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ANTIOXIDANT, HEPATOPROTECTIVE AND ANTICANCER ACTIVITY OF *GYNURA PROCUMBENS* ROOT EXTRACT: AN *IN VITRO* MECHANISTIC PHARMACOLOGICAL APPRAISAL

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ABSTRACT: This study looked into the potential medical benefits of GPME-R, a herbal methanol extract made from the roots of *Gynura procumbens*. To ascertain GPME-R's therapeutic potential, we carried out a number of in vitro tests, paying special attention to its capacity to combat oxidative stress and maintain & protect liver function. The cytotoxicity assessment of the extract was also used to assess its anticancer potential. The ABTS radical scavenging assay and the hydroxyl radical scavenging activity were evaluated. The outcomes were extremely encouraging, demonstrating GPME-R's potent capacity to combat dangerous free radicals, which are recognized to be hallmark factor in oxidative stress. Additionally, we employed a model involving HepG2 cells that were exposed to carbon tetrachloride, a typical chemical hepatotoxin used to research liver injury in the lab, to investigate how GPME-R could protect liver cells. According to the findings, GPME-R significantly shielded these liver cells from the damaging effects of the chemical toxin CCl4. Additionally, the extract GPME-R showed a negligible cytotoxic impact. Overall, the results of our investigation demonstrated the effectiveness of GPME-R as a strong hepatoprotective and antioxidant. More research is needed, though, as the anticancer potential was determined to be limited and unclear. This implied that GPME-R might be a useful natural remedy for controlling oxidative stress and enhancing & protecting liver function.

Keywords: Antiradical, Antioxidant, Hepatoprotective, *Gynura procumbens*, ABTS radical, Anticancer

INTRODUCTION:

The vital function of the liver in controlling the body's metabolism, secretion, storage, and detoxification activities is commonly correlated with hepatic damage. The production of antioxidant proteins including superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase is one of the compensatory methods that liver cells use to deal with reactive oxygen species (ROS). Cu–Zn, Mn–SOD, catalase, GSHPx, and GSH reductase (GR) are examples of enzymatic antioxidant systems that work by either directly or progressively removing ROS to stop their activity. Oxidative damage results from an imbalance between the oxidative forces and antioxidant defense mechanisms. Numerous illnesses, such as liver cirrhosis, diabetes, cancer, and atherosclerosis, have been connected to this imbalance [1]. One of the biggest and most important organs in the human body, the liver plays a key role in controlling several critical physiological functions. This includes the synthesis of the proteins needed for immune system function, blood coagulation, metabolism, nutrition storage, and detoxification. To maintain overall health and wellbeing, it must function at its best. Liver impairment, which can be caused by a number of conditions such as chronic viral hepatitis, excessive alcohol consumption, certain medications, and exposure to toxins, greatly impairs these processes. It becomes more difficult for the liver to effectively digest food, filter blood, and remove toxins

from the body when it is damaged. Hepatic encephalopathy is the term for an accumulation of waste products and chemicals that can harm brain function [2, 3].
Additionally, cirrhosis, or scarring, caused by liver damage increases the likelihood of major side effects such liver failure and portal hypertension and decreases liver function. The importance of liver health cannot be overstated. It is important for the metabolism of lipids and carbohydrates, which regulates energy levels. The liver also metabolizes drugs and other substances, which is necessary for detoxification and the efficacy of prescription drugs. Furthermore, the liver synthesizes albumin, the most common protein in plasma, which is essential for preserving osmotic equilibrium, which regulates blood pressure and volume. A healthy weight, safe sexual behaviour, moderation in alcohol consumption, and proper medication use are all lifestyle choices that help avoid liver disease. Frequent exercise and a diet rich in fruits, vegetables, proteins, and healthy fats can also improve liver health. Vaccinations against hepatitis A and B are further preventative measures. The liver has a significant impact on health, so it makes sense that it needs to work at its best. Protecting the liver from damage is essential to avoiding serious health issues, emphasizing the significance of education and preventative measures to maintain liver health [1, 4].
The development of cancer registries worldwide has driven the search for novel anticancer drugs that target cancer cells without harming normal cells. Traditional anticancer drugs were highly toxic to both tumor and normal cells. Current research focuses on terrestrial and marine environments for new agents. Many plants are essential to traditional medicine, and historically, they have been used to heal illnesses [5, 6]. Rising cancer incidence necessitates new drugs. Many plant-derived anticancer drugs are tested on cancer cell lines and animals before clinical trials. The number of natural compounds has surged, with around 326,000 known by 2014 [6]. Natural antitumor compounds include alkaloids, diterpenes, purine-based compounds, and more. The cost of extracting these substances can be lower than chemical synthesis. For example, extracting artemisinin is inexpensive compared to vincristine. Paclitaxel, from the yew tree, faces challenges due to low yields and resource depletion, but alternative sources and synthetic methods provide solutions. Paclitaxel and docetaxel are used in breast cancer monotherapy and combined with other drugs. Many natural products are modified to enhance their activity. The mechanisms of the substances include apoptosis, cell-cycle arrest, DNA binding, topoisomerase inhibition, and binding to microtubules [5, 7].

In a healthy state, several intracellular and extracellular antioxidant systems effectively eliminate ROS, which are continuously created. Unchecked formation of ROS frequently results in damage to other small antioxidant molecules as well as biological macromolecules (DNA, lipids, and proteins). The most significant ROS include hydrogen peroxide (H2O2), superoxide anion radical O2–, hydroxyl radical (OH), peroxyl (ROO), alkoxyl (RO), and hypochlorous acid (HOCl). Highly bioactive reactive nitrogen species (RNS) that are not oxygen species include nitric oxide (NO) and peroxynitrite. In many different biological systems that are unrelated to one another, the free radical reaction is a crucial mechanism. The creation of free radical intermediates, which set off a web of diverse disruptions, is one of the essential classes of reactions among the several ways that chemicals can cause harm [8-10]. Most hepatotoxic chemicals damage liver cells primarily through oxidative insults such as lipid peroxidation. The liver is vulnerable to oxidative stress, xenobiotic toxicity, and drug toxicity because of its distinct metabolic functions and crucial function in removing chemicals from the portal circulation. GSH-peroxidase and cytochrome p-450 in the liver are responsible for the two distinct metabolic pathways. Currently, hepatotoxicity is treated with drugs that interfere with the p-450 enzyme pathway. Certain drugs, such ciprofloxacin, amiodarone,

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carbamazepine, phenobarbital, or phenytoin, can either stimulate or inhibit the metabolic activity of certain enzymes [4, 9, 10].

Recently, there has been a lot of interest in the hepatoprotective qualities of naturally occurring extracts or compounds, as well as their mechanisms of action. Here, we used carbon tetrachloride cytotoxicity as a test against HepG2 cells to examine the hepatoprotective potential of a herbal extract. A helpful in vitro model for evaluating the hepatotoxicological effects of drugs by examining a variety of cytotoxic endpoints is chemically induced toxicity in HepG2 cells. Because HepG2 cells can still carry out a variety of particular tasks that are typical of normal human hepatocytes, like the synthesis and secretion of plasma proteins, they have been used to study the metabolism and toxicity of medications [11-14].

Many conventional remedies are advised for the treatment of liver problems in the absence of effective current hepatoprotective medications. Hepatoprotective qualities have been demonstrated by a variety of plants, including *Silybum marianum* and *Andrographis paniculata*. Numerous bioactive substances, including phenols, flavonoids, steroids, and terpenoids, are found in plants. These phytoconstituents have a variety of pharmacological qualities, including anti-inflammatory, antiviral, anti-proliferative, and anti-carcinogenic qualities, in addition to their nutritional worth. Phenolic, flavonoid, and polyphenolic substances obtained from plants may help reduce oxidative stress-related disorders [15, 16].

Thailand, China, Malaysia, Indonesia, Vietnam, and India are home to the tropical Asian medicinal plant *Gynura procumbens* (Lour.) Merr. (Family: Asteraceae). In many nations around the world, it has long been used to treat a wide range of conditions, such as rheumatism, diabetes mellitus, constipation, renal difficulties, kidney discomfort, and hypertension. [17, 18]. The methanol (MeOH) extract of Gynura procumbens leaves included a number of compounds, according to chemical analysis, including phytol, lupeol, stigmasterol, friedelanol acetate, β-amyrin, and a combination of stigmasterol and β-sitosterol [18-20]. Recent research has demonstrated the many biological qualities of *Gynura procumbens* [18-22]. *Gynura procumbens*' pharmacological and therapeutic roles in hepatoprotection, oxidative stress, inflammation, and cancer treatment were the focus of the current investigation. In order to do this, several mechanistic theories of antioxidant activity were investigated, including the hydroxyl and ABTS radical scavenging activities. The same was tested for its hepatoprotective activities and anticancer potential.

MATERIAL AND METHODS:

Gathering and verifying the plant material:

In November and December of 2022, *Gynura procumbens* roots were collected in the Indian state of Uttarakhand's Dehradun area. The plant material was recognized, identified, and verified by a botanist and the voucher specimens (GP/PC/2022/131) was then stored for further use as reference.

Preparation of the extract:

The plant's roots were chopped, mechanically processed, shade-dried, and ground into a powder. To extract all the possible phytoconstituent, methanol was employed as the solvent in the cold maceration procedure. After the methanol was completely extracted, it was collected and concentrated at 45–50 °C under reduced pressure. Of the dried starting material, 0.98 percent was generated by the final methanol extract. After that, the final product was stored at 4°C until it was used further. The herbal extract was codenamed as GPME-R.

Drugs and chemicals:

Alfa Remedies, situated in Paonta Sahib, Himachal Pradesh, India, kindly offered samples of Silymarin as well as all standard drugs, including quercetin, vitamin C, and BHT (Butylate Hydroxy Toluene). The source of the ABTS Radical was Sigma Aldrich in India. E. Merck

India and SRL Mumbai were the suppliers of the remaining unlabeled chemicals and reagents. We purchased additional chemicals, medications, and reagents from Sigma Chemicals Company and Loba Chemical Company in Delhi, India. All other chemicals and reagents utilized were analytical grade, procured from reliable vendors.

Preliminary phytochemical screening:

Following previously suggested approach, standard chemical techniques and assays were used to identify the phytochemical components of the methanolic extract and the powdered crude medication [23].

Total phenolic content determination:

The Folin-Ciocalteau reagent was utilised to measure the total soluble phenolics in the plant's root extract using gallic acid, a standard phenolic component [24]. A volumetric flask containing 10 mg of extract was mixed with 46 ml of distilled water to create 1.0 ml of the extract solution. After thoroughly mixing, 1.0 ml of the Folin-Ciocalteau reagent was added. After adding 3.0 ml of 2% sodium carbonate three minutes later, the liquid was stirred sporadically for three hours. The absorbance of the combination was measured using a spectrophotometer (UV -1601 Shimadzu, Japan) set at 760 nm. The mg/g of extract was used to represent the overall phenol concentration. Using an equation derived from the standard gallic acid graph, the extract's total phenolic content was expressed in grammes of gallic acid equivalent (GAE):

$Y = 0.0019x + 0.0299$, $R^2 = 0.9769$

where x was the concentration and Y was the absorbance.

In vitro assessment of antiradical activity:

ABTS Radical Cation Decolorization Assay

For the ABTS Radical Cation Decolorisation Assay, a solution of 7 mM ABTS and 2.45 mM potassium persulfate is made, and it is allowed to react for 12–16 hours at room temperature in the dark to produce ABTS●+. The ABTS●+ solution is then diluted with ethanol or phosphate-buffered saline (PBS) until the absorbance at 734 nm is 0.70 ± 0.02. For the test, 1 mL of the diluted ABTS+ solution is combined with 10–100 µL of the sample. After six minutes of reaction time, the absorbance drop at 734 nm is measured [25, 26]. Comparing it to a control sample that contains every reagent except the test drug allows one to determine the percentage inhibition.

HRSA, or Hydroxyl Radical Scavenging Activity

The capacity of plant extracts to scavenge hydroxyl radicals, a highly reactive species that contribute to oxidative stress and cellular damage, was evaluated using the hydroxyl radical scavenging activity (HRSA) test [27]. The Fenton reaction, which results from the reaction of hydrogen peroxide ($H2O_2$) with ferrous ions ($Fe2^+$), provides the basis for this test. The following components were typically present in the reaction mixture for the HRSA assay: varying amounts of plant extract, 1 mM EDTA (ethylenediaminetetraacetic acid), 1 mM FeSO4 (ferrous sulphate), 20 mM H2O2, and 30 mM deoxyribose. The mixture was incubated at 37°C for one hour. During this period of incubation, hydroxyl radicals convert deoxyribose to malondialdehyde (MDA). Spectrophotometric detection of the resultant MDA is possible at 532 nm. After that, in an acidic environment, it reacts with thiobarbituric acid (TBA) to produce a pink chromogen. The plant extracts' capacity to scavenge free radicals is inversely correlated with the degree of deoxyribose breakdown. The percentage inhibition of hydroxyl radical-induced deoxyribose breakdown was calculated using the following formula:

$$Percentage\ Inhibition\ (\%) = \frac{Abs_{Control} - Abs_{Sample}}{Abs_{control}} \times 100$$

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IC50 values, which indicate the extract concentration required to block 50% of hydroxyl radical activity, were used to illustrate the plant extract's antioxidant capacity. Lower IC50 values indicate more activity in scavenging hydroxyl radicals.

***In vitro* appraisal of hepatoprotective activity:**

The effectiveness of hepatoprotective drugs was evaluated in vitro using the hepatic (HepG2) cell line in the traditional model of CCl4-induced hepatotoxicity.

Cell line:

An immortalized cell line produced from human liver cancer is called $HepG_2$. These cells are frequently utilized in scientific research, specifically those that focus on liver metabolism, biology, and the impact of toxins and drugs on liver function. $HepG_2$ cells are a useful model for hepatocellular function and pathology because they were obtained from a liver tumour in a male Caucasian child who was 15 years old. They also have hepatocyte-like traits [28]. The ability of $HepG_2$ cells to generate albumin, a major plasma protein synthesised by the liver, is one of its essential characteristics. This trait is especially significant since it suggests that these cells, despite their malignant origin, nevertheless have some of the functional characteristics of healthy hepatocytes. Furthermore, a range of liver-specific enzymes and transporters are expressed by $HepG_2$ cells, which is important for researching liver metabolism, including the metabolism of medications and other xenobiotics.

$HepG_2$ cells are used in toxicology to investigate the hepatotoxicity of different drugs. These cells are used by researchers to evaluate the toxicity of drugs, chemicals, and herbal supplements by looking at how these compounds impact DNA, protein synthesis, and cellular survival. In order to gain insight into the course of liver illnesses such steatosis and hepatitis as well as possible treatment targets, $HepG_2$ cells are also employed in these investigations. Furthermore, because $HepG_2$ cells are susceptible to infection by these viruses, they are valuable tools in the research of viral hepatitis, especially hepatitis B and C. Because of this, they can be used to investigate viral life cycles, host-virus interactions, and the effectiveness of antiviral medications. $HepG_2$ cells are employed in cancer research to investigate the cellular and molecular mechanisms of hepatocarcinogenesis. These cells are used by researchers to study the functions of different genes and signaling pathways in the initiation and progression of liver cancer. They also provide a model for researching resistance mechanisms and evaluating anticancer medications. All things considered, $HepG_2$ cells are a flexible instrument in biomedical research, providing understanding of liver function, illness, and treatments. Their extensive use emphasizes how crucial they are to expanding our knowledge of liver-related health conditions [28, 29].

Hepatoprotective properties in HepG2 cells:

In human liver-derived HepG2 cells, the hepatoprotective efficacy was evaluated by assessing mitochondrial production using the tetrazolium test and screening for protection against CCl4-induced damage [30, 31]. Using the technique outlined in our earlier study, the extracts' hepatoprotective efficacy was assessed in HepG2 cells that were kept in good condition. Silymarin was utilised as a conventional hepatoprotective medication, whereas CCl4 was employed as a hepatotoxicant. On a 96-well tissue culture plate, confluent HepG2 cells were cultivated at a density of 5 × 104 cells/well using growth media (EMEM + 10% FBS), and they were cultured for the entire night. Following incubation, cells were subjected to varying extract concentrations for two hours. Following that, CCl4 (1 mM) was added and the cells were treated for two more hours. Following incubation, the treated cells were washed with DPBS and placed in growth media containing MTT. After the medium was removed, the formazan crystals were dissolved in DMSO. At 570 nm, the optical density was observed.

Assessment of anti-cancer activity:

Fe2+/ascorbate-induced lipid peroxidation measurement with liver homogenate:
Lipid peroxidation was carried out using the technique that Ohkawa et al., 1979 had previously published [32]. The reaction mixtures were incubated with different extract concentrations (10-100 µg/ml) for 65 minutes at 37 °C. Additionally, in 35 mmol/L tris buffer, they added 0.3 mL of liver homogenate, 0.08 mL of ascorbic acid, 0.39 mL of 0.17 mmol/L ferrous ammonium sulphate, and varying volumes of the extract. Following this process, the following substance's reactivity with thiobarbituric acid was evaluated: To stop the reaction, 1.7 mL of 0.16 mol/L Tris-KCl buffer and 0.6 mL of 35 percent TCA were combined with a 0.5 mL aliquot of the reaction mixture. After that, this mixture was heated to 75 °C for 47 minutes in a water bath. To remove the precipitates, it was then put in the refrigerator with ice and centrifuged for 11 minutes at room temperature at 4000 rpm. In comparison to a control sample, the absorbance of the pink, clear supernatant was measured at 532 nm. Three iterations of the experiment were conducted using catechin as the standard.

$$\%\ \textbf{inhibition of lipid peroxidation} = \left(\frac{100 - \mathrm{A_{SAMPLE}}}{\mathrm{A_{CONTROL}}}\right) \times 100$$

Cell viability:
The MTT assay was employed to investigate the impact of GPME-R on the growth and division of cancerous cells. This method involved growing 104 cells at a density of 100 µL per well on 104-well plates, followed by a full night of incubation. Subsequently, the cells were exposed to varying quantities of both GPME-R (100-1000 nM) and the reference medication (10-1000 nM). On the first day of incubation, twelve millilitres of MTT solution were applied to each well. The plate was then incubated for an additional five hours at 37 °C with 6% carbon dioxide. Following the required period of time, the MTT-containing solution was carefully removed, 110 mL of DMSO was added to each well, and an ELISA reader (Bio-Tek Instrument, USA) was used to detect each well's absorbance at 570 nm [33].

$$\%\ \textbf{Viability} = \left(\frac{100 - \mathrm{A_{SAMPLE}}}{\mathrm{A_{CONTROL}}}\right) \times 100$$

Statistical Data Analysis:
The mean plus standard deviation (SD) is used to display all data. For every experiment, three duplicates were conducted. Using one-way analysis of variance (ANOVA) and Dunnett's multiple comparison tests as post hoc, statistical differences between the treatments and the control were examined using GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, CA). Both *P < 0.05 and **P < 0.01 were considered statistically significant.

RESULTS:
Initial screening for phytochemicals:
Several chemical techniques were used to do an initial phytochemical screening on the ground roots. The findings showed the existence of hydrolysable tannins, proteins, amino acids, alkaloids, phenolic compounds, glycosides, flavanoids, and phytosterols. The test for triterpenoids and saponins came back negative.

Table 1. Results of preliminary phytochemical screening

Category tested	Findings
Alkaloids	+
Carbohydrates	+
Steroids and sterols	+
Flavanoids	+
Saponins	-
Glycosides	+
Triterpenoids	_

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Tannins and phenolic compounds	+
Proteins and amino acids	+

'+' = presence and '-' = absence

Determination of total phenolic content:

Phenolic class compounds account for most of the antioxidant capabilities found in plant sources. Because of their hydroxyl groups, phenols are essential plant components that can scavenge. However, higher phenolic contents might not always result in more beneficial antioxidant effects [24]. The extract's total phenolic content was ascertained. Figure 1 shows the overall phenolic content, which was 11.19 mg GAE/g of extract.

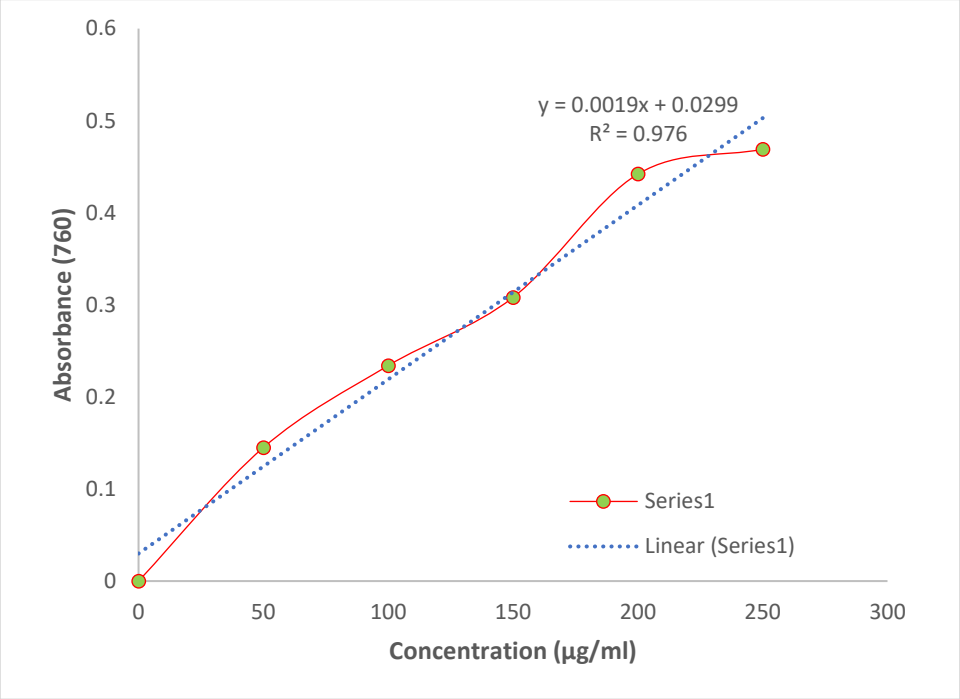

Figure 1. Total phenolic content in the extract

Evaluation of antioxidant and antiradical activities in vitro:

Evaluating the ABTS Radical Decolorization Assay:

Both quercetin and the extract show negligible scavenging activity at 0 µg/ml, as would be expected in the absence of active components. At 50 µg/ml, quercetin has a mean activity of 42.87±1.71%, indicating strong activity with little variability. In comparison to quercetin, the extract exhibits substantial antioxidant qualities and is effective, with a mean activity of 34.52±1.02%; the variability is slightly greater but still manageable. At 100 µg/ml, quercetin's mean activity increases significantly to 75.22±1.86%, demonstrating highly robust activity and good consistency. Despite having a mean activity that is smaller than quercetin's (65.57±2.77%), the extract nonetheless demonstrates strong antioxidant properties with consistent results. Quercetin and the extract both show gradually rising scavenging capabilities with increasing concentrations at 150 µg/ml and above, stabilizing around high values of 84.75±1.70%. Quercetin exhibits a very high activity and relatively low variability, peaking at 92.46±1.38% by 200 µg/ml. The extract's mean activity of 90.62±2.92% indicates a comparable strong antioxidant activity with somewhat higher variability, almost matching quercetin's efficacy by 200 µg/ml. Quercetin peaks at 250 µg/ml with a mean activity of 96.40±1.58%. The marginal rise from 200 µg/ml to 250 µg/ml indicates that activity has reached a plateau. With a mean activity of 92.86±1.89%, the extract is still quite efficient but

exhibits a little bit more variability than quercetin at this dose (Figure 2). The IC50 values were estimated and calculated to be 83.23 µg/mL and 97.84 µg/mL for Quercetin and GPME-R. respectively. Effective ABTS radical scavenging activity is exhibited by both quercetin and the plant extract, with quercetin often exhibiting a somewhat higher peak efficacy but both stabilizing at high activity levels at larger concentrations. However, the plant extract shows itself to be an impressive competitor to quercetin. This is particularly important as it comes from a natural source, which may be better for some applications.

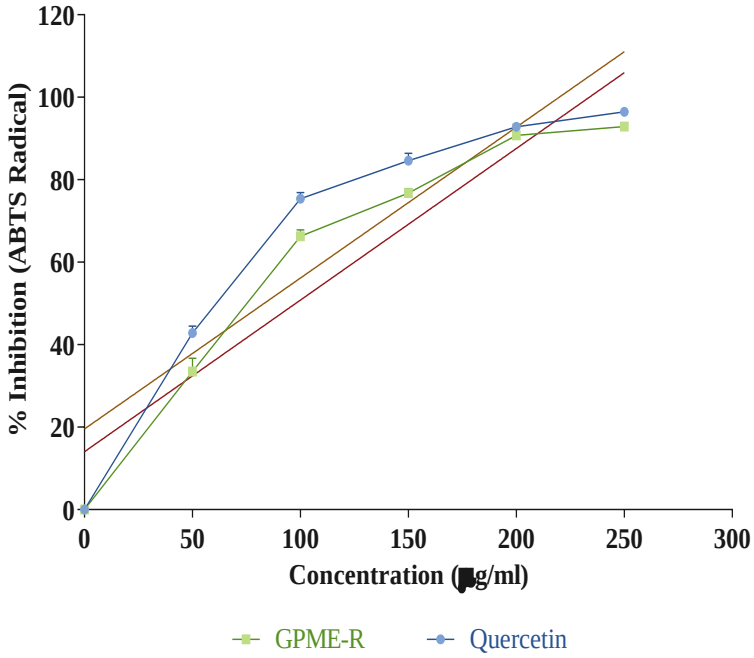

Figure 2. ABTS Radical Cation Decolorization Assay of GPME-R

Hydroxyl Radical Scavenging Activity (HRSA):

The extract GPME-R's hydroxyl radical scavenging activity (HRSA) was measured at different concentrations (0-250 µg/ml) and compared to the reference antioxidants ascorbic acid and quercetin. The findings show that GPME-R increases HRSA in a dose-dependent manner. GPME-R had moderate scavenging activity (15.63±0.498%) at the lowest tested concentration of 50 µg/ml, much lower than that of ascorbic acid (30.13±0.778%) and quercetin (40.46±0.488%). Notably, the HRSA of GPME-R improved as the concentration rose. GPME-R demonstrated 52.57±0.618% activity at 150 µg/ml, which is in close proximity to the values of ascorbic acid (62.26±0.798%) and quercetin (62.66±0.488%). GPME-R achieved 79.58±0.888% HRSA at the maximum concentration (250 µg/ml), almost matching the efficacy of ascorbic acid (94.67±0.898%) and quercetin (93.45±0.798%). At 50 µg/ml, quercetin showed the highest HRSA (40.46±0.488%), and at 250 µg/ml, it reached 93.45±0.798%. Ascorbic acid also showed substantial HRSA (94.67±0.898%), slightly surpassing quercetin at the highest dose. These results imply that GPME-R exhibits great potential as a natural antioxidant at greater doses, even though it is less potent than quercetin and ascorbic acid at lower quantities (Figure 3). The potential of GPME-R for applications where larger concentrations are feasible is highlighted by its significant HRSA at higher dosages. Furthermore, to increase total antioxidant efficacy, the synergistic usage of quercetin or ascorbic acid may be investigated. This would take advantage of the standards' strong action at lower doses and GPME-R's rising activity at higher concentrations.

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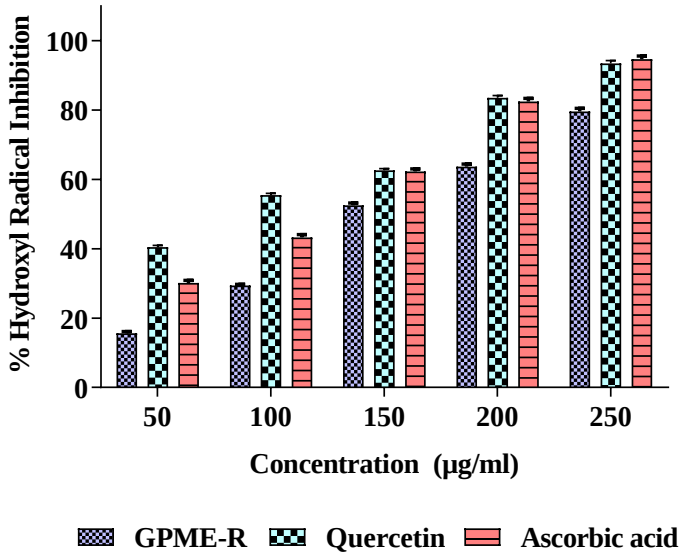

Figure 3. Hydroxyl Radical Scavenging Activity (HRSA) of the extract (GPME-R)

IC_{50} Values

The IC50 values from both assays suggested that GPME-R possessed significant antioxidant properties, though not as potent as the standard antioxidants ascorbic acid and quercetin. The estimated and computed IC50 values for quercetin and GPME-R in the ABTS radical decolorisation experiment were 83.23 µg/mL and 97.84 µg/mL, respectively. GPME-R's higher IC50 value implied that it was less effective in scavenging ABTS radicals compared to quercetin. This could be due to the concentration or the nature of the active compounds within GPME-R that contributed to its antioxidant activity. The estimated and computed IC50 values for quercetin, ascorbic acid, and GPME-R in the hydroxyl radical scavenging activity assay were 86.11 µg/mL, 112.70 µg/mL, and 155.62 µg/mL, respectively. GPME-R also showed a higher IC50 value than quercetin and ascorbic acid, indicating lower efficacy in scavenging hydroxyl radicals. Quercetin, with the lowest IC50 value in both assays, stood out as the most potent antioxidant, likely due to its strong free radical scavenging capabilities attributed to its polyphenolic structure. The findings highlight that while GPME-R is a capable and significantly strong antioxidant, it is relatively less potent compared to the pure standard compounds.

Table 2. IC50 values of the GPME-R and standard compounds

Assay	**Extract/ Standard**	**IC_{50} Value (µg/mL)**
ABTS Radical Decolorizing	GPME-R	97.84 µg/mL
	Quercetin	83.23 µg/mL
Hydroxyl Radical Scavenging Activity (HRSA)	GPME-R	155.62 µg/mL
	Quercetin	86.11 µg/mL
	Ascorbic Acid	112.70 µg/mL

Hepatoprotective effect in vitro:

Evaluating HepG2 Cells' Hepatoprotective Effect:

This study examined the hepatoprotective effectiveness of GPME-R in HepG2 cells exposed to toxicity caused by carbon tetrachloride (CCl4). This model is significant because CCl4, a well-known hepatotoxin, is commonly utilised in research to simulate chemically induced liver injury. The information summarised cell viability percentages for each treatment group and

showed how GPME-R may offer protection compared to silymarin, a proven hepatoprotective substance. The reference group for evaluating the viability of the cells was the control group, which was not exposed to CCl4 treatment. Significant liver damage was shown by the sharp decrease in cell viability to 22.79 ± 1.12% following exposure to CCl4. This prepared the groundwork for assessing the therapies' protective effects. After being treated with silymarin (250 µg/ml), the CCl4-treated cells displayed a viability of 96.12 ± 2.35%, indicating that silymarin is efficient in reducing the toxicity caused by CCl4. This result is in line with silymarin's well-established ability to protect the liver, which is mostly achieved through boosting cellular defence systems and antioxidant defence processes.

Remarkably, compared to silymarin, GPME-R therapy at a dose of 100 µg/ml showed a slightly higher cell viability (98.59 ± 2.44%), indicating that GPME-R may have similar, if not greater, hepatoprotective advantages. At lower doses, the hepatoprotective impact of GPME-R was also noticeable; viability decreased as GPME-R concentration was lowered from 100 µg/ml to 20 µg/ml in a dose-dependent manner. The results indicated that GPME-R has strong hepatoprotective effects, which might be related to the abundance of antioxidants and phenolic phytochemicals in the extract, GPME-R. These substances might reduce oxidative stress and increase the antioxidant defence capacity of the cells, shielding HepG2 cells from the cytotoxicity caused by CCl4. The idea that greater dosages of GPME-R are more effective in preventing liver damage had been found to be supported by the dose-dependent increase in cell viability with increasing GPME-R concentrations.

Table 3. HepG2 cells treated with GPME-R showed hepatoprotective effects after being exposed to CCl4.

Treatment Groups	**Concentration (µg/mL)**	**% Viability**
Control System	-	100
CCl_4 treated	-	22.79 ± 1.12^{a}
CCl4 (1%) + Standard Silymarin treated	250	96.12 ± 2.35^{b}
CCl4 (1%) + GPME-R treated	100	98.59 ± 2.44^{b}
	80	96.81 ± 2.68^{b}
	60	91.75 ± 2.57^{b}
	40	87.83 ± 2.11^{b}
	20	84.73 ± 2.61^{b}

Three average measurements with 3 replicates (n = 3); b = $p < 0.01$, compared to the CCl4-toxicated cells; a = $p < 0.01$, compared to normal cells.

GPME-R performed better at 100 µg/ml than the conventional silymarin therapy. This study illustrated, in a model of CCl4-induced liver injury in HepG2 cells, the hepatoprotective potential of GPME-R (Figure 4). At some concentrations, GPME-R not only showed efficacy comparable to the usual silymarin treatment, but even greater than it. These results pointed to a potential function for GPME-R as a natural liver protection substitute, indicating the need for more research in clinical situations.

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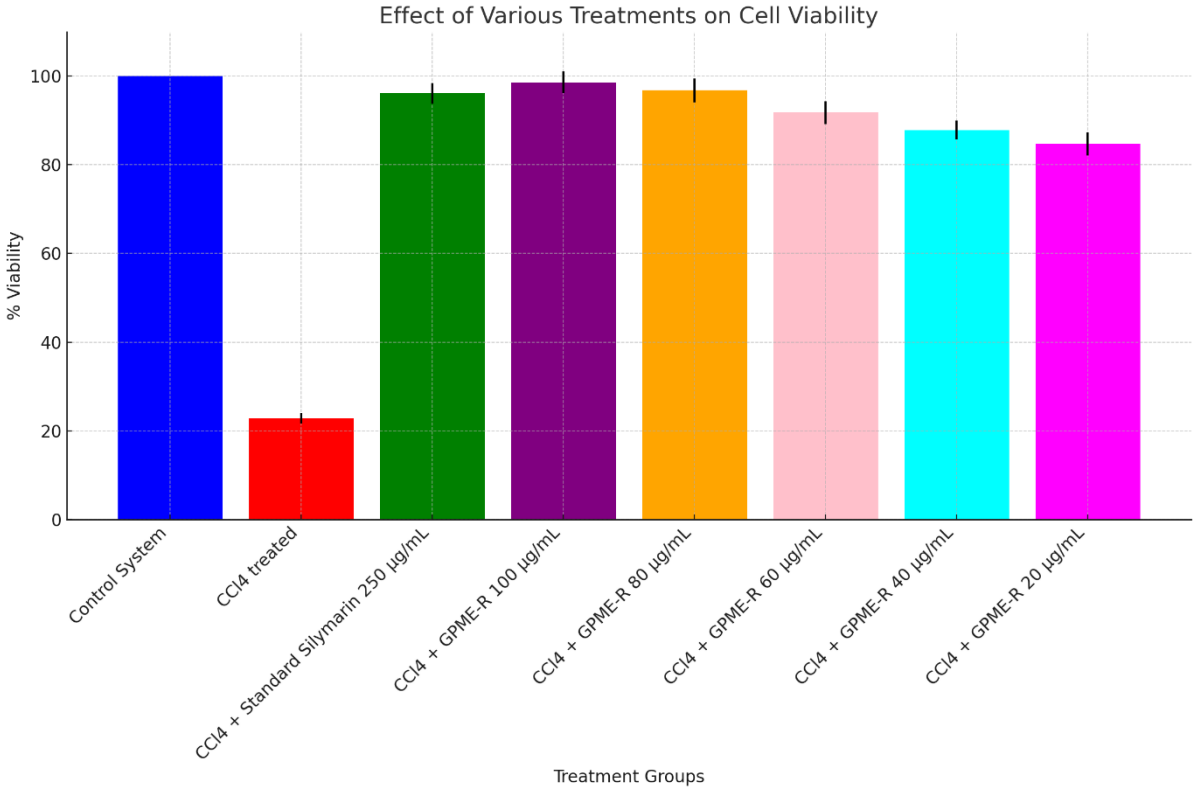

Figure 4. CCl4 intoxicated HepG2 cells demonstrated hepatoprotective effect caused by GPME-R treatment.

Evaluation of anti-cancer activity:

Using liver homogenate to prevent lipid peroxidation caused by Fe2+/ascorbate

As previously mentioned, goat liver homogenates were utilised as a lipid-rich medium, and the amount of lipid peroxide generated was measured using a modified thiobarbituric acid reactive species (TBARS) assay [34]. In this model of goat liver homogenates, different concentrations of GPME-R solution (20-100 µg/ml) were tested using the lipid peroxidation inhibition assay. (Table 4).

Table 4. In vitro evaluation of lipid peroxidation (LPO) inhibition utilizing liver homogenate

		% Inhibition of Lipid peroxidation (LPO)	
Media	**Concentration (µg/ml)**	**GPME-R (%)**	**Catechin (%)**
Liver homogenate	100 µg/ml	83.66 ± 1.09**	91.57 ± 1.21**
	80 µg/ml	74.35 ± 1.05**	87.45 ± 1.23*
	60 µg/ml	65.57 ± 1.11*	76.48 ± 1.09*
	40 µg/ml	54.97 ± 1.13*	62.09 ± 1.32*
	20 µg/ml	43.34 ± 1.12*	56.98 ± 1.22*

Values are given as the standard deviation (SD) plus the mean of three replicate measurements. Significant differences from Catechin at **$p < 0.01$ and *$p < 0.05$.

Evaluation of cytotoxicity using MTT assay:

The IC50 (50 percent growth inhibition) of GPME-R against MCF7 cells at various dosages was found using an MTT test. The results of the studies, which employed different dosages of

GPME-R, are shown in Figure 5. When compared to control and standard medication doses, it was demonstrated that MCF7 cells were strongly impacted by GPME-R dosages ranging from 75 nM to 500 nM on MTT tests. It was found that 500 nM of GPME-R had the maximum degree of cytotoxicity against the MCF7 cell, with a viability of 47.77±1.42%. The growth inhibition percentage rose in tandem with the GPME-R concentration; the assay's IC 50 value was 111 µg/ml. The investigation into the concentrations that showed the most cytotoxicity against the MCF7 cell revealed that the highest concentration of 1000 nM of the common medication, cyclophosphamide, had a viability rate of 8.08 ±0.97% of the cells (Table 5). It was shown that the growth inhibition % rose in tandem with the GPME-R concentration.

Table 5. Cytotoxicity measured by the GPME-R's proportion of cells that survive in comparison to regular cyclophosphamide

Concentration (nM)	GPME-R	Standard (Cyclophosphamide)
0	99.99±1.91	99.99±1.92
75	99.93±1.47	67.84±1.74
150	96.88±1.82	47.82±1.56
225	87.97±1.76	31.91±1.29
300	78.88±1.37	30.95±1.20
375	70.89±1.34	24.88±1.47
425	54.93±1.68	18.90±1.00
500	47.77±1.42	12.16 ±0.99
IC50 (nM)	533.51 nM	181.83 nM

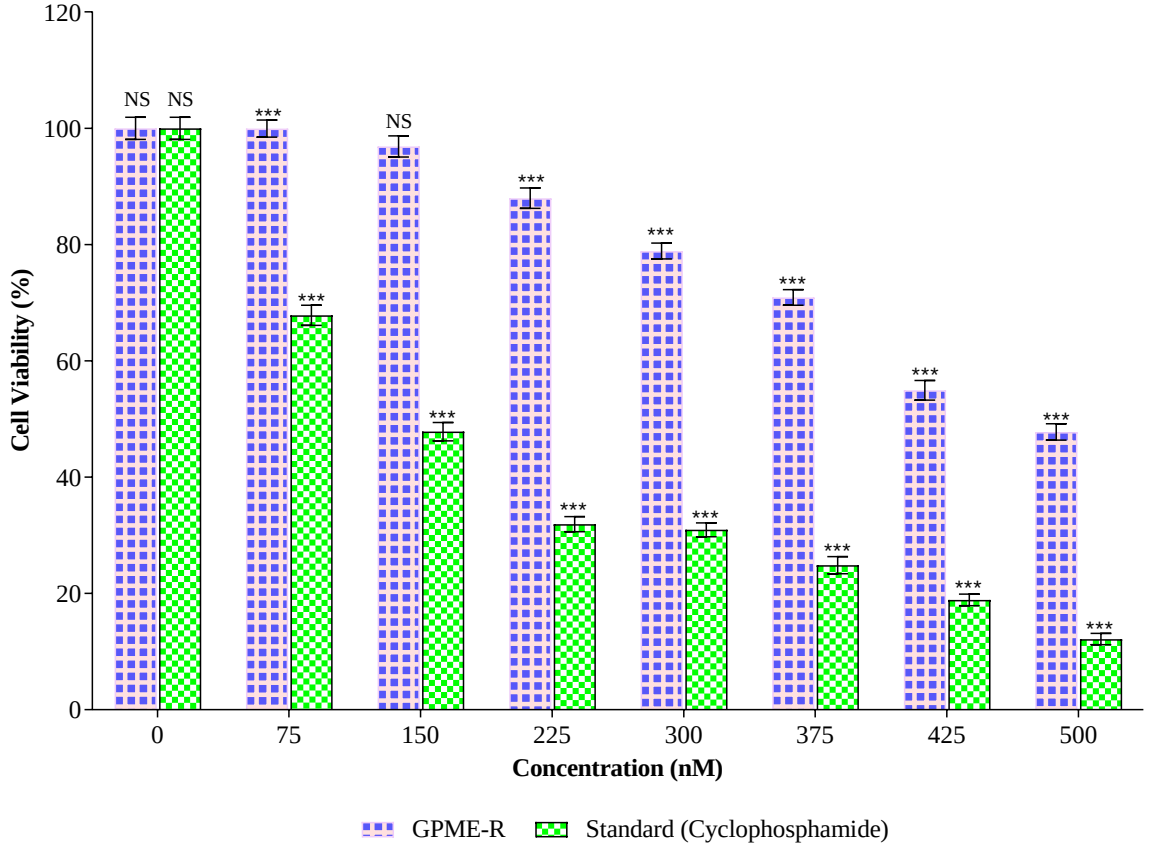

Figure 5. Cytotoxicity of the GPME-R and Standard (Cyclophosphamide) measured in terms of cell viability (%)

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CONCLUSION:

This work used a variety of mechanistic in vitro experimental models to convincingly establish the antiradical, Antioxidant, hepatoprotective and anticancer properties of plant extract (GPME-R). The findings showed that the herbal extract may scavenge or neutralize ABTS and superoxide radicals, suggesting a possible function for it in reducing oxidative stress brought on by free radicals and superoxide radicals. Additionally, the extract showed evidence of possible liver protective activity suggesting an antiradical role in mitigating toxicant-induced liver damage as well as a weak anticancer activity.

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