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Pharmacological Potential of Gynura Procumbens Root Extract: Role in Oxidative Stress, Inflammation and Nephroprotection

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ABSTRACT:

This study assessed the nephroprotective, anti-inflammatory, and antioxidant properties of *Gynura procumbens* methanol root extract (GPME-R). After the plant material was extracted, it underwent tests to determine its total phenolic content, reducing power, ability to scavenge radicals such as DPPH and superoxide, and ability to chelate metals. It was also determined whether the extract inhibited the enzymes 5-lipoxygenase (LOX), cyclooxygenase-1 (COX-1), and cyclooxygenase-2 (COX-2). *Gynura procumbens* root extract's nephroprotective potential was also assessed in HEK-293 cells exposed to cisplatin-induced damage. The extract has 0.177 mg/mL of gallic acid equivalents of phenolic compounds, according to the results. The extract showed less action in the reducing power assay at all doses when compared to quercetin and vitamin C. The extract was less effective than BHT for DPPH radical scavenging at higher doses, but more effective at lower concentrations. COX-1, COX-2, and LOX were all shown to be inhibited by the extract in a dose-dependent manner; LOX was most significantly inhibited (IC_{50} of 101.88 µg/mL). There was also notable metal chelating activity in the extract. Additionally, the extract showed notable dose-dependent nephroprotective efficacy in cisplatin-induced nephrotoxicity model in HEK-293 cells. According to these results, GPME-R has strong nephroprotective, anti-inflammatory, and antioxidant characteristics.

Keywords: Antioxidant activity, DPPH, Anti-inflammatory, Nephroprotective, Cyclooxygenase, *Gynura procumbens*

INTRODUCTION:

Oxidative stress and inflammation are interdependent biological processes that are important for preserving homeostasis and for advancing the pathophysiology of many illnesses. The body's natural reaction to damage or infection is inflammation, which it uses to get rid of unwanted stimuli and start the healing process for damaged tissue. Reactive oxygen species (ROS) are produced, cytokines are released, and immune cells are activated. Acute inflammation protects the body and goes away when the offending chemical is removed, but chronic inflammation can harm tissue and play a role in the emergence of long-term conditions like cancer, heart disease, and arthritis. [1, 2]. When there is an imbalance between the body's capacity to detoxify reactive intermediates (ROS) or heal the harm they cause, oxidative stress results. The production of ROS, such as hydroxyl radicals, superoxide anions, and hydrogen peroxide, is a consequence of regular cellular metabolism, especially during mitochondrial respiration. They are crucial for maintaining homeostasis and cell signalling. On the other hand, overproduction of ROS can harm lipids, proteins, and DNA within cells, which can result in cell

death and malfunction. [3]. Oxidative stress and inflammation interact in a complicated and reciprocal way. To eliminate infections, active immune cells like neutrophils and macrophages release a lot of ROS during inflammation. Although this generation of ROS is necessary for the removal of pathogens, if it is not appropriately controlled, it may potentially inadvertently harm host tissues. On the other hand, oxidative stress has the ability to trigger many signalling pathways, such as nuclear factor-kappa B (NF-κB), that encourage the production of genes that increase inflammation and maintain the inflammatory response. [4-8].

Inflammation and chronic oxidative stress combine to produce a vicious cycle that worsens tissue damage and speeds up the course of illness. For instance, oxidative alteration of low-density lipoproteins (LDL) in atherosclerosis causes the artery wall to become inflamed, which encourages the formation of plaque and raises the risk of cardiovascular events. Similar to this, oxidative stress and persistent inflammation cause neuronal damage and cognitive loss in neurodegenerative illnesses like Alzheimer's. Chaudhari, Talwar, Parimisetty, Lefebvre d'Hellencourt, & Ravanan, 2014). Thus, controlling inflammation and oxidative stress is essential for both preventing and treating chronic illnesses. Potential treatment approaches include antioxidants, which neutralise ROS, and anti-inflammatory drugs, which lessen the inflammatory response. Natural substances, such the polyphenols present in plants, have demonstrated potential in modifying these pathways, underscoring the significance of lifestyle and nutrition in preserving health and averting illness. [3, 9-11].

Thailand, China, Malaysia, Indonesia, Vietnam and India are home to the tropical Asian medicinal plant *Gynura procumbens* (Lour.) Merr. (Family: Asteraceae). In many nations across the world, it has long been used to treat a wide range of medical conditions, including rheumatism, diabetes mellitus, constipation, kidney issues, renal discomfort and hypertension [12, 13]. Many compounds were identified from the methanol (MeOH) extract of *Gynura procumbens* leaves through chemical investigation including phytol, lupeol, stigmasterol, friedelanol acetate, β-amyrin, and a combination of stigmasterol and β-sitosterol [13-15]. Recent research has demonstrated the many biological qualities of *Gynura procumbens* [13-17]. The goal of the current study is to investigate more about role of *Gynura procumbens* in inflammation, oxidative stress and nephroprotection. A number of mechanistic antioxidant activity models, including as the DPPH radical scavenging activity, superoxide radical scavenging activity, reducing power assay, and metal chelating activity were taken into consideration for this purpose. The anti-inflammatory properties of the same were assessed using tests for the inhibition of the enzymes lipoxygenase and cyclooxygenase.Furthermore,nephroprotective effect of *Gynura procumbens* root extract was also evaluated in cisplatin-induced toxicity in HEK-293 cells.

MATERIAL AND METHODS:

Collection and authentication of the Plant material:

The *Gynura procumbens*roots were collected in the Dehradun region in November and December of 2022. A botanist recognized, identified, and confirmed the plant material; the voucher specimens (GP/PC/2022/131) were then preserved for possible use at a later time.

Preparation of the extract:

The roots of the plant were shade-dried, sliced, mechanically processed and grounded into a powder. Methanol was used as the solvent in the cold maceration process to extract all the phytoconstituents. The methanol extract was collected and concentrated at 45–50 °C under reduced pressure once it had been fully extracted. The final methanol extract yielded 0.98 percent of the dried starting material. The finished product was then kept in storage at 4°C until it was required. The herbal extract's code was GPME-R.

Drugs and chemicals

DPPH (1, 1-diphenyl-2-picryl hydrazyl hydrate) was supplied by Loba Chemical Company, India. Samples of quercetin, vitamin C, and BHT (Butylate Hydroxy Toluene) were procure from Sigma Aldrich, India. All other unlabeled chemical and reagents were procured from SRL Mumbai and E. Merck India.

Determination of total phenolic compounds

The Folin-Ciocalteau method was used to determine the sample's total phenolic content. [18], which, in an alkaline environment, entails the chemical reduction of the Folin-Ciocalteau reagent by phenolic substances, yielding a blue complex that may be measured spectrophotometrically. Gallic acid was first dissolved in distilled water to create a standard curve with a concentration range of 0 to 200 µg/mL. One milliliter aliquots of each standard solution were put into different test tubes. One milliliter of the Folin-Ciocalteau reagent was added to each tube, and everything was well mixed. The reaction mixtures were incubated at room temperature for two hours in the dark after three minutes, during which time three milliliters of a 20% sodium carbonate solution were added to each tube. A test tube containing one milliliter of the sample extract was used for the sample preparation, and the same protocol as for the standards was adhered to. Using a spectrophotometer, the absorbance of each reaction mixture was measured at 765 nm. Distilled water was used to prepare a blank sample in place of the sample extract. Using the gallic acid standard curve, the total phenolic content was determined and reported as milligrams of gallic acid equivalents (GAE) per gramme of the sample. This technique made sure that the total phenolic components in different plant extracts were quantified accurately and consistently, which helped to understand their possible antioxidant qualities.:

$y = 0.0029x + 0.0845$

$R^2 = 0.9545$

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

Antioxidant activity:

Determination of DPPH (1, 1–diphenyl–2–picryl hydrazyl) radical scavenging activity:

Using the DPPH radical and the previously mentioned techniques, the extract's capacity to scavenge free radicals was assessed. [19]. Determining the DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity of compounds, including plant extracts, is a commonly employed technique for evaluating their antioxidant capacity. This test is based on the decrease of the stable free radical known as DPPH, which has a deep purple colour. Spectrophotometry can measure the reduction in colour intensity caused by antioxidants' ability to squelch this radical. A DPPH solution is first made in methanol or ethanol to provide an absorbance of roughly 0.8 to 1.0 at 517 nm, which is needed to perform the experiment. To stop deterioration, this solution must be made freshly and kept out of the light. To create a dose-response curve, the investigational samples are likewise produced in the same solvent as DPPH, but at different concentrations. In test tubes or microplate wells, equal amounts of the DPPH solution and the sample solutions are mixed. The solvent is used in the control setup rather than the sample. After mixing, the assay mixture is left to sit for 30 to 1 hour at room temperature in the dark, depending on the procedure or unique properties of the sample. At 517 nm, the absorbance is measured following incubation. The sample's capacity to scavenge radicals is indicated by the decrease in absorbance when compared to the control. A formula that determines the proportion of DPPH radical scavenging is used to quantify this activity and gives a clear indication of the sample's antioxidant capacity. It is normal procedure to include a recognised antioxidant as a standard, such as Trolox or ascorbic acid, for comparison and accuracy. The following formula was utilised to determine the DPPH• radical's % scavenging.:

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

where Ac is the absorbance of the control reaction system and At is the absorbance when the extract or reference is present.

Reducing power:

The extract's reduction power was determined using the previously described methodology [20]. In order to perform the reducing power assay with different extract concentrations (50-250 µg/mL), a methodical and comprehensive technique is followed. To make a total volume of 5.2 mL for each sample, each extract concentration is first mixed with 1.1% potassium ferricyanide [K3Fe(CN)6] solution and 0.3 M phosphate

buffer at pH 6.7 in 1.5 mL of distilled water. After that, this combination is incubated for 25 minutes at 50°C to allow the reduction reaction to take place, in which the reducing agents in the sample decrease potassium ferricyanide to potassium ferrocyanide.2.6 mL of 12% trichloroacetic acid is added to stop the reaction after it has been incubated; this precipitates proteins and puts an end to the reaction. The clear supernatant is then collected after the mixture is centrifuged for 12 minutes at 4000 rpm to separate the precipitated proteins. 2.6 mL of distilled water and 0.6 mL of a 0.2% ferric chloride solution are added to this supernatant. Ferric chloride is an essential ingredient because it combines with the ferrous ions created during reduction to create a coloured complex. A spectrophotometer (Shimadzu, Japan model 1601) is then used to measure the absorbance of this final mixture at 700 nm. The idea behind this measurement is that an extract sample's ability to give electrons is shown by its higher absorbance, which also signals a stronger reducing power. With the help of electron donation, this assay efficiently measures the samples' antioxidant capacity, indicating their potential to function as reducing agents by neutralising reactive species.

Evaluating superoxide radical ($O_2^{\bullet -}$) scavenging activity:

The assay for scavenging superoxide radical (O2•−) is an essential method [21] to evaluate the antioxidant potential of different substances, which is important in the fight against diseases associated with oxidative stress. This assay depends on antioxidants' capacity to counteract superoxide radicals produced in a test system, which usually produces radicals via the xanthine/xanthine oxidase system. Using this process, xanthine oxidase breaks down xanthine and produces superoxide and uric acid radicals. The indicator used in this reaction is nitroblue tetrazolium (NBT), which turns blue when reduced by superoxide radicals. First, a reaction mixture including xanthine at a predetermined concentration, xanthine oxidase to produce radicals, NBT to identify the radicals, and phosphate buffer to keep the pH at 7.4 is prepared. To measure the antioxidant's scavenging activity, the test sample containing the research antioxidant is introduced at different quantities. To enable the effective processing of several samples, this mixture is normally incubated for 20 to 30 minutes at room temperature in a 96-well plate. After the incubation period is up, the reaction is halted by adding a stop solution, which either chelate necessary divalent cations or buffer the pH to prevent xanthine oxidase from activating further. Next, spectrophotometric measurement of the formazan product's absorbance is performed at 560 nm. The scavenging activity is measured by the decrease in absorbance relative to a control sample that does not include the test antioxidant. A formula that compares the absorbance of the test sample and the control is used to compute this; a larger reduction indicates more effective scavenging action. Known antioxidants such as superoxide dismutase or ascorbic acid are employed as positive controls for comparison and validation, while xanthine oxidase-free blank, which contains all reagents but xanthine oxidase, helps account for non-enzymatic reductions of NBT. This assay is very useful for identifying compounds that may be able to neutralise superoxide radicals and lessen the consequences of oxidative stress, potentially leading to therapeutic advantages. The % inhibition of superoxide anion was calculated using the following formula.:

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

where Ac is the absorbance of the control (absent extract), and At is the absorbance in the presence of the standard or extract.

Metal Chelating activity:

The previously published method was used to test the chelating of ferrous ions by the methanolic extract, BHT, and EDTA [22, 23]. In summary, a solution of 2 mm FeCl2 (0.05 mL) was mixed with several quantities of extract (20, 40, and 60 µg/mL). Five millimetres (0.2 millilitres) of ferrocyanide were added to start the reaction, which was then violently agitated and allowed to stand at room temperature for ten minutes. The solution's absorbance was then determined using spectrophotometry in a spectrophotometer (8500 II; Bio-Crom Gmb, Zurich, Switzerland) at 562 nm. Every test and analysis were performed three times, then averaged. Using the following formula, the % chelating effect of standard chemicals and melatonin was determined:

Percentage metal chelating activity = $(A_c - A_t / A_c) \times 100$

where A1 is the absorbance when the extract sample or standard compounds are present, and Ao is the absorbance of the control reaction. There were no complex formation molecules or FeCl2 and ferrozine in the control.

Anti-inflammatory activity:

Cyclooxygenase–1 (COX–1) and cyclooxygenase–2 (COX–2) assays:

In order to assess a plant extract's inhibitory effect on the enzymes cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), particular assays are designed to account for the unique properties of plant proteins [24, 25]. First, the plant extract is prepared using suitable solvents such as methanol or ethanol; it is then dried and reconstituted at a predetermined concentration in an acceptable buffer. The purified COX-1 enzyme, which is typically obtained from cows, is prepared for the COX-1 assay by mixing it with arachidonic acid at a fixed concentration in an assay buffer. To allow for possible inhibition, the COX-1 enzyme is pre-incubated with the plant extract at 37 degrees Celsius. Arachidonic acid is added to initiate the reaction after this pre-incubation. After the reaction has been going on for ten to twenty minutes, a stopping solution, like hydrochloric acid, is added to stop it. Using a colorimetric technique, which gauges the change in absorbance brought on by PGH2's interaction with a chromogenic substrate, the resultant prostaglandin H2 (PGH2) is quantified. The COX-2 assay, which uses an inducible enzyme, frequently makes use of a cell-based methodology. In order to induce enzyme expression, cells that can express COX-2 when stimulated, such as macrophages, are grown and exposed to an inflammatory substance like lipopolysaccharide. The plant extract is then added to these cells, and its capacity to inhibit COX-2 is evaluated. Arachidonic acid is added to the culture to start the reaction after a pre-incubation time with the extract. A colorimetric method is used to assess PGH2 production, same like in the COX-1 assay. These techniques are essential for evaluating natural products for possible anti-inflammatory qualities and for supplying vital information about how well plant extracts inhibit important inflammatory enzymes [24, 25].

Effect on 5–lipoxygenase (LOX) enzyme:

The 5-LOX test was carried out utilising the previously documented protocol. [26]. Prior to performing the test, all required materials and reagents for 5-lipoxygenase (5-LOX) enzyme activity measurement were ready. The enzyme was obtained from human leukocytes or from recombinant versions that were sold commercially. Arachidonic acid, the substrate, was produced in a suitable buffer such as Tris-HCl at concentrations typically ranging from 10 to 100 µM. Antioxidants were added to the reaction mixture together with necessary cofactors like ATP and Ca2+ to guarantee maximum enzyme activity and avoid non-enzymatic oxidation of the substrate. The enzyme and the arachidonic acid substrate were combined in a reaction buffer for the assay setup, with the goal of achieving a total volume of 100–200 µL in a test tube or a microplate well. To stabilise the enzyme, this combination was pre-incubated for approximately ten minutes at 37°C. The substrate was added to the enzyme mixture that had been pre-incubated, and the enzymatic reaction was then allowed to run for 10 to 30 minutes at the same temperature. An ice-cold solution, like methanol, was added to stop the reaction and help precipitate proteins and remove reaction products. After the process, the leukotrienes were extracted from the aqueous phase using an organic solvent such as hexane or ethyl acetate. Using standards for calibration, the leukotriene concentrations were precisely measured using either Gas Chromatography-Mass Spectrometry (GC-MS) or High-Performance Liquid Chromatography (HPLC). The amount of product generated in a unit of time was used to compute the enzymatic activity, which indicated the catalytic efficiency of the enzyme under test conditions. To account for non-enzymatic reactions, negative controls were included, and the assay was run in duplicates to ensure that the results were reliable. Understanding the function of the enzyme and identifying possible inhibitors for use in therapeutic research against inflammatory illnesses required the application of this analytical methodology.

Nephroprotective activity:

Effect of TP-ME in cisplatin-induced toxicity in HEK-293 cells:

The study made use of the human kidney embryonic cell line (ATCC; HEK-293). The cells were cultivated in DMEM supplemented with 12% foetal bovine serum that had been heat-inactivated in an incubation chamber with 6% carbon dioxide at 37°C. Cells that were 80–90% confluent were subjected to trypsinization, and adequate media was provided to halt the trypsin's activity. After centrifuging the cells for five minutes at 1400 rpm, the pellet was reconstituted in medium and the cells were counted on a hemacytometer using the Trypan blue exclusion method. The supernatant was discarded. The cells were diluted with medium to yield the necessary number of cells. For cell growth tests, the final seeding density was kept at 9000 cells per well in a 104-well microtiter plate with a flat bottom. Following a 26-hour seeding period, the cells were either left untreated, treated with an additional treatment, or cotreated with 20µM cisplatin (CP) and TP-ME (10, 20, 50, 90, 140, and 200 µg/mL) for another 26 hours. The analysis for cell viability was conducted 26 hours following the initiation of treatment [27, 28].

Cell viability test:

The MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] experiment was used to test the vitality of the cells. The MTT assay provides a sensitive assessment of the usual metabolic state of cells. After treatment, MTT solution (6 mg/ml) was added to the matching treated cell wells, and the cells were incubated for three hours. After dissolving the formazan, which was ultimately generated in a dark blue colour, the absorbance at 572 nm was measured using a microtiter plate reader.

Statistical analysis:

Each set of data was displayed as mean ± SD (n = 3). Software named GraphPad Prism version 8 was used to do the statistical analyses, which included one-way analysis of variance (ANOVA) and *post hoc* "Dunnett's Multiple Comparison Test". A statistical significance level of 0.05 or less ($p<0.05$) was set up.

RESULTS AND DISCUSSIONS:

Total phenolic compounds determination:

An estimate of the total phenolic contents per gram of the extract was 0.177 mg/mL of gallic acid equivalents (GAE).

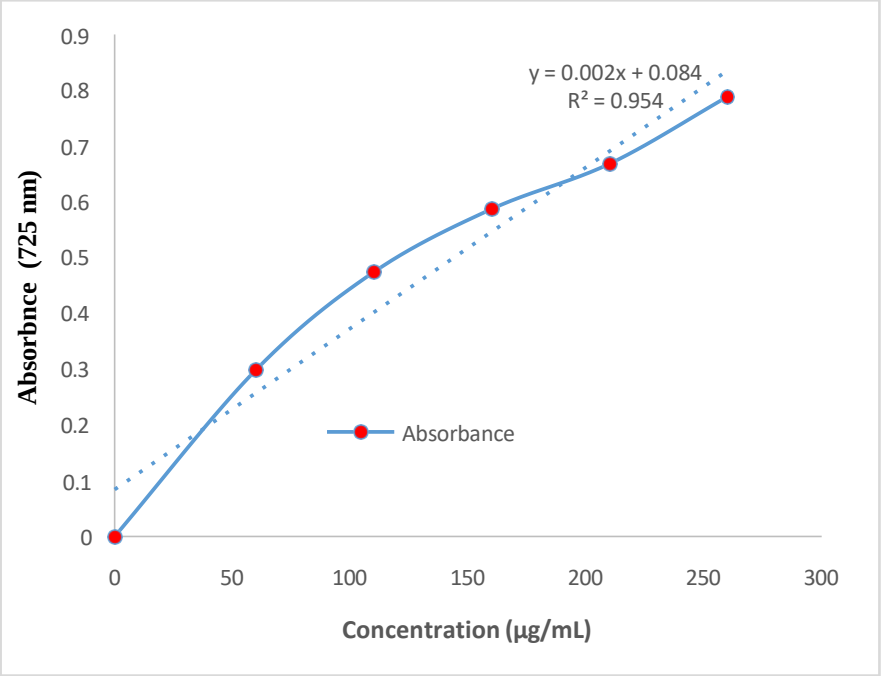

Figure 1. Estimation of total phenolic contents in GPME-R

Estimation of Antioxidant activity:

Evaluating the scavenging of DPPH radical:

The GPME-R and BHT (Butylated Hydroxytoluene) were tested at different concentrations for their ability to scavenge DPPH radicals, which shed light on their antioxidant properties. Antioxidants' capacity to scavenge free radicals is commonly assessed using the ability to scavenge DPPH radicals; larger percentages of scavenging ability indicate stronger scavenging ability. The extract exhibits a scavenging activity of 15.136% ± 1.31% at the lowest concentration tested (10 µg/mL), which is much higher than BHT's 10.621% ± 0.903%. This implies that the extract, as opposed to BHT, is more efficient at scavenging DPPH radicals at lower concentrations. BHT has greater scavenging action (43.956% ± 0.637%) at 50 µg/mL compared to the extract (37.437% ± 0.865%). This pattern is maintained at 100 µg/mL, where BHT shows 68.956% ± 1.010% scavenging activity, somewhat higher than 69.873% ± 1.408% for the extract. These findings suggest that at modest concentrations, BHT outperforms the extract in terms of effectiveness. The extract's and BHT's scavenging activities are extremely similar at greater doses of 200 µg/mL, with the extract at 91.741% ± 1.376% and BHT at 89.783% ± 0.752%. This resemblance implies that at this concentration, the extract and BHT are equally efficacious. BHT exhibits a marginally stronger scavenging activity (94.894% ± 1.149% and 95.690% ± 0.842%, respectively) at higher concentrations of 300 µg/mL and 500 µg/mL than the extract (93.966% ± 1.361% and 94.995% ± 1.763%). This suggests that BHT is just slightly more effective than the extract at scavenging DPPH radicals at very high doses. Compared to BHT, the extract exhibits greater scavenging activity at the lowest concentration (10 µg/mL). But at moderate to high concentrations (50 µg/mL and above), BHT shows more scavenging activity than the extract as the concentration rises. At 200 µg/mL, the extract and BHT start to function similarly, but at larger concentrations (300 µg/mL and 500 µg/mL), BHT starts to outperform the extract. At concentrations more than 50 µg/mL, BHT is generally a more effective DPPH radical scavenger than the extract (Figure 2).

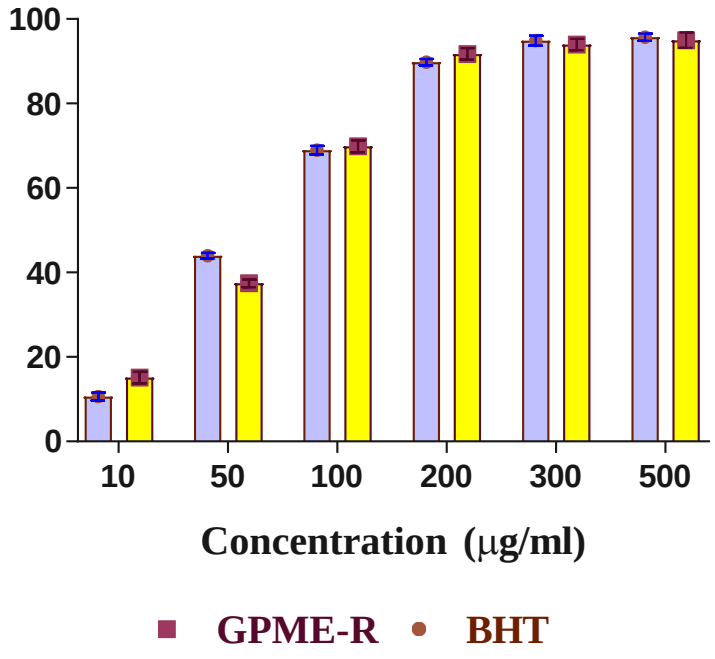

Figure 2. DPPH radical Scavenging of the GPME-R compared to BHT

Results from Reducing power measurement:

The data from the reducing power test sheds light on the GPME-R's antioxidant potential in relation to quercetin and vitamin C at various doses (Figure 3). The assay quantifies the compounds' capacity to transfer electrons and convert Fe3+ to Fe2+; higher absorbance values correspond to increased reducing power. The extract has a reducing power of 0.1734 ± 0.0041 at 50 µg/mL, which is less than that of vitamin C (0.2032 ± 0.00441) and

quercetin (0.2631 ± 0.00111). At 100 µg/mL, the extract exhibits an absorbance of 0.2555 ± 0.0031, which is lower than that of vitamin C at 0.3028 ± 0.00621 and quercetin at 0.3166 ± 0.00211. This tendency is sustained. The extract reaches 0.3566 ± 0.0021, nevertheless lagging behind vitamin C (0.4056 ± 0.0057) and quercetin (0.4622 ± 0.0021) at 150 µg/mL. At greater doses, the pattern of the extract having a reduced reducing power in comparison to quercetin and vitamin C continues. The extract has an absorbance of 0.4772 ± 0.0071 at 200 µg/mL, whereas the absorbances of quercetin and vitamin C are 0.5801 ± 0.00048 and 0.49660 ± 0.00141, respectively. Ultimately, the extract reaches a reduction power of 0.5232 ± 0.0041 at the highest concentration evaluated (250 µg/mL). Quercetin and vitamin C, on the other hand, have greater absorbances of 0.6822 ± 0.00201 and 0.6160 ± 0.00216, respectively. In conclusion, quercetin constantly shows the greatest reducing power at all concentrations, with vitamin C coming in second. When compared to the other two common antioxidants, the extract consistently exhibits the lowest but most notable reducing power, even though it does so at larger doses. This shows that although the extract has antioxidant activity, it is not as good as quercetin and vitamin C at donating electrons and converting Fe3+ to Fe2+ [20].

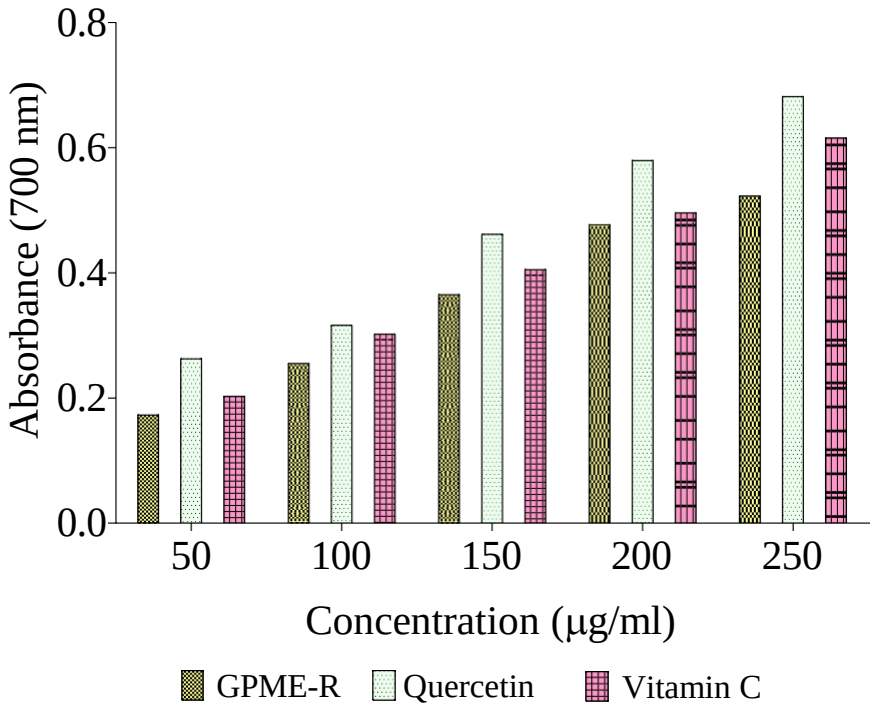

Figure 3. Reducing power assay compared to Quercetin and Vitamin C of GPME-R

Evaluating the scavenging of superoxide radical ($O_2^{\bullet -}$):

The data presented depicts the assay's capacity to neutralise superoxide radicals, a common reactive oxygen species, and shows that the GPME-R is more effective at scavenging superoxide radicals (O2•–) than Vitamin C (ascorbic acid) at different concentrations; higher percentages indicate greater efficacy. At the lowest concentration (50 µg/mL), the extract demonstrates a scavenging activity of 11.82% ± 0.9580%, while Vitamin C demonstrates a significantly higher activity of 55.89% ± 1.511%. This suggests that at this concentration, vitamin C is far more efficient than the extract at scavenging superoxide radicals. The extract's scavenging activity improves to 29.54% ± 0.9821% at a concentration of 100 µg/mL, but it still falls short of vitamin C's activity of 64.74% ± 1.155%. At 150 µg/mL, the extract reaches 45.80% ± 1.29%, continuing this trend, while vitamin C exhibits a substantially greater scavenging activity of 78.69% ± 1.0889%. The scavenging activity of the extract increases to 57.19% ± 1.225% at 200 µg/mL, although it is still less than that of vitamin C, which has an activity of 80.88% ± 1.897%. The extract reaches its maximal scavenging activity of 70.87% ± 1.416% at the highest concentration tested (250 µg/mL), while vitamin C reaches 85.91% ± 1.899%. At all investigated doses,

vitamin C exhibits a considerably greater superoxide radical scavenging activity than the extract. Vitamin C is more than four times more efficient than the extract at 50 µg/mL. The extract's scavenging activity continuously rises with concentration, although it always falls short of vitamin C. The extract's scavenging activity (70.87% ± 1.416%) is not as effective as vitamin C's (85.91% ± 1.899%) even at the greatest concentration (250 µg/mL). These findings indicate that the extract has a considerable superoxide radical scavenging activity; however, vitamin C is a more effective antioxidant in this situation than the extract (Figure 4) [29, 30].

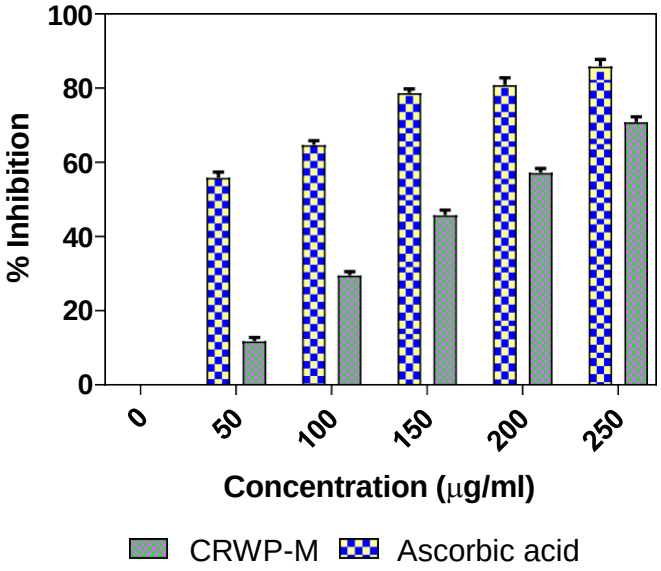

Figure 4. The GPME-R's ability to scavenge superoxide radicals (O2• –) in comparison to vitamin C (ascorbic acid)

Evaluating the metal chelating activity:

The proportion of metal chelating activity with increasing concentration (50-250 µg/ml) was demonstrated by the results of measuring the chelating activity of ferrous ions by GPME-R and standard compounds. It was clear from the data that GPME-R's metal-chelating activity depended on concentration. As GPME-R concentration rose, so did the percentage metal chelating activity (Figure 5). The reference chemical, EDTA, and GPME-R were found to have IC50 values of 125.27 µg/mL and 148.77 µg/mL, respectively.

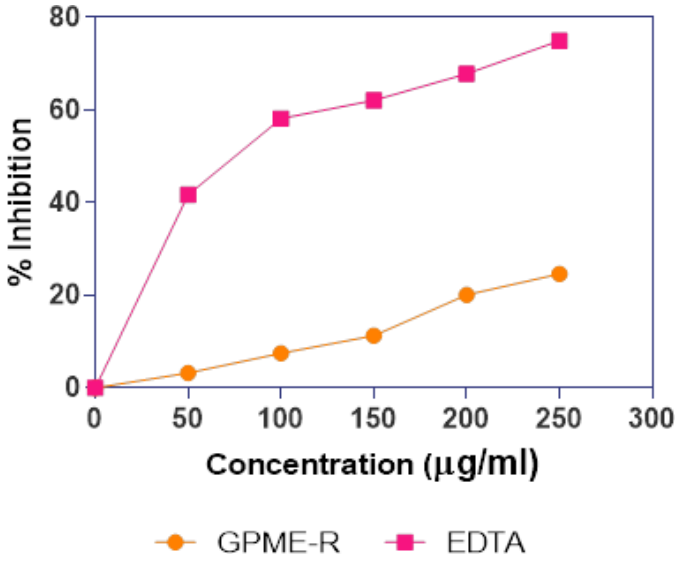

Figure 5. Metal chelating activityof GPME-R

Table 1. IC50 values for the scavenging activity of DPPH, superoxide radicals and metal chelating activity of GPME-R.

Drugs	**IC_{50} (µg·mL^{-1})**		
	DPPH radical	**Superoxide radical**	**Metal chelating**
GPME-R	97.88 µg/mL	117.91 µg/mL	148.77 µg/mL
BHT	68.56 µg/mL	-	-
Vitamin C (Ascorbic acid)	-	97.87 µg/mL	-
Ethylenediaminetetraacetic acid (EDTA)	-	-	125.27 µg/mL

Evaluation of Anti-inflammatory activity:

Assessing the COX-1 (Cyclooxygenase-1) and COX-2 (cyclooxygenase-2) enzymes inhibition:

With increasing concentration, the herbal extract (GPME-R) showed a progressive rise in COX-1 inhibition. The inhibition was rather low at 50 µg/mL (5.145 ± 0.022%), but at 250 µg/mL it increased dramatically to 96.669 ± 0.991%. The COX-1 IC50 value was 298.66 µg/mL, meaning that greater extract concentrations are needed to produce a 50% reduction in COX-1 activity. As with COX-1, the herbal extract's inhibition of COX-2 increased with concentration. At 50 µg/mL, the inhibition was 6.876 ± 0.432%, while at 250 µg/mL, it was 89.910 ± 1.033%. Compared to COX-1, COX-2's IC50 value was marginally higher at 369.87 µg/mL, indicating that COX-2 is less susceptible to GPME-R.

5-lipoxygenase (LOX) enzyme assay:

Even at lower concentrations, the herbal extract (GPME-R) demonstrated a robust reaction in its inhibition of LOX. At 50 µg/mL, the inhibition was 4.934 ± 0.055%; at 250 µg/mL, it was 69.878 ± 1.113%. Compared to COX-1 and COX-2, the extract is more effective at inhibiting LOX activity at lower doses, as evidenced by the much lower IC50 value for LOX (101.88 µg/mL). The COX-1, COX-2, and LOX enzymes are all inhibited by

the herbal extract in a dose-dependent manner. The extract has the lowest IC50 value (101.88 µg/mL), indicating increased sensitivity, and is most effective at suppressing LOX activity. Conversely, COX-2 inhibition is the least sensitive to the extract; it takes the highest dose for 50% inhibition (IC50 of 369.87 µg/mL). With an IC50 value of 298.66 µg/mL, COX-1 exhibits moderate sensitivity. These findings imply that, in comparison to COX-1 and COX-2, the herbal extract may be a stronger LOX inhibitor (Table 1).

Table 2. Percentage enzyme inhibition of the COX and LOX system by the herbal extract (GPME-R).

Concentration (µg/mL)	**% Enzyme Inhibition**		
	COX-1	**COX-2**	**LOX**
50	5.145 ± 0.022	6.876 ± 0.432	4.934 ± 0.055
100	9.288 ± 0.118	13.884 ± 0.677	9.789 ± 0.099
150	25.775 ± 0.887	30.911 ± 0.977	19.887 ± 1101
200	48.432 ± 0.977	48.673 ± 0.998	43.993 ± 1.078
250	96.669 ± 0.991	89.910 ± 1.033	69.878 ± 1.113
IC_{50}	298.66 µg/mL	369.87 µg/mL	101.88 µg/mL

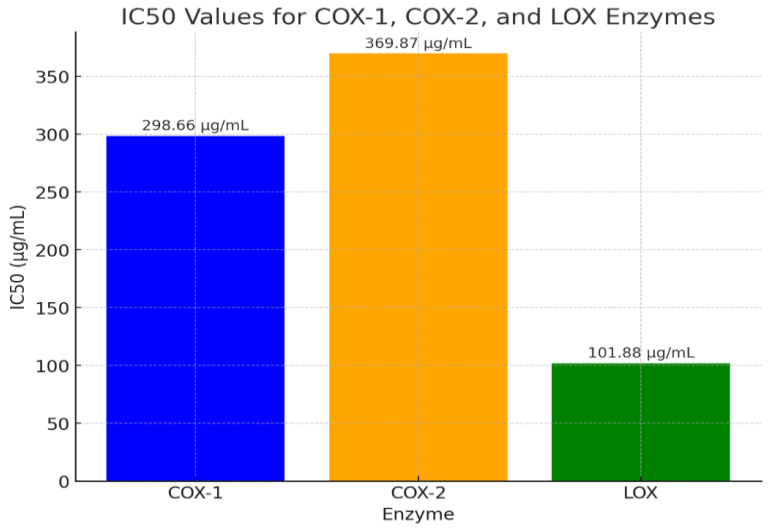

Figure 6. Percentage enzyme inhibition of the COX and LOX system by GPME-R.

Nephroprotective activity:

Result of impact of GPME-R in nephrotoxicity in HEK-293 cell Line induced by cisplatin:

The nephrotoxicity model in human embryonic kidney (HEK-293) cells induced by cisplatin was used to evaluate the efficacy of GPME-R in terms of nephroprotection. The assay for cell viability was used to ascertain the nephron-cytoprotective effect of GPME-R in human embryonic kidney cells treated with cisplatin. The cells were exposed to different concentrations of GPME-R (10, 20, 50, 90, 140, and 200 µg/mL) for a full day, either with or without 20 µM of Cisplatin. There are no discernible negative effects of the GPME-R treatment on cell viability on its own. When compared to a normal control, the cells' vitality was significantly decreased by cisplatin treatment ($P<0.001$). The vitality of cells treated with both GPME-R and CP rose significantly. Applying GPME-R therapy in place of cisplatin control resulted in a significant increase in cell viability. The EC50 was determined to be 11.231 µg/mL (Figure 7).

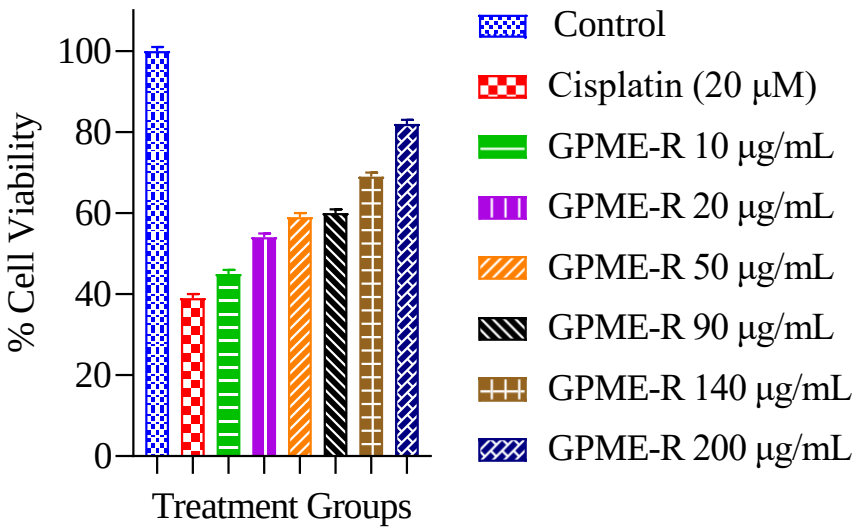

Figure 7. Effect of GPME-R in nephrotoxicity induced by cisplatin in HEK-293 cells.

CONCLUSIONS:

Gynura procumbens roots methanol extract (GPME-R) demonstrated significant antioxidant, anti-inflammatory, and nephroprotective activities. At higher concentrations, the extract showed a progressive increase in enzyme inhibition, radical scavenging, and nephroprotective activity. In the reducing power assay, GPME-R showed measurable activity, but it was consistently outperformed by Quercetin and Vitamin C; at lower concentrations, the extract's DPPH radical scavenging activity was higher than BHT, but at higher concentrations, it became less effective as a free radical scavenger. In the LOX enzyme inhibition assay, where GPME-R showed the lowest IC50 value, indicating a strong inhibition at lower concentrations, the anti-inflammatory activity of the compound was most prominent. Additionally, the extract was found to be a considerable nephroprotective in the model of nephrotoxicity induced by cisplatin. These findings demonstrated the potential of root extract from *Gynura procumbens* as a natural source of nephroprotective, anti-inflammatory, and antioxidant compounds.

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