Bulletin of Pure and Applied Sciences
Zoology (Animal Science), Vol.43B, No.1S,
January-June 2024: P.976-990

Print version ISSN 0970-0765
Online version ISSN 2320-3188

Original Research Article
Content Available online at: www.bpasjournals.com

INNOVATIVE EXTRACTION METHODS AND SOLVENT OPTIMIZATION FOR *PAEDERIA FOETIDA* IN VITRO ANALYSIS OF ANTIOXIDANT AND NEUROPROTECTIVE PROPERTIES

Gireesh Tripathi[1], Komal Kriti[2], Kavitha S[3*,] B.R. Asokan[4], Pankaj Mohan Pimpalshende[5], Dilip Kumar Roy[6], Anuva Samanta[7], Konda V V S Krishna[8],

[1]Department of Pharmacy, Shri Krishna University Vill Chowka Sagar Road Chhatarpur, Madhya Pradesh India Pin- 471301.
[2]Dhanbad College of Pharmacy, Shaktinagar, Dhanbad, Jharkhand, 826001.
[3]School of Allied Health Sciences, the Apollo University Murukambattu Chittoor Andhra Pradesh Pin-517001.
[4]Department of Pharmacology, Aarupadai Veedu Medical College & Hospital,Vinayaka Mission's Research Foundation,(DU), Puducherry Campus,607403.
[5]Department of Pharmaceutics, Hi-Tech college of Pharmacy Chandrapur Pin Code: -442406.
[6 7]Department of Pharmaceutical Technology, JIS University 81, Nilgunj Rd, Jagarata Pally, Deshpriya Nagar, Agarpara, Kolkata, West Bengal 700109.
[8]Department of Pharmacy, Government Polytechnic for Women, Srikakulam, Andhra Pradesh, India. Pin code- 532 005.

Corresponding Author
Kavitha S
School of Allied Health Sciences, the Apollo University Murukambattu Chittoor Andhra Pradesh Pin-517001.

ABSTRACT

The goal of the current study is to determine the best extraction method and solvent for the extraction of *Paederia foetida*, a traditional medicinal herb. Additionally, the study looked into the medicinal plant extract's overall capacity to prevent the lipid peroxidation process as well as its neuroprotective properties. Furthermore, the study aimed to evaluate the phytochemical properties and antioxidant potential of *Paederia foetida* extracts obtained using various extraction techniques. The extraction methods included Soxhlet extraction, cold maceration, and microwave-assisted extraction using solvents such as methanol, ethanol, chloroform, and water. The extracts underwent preliminary phytochemical screening, revealing the presence of alkaloids, flavonoids, glycosides, saponins, phenols, phytosterols, terpenoids, carbohydrates, and proteins. The lipid peroxidation inhibition (LPO) assay was conducted using egg yolk, rat liver, and rat brain homogenates to assess antioxidant activity. The *Paederia foetida* methanol extract (PFWP-ME) exhibited significant LPO inhibition in all models, with 82.79% in egg yolk, 78.97% in rat liver, and 79.81% in rat brain homogenates. Additionally, the neuroprotective activity of PFWP-ME was evaluated by measuring intracellular reactive oxygen species (ROS) levels in SK-N-SH cells exposed to oxidative stress. The extract showed

a dose-dependent reduction in ROS levels, indicating strong antioxidant properties. These findings suggest that *Paederia foetida* is a valuable source of natural antioxidants with potential therapeutic applications.
Keywords: Extraction methods, Lipid peroxidation inhibition, *Paederia foetida,* Neuroprotective, intracellular reactive oxygens species

INTRODUCTION

Lipid peroxidation is a critical biochemical process involving the oxidative degradation of lipids, particularly polyunsaturated fatty acids. It is initiated when reactive oxygen species (ROS) attack these lipids, leading to the formation of lipid radicals. This process is significant because it can result in cell membrane damage, affecting cell integrity and function, and is implicated in various pathological conditions, including atherosclerosis, neurodegenerative diseases, and cancer (Khoshakhlagh et al., 2023, Luper, 1999, Mendoza-Fernández et al., 2023). The lipid peroxidation process occurs in three stages: initiation, propagation, and termination. During the initiation phase, ROS such as hydroxyl radicals, singlet oxygen, or peroxynitrite interact with polyunsaturated fatty acids in cell membranes, abstracting hydrogen atoms and creating lipid radicals (Tung et al., 2019). These lipid radicals react with molecular oxygen to form peroxyl radicals, which propagate the chain reaction by attacking adjacent fatty acids, leading to the formation of new lipid radicals and lipid hydroperoxides. Propagation involves the continuous reaction of peroxyl radicals with other lipids, perpetuating the cycle and amplifying the damage. This stage results in the accumulation of lipid hydroperoxides and secondary by-products like malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). These aldehydes are highly reactive and can form adducts with proteins, nucleic acids, and other biomolecules, disrupting their normal function and contributing to cellular dysfunction and toxicity. Termination of lipid peroxidation occurs when antioxidants such as vitamin E, glutathione, and enzymes like superoxide dismutase (SOD), catalase, and glutathione peroxidase neutralize the lipid radicals, thus preventing further propagation of the oxidative chain reaction. These antioxidants donate electrons to lipid radicals, stabilizing them and halting the peroxidation process (Jangam et al., 2024, Luper, 1999, Tung et al., 2019).

The measurement of lipid peroxidation is often carried out using assays that detect MDA and 4-HNE, as these by-products serve as reliable markers of oxidative stress and lipid damage. The thiobarbituric acid reactive substances (TBARS) assay is a commonly used method for quantifying MDA levels, providing insights into the extent of lipid peroxidation and oxidative damage in biological samples. Lipid peroxidation plays a crucial role in aging, inflammation, and the pathogenesis of numerous diseases. Understanding its mechanisms and developing strategies to mitigate lipid peroxidation through antioxidants or other therapeutic agents is vital for improving health outcomes and managing oxidative stress-related conditions (Jangam et al., 2024, Luper, 1999, Tung et al., 2019).

Reactive oxygen species (ROS) are highly reactive molecules containing oxygen. These include free radicals like superoxide anion (O2•-), hydroxyl radical (•OH), and non-radical molecules such as hydrogen peroxide (H2O2). ROS are natural by-products of cellular metabolism, particularly within the mitochondria during aerobic respiration (Khoshakhlagh et al., 2023, Luper, 1999, Mendoza-Fernández et al., 2023). ROS play dual roles in biological systems (Mohyeldin et al., 2023, Sharma et al., 2021, Singh et al., 2018, Steven et al., 2019).

At low to moderate levels, they function as signalling molecules, regulating processes such as cell proliferation, apoptosis, and immune responses. However, excessive ROS production leads to oxidative stress, a condition where the balance between ROS and antioxidants is disrupted. This imbalance can cause significant damage to cellular components, including lipids, proteins, and DNA (Khoshakhlagh et al., 2023, Luper, 1999, Mendoza-Fernández et al., 2023, Saha et al., 2023).

Oxidative stress is implicated in the pathogenesis of numerous diseases, such as cancer, cardiovascular diseases, neurodegenerative disorders (e.g., Alzheimer's and Parkinson's diseases), and diabetes. The body employs a range of antioxidants, including enzymatic antioxidants like superoxide dismutase (SOD), catalase, and glutathione peroxidase, as well as non-enzymatic antioxidants like vitamin C, vitamin E, and glutathione, to neutralize excess ROS and mitigate their harmful effects (Jangam et al., 2024, Luper, 1999, Tung et al., 2019). Understanding ROS dynamics and managing oxidative stress through antioxidant therapies or lifestyle modifications are crucial for maintaining cellular health and preventing ROS-related diseases (Mohyeldin et al., 2023, Sharma et al., 2021, Singh et al., 2018, Steven et al., 2019, Khoshakhlagh et al., 2023, Saliani et al., 2023, Thabrew et al., 1997, Tung et al., 2019).

Paederia foetida, commonly known as skunkvine or Chinese fever vine, is a perennial climbing plant from the Rubiaceae family, native to Southeast Asia and also profusely available in India. The plant is easily identifiable by its strong, unpleasant odour (Khushbu et al., 2010, Dutta et al., 2023, Chanda et al., 2013). It has been widely used in traditional medicine across various cultures for its extensive therapeutic properties. *Paederia foetida* is particularly valued for its ability to treat gastrointestinal disorders such as diarrhoea and dysentery, owing to its potent antibacterial and anti-inflammatory effects (Dutta et al., 2023, Chanda et al., 2013). Additionally, it is employed in the management of rheumatic pain, fever, and respiratory issues, with the leaves and roots often prepared as decoctions, infusions, or in powdered form. The medicinal benefits of *Paederia foetida* are largely attributed to its rich phytochemical composition (Luper, 1999, Maleki et al., 2019, Steven et al., 2019, Dutta et al., 2023). Thus, the goal of the current study was to investigate *Paederia foetida*, a traditional medicinal plant (Kirtikar and Basu, 1935, Chopra et al., 1956, Dutta et al., 2023, Chanda et al., 2013). Another goal of this research was to close the knowledge gap between contemporary science and traditional medicine. Additionally, the current study sought to optimize the extraction process and assess *Paederia foetida*, a traditional medicinal plant, for neuroprotective potential, lipid peroxidation inhibition, and its function in oxidative stress using a mechanistic approach.

MATERIAL AND METHODS

Plant Material and Authentication

Global Herbs, located in Khari Bhaoli, New Delhi, and Herbal Team from Himachal Pradesh, supplied plant material as they are authorized and pre-validated herbal crude drug suppliers. The plant materials were identified botanically and herbarium was deposited for future reference.

Pilot level extraction: choosing an appropriate extraction technique and solvent

Pilot level extraction involves selecting a suitable solvent and extraction method. There are many solvents available for extracting medicinal plants, but choosing the right one is crucial. The selection depends on the chemical characteristics of the active ingredients and the quality of the solvent. Solvents vary in polarity and boiling points, which affects their ability to extract

different compounds. Additionally, solvents can interact differently with other substances they encounter. Therefore, choosing the right solvent is the first step towards successful extraction of medicinal herbs. This decision ensures that the active ingredients are effectively extracted, preserving their medicinal properties. The method of extraction also plays a significant role, as it must be compatible with the solvent and the plant material. Together, the right solvent and method lead to a more efficient and effective extraction process, yielding high-quality extracts for further use (Sasidharan et al., 2011).

Treatment of Plant Material and Selection of a suitable solvent

The treatment of plant material and the selection of a suitable solvent is a critical process. After being shade-dried, the plant materials were ground into a fine powder using an electric grinder. One gram of the powdered plant material was then dissolved in 10 ml of various solvents in test tubes. These solvents varied in polarity from low to high and included ethanol, HPLC grade water, aqueous methanol, aqueous ethanol, ethyl acetate, chloroform, and acetone. The test tubes were sealed with aluminum foil and left undisturbed for a day. The next day, the contents of the test tubes were filtered. The filtrate obtained was then subjected to preliminary phytochemical tests. The results of these tests were crucial in determining the most suitable solvent for further extractions. This step ensured that the chosen solvent effectively extracted the active ingredients from the plant material, leading to efficient and high-quality extracts for subsequent use.

Studying the phytochemistry of extracts at the pilot level

The phytochemical investigation of pilot level extracts involved a series of tests to determine their chemical composition. This screening aimed to identify various compounds such as alkaloids, flavonoids, glycosides, saponins, phenols, phytosterols, terpenoids, carbohydrates, and proteins within the extract. Each test provided insights into the presence of these components in the prepared samples. This comprehensive analysis not only confirmed the existence of these bioactive compounds which is crucial for understanding the medicinal potential of the plant extracts.

Choosing an appropriate extraction technique

Cold maceration

The cold maceration technique was used to extract phytochemicals from the plants. For this process, dry drug powder was macerated in 80% methanol at room temperature for seven days. During this period, the mixture was occasionally shaken and stirred to enhance the extraction. After maceration, the mixture was filtered to separate the liquid extract. The obtained filtrate was then concentrated by heating it in a water bath, which helped to reduce its volume. Finally, the extract was further concentrated over the water bath to obtain the final, potent extract, ready for further analysis or use (Sasidharan et al., 2011).

Soxhlet extraction

The Soxhlet extraction technique was employed to fully extract phytochemicals from coarsely ground plant materials. Solvents such as petroleum ether, chloroform, ethyl acetate, methanol, and water were used in the process. The extracts obtained were then dried using a rotary evaporator at 45 degrees Celsius. Once dried, the extracts were stored in vacuum desiccators containing anhydrous silica gel to prevent moisture absorption. This method ensured thorough extraction of the desired compounds, providing high-quality extracts for further analysis and use in various applications. The Soxhlet apparatus is particularly effective for continuous

extraction, maximizing yield and efficiency.

Microwave assisted technique

The microwave-assisted extraction technique was employed to extract phytochemicals from the plants. In this process, five grams of dry plant powder were mixed with one hundred milliliters of 90% methanol. This mixture was then subjected to microwave irradiation at a power of 160 watts. The extraction process was carried out in intervals; initially, the mixture was microwaved for a few minutes, followed by a period where the temperature was allowed to drop to room temperature. The microwave was then turned on for an additional minute to complete the extraction. After the microwave process, the mixture was filtered to separate the liquid extract from the plant residue. The filtrate was then concentrated using a water bath to reduce its volume, ensuring the final extract was potent and ready for further analysis or use. This method is efficient and time-saving, providing high-quality extracts with minimal solvent use and energy consumption (Mandal et al., 2007).

Preliminary Phytochemical Screening

Three different extraction techniques were used to produce the extracts. To identify the presence of alkaloids, flavonoids, glycosides, saponins, phenols, phytosterols, carbohydrates, and proteins, a preliminary phytochemical screening was conducted on all the extracts. Based on the results of this screening, the extraction method that yielded the highest quality and quantity of desired phytochemicals was selected for further production of the final extract. This step ensured that the most effective extraction technique was used, optimizing the extraction process for the best possible results.

Mass extraction and Extractive yield

For the mass extraction of each plant or plant part, one of the three techniques—cold maceration, microwave-assisted extraction, or Soxhlet extraction—was used with the appropriate solvents (as detailed in Table 1). This approach ensured the efficient extraction of phytochemicals on a larger scale, providing a substantial yield for further analysis and use (Sasidharan et al., 2011, Mandal et al., 2007). The yield of the extracts produced with various extraction techniques was calculated.

Qualitative phytochemical evaluation

A preliminary phytochemical screening was performed on the extracts obtained from different extraction techniques to identify the presence of proteins, carbohydrates, phytosterols, alkaloids, flavonoids, glycosides, saponins, and phenols. This analysis helped determine the variety of bioactive compounds in each extract, ensuring their potential therapeutic benefits (Harborne, 1998).

In vitro Lipid Peroxidation Inhibition **(LPO_ inhibition using egg yolk, rat liver and rat brain homogenates**

An enhanced thiobarbituric acid reactive species (TBARS) assay was employed to quantify lipid peroxides generated in lipid-fortified mediums using egg yolk, rat liver, and rat brain homogenates. This method, as reported by Ruberto et al. in 2000 (Ruberto et al., 2000)., is based on the formation of TBARS when lipids undergo peroxidation. During the assay, thiobarbituric acid (TBA) reacts with TBARS to form a complex. The extent of lipid peroxidation is then estimated by measuring the absorbance of this complex at 532 nm using a spectrophotometer. Higher absorbance levels indicate more lipid peroxidation, reflecting a greater presence of TBARS. By comparing the absorbance levels of treated samples to those

of untreated samples, we can determine the effectiveness of antioxidants in inhibiting lipid peroxidation. Treated samples with lower TBARS levels indicate that the antioxidants are successful in preventing lipid peroxidation. This assay is crucial for understanding the protective effects of antioxidants against oxidative damage in biological systems, providing insights into their potential therapeutic applications (Ruberto et al., 2000).

Cell culture

The SK-N-SH and RAW264.7 cell lines were cultivated using RPMI1640 medium, which was supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, and 10% fetal bovine serum. The cells were maintained in an incubator set to 37°C, with an atmosphere of 5% carbon dioxide and humidified air. This environment ensured optimal growth conditions for the cell cultures, providing necessary nutrients and maintaining sterility to support their proliferation and viability for further experimental procedures.

Neuroprotective activity

Cell culture and treatment

The human neuroblastoma cell line, SK-N-SH, obtained from the American Type Culture Collection (ATCC), was used to investigate the neuroprotective effects of WS. These cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin. For the experiment, SK-N-SH cells were plated in 96-well plates (Corning) at a density of 1.5 × 10^4 cells per well and incubated at 37°C in a humidified atmosphere with 5% carbon dioxide. After 24 hours of incubation, the medium was completely withdrawn, and the cells were maintained in DMEM containing antibiotics but without serum. This step ensured the removal of serum factors that could interfere with the treatment. Following serum deprivation, the cells were exposed to acrolein at a toxic concentration of 20 µM. This treatment aimed to induce neurotoxicity, allowing the assessment of KONIGU's protective effects against such damage. The setup provided a controlled environment to study the interactions between the neurotoxin and potential neuroprotective agents, paving the way for understanding the therapeutic benefits of WS in neuroprotection (Thummayot et al., 2016, Thummayot et al., 2014).

Intracellular reactive oxygen species level

The antioxidant activity of KONIGU was evaluated by measuring its ability to scavenge reactive oxygen species (ROS) in SK-N-SH cells using the fluorescent dye DCF-DA. This method, previously described in scientific literature, involves the use of DCF-DA, a cell-permeable dye that is enzymatically converted to the highly fluorescent molecule DCF in the presence of intracellular ROS. The fluorescence intensity of DCF directly correlates with the level of ROS within the cells. Higher fluorescence indicates higher levels of ROS, while lower fluorescence suggests effective scavenging of ROS by the antioxidant. By comparing the fluorescence intensity in treated and untreated cells, the efficacy of KONIGU in reducing oxidative stress within SK-N-SH cells was confirmed, demonstrating its potential as a potent antioxidant (Thummayot et al., 2014, Ramassamy and Singh, 2017).

Statistical analysis

One-way ANOVA was used to analyze the experimental outcomes statistically. Dunnett's test was performed as a post hoc analysis to further examine the ANOVA results. A P value of 0.05 or less was considered statistically significant. All data are presented as mean ± standard deviation (SD). GraphPad Prism Software (Version 8) was used for all graphing, plotting, and

statistical analysis, ensuring precise and clear visualization of the results.

RESULTS AND DISCUSSIONS

Choosing an Appropriate Solvent and Extraction Technique

When trying to extract phytochemicals from medicinal plants, choosing the appropriate solvent and extraction technique is crucial. It facilitates the majority of phytochemicals to be extracted from the plants. *Paederia foetida* was extracted for this investigation. The selection of solvent and technique is an important aspect since it influences the extraction process of the phytochemicals from these plants.

Solvent Selection and Extraction Method Selection:

Table 1 lists the most effective extraction solvent and extraction technique for the plant after they have been assessed.

Table 1. Indicating the plant portion that was used, the extraction technique, and the appropriate solvent.

Plant Name	**Parts used**	**Solvent selected**	**Extraction method Selected**
Paederia foetida	Whole plant	Methanol	Cold maceration

Extractive yield and Qualitative phytochemical assessment

From 1 kilogram of *Paederia foetida*, an extractive weight of 73.87 grams was obtained. This results in an extractive value of 7.387%. This yield indicates the efficiency of the extraction process and the amount of bioactive compounds that can be derived from *Paederia foetida*. A 7.387% extractive value signifies a substantial yield, suggesting that *Paederia foetida* is a rich source of extractable phytochemicals, which can be further utilized for their medicinal properties.

Table 2. The yield of the extracts obtained

Medicinal Plant Names		**Extractive Weight in grams and Values in Percentage**
Paederia foetida (1 kg)	Extractive weight	73.87 gm
	Extractive value	7.387 %

The preliminary phytochemical screening of the extracts from *Paederia foetida* revealed the presence of several bioactive compounds. The analysis indicated that flavonoids, sterols, carbohydrates, tannins, glycosides, saponins, terpenoids, and fatty acids are present in the extracts. Each of these phytoconstituents contributes to the medicinal properties of *Paederia foetida*, highlighting its potential therapeutic benefits. The presence of these compounds suggests that *Paederia foetida* could be a valuable source of natural bioactive substances with various health-promoting effects. This screening provides a foundation for further investigation into the specific activities and potential uses of these phytochemicals in medical and pharmaceutical applications (Table 3) (Harborne, 1998).

Table 3. Preliminary phytochemical screening of the extract

Phytoconstituents	***Paederia foetida* (PFWP-ME)**

Flavanoids	√
Sterols	√
Carbohydrates	√
Tannins	√
Glycosides	√
Saponins	√
Terpenoid	√
Fatty acid	√

'√'= Present and '-' = Absent

Lipid peroxidation inhibition employing homogenates of rat brain, liver, and egg yolk

As indicated earlier, the amount of lipid peroxide produced was measured using a modified thiobarbituric acid reactive species (TBARS) method (Badmus et al., 2011). The in vitro lipid peroxidation inhibition (LPO) results for the egg yolk system indicate significant antioxidant activity of the extracts. The extract from *Paederia foetida*, labeled PFWP-ME, demonstrated an LPO inhibition of 82.79% ± 1.21, which is statistically significant with a p-value of less than 0.01. This shows that *Paederia foetida* has a strong ability to inhibit lipid peroxidation.

For comparison, the standards used were Quercetin and Catechin, which exhibited LPO inhibition values of 93.72% ± 1.89 and 97.46% ± 1.68, respectively. Although the *Paederia foetida* extract showed slightly lower inhibition than these standards, its high percentage indicates a potent antioxidant capacity, making it a promising candidate for further research and potential therapeutic applications.

Table 4. Findings for the in vitro inhibition of lipid peroxidation (LPO) in the egg yolk system

Plant NAME	**Extract Name**	**% Inhibition of LPO**
Paederia foetida	PFWP-ME	82.79±1.21**
Standard 1	Quercetin	93.72±1.89
Standard 2	Catechin	97.46±1.68

***p < 0.01*

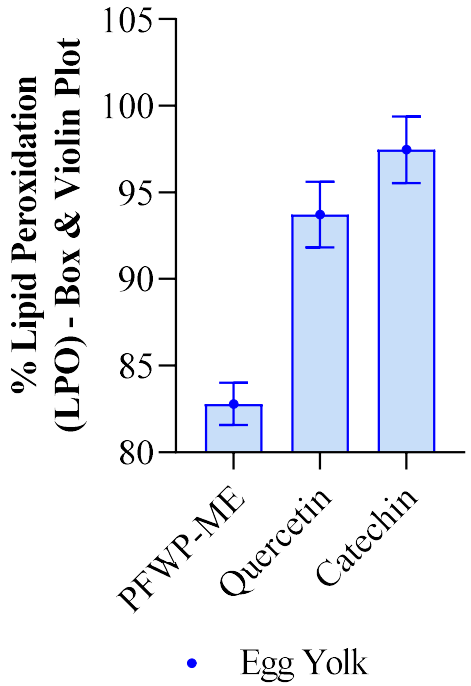

Figure 1. Egg yolk model: in vitro inhibition of lipid peroxidation (LPO)

The in vitro lipid peroxidation inhibition (LPO) results for the rat liver homogenate system show significant antioxidant activity of the *Paederia foetida* extract. The extract, labeled PFWP-ME, demonstrated an LPO inhibition of 78.97% ± 1.44, with a statistically significant p-value of less than 0.01. This indicates that *Paederia foetida* effectively inhibits lipid peroxidation in rat liver homogenate. For comparison, the standards Quercetin and Catechin showed higher LPO inhibition values of 93.72% ± 1.79 and 97.46% ± 1.92, respectively. While the *Paederia foetida* extract has a slightly lower inhibition rate compared to these standards, it still exhibits a strong antioxidant capacity. This finding highlights the potential of *Paederia foetida* as a valuable source of natural antioxidants, suitable for further research and potential therapeutic use.

Table 5. Rat liver homogenate was used to test the effects of lipid peroxidation inhibition (LPO) in vitro.

Plant NAME	**Extract Name**	**% Inhibition of LPO**
Paederia foetida	PFWP-ME	78.97±1.44**
Standard 1	Quercetin	93.72±1.79
Standard 2	Catechin	97.46±1.92

$^{**}p < 0.01$

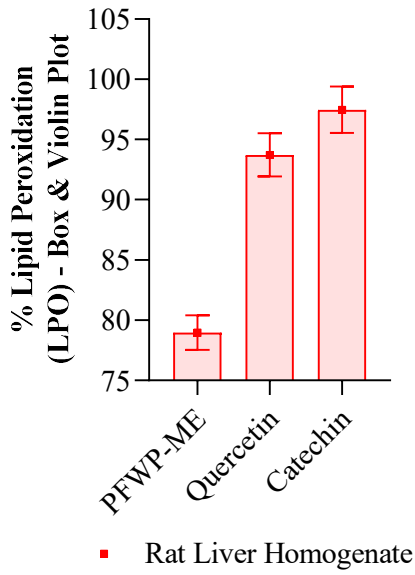

Figure 2. Rat liver homogenate model: in vitro inhibition of lipid peroxidation (LPO)

The in vitro lipid peroxidation inhibition (LPO) results for the rat brain homogenate system indicate significant antioxidant activity of the *Paederia foetida* extract. The extract, labeled PFWP-ME, demonstrated an LPO inhibition of 79.81% ± 1.87, with a statistically significant p-value of less than 0.01. This shows that *Paederia foetida* has a substantial ability to inhibit lipid peroxidation in rat brain homogenate. Comparatively, the standards Quercetin and Catechin showed higher LPO inhibition values of 93.72% ± 1.69 and 97.47% ± 1.75, respectively. Although the *Paederia foetida* extract exhibits a slightly lower inhibition rate than these standards, it still presents a robust antioxidant capacity. These results underscore the potential of *Paederia foetida* as a valuable natural antioxidant, warranting further research and exploration for therapeutic applications.

Table 6. Result for *in vitro* Lipid peroxidation Inhibition (LPO) in rat brain homogenate.

Plant NAME	**Extract Name**	**% Inhibition of LPO**
Paederia foetida	PFWP-ME	79.81±1.87**
Standard 1	Quercetin	93.72±1.69
Standard 2	Catechin	97.47±1.75

$^{**}p < 0.01$

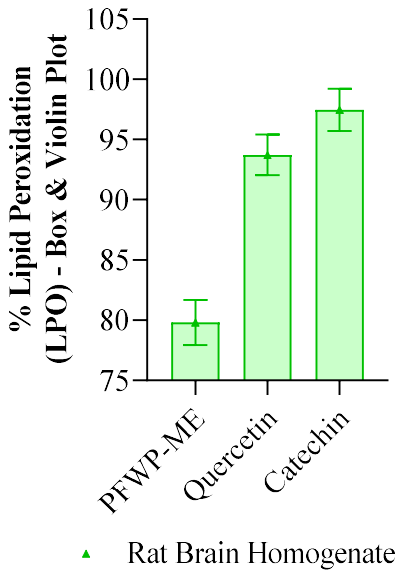

Figure 3. In vitro Inhibition of Lipid Peroxidation (LPO) in a Rat Brain Homogenate Model

The in vitro lipid peroxidation inhibition (LPO) results for *Paederia foetida* extracts across three different systems—egg yolk, rat liver, and rat brain homogenates—demonstrate significant antioxidant activity. In the egg yolk system, the *Paederia foetida* extract (PFWP-ME) showed an LPO inhibition of 82.79% ± 1.21, which, while slightly lower than the standards Quercetin (93.72% ± 1.89) and Catechin (97.46% ± 1.68), still indicated strong antioxidant capacity. In the rat liver homogenate system, the extract maintained a high inhibition rate of 78.97% ± 1.44, confirming its efficacy despite being somewhat lower than Quercetin (93.72% ± 1.79) and Catechin (97.46% ± 1.92). Similarly, in the rat brain homogenate system, the extract demonstrated substantial LPO inhibition at 79.81% ± 1.87, again slightly less effective than the standards Quercetin (93.72% ± 1.69) and Catechin (97.47% ± 1.75). Across all three systems, *Paederia foetida* exhibited robust antioxidant properties with significant LPO inhibition percentages, highlighting its potential as a valuable natural antioxidant. These findings support further investigation into *Paederia foetida* for therapeutic applications.

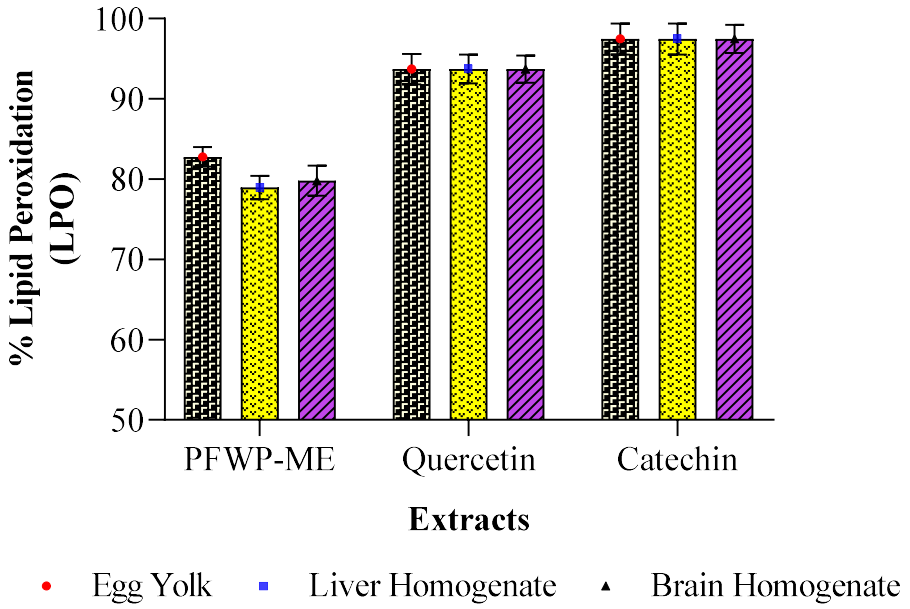

Figure 4. A relative comparison of *in vitro* Lipid peroxidation (LPO) Inhibition of the extracts in different models

Neuroprotective activity

The extract decreases intracellular reactive oxygen species level

The data presented in Table 7 highlights the neuroprotective activity of *Paederia foetida* extract (PFWP-ME) by demonstrating its ability to decrease intracellular reactive oxygen species (ROS) levels in a dose-dependent manner. The control group maintained a baseline ROS level of 100 ± 2.91 µM. Upon treatment with 500 µM hydrogen peroxide (H2O2), a significant increase in ROS levels to 291 ± 7.54 µM was observed, indicating oxidative stress. When cells were treated with varying concentrations of PFWP-ME, a notable reduction in ROS levels was seen. At a concentration of 3 µg/ml, the ROS level slightly decreased to 289 ± 6.65 µM. As the concentration of PFWP-ME increased, the ROS levels continued to drop, reaching 168 ± 3.09 µM at the highest concentration of 48 µg/ml. This indicates that PFWP-ME effectively scavenges ROS, thereby reducing oxidative stress in the cells. The dose-dependent reduction in ROS levels suggests that the extract possesses significant antioxidant properties. By lowering the intracellular ROS levels, PFWP-ME helps protect the cells from oxidative damage, which is a key factor in neuroprotection. Oxidative stress is known to contribute to neuronal damage and is implicated in various neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Therefore, the ability of PFWP-ME to mitigate ROS levels highlights its potential as a therapeutic agent in preventing or slowing the progression of such conditions. In summary, the extract of *Paederia foetida* (PFWP-ME) shows promising neuroprotective effects by significantly reducing ROS levels in cells exposed to oxidative stress. These findings support further research into its potential use in treating or preventing neurodegenerative diseases, emphasizing the need to explore its active components and mechanisms of action.

Table 7. Impact of PFWP-ME in intracellular reactive oxygen species level

Control	**H_2O_2**	**PFWP-ME Concentration (µg/ml)**

	500 µm	3	6	12	24	48
100	291±7.54	289±6.65	250±6.75	232±6.27	172±4.22	168±3.09

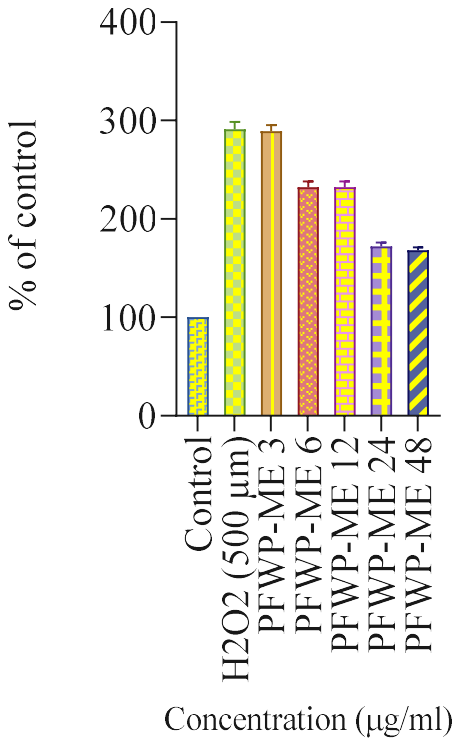

Figure 5. The amount of intracellular reactive oxygen species is decreased by PFWP-ME.

CONCLUSIONS

The study successfully demonstrated the phytochemical power and significant antioxidant potential of *Paederia foetida* whole plant extract obtained through various extraction techniques. The preliminary phytochemical screening identified several bioactive compounds, including flavonoids, sterols, carbohydrates, tannins, glycosides, saponins, terpenoids, and fatty acids, highlighting the plant's medicinal properties. The lipid peroxidation inhibition assays confirmed the strong antioxidant capacity of the methanol extract (PFWP-ME) across different biological models, including egg yolk, rat liver, and rat brain homogenates. Furthermore, the neuroprotective activity assessment revealed a dose-dependent decrease in intracellular reactive oxygen species levels in SK-N-SH cells, indicating the extract's potential to mitigate oxidative stress and protect against neurodegenerative damage. These results underscore the therapeutic potential of *Paederia foetida* as a natural antioxidant source. Future research should focus on isolating and characterizing specific active compounds and exploring their mechanisms of action to further understand the plant's medicinal benefits and applications in preventing or treating oxidative stress-related diseases.

REFERENCE

BADMUS, J. A., ADEDOSU, T. O., FATOKI, J. O., ADEGBITE, V. A., ADARAMOYE, O. A. & ODUNOLA, O. A. 2011. Lipid Peroxidation Inhibition and Antiradical Activities of Some Leaf Fractions of *Mangifera Indica*. *Acta Poloniae Pharmaceutica - Drug*

Research, 68**,** 23-29.
CHANDA, S., SARETHY, I. P., DE, B. & SINGH, K. 2013. Paederia foetida—a promising ethno-medicinal tribal plant of northeastern India. *Journal of Forestry Research,* 24**,** 801-808.
CHOPRA, R. N., NAYAR, S. L. & CHOPRA, I. C. 1956. *Glossary of Indian medicinal plants*, Council of Scientific & Industrial Research New Delhi.
DUTTA, P. P., MARBANIANG, K., SEN, S., DEY, B. K. & TALUKDAR, N. C. 2023. A review on phytochemistry of Paederia foetida Linn. *Phytomedicine Plus,* 3**,** 100411.
HARBORNE, A. 1998. *Phytochemical methods a guide to modern techniques of plant analysis*, springer science & business media.
JANGAM, A., KOTIPALLI, R. S. S., PATNAIK, S. S., KASIREDDY, B., GAJA, S. K., SREEDHAR, B., JERALD, M. K., ADDLAGATTA, A. & MURALIDHARAN, K. 2024. Gymnema sylvestre extract improves PCOS by altering the YAP1 protein in the mouse ovary via mitochondrial changes. *Phytomedicine Plus,* 4**,** 100515.
KHOSHAKHLAGH, M., BUTLER, A. E., JAMIALAHMADI, T. & SAHEBKAR, A. 2023. Protective Effects of Curcumin Against Alcoholic Fatty Liver. *Curr Med Chem*.
KHUSHBU, C., ANAR, P., MAYUREE, P., CAROL, M., ROSHNI, S. & SUBODH, A. 2010. Paederia foetida Linn. As a potential medicinal plant: A Review. *Journal of Pharmacy Research,* 3**,** 3135-3137.
KIRTIKAR, K. & BASU, B. 1935. *Indian Medicinal Plants,* Allahbad, Lalit Mohan Basu.
LUPER, S. 1999. A review of plants used in the treatment of liver disease: part two. *Altern Med Rev,* 4**,** 178-88.
MALEKI, S. J., CRESPO, J. F. & CABANILLAS, B. 2019. Anti-inflammatory effects of flavonoids. *Food chemistry,* 299**,** 125124.
MANDAL, V., MOHAN, Y. & HEMALATHA, S. 2007. Microwave assisted extraction—an innovative and promising extraction tool for medicinal plant research. *Pharmacognosy reviews,* 1**,** 7-18.
MENDOZA-FERNÁNDEZ, P. D., SILVA-CORREA, C. R., TORRE, V. E. V., ASPAJO-VILLALAZ, C. L., CALDERÓN-PEÑA, A. A. & ROSARIO-CHÁVARRI, J. D. 2023. Hepatoprotective and nephroprotective effects of Tessaria integrifolia Ruiz and Pav. on diclofenac-induced toxicity in rats. *Vet World,* 16**,** 1933-1939.
MOHYELDIN, R. H., ALAAELDIN, R., SHARATA, E. E., ATTYA, M. E., ELHAMADANY, E. Y. & FATHY, M. 2023. LCZ696 attenuates sepsis-induced liver dysfunction in rats; the role of oxidative stress, apoptosis, and JNK1/2-P38 signaling pathways. *Life Sci,* 334**,** 122210.
RAMASSAMY, C. & SINGH, M. 2017. In vitro screening of neuroprotective activity of Indian medicinal plant Withania somnifera. *Journal of Nutritional Science,* 6**,** e54.
RUBERTO, G., BARATTA, M. T., DEANS, S. G. & DORMAN, H. D. 2000. Antioxidant and antimicrobial activity of Foeniculum vulgare and Crithmum maritimum essential oils. *Planta medica,* 66**,** 687-693.
SAHA, J., MONDAL, M. I. H., AHMED, F. & RAHMAN, M. 2023. Extraction, characterization and functionality assessment of Aloe vera, chitosan and silk sericin. *Arabian Journal of Chemistry,* 16**,** 105087.
SALIANI, N., MONTASSER KOUHSARI, S. & IZAD, M. 2023. The Potential

epatoprotective Effect of Vaccinium arctostaphylos L. Fruit Extract in Diabetic Rat. *Cell J,* 25**,** 717-726.

SASIDHARAN, S., CHEN, Y., SARAVANAN, D., SUNDRAM, K. & LATHA, L. Y. 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines,* 8.

SHARMA, V. K., SINGH, T. G., GARG, N., DHIMAN, S., GUPTA, S., RAHMAN, M. H., NAJDA, A., WALASEK-JANUSZ, M., KAMEL, M., ALBADRANI, G. M., AKHTAR, M. F., SALEEM, A., ALTYAR, A. E. & ABDEL-DAIM, M. M. 2021. Dysbiosis and Alzheimer's Disease: A Role for Chronic Stress? *Biomolecules,* 11.

SINGH, M. P., CHAUHAN, A. K. & KANG, S. C. 2018. Morin hydrate ameliorates cisplatin-induced ER stress, inflammation and autophagy in HEK-293 cells and mice kidney via PARP-1 regulation. *International Immunopharmacology,* 56**,** 156-167.

STEVEN, S., FRENIS, K., OELZE, M., KALINOVIC, S., KUNTIC, M., BAYO JIMENEZ, M. T., VUJACIC-MIRSKI, K., HELMSTÄDTER, J., KRÖLLER-SCHÖN, S. & MÜNZEL, T. 2019. Vascular inflammation and oxidative stress: major triggers for cardiovascular disease. *Oxidative medicine and cellular longevity,* 2019.

THABREW, M. I., HUGHES, R. D. & MCFARLANE, I. G. 1997. Screening of hepatoprotective plant components using a HepG2 cell cytotoxicity assay. *J Pharm Pharmacol,* 49**,** 1132-5.

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.

THUMMAYOT, S., TOCHARUS, C., SUKSAMRARN, A. & TOCHARUS, J. 2016. Neuroprotective effects of cyanidin against Aβ-induced oxidative and ER stress in SK-N-SH cells. *Neurochemistry International,* 101**,** 15-21.

TUNG, B. T., NHAM, D. T., HAI, N. T. & THU, D. K. 2019. Chapter 10 - Curcuma longa, the Polyphenolic Curcumin Compound and Pharmacological Effects on Liver. *In:* WATSON, R. R. & PREEDY, V. R. (eds.) *Dietary Interventions in Liver Disease.* Academic Press.