

Phytochemical And Pharmacological Assesment Of Carica Papaya Seed Extract Revealed That It Shows Neuroprotection Reveses Oxidative Damage And Neutralized Free Radical

Senthil Kumar Raju¹, Savitha H S², Ashok Kumar^{3*}, Sandhya Yadav⁴, Megha Rai⁵, Ajay Kumar Yadav⁶, Rajesh Kumar Mukherjee⁷, Sachhidananda Mahapatra⁸

¹Professor and Head, Department of Pharmaceutical Chemistry, Swamy Vivekanandha College of Pharmacy, Tiruchengode, Namakkal Dt, Tamilnadu, India. 637205

²Assistant professor, Department of chemistry, SJB Institute Of Technology Bengaluru. 560060

³Associate Professor, Faculty of Pharmaceutical Sciences, The ICFAI University, Baddi, Solan, Himachal Pradesh. 174103

⁴Assistant Professor, Tahira Institute of Medical Sciences, BL-2, Sector -7,GIDA, Gorakhpur, Uttar Pradesh. 273209

⁵Assistant Professor, Technocrats Institute Of Technology And Science Pharmacy, Bhopal, Madhya Pradesh. 462021

⁶Associate Professor, Tahira Institute of Medical Sciences, BL-2, Sector -7,GIDA, Gorakhpur, Uttar Pradesh. 273209

⁷Assistant Professor, Brainware University, Department of Pharmaceutical Technology, 398, Ramkrishnapur Rd, Near Jagadighata Market, Barasat, Kolkata, West Bengal. 700125

⁸Assistant Professor, Pharmacology, The Pharmaceutical College, Tingipali, Barpali, Odisha. 768029

***Corresponding Author:** Ashok Kumar

*Associate Professor, Faculty of Pharmaceutical Sciences, The ICFAI University, Baddi, Solan, Himachal Pradesh. 174103

ABSTRACT

This study investigates the pharmacological, phytochemical, antioxidant and neuroprotective properties of Carica papaya seed extract, focusing on its potential to combat oxidative stress-related diseases. One of the main causes of the advancement of many chronic illnesses, especially neurological diseases, is oxidative stress. Exploring natural antioxidant sources, such as medicinal plants, is becoming more popular due to the drawbacks of synthetic antioxidants. Carica papaya seeds, traditionally used in various medicinal applications, are known to contain bioactive compounds with potential health benefits. In this study, we performed a comprehensive analysis of the phytochemical composition of the seed extract, evaluated its antioxidant capacity through standard assays, and explored its neuroprotective pharmacological effects. According to the findings, Carica papaya seed extract has strong antioxidant and neuroprotective properties since it contains a variety of bioactive substances, such as terpenoids, phenolic acids, and flavonoids. According to these results, Carica papaya seed extract may be a useful natural source of antioxidants with potential therapeutic uses in the management and prevention of disorders linked to oxidative stress.

Keywords: Phytochemical screening, Oxidative stress, Niric oxide, Antioxidant activity, Carica papaya, Neuroprotective.

INTRODUCTION

Reactive oxygen species (ROS) generation and the body's capacity to eliminate these reactive intermediates or heal the consequent damage are out of balance, which leads to oxidative stress. ROS are byproducts of regular cellular metabolism, especially in the mitochondria. They include free radicals like superoxide anion (O_2^-) and hydroxyl radical ($OH^\cdot$), as well as non-radical species such hydrogen peroxide (H_2O_2). Under typical physiological circumstances, ROS are essential for homeostasis and cell signalling. However, oxidative stress results from their overproduction, which damages cells and molecules by overwhelming the body's antioxidant defences.. Many illnesses have been linked to oxidative stress, especially those that are associated with ageing and chronic inflammation. These comprise diabetes, cancer, heart disease, and neurological conditions. Oxidative stress has a major role in the neurodegenerative illnesses that cause amyotrophic lateral sclerosis (ALS), Parkinson's disease, and Alzheimer's disease by causing neuronal damage and death (Deore, Kide, Baviskar, Khadabadi, & Shende, 2023; Gurib-Fakim, 2006; Petrovska, 2012).

Because of its large lipid content, rapid oxygen consumption, and weak antioxidant defences, the brain is especially susceptible to oxidative stress. The main nervous system cells, neurones, are extremely vulnerable to ROS damage, which can result in DNA damage, lipid peroxidation, and protein oxidation. The progressive character of neurodegenerative illnesses can be attributed to these mechanisms, which can impair cellular function and cause cell death. The term "neuroprotection" describes tactics and systems that guard against damage and deterioration of the nervous system. Antioxidants, or compounds that neutralise reactive oxygen species (ROS), are the main target of neuroprotective treatments because of the role that oxidative stress plays in neurodegenerative illnesses. Antioxidants can come from exogenous sources like vitamins C and E, polyphenols, and synthetic chemicals, or they can come from endogenous sources like glutathione, catalase, and superoxide dismutase (SOD). By scavenging reactive oxygen species (ROS), these

antioxidants prevent oxidative damage and maintain neuronal function (Azeez & Lunghar, 2021; Gurib-Fakim, 2006; Malvi, Jain, Khatri, Patel, & Mishra, 2011; Sarkhel, 2014).

Recent research has highlighted the potential of various natural compounds with antioxidant properties for neuroprotection. For instance, polyphenols found in green tea, red wine, and various fruits have shown promise in reducing oxidative damage in neuronal cells. Additionally, compounds such as curcumin, resveratrol, and flavonoids are being investigated for their ability to modulate oxidative stress pathways and confer neuroprotection. In assumption, oxidative stress plays a critical role in the development of various diseases, particularly neurodegenerative disorders. The brain's susceptibility to oxidative damage underscores the importance of neuroprotective strategies that target oxidative stress. Enhancing the body's antioxidant defenses through dietary, pharmacological, or lifestyle interventions offers a promising avenue for mitigating the impact of oxidative stress and preserving cognitive and neurological health. Continued research into antioxidant therapies holds potential for improving outcomes in neurodegenerative diseases and enhancing overall brain health (Chio et al., 2022; Ovia, Yasasve, & Ansel Vishal, 2021; Rajendran et al., 2024; Shu et al., 2024; Sies, 2020).

The investigation of medicinal plants for antioxidant activity and neuroprotection is grounded in a growing recognition of the limitations and side effects of synthetic drugs, alongside the vast untapped potential of natural compounds. Medicinal plants have been used for centuries in traditional medicine, offering a rich source of bioactive compounds with therapeutic properties. In recent years, scientific interest in these plants has surged, driven by the need to discover new, safer, and more effective treatments for oxidative stress-related diseases, particularly neurodegenerative disorders (Akbari, Baghaei-Yazdi, Bahmaie, & Mahdavi Abhari, 2022; Jang, 2022; Monageng, Offor, Takalani, Mohlala, & Opuwari, 2023; Wang et al., 2023). Numerous chronic illnesses, including neurological disorders like Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis (ALS), are mostly caused by oxidative stress. The hallmark of these conditions is the gradual loss of neuronal structure and function, which is frequently connected to the build-up of reactive oxygen species (ROS), which harm lipids, proteins, and DNA among other cellular constituents. Although synthetic antioxidants have been created to fight oxidative stress, they frequently have drawbacks such as low bioavailability, possible toxicity at high dosages, and ineffectiveness in intricate biological systems (Akbari et al., 2022; Jang, 2022; Monageng et al., 2023; Wang et al., 2023).

Medicinal plants, on the other hand, offer a diverse array of natural antioxidants that may overcome these limitations. These plants contain various phytochemicals, such as flavonoids, phenolic acids, tannins, and alkaloids, which have demonstrated potent antioxidant properties. The synergistic interactions among these compounds within the plant matrix may enhance their effectiveness and reduce the risk of side effects. Furthermore, many medicinal plants are recognized for their neuroprotective effects, suggesting that they may offer dual benefits by both scavenging ROS and directly protecting neuronal cells from damage. The rationale for exploring medicinal plants as sources of new antioxidant and neuroprotective agents also lies in the urgent need for novel therapeutic strategies to address the growing burden of neurodegenerative diseases. Current treatments for these conditions are largely symptomatic, offering limited ability to halt or reverse disease progression (Ebrahimi, Soukhtanloo, & Mostafavi-Pour, 2023; Hassan et al., 2017; Jafaripour, Sohrabi Zadeh, Jafaripour, Ahmadvand, & Asadi-Shekaari, 2023; Kang & Yang, 2020; Voufo et al., 2023). The discovery of plant-derived compounds that can mitigate oxidative damage and support neuronal health could lead to significant advances in the prevention and treatment of neurodegenerative disorders. Moreover, the biodiversity of medicinal plants provides a vast and largely untapped reservoir of potential therapeutic agents. Each plant species contains a unique profile of bioactive compounds, offering numerous possibilities for discovering new drugs with antioxidant and neuroprotective properties. Investigating these plants not only holds promise for developing new treatments but also contributes to the preservation of traditional knowledge and biodiversity. In closing, the investigation of medicinal plants for antioxidant activity and neuroprotection is a promising area of research with the potential to yield safer, more effective therapies for oxidative stress-related diseases. As the global burden of neurodegenerative diseases continues to rise, the search for natural compounds that can protect against oxidative damage and support neuronal health is more critical than ever (Hassan et al., 2017).

To effectively treat and prevent illnesses linked to oxidative stress, more study must be done on the antioxidant processes and therapeutic potential of medicinal plants. There is a rising need to investigate natural sources of antioxidants due to our expanding awareness of the role that oxidative stress plays in a variety of chronic disorders, including neurological diseases. With their wide variety of bioactive chemicals, medicinal plants provide exciting opportunities for the discovery of novel, strong antioxidants that might serve as safer and more efficient substitutes for synthetic medications. The goal of the current study was to assess the antioxidant, phytochemical, and pharmacological characteristics of *Carica papaya* seed extract in light of these factors. Papaya carica seeds are known to contain a variety of bioactive chemicals with potential health advantages, and the fruit has long been utilised for medical purposes. This study intends to shed light on the seed extract's potential as a natural antioxidant source, which may help prevent and cure disorders connected to oxidative stress by doing a thorough evaluation of the extract. The primary goals of the study are to identify and measure the important phytochemicals in the seed extract, evaluate its antioxidant potential using a variety of tests, and investigate any pharmacological implications. By using a multidisciplinary approach, the research aims to further knowledge of the therapeutic potential of *Carica papaya* seeds and their function in preventing oxidative stress, which will ultimately aid in the creation of new antioxidant treatments.

MATERIAL AND METHODS

Collection, Identification and Extraction

The fruits of *Carica papaya* were collected from the local markets of Karnal region in Haryana, India. These fruits were thoroughly authenticated by a qualified botanist to confirm their identity. A voucher specimen, labeled as BKS/COG/2021-34/229, has been carefully preserved for any future reference and verification. Following collection, the fruits were meticulously washed to remove any adhering dirt and impurities. Then seeds were separated and collected and were then dried under natural shade for a duration of four weeks, ensuring that the phytochemical integrity of the materials was maintained. This drying process was conducted under controlled conditions to prepare the seeds for subsequent experimental use and analysis.

Preparation of extract by cold maceration

To prepare the extract through consecutive solvent extraction, 200 g of powdered seeds were subjected to cold maceration using methanol for a period of 10 days. Prior to each extraction with a fresh solvent, the powdered plant material was thoroughly dried overnight to remove any residual moisture that could affect the extraction process. The resulting methanol extract, labeled as CPSE-M, underwent solvent removal using a rotary vacuum evaporator at a controlled temperature of 45-50°C to concentrate the extract. Afterward, the extract was allowed to air-dry to remove any remaining solvent traces. During the process, the coloration and consistency of the extracts were carefully observed and recorded. The final methanol extract was then utilized for further phytochemical analysis and experimental studies to evaluate its antioxidant properties. This methodical approach ensured that the extract retained its bioactive compounds, making it suitable for subsequent analysis and testing (Shah, 2013).

Qualitative Phytochemical Analysis:

To find the main bioactive components in the methanol extract, a qualitative phytochemical study was performed. Using Mayer's, Wagner's, and Dragendorff's reagents, alkaloids were found, and flavonoids were identified using the Shinoda test, which revealed a yellow to orange colour. Tannins were confirmed by the Ferric Chloride test, producing a dark blue or greenish-black color. Saponins were detected by the froth test, forming a stable froth. Glycosides were identified using the Keller-Kiliani test, indicated by a reddish-brown ring. Phenolic compounds were confirmed with the Ferric Chloride test, turning deep blue or green. Terpenoids were detected by the Salkowski test, showing a reddish-brown color, and steroids were identified by the Libermann-Burchard test, resulting in a greenish color. This analysis provided a comprehensive profile of the phytochemicals in the extract, indicating its potential medicinal value. (Harborne, 1998).

Antioxidant activity by reducing power assay

The reducing power test was used to assess the antioxidant activity of CPSE-M in accordance with a previously defined protocol. A range of CPSE-M concentrations (from 50 to 250 µg/mL) were produced in 1 millilitre of distilled water, and then combined with 3 millilitres of phosphate buffer (0.3 mol/L, pH 6.5) and 3 millilitres of potassium ferricyanide ($K_3Fe(CN)_6$), 1.5%. After that, the mixtures were incubated for 15 minutes at 55°C to promote the reduction reaction. After incubation, the mixtures were centrifuged for 12 minutes at 4000 rpm in order to terminate the reaction, and 3 ml of 11% trichloroacetic acid was added. After carefully collecting the supernatant (3 ml), 0.6 ml of 0.2% ferric chloride ($FeCl_3$) and 3 ml of distilled water were combined. Using a UV spectrophotometer (UV-1601 Shimadzu, Japan), the absorbance of the resultant solution was determined at 700 nm. Greater reducing power, which is correlated with increased antioxidant activity of the CPSE-M extract, was evidenced by a higher absorbance value. This test added to our knowledge of the extract's potential therapeutic effects by revealing important details about its antioxidant capacity (Harborne, 1998; Pulido, Bravo, & Saura-Calixto, 2000).

Antioxidant activity by Nitric Oxide scavenging assay

The nitric oxide scavenging assay was conducted to assess the ability of the methanol extract of *Carica papaya* seeds (CPSE-M) to neutralize nitric oxide radicals. The assay's foundation is the idea that sodium nitroprusside spontaneously produces nitric oxide at physiological pH when dissolved in phosphate-buffered saline (PBS) (Sarma Katakai et al., 2012). This nitric oxide then reacts with oxygen to form nitrite ions, which can be quantitatively measured using the Griess reagent. The presence of antioxidant compounds in the extract inhibits the formation of nitrite ions, thereby indicating its nitric oxide scavenging activity. To begin the assay, various concentrations of CPSE-M were prepared by dissolving the extract in methanol to achieve a range from 10 µg/mL to 100 µg/mL. Each concentration of the extract was then mixed with an equal volume of sodium nitroprusside solution (10 mM) in PBS. The reaction mixtures were incubated at 25°C for 150 minutes, allowing for the generation of nitric oxide. Following the incubation period, 1 mL of the Griess reagent, consisting of 1% sulfanilamide in 5% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride, was added to each mixture. The reaction was allowed to proceed at room temperature for 10 minutes, during which a pink chromophore developed as a result of the interaction between nitrite ions and the Griess reagent. With the use of a UV-visible spectrophotometer, the absorbance of the resultant solution was determined at 546 nanometres. The amount of nitric oxide scavenging by CPSE-M was measured by the decrease in absorbance when compared to a control that included all of the reagents except the extract. The same method was used to test the scavenging activity of ascorbic acid,

which served as a reference antioxidant for comparison. Using the formula, the absorbance of the sample and the control were compared to determine the percentage of nitric oxide scavenging activity: Inhibition (%) = (Absorbance of Control – Absorbance of Sample) / (Absorbance of Control) × 100. The results were expressed as IC₅₀ values, which represent the concentration of the extract required to inhibit 50% of nitric oxide generation. The IC₅₀ values were determined by plotting the percentage inhibition against the concentration of CPSE-M. This assay provided valuable insights into the antioxidant potential of CPSE-M, highlighting its effectiveness in scavenging nitric oxide radicals, which are associated with oxidative stress and inflammation.

Neuroprotective activity

Cell culture and treatment

Carica papaya seed extract was investigated for its potential neuroprotective properties using the human neuroblastoma cell line SK-N-SH. The American Type Culture Collection (ATCC) provided this cell line, which was cultivated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% foetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin. In the experiment, 1.5×10^4 SK-N-SH cells were seeded into each well of 96-well Corning plates, and the plates were then incubated at 37°C in a humidified environment with 5% carbon dioxide. Following a 24-hour incubation period, the growth media was entirely eliminated, and the cells were kept in DMEM that was enhanced with antibiotics but did not include serum. In order to prevent serum factors from potentially interfering with the experimental therapy, it was imperative to take this step. The cells were subjected to acrolein, a recognised neurotoxin, at a dose of 20 µM after being denied serum to cause neurotoxicity. The purpose of this therapy was to simulate a neurotoxic environment so that the neuroprotective effects of WS against the generated damage could be assessed. The evaluation of extract's therapeutic potential in neuroprotection was made easier by this experimental design, which offered a controlled environment to explore the interactions between the neurotoxic and possible neuroprotective drugs. The goal of the study was to clarify the processes via which CPSE-M may lessen neurotoxic effects, advancing the possibility of using it in treatments for neurodegenerative diseases (Thummayot et al., 2014; Thummayot, Tocharus, Suksamrarn, & Tocharus, 2016).

Estimating the Intracellular reactive oxygen species level

By using the fluorescent dye DCF-DA to scavenge reactive oxygen species (ROS) in SK-N-SH cells, the antioxidant activity of CPSE-M was evaluated. This technique, which has been extensively studied in scientific literature, uses DCF-DA, a cell-permeable dye that, when within the cell, is enzymatically transformed into the highly luminous chemical DCF when intracellular ROS are present. The amount of ROS within the cells may be determined by measuring the intensity of DCF fluorescence; higher fluorescence implies increasing ROS levels, whereas lower fluorescence shows efficient ROS scavenging by the antioxidant. This investigation compared the fluorescence intensity of DCF in treated cells to that in control, untreated cells. By scavenging ROS, CPSE-M substantially decreased oxidative stress in the treated cells, as evidenced by a considerable decrease in fluorescence intensity. This demonstrated CPSE-M's potential as a strong antioxidant and demonstrated its capacity to shield cells from oxidative damage (Ramassamy & Singh, 2017; Thummayot et al., 2014).

Statistical analysis

Three duplicates of each quantitative test method were carried out to guarantee the precision and dependability of the outcomes. With a sample size of three (n=3), the results were represented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used in the statistical analysis to assess the differences between the samples, and Dunnett's Multiple Comparison Test was used in the post hoc analysis. The statistical method was used with the aid of the software program GraphPad Prism. A statistically significant p-value was defined as $P < 0.05$, signifying that the observed differences were not the result of chance.

RESULTS AND DISCUSSION

Preliminary Phytochemical investigations and percentage yield

The preliminary phytochemical screening results presented in Table 1 indicate that the methanol extract of the plant material (CPSE-M) contains a wide range of phytoconstituents. Specifically, the presence of carbohydrates, sterols, glycosides, fatty acids, tannins, flavonoids, terpenoids, and saponins was confirmed. This comprehensive profile suggests that CPSE-M is rich in diverse bioactive compounds, each of which may contribute to the extract's potential therapeutic properties. The presence of these constituents points to the extract's possible roles in antioxidant, anti-inflammatory, and other health-promoting activities. The percentage yield, of extract was shown in table 2.

Table 1. Preliminary phytochemical screening results

Phytoconstituents	CPSE-M
Carbohydrates	√
Sterols	√
Glycosides	√
Fatty acid	√
Tannins	√
Flavanoids	√
Terpenoid	√
Saponins	√

‘√’= Present and ‘-’ = Absent

Table 2: Percentage yield and characteristics of extracts of seeds of *Carica papaya*.

Extract	% Yield	Colour/Consistency
Methanol (CPSE-M)	11.57%	Greenish Brown / Sticky mass in nature

Antioxidant activity by reducing power assay

The results of the reducing power assay for the CPSE-M extract, in comparison with the standard antioxidant Butylated Hydroxytoluene (BHT), are presented in Figure 1. This assay assessed the antioxidant capacity of CPSE-M by measuring its ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), a process that reflects the extract's electron-donating and free radical neutralizing capabilities. The half-maximal inhibitory concentration (IC_{50}) values for CPSE-M and BHT were determined to be $92.64 \mu\text{g/mL}$ and $89.78 \mu\text{g/mL}$, respectively, indicating that CPSE-M has a reducing capacity comparable to that of BHT. At a concentration of $250 \mu\text{g/mL}$, CPSE-M achieved an absorbance of 0.588 ± 0.0041 , demonstrating its effectiveness in donating electrons to reduce oxidative stress. In comparison, BHT, known for its potent antioxidant properties, exhibited a higher absorbance of 1.081 ± 0.009 at the same concentration, reflecting its superior reducing power. Nonetheless, the significant reducing capacity of CPSE-M highlights its robust antioxidant potential, likely attributable to its rich content of bioactive compounds such as phenols, flavonoids, and terpenoids. The presence of these phytochemicals in CPSE-M suggests that it may play a crucial role in combating oxidative stress and associated diseases, such as neurodegenerative disorders, cardiovascular diseases, and other conditions linked to oxidative damage. The comparable performance of CPSE-M to BHT underscores its potential as a natural alternative to synthetic antioxidants, which are commonly used in various therapeutic and preventive applications. To further substantiate these findings, additional research is necessary to elucidate the specific mechanisms through which CPSE-M exerts its antioxidant effects. This includes identifying the key active compounds within the extract and understanding their individual contributions to the overall antioxidant activity. Such studies could pave the way for the development of CPSE-M as a natural therapeutic agent for managing oxidative stress-related conditions, offering a promising alternative to conventional antioxidant treatments.

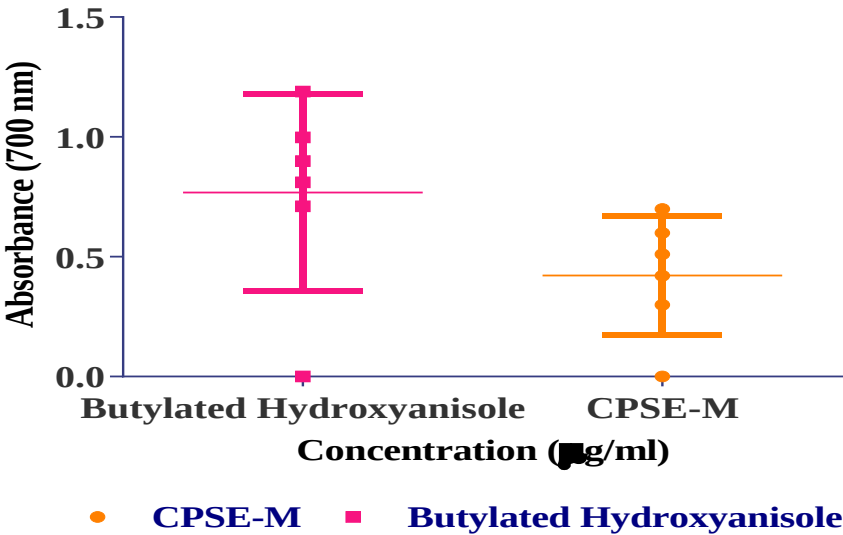


Figure 1. Reducing power assay of the extract

Antioxidant activity by Nitric Oxide scavenging assay

The nitric oxide scavenging assay results indicate that the methanol extract of *Carica papaya* seeds (CPSE-M) exhibits notable antioxidant activity, although it is less potent than the standard antioxidant, ascorbic acid. The data reveal a concentration-dependent increase in nitric oxide scavenging activity for both CPSE-M and ascorbic acid. At the lowest concentration, CPSE-M showed a scavenging activity of $24.64\% \pm 0.63$, compared to $38.56\% \pm 0.312$ for ascorbic acid. This trend continued across the concentrations tested, with CPSE-M demonstrating a maximum scavenging activity of $66.76\% \pm 0.249$ at the highest concentration, while ascorbic acid achieved $86.02\% \pm 0.853$. Although CPSE-M consistently showed lower scavenging activity than ascorbic acid at all tested concentrations, its ability to inhibit nitric oxide formation significantly increased with higher concentrations. These results suggest that CPSE-M possesses a substantial antioxidant capacity, capable of scavenging nitric oxide radicals effectively, albeit not as efficiently as ascorbic acid. The presence of bioactive compounds such as flavonoids, tannins, and terpenoids in CPSE-M likely contributes to its observed antioxidant activity. While ascorbic acid remains the more potent antioxidant in this assay, CPSE-M's significant nitric oxide scavenging activity underscores its potential as a natural source of antioxidants, which could be beneficial in managing oxidative stress-related conditions. Further studies could explore the specific compounds in CPSE-M responsible for this activity and their possible therapeutic applications.

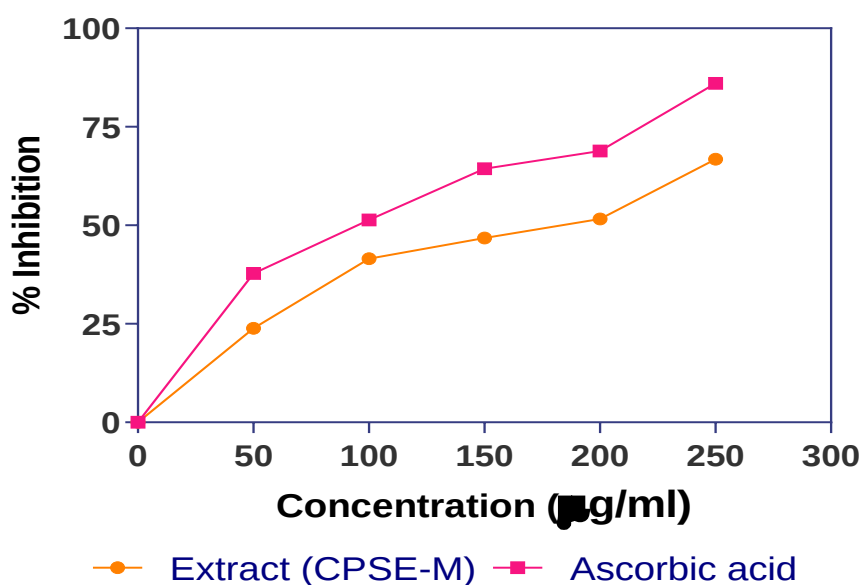


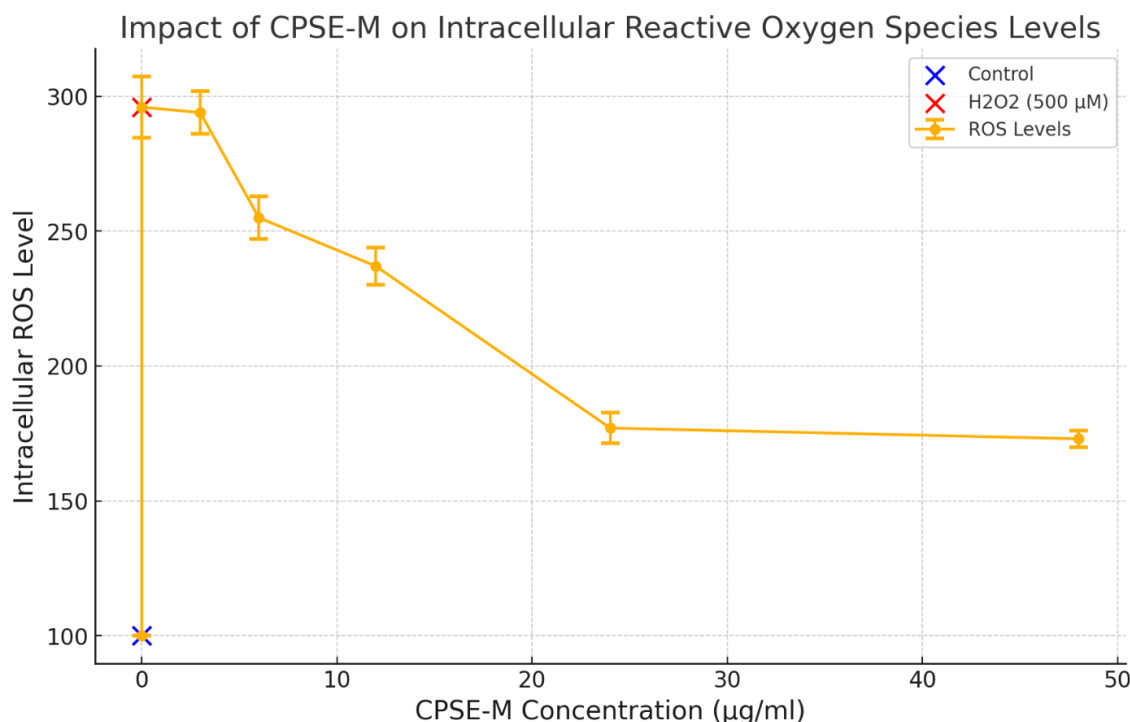
Figure 2. Nitric Oxide scavenging assay of the extract

Neuroprotective effect: The extract lowers the amount of reactive oxygen species inside cells

The effects of CPSE-M on intracellular reactive oxygen species (ROS) levels are shown in Table 3 relative to a control and H_2O_2 treatment. The baseline ROS level of the control group is 100. However, exposure to $500 \mu\text{M}$ H_2O_2 dramatically raises ROS levels to 296 ± 11.32 , suggesting oxidative stress that has been generated. ROS levels are seen to decrease dose-dependently with increasing CPSE-M doses ($3 \mu\text{g/mL}$ to $48 \mu\text{g/mL}$). ROS levels drop to 294 ± 7.98 at the lowest dose of $3 \mu\text{g/mL}$, indicating a minor decrease in comparison to the H_2O_2 group. As the CPSE-M concentration increases, the ROS levels continue to drop, with the highest concentration of $48 \mu\text{g/mL}$ reducing ROS to 173 ± 3.11 , which is a substantial decrease compared to the H_2O_2 group. The data suggest that CPSE-M exhibits a strong antioxidant effect by effectively reducing intracellular ROS levels induced by H_2O_2 . This reduction is concentration-dependent, with higher doses of CPSE-M resulting in greater decreases in ROS levels. The ability of CPSE-M to lower ROS at all tested concentrations indicates its potential to mitigate oxidative stress within cells. The extract is effective at neutralising free radicals, as demonstrated by the significant reduction in ROS at the highest CPSE-M concentration ($48 \mu\text{g/mL}$). This reduction may be due to the presence of bioactive compounds, such as phenols, flavonoids, and terpenoids, which were identified during the preliminary phytochemical screening. Because of these chemicals' well-known antioxidant qualities, there has probably been a reduction in ROS. This discovery highlights the possible therapeutic use of CPSE-M in circumstances with high levels of oxidative stress. CPSE-M may assist in shielding cells against oxidative damage, which is linked to a number of illnesses, such as cancer, cardiovascular disease, and neurological disorders, by reducing intracellular ROS levels. Subsequent research ought to investigate the precise processes by which CPSE-M demonstrates its antioxidant properties and pinpoint the principal active ingredients accountable for this phenomenon.

Table 3. Effect of CPSE-M on the amount of intracellular reactive oxygen species

Control	H ₂ O ₂	CPSE-M Concentration (µg/ml)				
		3	6	12	24	48
100	296±11.32	294±7.98	255±7.89	237±6.99	177±5.65	173±3.11

**Figure 3.** Reactive oxygen species inside cells are decreased by CPSE-M.

CONCLUSIONS

The results of this investigation show that the extensive phytochemical composition of *Carica papaya* seed extract contributes to its notable antioxidant and neuroprotective properties. Flavonoids, phenolic acids, and terpenoids are examples of bioactive chemicals that highlight the extract's ability to reduce oxidative stress and guard against oxidative damage. According to these findings, *Carica papaya* seed extract shows promise as a natural antioxidant treatment, especially for neurological illnesses and other disorders where oxidative stress is a major factor. To prepare the way for the extract's possible usage in clinical applications, more investigation is necessary to determine the precise mechanisms of action and assess the extract's effectiveness in vivo.

REFERENCES

1. Akbari, B., Baghaei-Yazdi, N., Bahmaie, M., & Mahdavi Abhari, F. (2022). The role of plant-derived natural antioxidants in reduction of oxidative stress. *Biofactors*, 48(3), 611-633. doi:10.1002/biof.1831
2. Azeez, T. B., & Lunghar, J. (2021). Antiinflammatory effects of turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*). *Inflammation and Natural Products*, 83-102.
3. Chio, J. C. T., Punjani, N., Hejrati, N., Zavvarian, M. M., Hong, J., & Fehlings, M. G. (2022). Extracellular Matrix and Oxidative Stress Following Traumatic Spinal Cord Injury: Physiological and Pathophysiological Roles and Opportunities for Therapeutic Intervention. *Antioxid Redox Signal*, 37(1-3), 184-207. doi:10.1089/ars.2021.0120
4. Deore, S. L., Kide, A. A., Baviskar, B. A., Khadabadi, S. S., & Shende, B. A. (2023). Memory Protecting Herbs: *Centella asiatica* and *Bacopa monnieri* in the Fight Against Alzheimer's Disease. *Revista Brasileira de Farmacognosia*, 33(6), 1263-1273.
5. Ebrahimi, S., Soukhtanloo, M., & Mostafavi-Pour, Z. (2023). Anti-tumor effects of Auraptene through induction of apoptosis and oxidative stress in a mouse model of colorectal cancer. *Tissue Cell*, 81, 102004. doi:10.1016/j.tice.2022.102004
6. Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular aspects of Medicine*, 27(1), 1-93.
7. Harborne, A. J. (1998). *Phytochemical Methods A Guide to Modern Techniques of Plant Analysis*: Springer Netherlands.

8. Hassan, W., Noreen, H., Rehman, S., Gul, S., Amjad Kamal, M., Paul Kamdem, J., . . . Bt da Rocha, J. (2017). Oxidative stress and antioxidant potential of one hundred medicinal plants. *Current topics in medicinal chemistry*, 17(12), 1336-1370.
9. Jafaripour, L., Sohrabi Zadeh, B., Jafaripour, E., Ahmadvand, H., & Asadi-Shekaari, M. (2023). Gallic acid improves liver cirrhosis by reducing oxidative stress and fibrogenesis in the liver of rats induced by bile duct ligation. *Scand J Gastroenterol*, 58(12), 1474-1483. doi:10.1080/00365521.2023.2229929
10. Jang, E. (2022). Hyperoside as a Potential Natural Product Targeting Oxidative Stress in Liver Diseases. *Antioxidants (Basel)*, 11(8). doi:10.3390/antiox11081437
11. Kang, Q., & Yang, C. (2020). Oxidative stress and diabetic retinopathy: Molecular mechanisms, pathogenetic role and therapeutic implications. *Redox Biology*, 37, 101799.
12. Malvi, R., Jain, S., Khatri, S., Patel, A., & Mishra, S. (2011). A review on antidiabetic medicinal plants and marketed herbal formulations. *Int J Pharm Biol Arch*, 2, 1344-1355.
13. Monageng, E., Offor, U., Takalani, N. B., Mohlala, K., & Opuwari, C. S. (2023). A Review on the Impact of Oxidative Stress and Medicinal Plants on Leydig Cells. *Antioxidants (Basel)*, 12(8). doi:10.3390/antiox12081559
14. Ovia, M., Yasasve, M., & Ansel Vishal, L. (2021). Role of Indian Herbal Medicine in the Treatment of Pulmonary Diseases. *Medicinal Plants for Lung Diseases: A Pharmacological and Immunological Perspective*, 85-102.
15. Petrovska, B. B. (2012). Historical review of medicinal plants' usage. *Pharmacognosy reviews*, 6(11), 1.
16. Pulido, R., Bravo, L., & Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of agricultural and food chemistry*, 48(8), 3396-3402.
17. Rajendran, P., Al-Saeedi, F. J., Ammar, R. B., Abdallah, B. M., Ali, E. M., Al Abdulsalam, N. K., . . . Ahmed, E. A. (2024). Geraniol attenuates oxidative stress and neuroinflammation-mediated cognitive impairment in D galactose-induced mouse aging model. *Aging (Albany NY)*, 16(6), 5000-5026. doi:10.18632/aging.205677
18. Ramassamy, C., & Singh, M. (2017). In vitro screening of neuroprotective activity of Indian medicinal plant *Withania somnifera*. *Journal of Nutritional Science*, 6, e54. doi:10.1017/jns.2017.48
19. Sarkhel, S. (2014). Ethnobotanical survey of folklore plants used in treatment of snakebite in Paschim Medinipur district, West Bengal. *Asian pacific journal of tropical biomedicine*, 4(5), 416-420.
20. Sarma Katak, M., Murugamani, V., Rajkumari, A., Singh Mehra, P., Awasthi, D., & Shankar Yadav, R. (2012). Antioxidant, hepatoprotective, and anthelmintic activities of methanol extract of *Urtica dioica* L. leaves. *Pharmaceutical Crops*, 3(1).
21. Shah, B. (2013). *Textbook of Pharmacognosy & Phytochemistry*: Elsevier India.
22. Shu, P., Mo, J., Li, Z., Li, M., Zhu, W., & Du, Z. (2024). Ferulic acid in synergy with retinol alleviates oxidative injury of HaCaT cells during UVB-induced photoaging. *Aging (Albany NY)*, 16. doi:10.18632/aging.205749
23. Sies, H. (2020). Oxidative stress: Concept and some practical aspects. *Antioxidants*, 9(9), 852.
24. Thummayot, S., Tocharus, C., Pinkaew, D., Viwatpinyo, K., Sringarm, K., & Tocharus, J. (2014). Neuroprotective effect of purple rice extract and its constituent against amyloid beta-induced neuronal cell death in SK-N-SH cells. *Neurotoxicology*, 45, 149-158.
25. Thummayot, S., Tocharus, C., Suksamrarn, A., & Tocharus, J. (2016). Neuroprotective effects of cyanidin against A β -induced oxidative and ER stress in SK-N-SH cells. *Neurochemistry International*, 101, 15-21.
26. Voufo, R. A., Kouotou, A. E., Tatah, N. J., TeTo, G., Gueguim, C., Ngondé, C. M. E., . . . Anatole, P. C. (2023). Relation between interleukin-6 concentrations and oxidative status of HIV infected patients with /or at risk of Kaposi disease in Yaounde. *Virol J*, 20(1), 165. doi:10.1186/s12985-023-02109-9
27. Wang, Y., Cai, Z., Zhan, G., Li, X., Li, S., Wang, X., . . . Luo, A. (2023). Caffeic Acid Phenethyl Ester Suppresses Oxidative Stress and Regulates M1/M2 Microglia Polarization via Sirt6/Nrf2 Pathway to Mitigate Cognitive Impairment in Aged Mice following Anesthesia and Surgery. *Antioxidants (Basel)*, 12(3). doi:10.3390/antiox12030714