Pharmacological Evaluation of Anti-Inflammatory and Antioxidant Potential of Cuscuta Reflexa Methanol Extract: Role in Inhibition of Cyclooxygenase and Lipooxygenase

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KEYWORDS
Cuscuta reflexa, Antioxidnt activity, Anti-inflammatory, DPPH, Cyclooxygenase.

ABSTRACT
In this study, the antioxidant and anti-inflammatory properties of the methanol extract of Cuscuta reflexa (CRWP-M) were evaluated. The plant material was collected from Dehradun and processed to obtain the extract, which was tested for its total phenolic content, reducing power, and scavenging activities against DPPH and superoxide radicals. The extract's inhibition of cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), and 5-lipoxygenase (LOX) enzymes was also assessed. Results showed that the extract contained 0.132 mg/mL of gallic acid equivalents of phenolic compounds. In the reducing power assay, the extract demonstrated lower activity compared to Quercetin and Vitamin C across all concentrations. For DPPH radical scavenging, the extract was more effective than BHT at low concentrations but less effective at higher concentrations. The extract exhibited a dose-dependent inhibition of COX-1, COX-2, and LOX, with the most significant effect on LOX (IC50 of 106.49 µg/mL). In superoxide radical scavenging, Vitamin C consistently outperformed the extract at all tested concentrations. These findings suggest that while CRWP-M has notable antioxidant and anti-inflammatory properties, its efficacy varies across different assays and is generally lower than that of standard antioxidants like Quercetin and Vitamin C.

1. Introduction

Inflammation and oxidative stress are interlinked biological processes that play crucial roles in maintaining homeostasis and contributing to the pathology of various diseases. Inflammation is the body's natural response to injury or infection, aimed at eliminating harmful stimuli and initiating tissue repair. It involves the activation of immune cells, the release of cytokines, and the production of reactive oxygen species (ROS). While acute inflammation is protective and resolves after the elimination of the offending agent, chronic inflammation can lead to tissue damage and contribute to the development of chronic diseases such as arthritis, cardiovascular diseases, and cancer (Lisa M. Coussens & Zena Werb, 2002; Ocampo-Gallego et al., 2023). Oxidative stress occurs when there is an imbalance between the production of ROS and the body's ability to detoxify these reactive intermediates or repair the resulting damage. ROS, including superoxide anions, hydrogen peroxide, and hydroxyl radicals, are generated as byproducts of normal cellular metabolism, particularly during mitochondrial respiration. They play important roles in cell signalling and homeostasis. However, excessive ROS production can damage cellular components, including lipids, proteins, and DNA, leading to cell dysfunction and death (Federico, Morgillo, Tuccillo, Ciardiello, & Loguercio, 2007). The interplay between inflammation and oxidative stress is complex and bidirectional. During inflammation, activated immune cells such as neutrophils and macrophages produce large amounts of ROS to destroy pathogens. This ROS production, while essential for pathogen clearance, can also cause collateral damage to host tissues if not properly regulated. Conversely, oxidative stress can activate various signaling pathways, including nuclear factor-kappa B (NF-κB), which promote the expression of pro-inflammatory genes and sustain the inflammatory response (Amaral et al.,

2019; Bozin & Mimica-Dukić, 2007; El-Hadary, Elsanhoty, & Ramadan, 2019; Khare, 2007; Kontogianni et al., 2013).

Chronic oxidative stress and inflammation create a vicious cycle that exacerbates tissue damage and contributes to disease progression. For example, in atherosclerosis, oxidative modification of low-density lipoproteins (LDL) triggers an inflammatory response in the arterial wall, promoting plaque formation and increasing the risk of cardiovascular events. Similarly, in neurodegenerative diseases like Alzheimer's, chronic inflammation and oxidative stress lead to neuronal damage and cognitive decline Chaudhari, Talwar, Parimisetty, Lefebvre d'Hellencourt, & Ravanan, 2014). Managing oxidative stress and inflammation is therefore crucial for preventing and treating chronic diseases. Antioxidants, which neutralize ROS, and anti-inflammatory agents, which reduce the inflammatory response, are potential therapeutic strategies. Natural compounds, such as polyphenols found in plants, have shown promise in modulating these pathways, highlighting the importance of diet and lifestyle in maintaining health and preventing disease (Ahmed, 2011; Chaudhari, Talwar, Parimisetty, Lefebvre d'Hellencourt, & Ravanan, 2014; Federico et al., 2007; Hassan et al., 2017).

Cuscuta reflexa, commonly known as dodder and referred to as "Amarbel" in Indian traditional medicine, is a parasitic plant belonging to the family Convolvulaceae (Saini, Mithal, & Menghani, 2015). This leafless, stemless plant relies entirely on its host for nutrition, wrapping around and penetrating the host plant's tissues to draw water and nutrients. Predominantly found in tropical and subtropical regions, Cuscuta reflexa spreads rapidly, significantly impacting agricultural crops and wild vegetation (Saini et al., 2015; Vijikumar, Ramanathan, & Devi, 2011). Despite its parasitic nature, Cuscuta reflexa has been recognized for its medicinal properties in traditional Indian medicine (Ayurveda). It has been used to treat a variety of ailments, including liver disorders, jaundice, hypertension, and inflammatory conditions. The plant is rich in bioactive compounds such as flavonoids, alkaloids, and glycosides, which contribute to its therapeutic effects (Gilani & Aftab, 1992). Recent scientific studies have highlighted the antioxidant and anti-inflammatory potential of Cuscuta reflexa. Its methanol extract has demonstrated significant free radical scavenging activity and the ability to inhibit key enzymes involved in inflammation, such as cyclooxygenase (COX) and lipoxygenase (LOX). These properties make it a promising candidate for developing natural remedies for oxidative stress and inflammation-related diseases (Gilani & Aftab, 1992; Patel, Sharma, Chauhan, & Dixit, 2012).

Overall, Cuscuta reflexa represents a unique paradox in nature, being both a parasitic threat to agriculture and a potential source of valuable medicinal compounds. Its traditional usage in Indian medicine underscores its significance, and further research is needed to fully understand its pharmacological benefits and to explore its potential in modern medicine (Adzet, Caiñigueral, & Iglesias, 1988; Bouaziz, Yangui, Sayadi, & Dhouib, 2009; Chaudhari et al., 2014; Khare, 2007; Steven et al., 2019). The present study was designed to investigate the role of this plant, Cuscuta reflexa in oxidative stress and inflammations. For the purpose several mechanistic antioxidant activity models were considered which included reducing power assay, DPPH radical scavenging activity and Superoxide radical scavenging activity assay. For the same, antiinflammatory activity was measured in terms of cyclooxygenase and lipoxygenase enzyme inhibition assays.

2. Material and Methods

Assortment and Authentication of The Plants: Preparation of the Extracts

In the Dehradun region, the whole Cuscuta reflexa plant was collected between October and December of 2022. The plant material was recognised, identified, and verified by a botanist, and the voucher specimens (MK/ACP/2022/328) were stored for potential future use. The entire plant was chopped, mechanically processed, shade-dried, and ground into a powder. To extract all of the phytoconstituents, the cold maceration method was employed. After the methanol extract was completely extracted, it was gathered and concentrated at lowered pressure at 45–50 °C. Of the dried starting material, 0.83 percent was obtained by the final methanol extract. After that, the completed item was stored at 4°C until needed. The code for the herbal extract was CRWP-M.

Drugs and Chemicals

The supplier of DPPH (1, 1-diphenyl-2-picryl hydrazyl hydrate) was Loba Chemical Company in India. Samples of quercetin, BHT (Butylate Hydroxy Toluene), and vitamin C were sent as gifts from Resht Pharmaceuticals in Haryana. Every other unlabeled chemical and reagent that was found in the market was easily accessible and of analytical quality (SRL Mumbai, E. Merck India).

Determination of Total Phenolic Compounds

The determination of total phenolic content in the sample was conducted using the Folin-Ciocalteau method (Slinkard & Singleton, 1977), which involves the chemical reduction of the Folin-Ciocalteau reagent by phenolic compounds under alkaline conditions, producing a blue complex measurable spectrophotometrically. Initially, a standard curve was prepared by dissolving gallic acid in distilled water to achieve a concentration range from 0 to 200 µg/mL. Aliquots of 1 mL from each standard solution were transferred to separate test tubes. Each tube received 1 mL of Folin-Ciocalteau reagent, followed by thorough mixing. After 3 minutes, 3 mL of 20% sodium carbonate solution was added to each tube, and the reaction mixtures were incubated at room temperature for 2 hours in the dark. For the sample preparation, 1 mL of the sample extract was placed in a test tube, and the same procedure was followed as with the standards. The absorbance of all reaction mixtures was measured at 765 nm using a spectrophotometer, with a blank sample prepared using distilled water instead of the sample extract. The total phenolic content was calculated using the gallic acid standard curve and expressed as mg of gallic acid equivalents (GAE) per gram of the sample. This method ensured accurate and reliable quantification of total phenolic compounds in various plant extracts, aiding in understanding their potential antioxidant properties:

$y = 0.0021x + 0.0158$

$R^2 = 0.9916$

Where, x was the concentration, and y was the absorbance.

Anti-Inflammatory Activity

Cyclooxygenase–1 (COX–1) and Cyclooxygenase–2 (COX–2) Assays

To evaluate the inhibitory activity of a plant extract on cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes, specific assays are tailored to accommodate the characteristics of plant extracts (Aguilar et al., 2002; Redl, Breu, Davis, & Bauer, 1994). The process begins with the preparation of the plant extract using appropriate solvents like methanol or ethanol; the extract is then dried and reconstituted in a suitable buffer at a defined concentration. For the COX-1 assay, the method involves preparing a purified COX-1 enzyme, usually sourced from bovine, in an assay buffer along with arachidonic acid at a predetermined concentration. The plant extract is pre-incubated with the COX-1 enzyme at 37 degrees Celsius to allow potential inhibition. After this pre-incubation, arachidonic acid is introduced to start the reaction. The reaction proceeds for about 10 to 20 minutes and is halted by adding a stopping solution such as hydrochloric acid. The resulting prostaglandin H2 (PGH2) is quantified using a colorimetric method that measures the change in absorbance due to PGH2's interaction with a chromogenic substrate. For the COX-2 assay, which involves an inducible enzyme, a cell-based approach is often employed. Cells capable of expressing COX-2 upon stimulation, such as macrophages, are cultured and treated with an inflammatory agent like lipopolysaccharide to induce enzyme expression. These cells are then exposed to the plant extract, which is tested for its ability to inhibit COX-2. Following a pre-incubation period with the extract, arachidonic acid is added to the culture to initiate the reaction. Similar to the COX-1 assay, PGH2 production is measured using a colorimetric approach. These methods are integral to screening natural products for potential anti-inflammatory properties, providing crucial data on the effectiveness of plant extracts in inhibiting key enzymes involved in the inflammatory process (Aguilar et al., 2002; Redl et al., 1994).

Effect on 5–lipoxygenase (LOX) Enzyme

The 5-LOX assay was conducted using the previously reported methodology (Kulkarni, Mitra, Chaudhuri, Byczkowski, & Richards, 1990). To conduct an assay for measuring the activity of the 5-lipoxygenase (5-LOX) enzyme, all necessary reagents and samples were prepared initially. The enzyme was sourced either from human leukocytes or through commercially available recombinant forms. The substrate, arachidonic acid, was prepared at concentrations typically ranging from 10 to 100 µM in a suitable buffer like Tris-HCl. Essential cofactors such as Ca2+ and ATP were included in the reaction mixture to ensure optimal enzyme activity, alongside antioxidants to prevent non-enzymatic oxidation of the substrate. For the assay setup, the enzyme was mixed with the arachidonic acid substrate in a reaction buffer, aiming for a total volume of 100-200 µL in either a test tube or a microplate well. This mixture was pre-incubated at 37°C for about 10 minutes to stabilize the enzyme. The enzymatic reaction was initiated by adding the substrate to the pre-incubated enzyme mixture, allowing the reaction to proceed for 10-30 minutes at the same temperature. The reaction was terminated by adding an ice-cold solution such as methanol, which also aided in precipitating proteins and extracting the reaction products.

Following the reaction, the leukotrienes were extracted using an organic solvent like ethyl acetate or hexane, separating them from the aqueous phase. The leukotrienes were quantified using High-Peformance Liquid Chromatography (HPLC) or Gas Chromatography-Mass Spectrometry (GC-MS), using standards for calibration to accurately measure the concentrations. The enzymatic activity was calculated based on the amount of product formed per unit time, which reflected the enzyme's catalytic efficiency under the tested conditions. Negative controls were included to account for non-enzymatic reactions and the assay was performed in replicates to validate the results. This methodological approach was crucial for understanding the enzyme's functionality or screening potential inhibitors in therapeutic research against inflammatory diseases.

Antioxidant Activity

Determination of DPPH (1, 1–Diphenyl–2–Picryl Hydrazyl) Radical Scavenging Activity

The free radical scavenging ability of the extract was evaluated using DPPH radical and the previously described methods (Shimada, Fujikawa, Yahara, & Nakamura, 1992). The determination of DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity is a widely used method to assess the antioxidant capacity of substances such as plant extracts. This assay based on the reduction of the DPPH radical, a stable free radical known for its deep purple color. Antioxidants can quench this radical, leading to a decrease in color intensity, which is quantifiable through spectrophotometry. To conduct the assay, a DPPH solution is initially prepared in methanol or ethanol to achieve an absorbance of about 0.8 to 1.0 at 517 nm. It is essential to prepare this solution freshly and to protect it from light to prevent degradation. The samples under investigation are also prepared at varying concentrations in the same solvent as DPPH to establish a dose-response curve. The assay itself involves mixing equal volumes of the DPPH solution and the sample solutions in test tubes or microplate wells, with a control setup using the solvent instead of the sample. Following mixing, the assay mixture is incubated in the dark at room temperature for 30 minutes to an hour, depending on the protocol or specific sample characteristics. After incubation, the absorbance is measured at 517 nm. The decrease in absorbance relative to the control indicates the radical scavenging activity of the sample. This activity is quantified using a formula that calculates the percentage of DPPH radical scavenging, providing a direct measure of the antioxidant capacity of the sample. For accuracy and comparison, it is common practice to include a known antioxidant, like ascorbic acid or Trolox, as a standard. The following formula was used for the calculation of percentage scavenging of DPPH• radical:

Percentage scavenging of DPPH$^{\bullet}$ radical = $[(A_c - A_t / A_c) \times 100]$

Where A_t = absorbance when the extract or reference is present and A_c = absorbance of the control reaction system.

Reducing Power

The previously outlined procedure was used to calculate the reduction power of the extract. (Oyaizu, 1986). To conduct the reducing power assay using various concentrations of the extract (50-250 µg/mL), a detailed and systematic procedure is followed. Initially, each extract concentration is combined with potassium ferricyanide [K3Fe(CN)6] solution (1.1% concentration) and 0.3 M phosphate buffer at pH 6.7 in 1.5 mL of distilled water, resulting in a total volume of 5.2 mL for each sample. This mixture is then incubated at 50°C for 25 minutes, allowing the reduction reaction to occur, where potassium ferricyanide is reduced to potassium ferrocyanide by the reducing agents in the sample. Following the incubation, the reaction is terminated by adding 2.6 mL of 12% trichloroacetic acid, which precipitates proteins and stops further reaction. The mixture is subsequently centrifuged at 4000 rpm for 12 minutes to separate the precipitated proteins, and the clear supernatant is collected. To this supernatant, 0.6 mL of 0.2% ferric chloride solution is added along with 2.6 mL of distilled water. The addition of ferric chloride is crucial as it reacts with the ferrous ions formed during the reduction to produce a coloured complex. The absorbance of this final mixture is then measured at 700 nm using a spectrophotometer (Shimadzu, Japan model 1601). The principle behind this measurement is that a higher absorbance indicates a greater reducing power, signifying a higher capability of the extract sample to donate electrons. This assay effectively quantifies the antioxidant capacity of the samples, reflecting their potential to act as reducing agents by neutralizing reactive species through electron donation.

Evaluating Superoxide Radical ($O_2^{\bullet -}$) Scavenging Activity

The superoxide radical (O2•−) scavenging activity assay (Beauchamp & Fridovich, 1971) is an essential method for assessing the antioxidant capacity of various substances, crucial for combating oxidative stress-related diseases. This assay relies on the ability of antioxidants to neutralize superoxide radicals generated in a test

system, typically using the xanthine/xanthine oxidase system for radical production. In this method, xanthine is metabolized by xanthine oxidase, producing both uric acid and superoxide radicals. Nitroblue tetrazolium (NBT) serves as the indicator in this reaction, changing to a blue-colored formazan product when reduced by superoxide radicals. The procedure begins by preparing a reaction mixture that includes xanthine at a specified concentration, xanthine oxidase to generate the radicals, NBT to detect the radicals, and a phosphate buffer to maintain the pH around 7.4. The test sample, containing the antioxidant under investigation, is added in varying concentrations to assess its scavenging activity. This mixture is incubated at room temperature for 20 to 30 minutes, typically in a 96-well plate to facilitate handling multiple samples efficiently. Once the incubation is complete, the reaction is stopped by adding a stop solution that can either buffer the pH or chelate essential divalent cations, inhibiting further activity of xanthine oxidase. The absorbance of the formazan product is then measured spectrophotometrically at 560 nm. The reduction in absorbance compared to a control sample, which lacks the test antioxidant, quantifies the scavenging activity. This is calculated using a formula that compares the absorbance of the control to that of the test sample, with a higher reduction indicating more potent scavenging activity. For validation and comparison, known antioxidants like ascorbic acid or superoxide dismutase are used as positive controls, while a blank containing all reagents except xanthine oxidase helps account for non-enzymatic reductions of NBT. This assay is highly valuable for screening substances that could mitigate the effects of oxidative stress by neutralizing superoxide radicals, thereby offering potential therapeutic benefits. The following equation was used for the calculation of percentage inhibition of superoxide anion:

Percentage inhibition of superoxide anion = $(A_c - A_t / A_c) \times 100$

Where A_t = the absorbance in presence of standard or extract, and A_c represents the control's absorption (without extract).

Statistical Analysis

All data was presented as mean ± SD (n = 3). The statistical analyses, which included post hoc "Dunnett's Multiple Comparison Test" and one-way analysis of variance (ANOVA), were carried out using the software application GraphPad Prism. A statistical significance threshold of 0.05 or less, represented as $p<0.05$, was used to calculate the P values.

3. Results and findings

3.1 Total Phenolic Compounds Determination

The total phenolic contents were estimated to be 0.132 mg/mL of gallic acid equivalents (GAE) per gram of the extract.

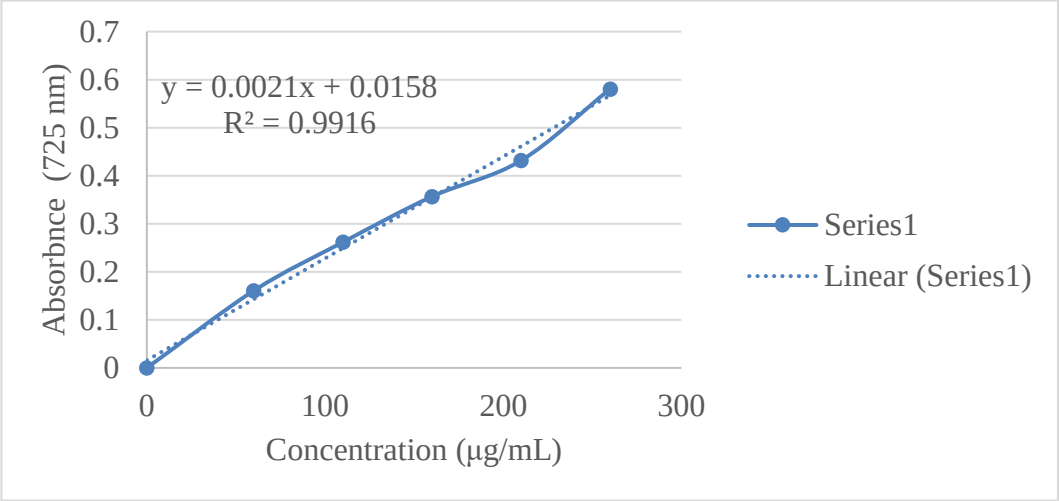

Figure 1. Estimation of total phenolic contents in CRWP-M

3.2 Evaluation of Anti-Inflammatory Activity

3.2.1 Assessing The COX-1 (Cyclooxygenase-1) And COX-2 (Cyclooxygenase-2) Enzymes Inhibition

The herbal extract (CRWP-M) exhibited a gradual increase in COX-1 inhibition with increasing concentration.

At 50 µg/mL, the inhibition was relatively low (6.04%), but it sharply increased to 98.90% at 250 µg/mL. The IC50 value for COX-1 was 303.13 µg/mL, indicating that higher concentrations of the extract are required to achieve 50% inhibition of COX-1 activity. Similar to COX-1, COX-2 inhibition by the herbal extract also increased with concentration. Starting at 7.99% inhibition at 50 µg/mL, it reached 91.48% at 250 µg/mL. The IC50 value for COX-2 was slightly higher at 375.74 µg/mL, suggesting that COX-2 is less sensitive to the extract compared to COX-1.

3.2.2 5-Lipoxygenase (LOX) Enzyme Assay

The inhibition of LOX by the herbal extract (CRWP-M) showed a strong response even at lower concentrations. Starting at 5.39% inhibition at 50 µg/mL, the inhibition reached 71.09% at 250 µg/mL. The IC50 value for LOX was significantly lower at 106.49 µg/mL, indicating that the extract is particularly effective in inhibiting LOX activity at lower concentrations compared to COX-1 and COX-2. The herbal extract exhibits a dose-dependent inhibition of COX-1, COX-2, and LOX enzymes. The extract is most effective in inhibiting LOX activity, with the lowest IC50 value (106.49 µg/mL), indicating higher sensitivity. In contrast, COX-2 inhibition requires the highest concentration for 50% inhibition (IC50 of 375.74 µg/mL), indicating it is the least sensitive to the extract. COX-1 shows moderate sensitivity with an IC50 value of 303.13 µg/mL. These results suggest that the herbal extract could potentially be a more effective inhibitor for LOX compared to COX-1 and COX-2 (Table 1).

Table 1. Percentage enzyme inhibition of the COX and LOX system by the herbal extract (CRWP-M).

Concentration (µg/mL)	% Enzyme Inhibition		
	COX-1	COX-2	LOX
50	6.038 ± 0.041	7.991 ± 0.128	5.394 ± 0.018
100	10.179 ± 0.184	15.433 ± 0.139	10.007 ± 0.063
150	27.123 ± 0.065	32.847 ± 0.194	21.893 ± 0.101
200	50.107 ± 0.077	50.077 ± 0.198	45.971 ± 0.178
250	98.898 ± 0.081	91.483 ± 0.173	71.087 ± 0.473
IC_{50}	303.13 µg/mL	375.74 µg/mL	106.49 µg/mL

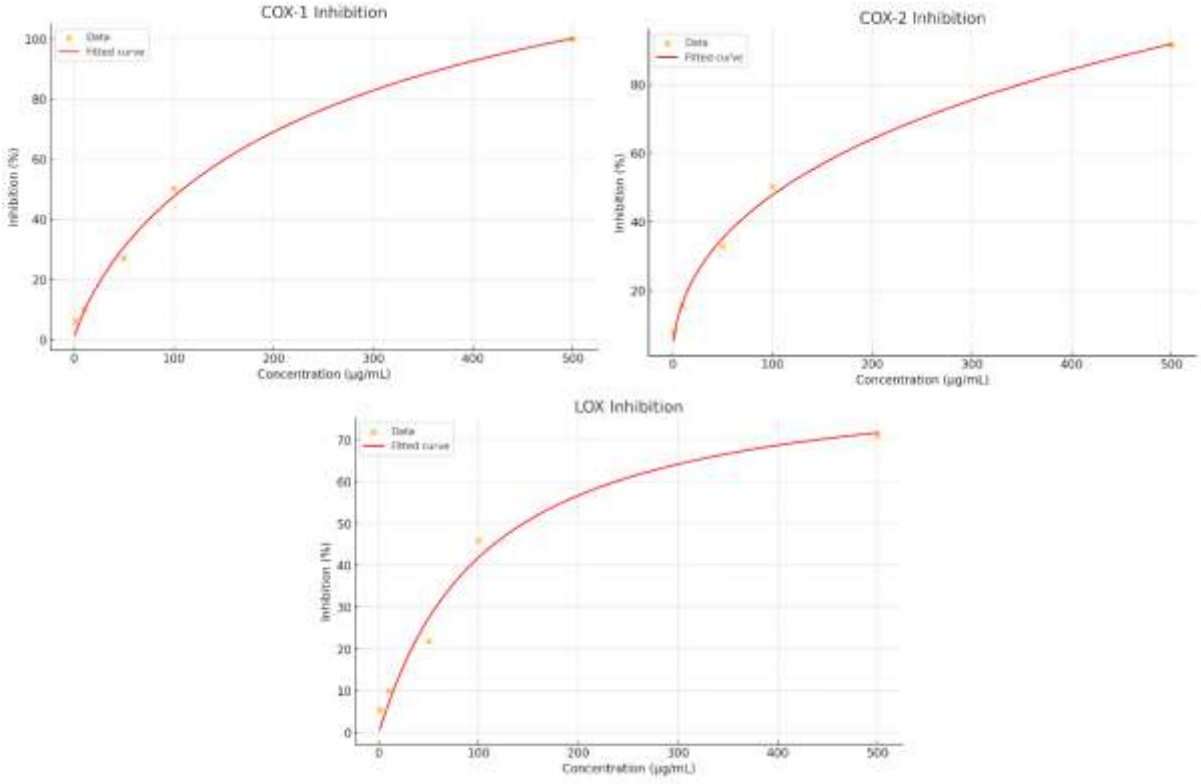

Figure 2. Percentage enzyme inhibition of the COX and LOX system by the herbal extract (CRWP-M).

Estimation of Antioxidant Activity

Results from Reducing Power Measurement

The reducing power assay data provides insight into the antioxidant capabilities of the CRWP-M compared to

Quercetin and Vitamin C across a range of concentrations. The assay measures the ability of the substances to donate electrons and reduce Fe3+ to Fe2+, with higher absorbance values indicating greater reducing power. At a concentration of 50 µg/mL, the extract shows a reducing power of 0.1905 ± 0.00491, which is lower than both Quercetin (0.2855 ± 0.00141) and Vitamin C (0.2102 ± 0.00491). This trend continues at 100 µg/mL, where the extract has an absorbance of 0.2785 ± 0.00401, compared to Quercetin's 0.3375 ± 0.00311 and Vitamin C's 0.3112 ± 0.00681. At 150 µg/mL, the extract reaches 0.3825 ± 0.00221, still trailing behind Quercetin (0.4815 ± 0.00621) and Vitamin C (0.4138 ± 0.00951). The pattern of lower reducing power for the extract relative to Quercetin and Vitamin C persists at higher concentrations. At 200 µg/mL, the extract records an absorbance of 0.4923 ± 0.00801, while Quercetin and Vitamin C show absorbances of 0.6016 ± 0.00088 and 0.5048 ± 0.00311, respectively. Finally, at the highest concentration tested (250 µg/mL), the extract achieves a reducing power of 0.5405 ± 0.00561. In comparison, Quercetin and Vitamin C reach higher absorbances of 0.7086 ± 0.00301 and 0.6265 ± 0.00281, respectively. In summary, Quercetin consistently exhibits the highest reducing power across all concentrations, followed by Vitamin C. The extract, while showing increasing reducing power with higher concentrations, consistently demonstrates the lowest but significant reducing power compared to the other two standard antioxidants. This suggests that, while the extract does possess antioxidant activity, it is less effective at donating electrons and reducing Fe3+ to Fe2+ compared to Quercetin and Vitamin C (Oyaizu, 1986).

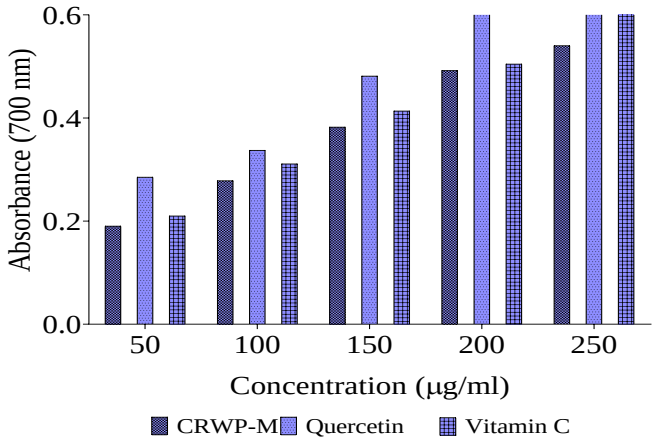

Figure 3. Reducing power assay compared to Quercetin and Vitamin C of CRWP-M

Evaluating the Scavenging of DPPH Radical

The scavenging of DPPH radicals by the CRWP-M and BHT (Butylated Hydroxytoluene) was measured across various concentrations, providing insight into their antioxidant activities. The ability to scavenge DPPH radicals is a common method to evaluate the free radical scavenging activity of antioxidants, with higher percentages indicating greater scavenging ability. At the lowest concentration tested (10 µg/mL), the extract shows a scavenging activity of 16.51% ± 1.53%, which is significantly higher than BHT's 9.36% ± 0.603%. This suggests that at lower concentrations, the extract is more effective at scavenging DPPH radicals compared to BHT. At 50 µg/mL, BHT demonstrates higher scavenging activity (42.69% ± 0.564%) than the extract (36.47% ± 0.86%). This trend continues at 100 µg/mL, where BHT exhibits a scavenging activity of 69.89% ± 1.051%, slightly surpassing the extract's 68.79% ± 1.29%. These results indicate that BHT becomes more effective than the extract at moderate concentrations. At higher concentrations of 200 µg/mL, the scavenging activities of the extract and BHT are very close, with the extract at 90.87% ± 1.64% and BHT at 90.33% ± 0.575%. This similarity suggests that both the extract and BHT are equally effective at this concentration. As the concentration increases further to 300 µg/mL and 500 µg/mL, BHT maintains a slightly higher scavenging activity with 93.29% ± 1.112% and 94.86% ± 0.638%, respectively, compared to the extract's 91.89% ± 1.64% and 92.92% ± 1.61%. This indicates that at very high concentrations, BHT is marginally more effective than the extract in scavenging DPPH radicals. The extract demonstrates a higher scavenging activity than BHT at the lowest concentration (10 µg/mL). However, as the concentration increases, BHT exhibits greater scavenging activity compared to the extract at moderate to high concentrations (50 µg/mL and above). The effectiveness of both the extract and BHT becomes comparable at 200 µg/mL, but BHT slightly surpasses the extract at higher concentrations (300 µg/mL and 500 µg/mL). Overall, BHT is a more potent DPPH radical scavenger than the extract at concentrations above 50 µg/mL (Table 2 and Figure 3).

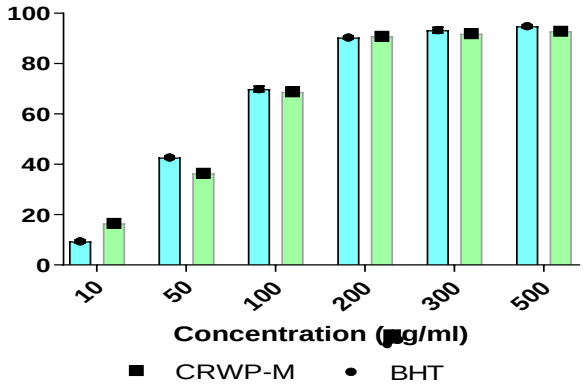

Figure 4. Scavenging of DPPH radical of the CRWP-M compared to BHT

Evaluating the Scavenging of Superoxide Radical ($O_2^{\bullet -}$)

The provided data illustrates the superoxide radical (O2•–) scavenging activity of the CRWP-M compared to Vitamin C (ascorbic acid) at various concentrations. This assay measures the ability of the substances to neutralize superoxide radicals, a common reactive oxygen species, with higher percentages indicating greater scavenging activity. At the lowest concentration (50 µg/mL), the extract exhibits a scavenging activity of 13.63% ± 0.535%, while Vitamin C shows a significantly higher activity of 56.91% ± 0.407%. This indicates that Vitamin C is much more effective at scavenging superoxide radicals than the extract at this concentration. As the concentration increases to 100 µg/mL, the scavenging activity of the extract improves to 31.35% ± 0.911%, but it remains lower than Vitamin C, which has an activity of 65.26% ± 0.169%. This trend continues at 150 µg/mL, where the extract reaches 47.61% ± 0.409%, while Vitamin C shows a much higher scavenging activity of 79.77% ± 0.502%. At 200 µg/mL, the extract's scavenging activity increases further to 59.88% ± 0.403%, but it is still lower than Vitamin C, which has an activity of 81.91% ± 0.758%. At the highest concentration tested (250 µg/mL), the extract achieves its maximum scavenging activity of 72.91% ± 0.378%, whereas Vitamin C reaches 86.77% ± 0.833%. Vitamin C demonstrates a significantly higher superoxide radical scavenging activity compared to the extract at all tested concentrations. At 50 µg/mL, Vitamin C is more than four times as effective as the extract. Although the scavenging activity of the extract increases steadily with higher concentrations, it consistently lags behind Vitamin C. Even at the highest concentration (250 µg/mL), the extract's scavenging activity (72.91%) does not match the efficacy of Vitamin C (86.77%). These results suggest that the extract demonstrates significant superoxide radical scavenging effect but Vitamin C is a far more potent superoxide radical scavenger than the extract, making it a superior antioxidant in this context (Figure 4 and Table 2) (Ashokkumar, Thamilselvan, GP, Mazumder, & Gupta, 2008; Erasto, Grierson, & Afolayan, 2007).

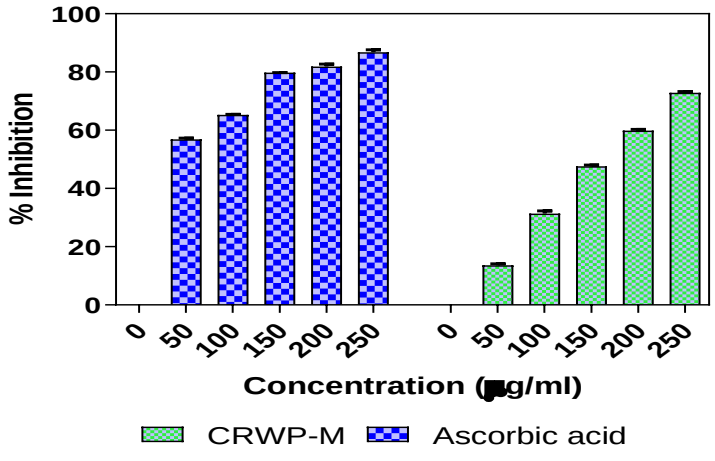

Figure 5. Superoxide radical (O2• –) scavenging activity of the CRWP-M compared to Vitamin C (Ascorbic acid)

Table 2. Calculated IC_{50} values for scavenging activity of DPPH and superoxide radicals by the CRWP-M (mean ± SD, n = 3)

Drugs	IC_{50} (μg·mL^{-1})	
	DPPH radical	Superoxide radical
CRWP-M	91.72 ± 0.99	122.71 ± 0.98
BHT	66.84 ± 0.98	-
Vitamin C (Ascorbic acid)	-	99.94± 0.99

4. Conclusions

The methanol extract of Cuscuta reflexa (CRWP-M) exhibits significant antioxidant and anti-inflammatory activities, although its efficacy is generally lower compared to standard antioxidants like Quercetin and Vitamin C. The extract showed a progressive increase in enzyme inhibition and radical scavenging activities with increasing concentrations. In the reducing power assay, CRWP-M demonstrated measurable activity, yet it was consistently outperformed by Quercetin and Vitamin C. The extract's DPPH radical scavenging activity was higher than BHT at low concentrations but fell behind at higher concentrations, indicating its moderate efficacy as a free radical scavenger. The most notable anti-inflammatory effect of CRWP-M was observed in the LOX enzyme inhibition assay, where it displayed the lowest IC50 value, suggesting a potent inhibition at lower concentrations. Despite the extract's promising antioxidant capabilities, particularly in scavenging superoxide radicals, Vitamin C proved to be significantly more effective. These results highlight the potential of Cuscuta reflexa extract as a natural source of antioxidants and anti-inflammatory agents, though its comparative lower efficacy suggests that it might be more beneficial when used in combination with other potent antioxidants for therapeutic applications.

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