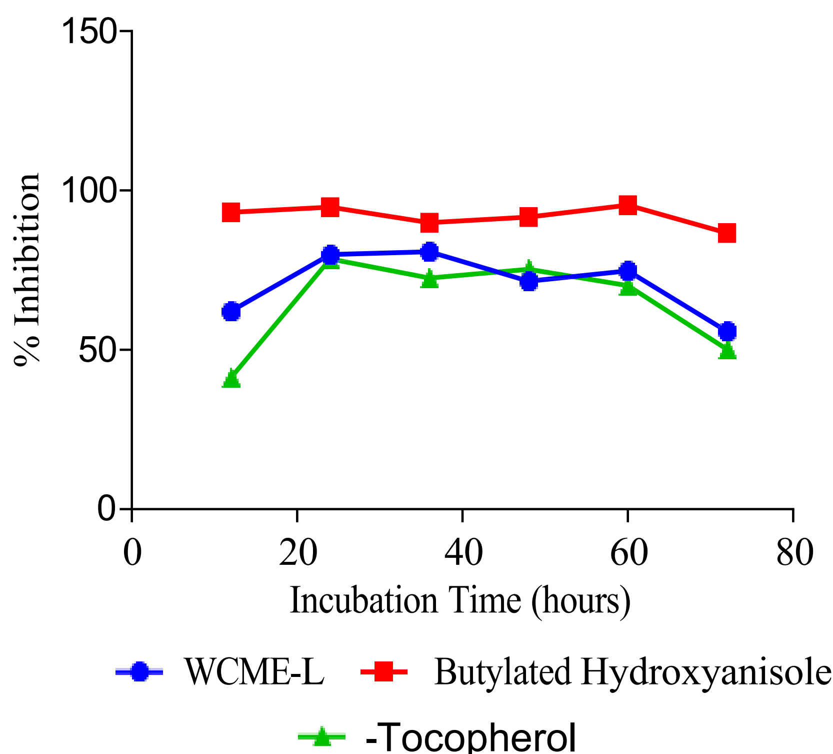


**Figure 2.** Estimating the total antioxidant activity in linoleic acid system

***Effect on reducing power assay***

The reducing power of the extract, BHA, and α-Tocopherol is illustrated in Figure 2. Using Oyaizu's method (Oyaizu, 1986), which measures the transformation of Fe3+ to Fe2+ in the presence of the extract, the study evaluated the reductive ability of the samples. The findings revealed that the extract's reducing power increased with its concentration. Across all concentrations examined, the extract exhibited a higher reducing power than α-Tocopherol but a lower one than BHA. Specifically, the reducing capability followed the order: BHA > Extract > α-Tocopherol. This indicated that while the extract is effective in its reducing power, making it a potent antioxidant, it is not as strong as BHA, a well-known antioxidant standard. However, its effectiveness surpasses that of α-Tocopherol, another common antioxidant. These results suggest that the extract could be a valuable natural antioxidant source, with potential applications in food preservation and health supplements to combat oxidative stress. .

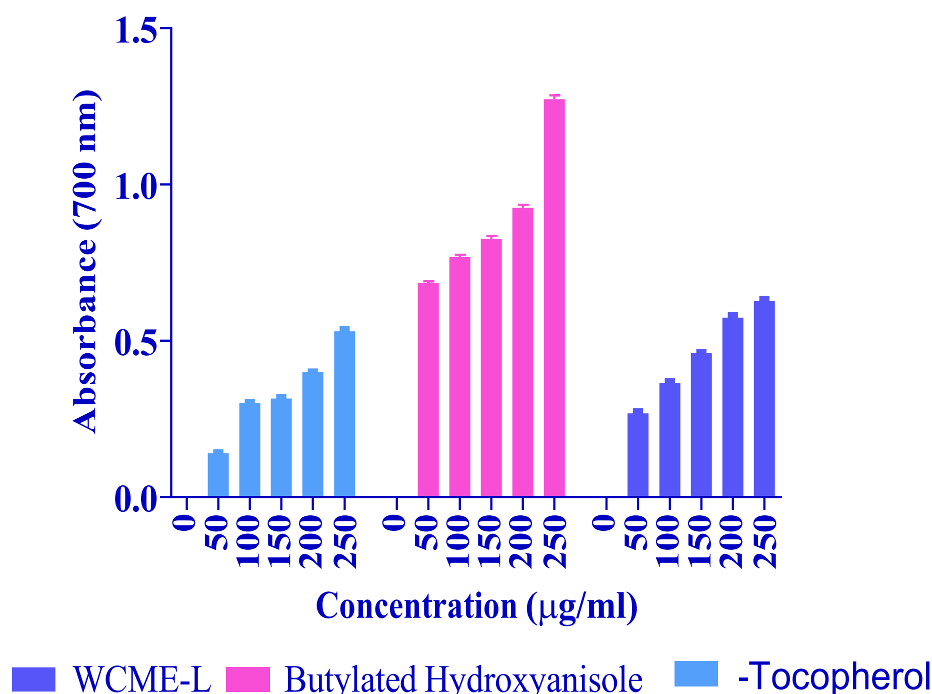


**Figure 3.** Estimating the reducing power

***Effect on DPPH radical scavenging activity***

The evaluation of antioxidant activities using the scavenging capability of the stable DPPH radical is a commonly employed method due to its efficiency and speed. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical that accepts an electron or hydrogen radical, transforming into a stable diamagnetic molecule. When antioxidants scavenge DPPH radicals, there is a decrease in absorbance at 517 nm, making DPPH a useful substrate for assessing the antioxidant activity of various compounds. In this study, Quercetin was used as a standard radical scavenger. Figure 4 demonstrates the reduction in DPPH radical concentration due to the scavenging capabilities of both the plant extract and Quercetin across different concentrations (50-250 µg/ml). The extract exhibited a slightly lower scavenging ability compared to Quercetin. Specifically, at a concentration of 250 µg/ml, the percent DPPH scavenging effect of the extract and Quercetin were 94.89% and 97.96%, respectively. These results indicate that the plant extract is a strong DPPH radical scavenger, nearly as effective as the standard Quercetin. The IC50 values, representing the concentration required to inhibit 50% of the DPPH radicals, were calculated using linear regression analysis. The IC50 values for the extract and Quercetin were found to be 108.76 µg/ml and 87.44 µg/ml, respectively. These findings suggest that the plant extract has a substantial free radical scavenging capability, though slightly less potent than Quercetin. Oxidative stress occurs when the production of free radicals surpasses the body's antioxidant defenses, leading to cellular damage and contributing to chronic conditions (Jainu and Devi, 2005). The data from this study indicate that the plant extract is a powerful free radical scavenger, capable of reducing or reversing the damage caused by oxidative stress. This highlights the potential of the plant extract as a natural antioxidant, which could be beneficial in preventing or mitigating the effects of oxidative stress-related diseases.

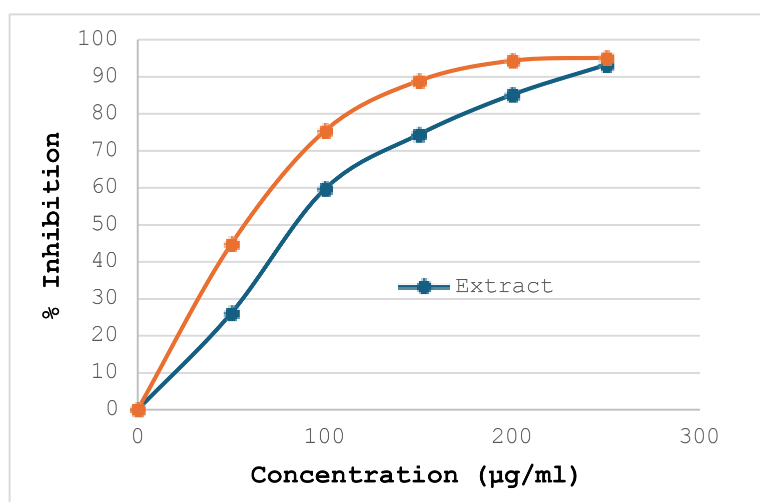


**Figure 4.** Estimating the DPPH radical scavenging activity

***Effect on Superoxide anion scavenging activity***

Phenolic compounds, especially flavonoids and catechins, are recognized as important antioxidants and superoxide scavengers. Their scavenging efficiency largely depends on the phenol concentration and the number and position of hydroxyl groups. Superoxide anion, a highly toxic species, is produced through various biological reactions within the physiological system. In this study, a decrease in absorbance at 590 nm in the presence of antioxidants indicated the consumption of superoxide anions in the reaction mixture. Figure 4 illustrates the increase in the percentage inhibition of superoxide radical generation with rising concentrations of both the plant extract and the standard compound, ascorbic acid. The plant extract demonstrated significant superoxide radical scavenging activity (Erasto et al., 2007, Ashokkumar et al., 2008). However, its scavenging activity was slightly lower than that of ascorbic acid. Specifically, the percentage inhibition of superoxide radicals by the extract and ascorbic acid was 72.95% and 87.59%, respectively. These findings suggest that while the plant extract is a potent superoxide radical scavenger, ascorbic acid remains a more effective standard. The results underscore the potential of the plant extract as a natural antioxidant, which could be utilized to combat oxidative stress and mitigate its harmful effects on the body.

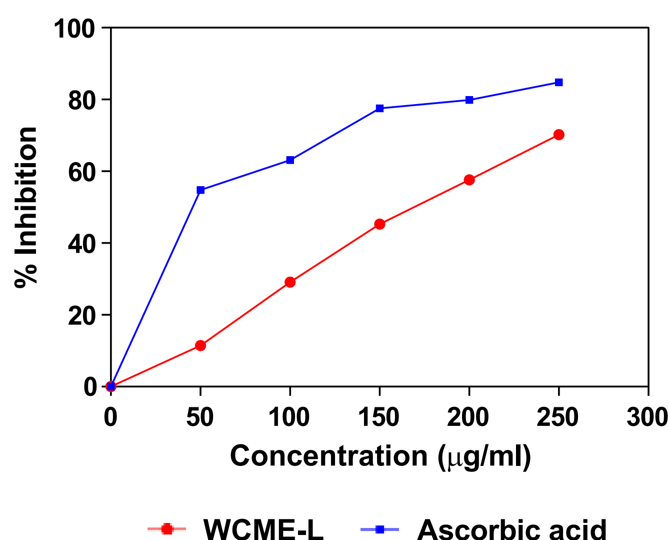


**Figure 5.** Estimating the Superoxide anion scavenging activity

***Effect on Metal chelating activity***

The method for determining metal chelating ability relies on the interaction of Fe2+ ions with the ferrozine reagent, resulting in the formation of a coloured complex that can be measured spectrophotometrically (Dinis et al., 1994, Kumaran and Joel Karunakaran, 2006). When a substance with metal chelating properties is present, it interferes with the formation of this complex, leading to a decrease in absorbance. Thus, by measuring the reduction in absorbance, one can estimate the chelating activity of the substance. In the current experimental setup, the formation of the ferrous-ferrozine complex was disrupted by both the plant extract and the standard chelating agent, EDTA. Figure 6 depicts the chelating activity of the extract and EDTA, showing the percentage of metal chelation at various concentrations (50-250 µg/ml). The results clearly indicate that the metal chelating activity of the extract is concentration-dependent, with higher concentration leading to increased chelation. The experiment demonstrated that as the concentration of the extract increased, its ability to chelate metal ions also increased. This was evidenced by the progressive reduction in absorbance of the ferrous-ferrozine complex, reflecting the extract's effectiveness in binding Fe2+ ions. The extract's performance was compared to that of EDTA, a well-known metal chelator, providing a benchmark for its efficacy. The study's findings suggest that the plant extract possesses significant metal chelating properties, which enhance with increasing concentration. This property is valuable as metal chelation plays a crucial role in reducing oxidative stress by binding transition metals that catalyze the formation of reactive oxygen species. Consequently, the plant extract could be a potent natural alternative for managing metal-induced oxidative damage, contributing to its potential use in therapeutic applications aimed at mitigating oxidative stress and related disorders.

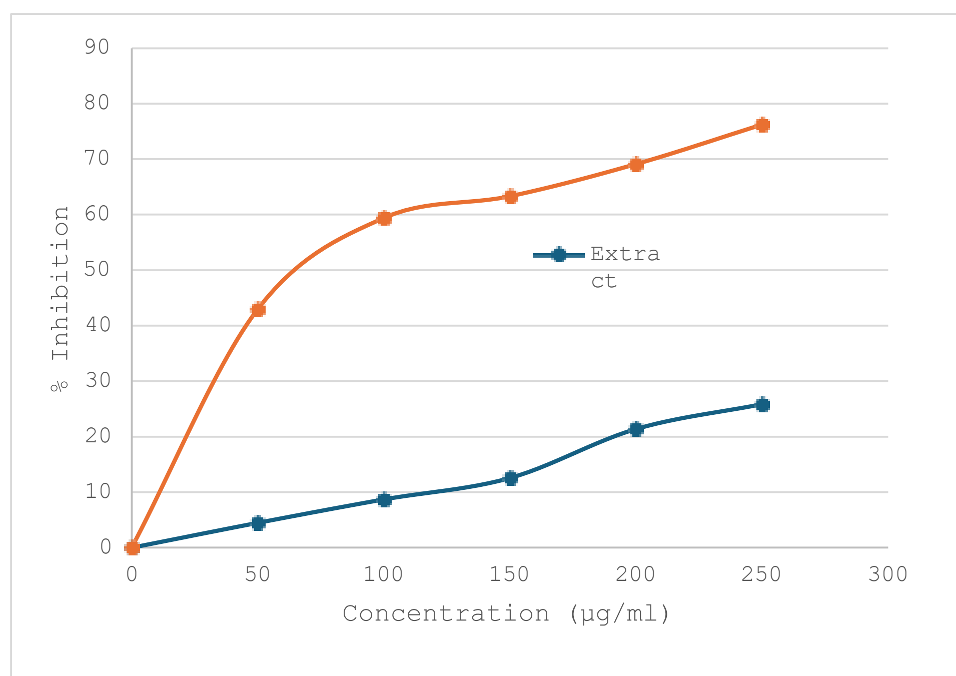


**Figure 6.** Estimating the Metal chelating activity

**Table 2.** The estimated IC50 values of WCME-L for the antioxidant models

| Assay Type | Substance | IC50 Value |
|---|---|---|
| DPPH | Quercetin | 87.44 µg/ml |
| | WCME-L | 108.76 µg/ml |
| Superoxide anion | Ascorbic acid | 115.93 µg/ml |

| | | |
|---|---|---|
| | WCME-L | 142.12 μg/ml |
| Metal chelating | EDTA | 134.84 μg/ml |
| | WCME-L | 198.72 μg/ml |

**Antidiabetic activity**

***Alpha-Amylase Inhibition Assay***

In the Alpha-Amylase Inhibition Assay, the extract shows a clear dose-response relationship, with its inhibitory activity against alpha-amylase, an enzyme crucial for starch breakdown, increasing with higher concentrations. At 0.1 mg/mL, the extract demonstrates initial inhibitory activity at 10.88±0.97%, indicating a modest effect. This marks the starting point of the extract's potential to interfere with the enzyme's function. As the concentration increases to 0.5 mg/mL, inhibition significantly rises to 25.65±0.98%, more than doubling the initial activity. This suggests the extract's components are effective at low doses and their effects enhance with higher doses. At 1.0 mg/mL, the inhibitory activity nearly doubles again to 45.87±1.11%, showing substantial effectiveness at moderate concentrations. At 2.5 mg/mL, inhibition climbs to 71.87±1.08%, indicating a robust interaction between the extract and the enzyme. At the highest tested concentration of 5.0 mg/mL, the extract exhibits very high inhibitory activity at 86.92±1.15%, approaching near-complete inhibition of alpha-amylase. This high percentage demonstrates the extract's potent efficacy at higher concentrations, making it a strong candidate for applications requiring control over enzymatic activity, such as managing blood sugar levels in diabetes. The standard deviations across all concentrations are consistently low, ranging from 0.97% to 1.15%, indicating precise and reproducible results. This low variability supports the reliability of the extract's effectiveness in inhibiting alpha-amylase. Overall, the alpha-amylase inhibition assay results suggest that the extract is a powerful inhibitor of the enzyme, with effectiveness increasing dramatically with concentration. Given its potent inhibitory action, the extract could be considered for therapeutic applications, particularly in conditions where starch digestion needs to be controlled or slowed, such as in diabetes management.

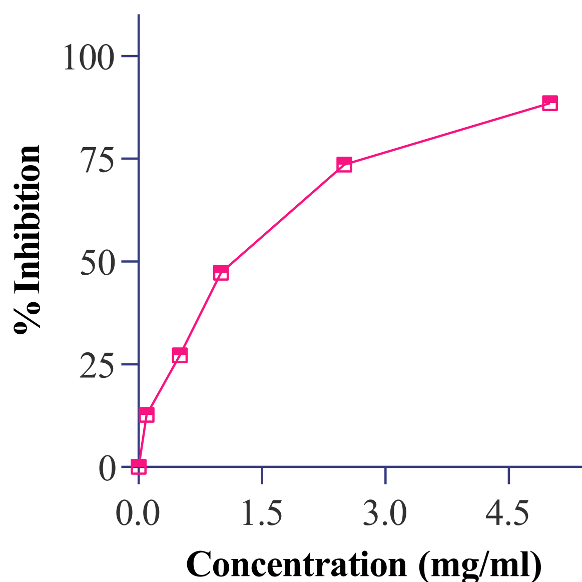


**Figure 7.** Alpha-Amylase Inhibition Assay of WCME-L

***Alpha-Glucosidase Inhibition Assay***

In the Alpha-Glucosidase Inhibition Assay, the extract shows effective inhibition of alpha-glucosidase, an enzyme that plays a crucial role in carbohydrate digestion by breaking down disaccharides to glucose. The increasing concentration of the extract correlates with increased inhibitory activity. Here's an interpretation of the data provided: At 0.1 mg/mL, the extract begins with a modest inhibition of 14.76±0.99%, indicating some initial capability to inhibit alpha-glucosidase activity at low concentrations. This initial activity is crucial for demonstrating the extract's potential effectiveness. At 0.5 mg/mL, inhibition significantly increases to 35.87±0.98%. This marked increase suggests that the extract contains compounds that are more effectively interacting with alpha-glucosidase as the concentration rises. At 1.0 mg/mL, the extract's inhibitory activity rises to 56.91±0.98%. Nearly doubling the inhibition from the 0.5 mg/mL concentration, this level shows a strong efficacy in moderate concentrations, impacting the enzyme's ability to process sugars significantly. At 2.5 mg/mL, the inhibition progresses to 75.78±1.12%. The continued increase at this concentration indicates a robust capability of the extract to inhibit alpha-glucosidase, suggesting that it might be highly effective in therapeutic contexts where slowing glucose absorption is desired. At 5.0 mg/mL, the extract shows very high inhibitory activity at 91.99±1.21%, nearing complete inhibition. This demonstrates the extract's potent efficacy at higher concentrations, making it a potential candidate for applications requiring substantial modulation of carbohydrate digestion, such as managing post-prandial glucose levels in diabetes management. The standard deviations across the concentrations are consistently low (ranging from 0.98% to 1.21%), which indicates that the measurements are precise and reproducible. This low variability adds credibility to the effectiveness of the extract in inhibiting alpha-glucosidase in a dose-dependent manner. Overall, the alpha-glucosidase inhibition assay results suggest that the extract is an effective inhibitor of the enzyme, with its ability to inhibit alpha-glucosidase increasing significantly as the concentration increases. This performance indicates potential therapeutic applications, particularly for managing glucose absorption and assisting in the control of blood sugar levels in metabolic disorders such as diabetes.

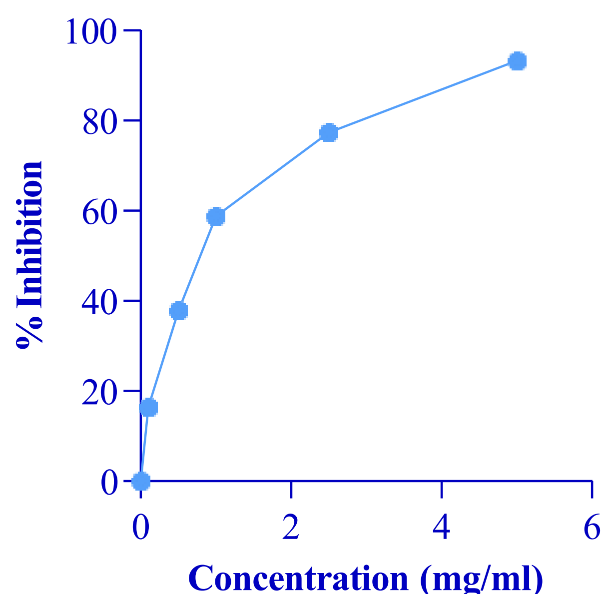


**Figure 8.** Alpha-Glucosidase Inhibition Assay of WCME-L

**Table 3.** The estimated IC50 values of WCME-L for the antidiabetic models

| Assay Type | Hill Slope | IC50 (mg/mL) |
|---|---|---|
| Alpha-Amylase Inhibition Assay | 1.23 | 1.16 |
| Alpha-Glucosidase Inhibition Assay | 1.10 | 0.98 |

## CONCLUSION

The comprehensive phytochemical screening of WCME-L highlights its potential as a source of diverse bioactive compounds. High concentrations of flavonoids and phenols, known for their antioxidant properties, suggest that the extract could be effective in neutralizing free radicals and reducing oxidative stress, which is beneficial in preventing cardiovascular diseases and certain cancers. The significant presence of saponins indicates additional benefits, including cholesterol-lowering effects, immune enhancement, and anti-cancer activities. Alkaloids, with their analgesic, anti-inflammatory, and antimalarial properties, further enhance the therapeutic potential of the extract. Despite the high phenomenon, the study did not find a direct correlation with antioxidant activity, suggesting that other compounds within the extract contribute significantly to its overall antioxidant capacity. The thiocyanate method confirmed the extract's potent antioxidant properties, showing higher activity than α-Tocopherol but lower than BHA. The reducing power assay reinforced the extract's strong antioxidant capacity, placing it between BHA and α-Tocopherol in effectiveness. In the DPPH and superoxide anion scavenging assays, the extract demonstrated significant efficacy, although slightly less potent than BHA and ascorbic acid, respectively. This suggests that while the extract is a strong antioxidant, it might be complemented by other standards for maximum efficacy. The alpha-amylase and alpha-glucosidase inhibition assays revealed a clear dose-response relationship, indicating the extract's potential in managing blood sugar levels. At higher concentrations, the extract exhibited near-complete inhibition of these enzymes, making it a promising candidate for diabetes management. Overall, the study underscores WCME-L as a potent natural antioxidant with a broad spectrum of bioactive compounds. Its diverse phytochemical profile and significant inhibitory activities against oxidative stress and carbohydrate-digesting enzymes highlighted its potential therapeutic applications. Future research should explore its clinical efficacy and safety, paving the way for its use in preventing and managing oxidative stress-related diseases and metabolic disorders.

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