

IN VITRO CARDIOPROTECTIVE POTENTIAL OF *Ruellia prostrata* LEAF EXTRACT AGAINST H₂O₂ INDUCED OXIDATIVE STRESS IN H9C2 CARDIOMYOCYTES

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ABSTRACT

The global incidence of cardiovascular disease (CVD) continues to remain a leading cause of disease and death, with oxidative stress being an important driver that drives damage to cardiomyocytes. The present investigation evaluated the cardioprotective power a hydroethanolic leaf extract of *Ruellia prostrata* Poir. The study tested the effect of an extract from *Acanthaceae* on a rat cell line. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric assay used to measure the cytotoxicity and cytoprotection. It was further confirmed by phase-contrast microscopy. H₂O₂ (150 µM) was used to induce the oxidative stress model. The cell viability of untreated stressed cells was found to be 52.3%. The viability restoration as a result of sequential treatment with the extract at various concentrations (2.5, 5, 10, 20 and 40 µg/mL) was 54.88%, 57.25%, 61.08%, 65.52% and 74.50%, respectively. Quercetin (20 µg/mL) was used as a standard reference which showed 92.66% viability restoration. Analysis of the morphology confirmed that cardiomyocytes treated dose dependently recover the characteristic spindle morphology. Because *prostrata* leaf extract contains phytoconstituents such as flavonoids, tannins, and phenolic acids, it has a strong cytoprotective effect. In order to comprehend other cardioprotective mechanisms of action, this extract deserves more research.

Keywords: *Ruellia prostrata*, H9C2 cardiomyocytes, hydrogen peroxide, oxidative stress, MTT assay, cytoprotection, cardioprotection

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1. INTRODUCTION

Cardiovascular illness places an enormous burden on the health care system and more than 17 million people die of these diseases every year. Take this cardio quiz to test your knowledge about cardiovascular diseases and their causes and effects. The mechanism of cardiac injury includes oxidative stress, which appears to be a common pathology linking myocardial ischemia-reperfusion, dilated cardiomyopathy, hypertension remodeling, and heart failure (Jin & Kang, 2024). The heart is particularly sensitive to redox imbalance due to its exceedingly high metabolic demand, relatively small endogenous antioxidant pool, and the abundance of mitochondria, where most reactive oxygen species (ROS) are formed.

All types of biological macromolecules are harmed by stressors that produce reactive oxygen species (ROS), such as superoxide anion (O₂^{•-}), hydrogen peroxide (H₂O₂), and the highly reactive hydroxyl radical (•OH). Increased intracellular ROS

can change calcium homeostasis, peroxidize membrane lipids, inactivate critical enzyme proteins, and damage DNA. Necrosis and apoptosis are brought on by these events (Peoples et al., 2019). Particularly well-characterized is the mitochondria-mediated/apoptotic pathway. Excessive ROS production causes an irreversible loss of cardiomyocytes by dissipating the mitochondrial membrane potential (ΔΨ_m), releasing cytochrome c into the cytosol, activating initiator caspase-9 and effector caspase-3 sequentially, and cleaving poly(ADP-ribose) polymerase (PARP) (Ryter et al. 2007).

H9C2 cell line is a clonal derivative of embryonic rat ventricular tissue (ATCC CRL-1446) and most widely adopted in vitro model in cardiovascular research (Tan et al., 2021). According to Shen and co-workers in 2022 this cell line refers to H9C2. They respond to H₂O₂ treatment and induce attenuation of

different cell functions and cell death like ischemia and reperfusion injuries.

The use of *Ruellia prostrata* Poir is a long-standing ethnopharmacological tradition in southern Asia and sub-Saharan Africa. The Acanthaceae family is utilized to treat several medicinal purposes. These include being used as a diuretic, wound healer, hypoglycaemic agent, and in the treatment of gonorrhoea and alopecia (Alhashim & Lombardo, 2021). Modern studies were carried out on *L. leucocephala* to see its bioactive properties. *L. leucocephala* contains a number of beneficial secondary metabolites. These include phenolic acids, flavonoids mainly apigenin, luteolin, and quercetin derivatives, tannins, triterpenoids, saponins, steroids and cardiac glycosides (Alhashim & Lombardo, 2021; Niyonzima et al., 2022). Certain compound classes possess established antioxidant and anti-apoptotic credentials, leading to R hypotheses. The extract from *prostrata* leaves may offer significant protection to cardiomyocytes.

Ruellia species have undergone a great deal of research on their antioxidant, anti-inflammatory and anti-cancer activities. But nothing documented is available on the cardioprotective activity of this particular *Ruellia* species. The cellular level impact of *prostrata* is unknown. Therefore the present study was designed to: (i) develop a standardised hydroethanolic extract of *R. prostrata*; (ii) assessed its cytoprotective activity under H₂O₂-induced stress on H9C2 cardiomyocytes by MTT assay; and (iii) confirmed the quantitative findings by phase contrast morphological study.

2. MATERIALS AND METHODS

Collection and Authentication of Plant Material

Leaves of *Ruellia prostrata* collected from Sengipatti village, Thanjavur District, Tamil Nadu, India (10.77°N, 79.12°E) during December 2023. The confirmation on the identity of the plant was done against authenticated herbarium specimens in the Department of Botany, VISTAS, Chennai. The leaf materials collected were washed thoroughly in running tap water to remove surface associated soil and debris, followed by a wash in distilled water. The leaves were cut into uniform pieces and spread on clean paper trays for shade drying at ambient temperature for 3 weeks until a constant weight was obtained. Sample was grained fine powder using a mixer grinder available in the laboratory. The powder was passed through a mesh for uniformity in particle size and was kept in amber glass bottles under airtight conditions at room temperature away from direct light until extraction.

Preparation of Hydroethanolic Leaf Extract.

Cold maceration, chosen to limit thermal degradation of heat-labile phenolic and flavonoid constituents, was carried out for extraction. In short, *R. Rehmannia* was dried to a weight of 10 grams. The *prostrata* leaves

powder mixed with a hydro-ethanolic solvent system (ethanol: distilled water, 70:30 v/v) at the ratio of 1:100 (g:mL) for 24 hours at room temperature with intermittent agitation to permit diffusion of solutes into the extracting solvent at intervals of 2 hours. The clarified filtrate underwent vacuum-filtration for passing the macerate through filter paper and it was used directly for bioassay studies. The amount of extract was recorded in µg/mL. The experiment was conducted in various dilutions, specifically 2.5, 5, 10, 20, and 40 µg/mL. Utilising the DMEM solution, each day of the experiment the dilutions were prepared freshly.

Cell Culture and Maintenance.

H9C2 rat cardiomyoblast cells (ATCC CRL-1446) were bought from National Center for Cell Science (NCCS), Pune, India. The culture medium for the cells comprised 10% (v/v) heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 44 mM sodium bicarbonate and an antibiotic-antimycotic mixture of penicillin G (100 IU/mL), streptomycin sulphate (100 µg/mL) and amphotericin B (2.5 µg/mL) in DMEM. The culture flasks (75 cm²) were kept in a humidified incubator at 37 °C in 5 % CO₂. The cultures were trypsinized with 0.25% trypsin-EDTA when they reached 80-85% confluence. To maintain a consistent cellular phenotype, we utilized only passages 5–15 for our experimental work.

Oxidative Stress Model

The addition of 150 µM H₂O₂ (30% stock solution; Sigma-Aldrich) in serum-free DMEM to the confluent H9C2 monolayers induced oxidative stress. This concentration was selected based on published dose-response data for H9C2 cells that reliably produces approximately 50% cell death without full cytotoxic activity thus allowing a useful cytoprotective rescue to be detected (Shen et al., 2022; Mojarrab et al., 2013). After pre-incubation with the extract, H₂O₂ was added to the cells and the treatment was kept for 6 hours before assessing viability.

Cytoprotection Study Using MTT

The cytoprotective action was analysed using the MTT colourimetric assay. The well-known assays provide for their reduction of a tetrazolium salt by metabolically active cells through mitochondrial pathways to insoluble purple formazan crystals (Mosmann, 1983; Alley et al., 1986). In 96 well flat-bottom microplates, cells (2 × 10⁴ cells/well in 200 µL complete DMEM) were seeded and their attachment was allowed overnight (18 hours) at 37°C/5% CO₂. There were four treatment groups in this study; (1) blank control – medium with no cells; (2) negative (vehicle) control – cells were left untreated; (3) cell challenged with H₂O₂ and receiving each concentration of the R.

The positive control to H₂O₂-challenge cells, which received quercetin (20 µg/mL) and *prostrata* extract

(2.5, 5, 10, 20 and 40 µg/mL). After four hours of pre-treatment with the extract, addition of 150 µM H₂O₂ was made and the cells were incubated for another six hours. Upon the aspiration of the culture media, 20 µL volume (5 mg/mL in phosphate-buffered saline) of MTT reagent was added to each well to develop a working concentration of 0.5 mg/mL. To promote formazan formation, the plates were covered in aluminium foil and placed in an incubator for 3 hours at 37 °C. Subsequently, we discarded the MTT solution and added DMSO (100 µL) and agitated gently on a plate shaker for 10 minutes to solubilise the formazan crystals. The microplate was used to read absorbance at 570 nm (reference wavelength 630 nm). The calculation for the estimate of cell viability, in terms of cell viability and absorbance, will be given as Cell Viability(%) = (Absorbancesample / Absorbancecontrol) × 100. The linear regression of the derived dose-response curve (Y = MX + C; where Y equal to 50), will be used to extrapolate the IC₅₀ value.

Morphological Evaluation through Phase Contrast

After treatment for 24 hours, cellular morphology was evaluated on an inverted phase-contrast microscope with Optika Pro5 CCD camera. Images taken at 100× magnification show representative fields of view. The parameters observed included the density of the cells, the maintenance of the characteristic spindle-shaped morphology of the cells, the presence of cytoplasmic granulation of the cells, the roundness of the cells, blebbing of the membrane, and the degree of pull-off of the cells.

Statistical Analysis

Every experiment was run on three separate occasions, and those repeats were then administered separately on three occasions (n=9 per treatment). The findings have been expressed as mean ± SD. The statistical significance of intergroup differences was evaluated using one-way analysis of variance (ANOVA) tests, followed by post hoc Tukey's multiple comparison analysis using GraphPad InStat version 3. p < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Cytoprotective Activity Determined by MTT Assay

Experimental results for viability of H9C2 cardiomyocyte cells exposed to *prostrata* leaf extract against oxidative stress induced using 150 µM H₂O₂ are stated in Table 1. The untreated control cells had virtually 100% viability whereas the 150 H₂O₂ treated cells showed reduced viability of 52.30% confirming oxidative injury as effective. Use of the extract caused a statistically significant, concentration-dependent improvement in cell viability across the range tested (2.5–40 µg/mL), from 54.88% to 74.50%. According to the test results, quercetin (at the standard concentration

of 20 µg/mL) is the positive control used and gives 92.66% viability.

Table.1: Effect of varying concentrations of extract on cytoprotective of H9C2 (Rat cardio myoblast) cell line as determined by MTT assay.

Act ivit y	H9C2 (Human synovial sarcoma cell line)							Stan dar d (Qu erce tin 20µ g/ml)
	Ce ll co nt rol	Co ntr ol + H ₂ O ₂	2.5 (µg /m L)	5 (µg /m L)	10 (µg /m L)	20 (µg /m L)	40 (µg /m L)	
Via bili ty (%)	100	52.30	54.88	57.25	61.08	65.52	74.50	92.66

% Cell viability = A 570 of treated cells / A 570 of control cells × 100%.

Figure.1: Effect of varying concentrations of extract on cytoprotective activity of H9C2 (Rat cardio myoblast) cell line as determined by MTT assay.

The profile of cytoprotection observed in this study which is dependant on concentration is similar to what has been previously reported for other medicinal plant extracts tested in the H9C2/H₂O₂ model. In H9C2 cells exposed to H₂O₂, leaf extract of *Malus doumeri* may enhance cell death. Antioxidant capacity was linked to reduced malondialdehyde formation and restoration of superoxide dismutase and catalase activity (Shen et al., 2022). Likewise, Mojarrab et al. (2013) reported that H9c2 cytotoxicity due to H₂O₂ can be reduced by extracting anethole. Current results extend this literature by showing that *R. Prostrata* matches cytoprotective effects.

Cytoprotection at 40 µg/mL presented a 74.50% magnitude of action (52.30% in the H₂O₂ control), meaning a +22.2 percentage points in viability and a relative rescue fraction of about 46.9%. Although this effect is inferior to the reference standard quercetin, it is nonetheless remarkable in view of the unfractionated mixture that the extract represents, and further purification of its active constituents may yield substantially more potent fractions. According to Chang et al. (2016), via modulation of the Akt–p53/Mdm2 axis, quercetin itself (a major kaempferol flavonoid)

prevents H₂O₂-induced apoptosis in H9C2 cells. It probably shares mechanistic pathways with other quercetin-derivative compounds present in the *R. Prostrata* Extract.

The chemical nature of *R. prostrata* may support heart by multiple beneficial methods to the heart. Flavonoids, along with phenolic acids, have the ability to act as direct scavengers that are able to purge free radicals. In addition, they are able to chelate redox-active metal ions (especially Fe²⁺ and Cu⁺) that act as catalysts in the formation of hydroxyl radicals via Fenton chemistry. Not only that, flavonoids are also able to modulate Nrf2/Keap1 antioxidant response element signalling to help upregulate the expression of endogenous antioxidant enzymes (Morariu-Briciu et al., 2026). The extract's triterpenoids and saponins may have complementary anti-inflammatory and membrane-stabilising effects. The plant's phytochemical profile also reveals the presence of cardiac glycosides which could help in maintaining the integrity of the sarcolemma under conditions of oxidative stress (Alhashim& Lombardo, 2021).

3.2 Morphological Assessment

The MTT data were confirmed qualitatively by phase contrast microscopy. The negative control group containing untreated H9C2 cells showed their characteristic spindle-shaped morphology as cells form dense, well-organized monolayers attached to the culture substrate. The effects of 150 µM H₂O₂ on cells were tested. The cells showed characteristics of oxidative damage and early apoptosis. The characteristics included loss of spindle shape and rounding and shrinkage of the cell. Also, cytoplasmic vacuolation and granulation due to organelle damage. Moreover, membrane blebbing occurred. Most of the adherent cells showed a marked reduction. It was probably due to the detachment of dead and dying cells. These morphological changes are consistent with the known pro-apoptotic effect of H₂O₂ in H9C2 cells (Zhang et al. 2013).

When cells are treated with *R. Morphological* recovery was encountered in a dose-dependent fashion. At 2.5 µg/mL, some spindle morphology was preserved, though rounding and lesser density persisted. At the concentration of 10–20 µg/mL, cell density became similar to that of controls, and spindle morphology was well-conserved. When cells were treated with 40 µg/mL, they appear morphologically organized, cellular density and attachment. They had a viability of 74.50 % at this concentration. As previously reported by Shen et al. (2022) and in other similar H₂O₂-rescue models, our observations recapitulate these patterns, collectively suggesting that extract inhibits both the necrotic and apoptotic components of oxidative cardiomyocyte injury.

Figure 2: Effect of varying concentrations of extract on cytoprotective activity of H9C2 (Rat cardio myoblast) cell line as determined by MTT assay

3.3 Mechanistic Considerations and Phytochemical Basis

When the generation of reactive oxygen species (ROS) occurs at a greater rate than our endogenous antioxidant systems can buffer, this creates oxidative stress. Specifically, the mitochondria, NOX2 and NOX4, and xanthine oxidase generate ROS (superoxide) with the help of SOD, CAT, glutathione peroxidase (GPx), and the thioredoxin system (Peoples et al., 2019; Jin & Kang, 2024). H₂O₂ forms a product as well as a substrate in the network: being a relatively stable, membrane-permeable oxidant, it can passively diffuse across membranes and generate the highly toxic hydroxyl radical via iron-catalysed Fenton reactions. The downstream effects comprise lipid peroxidation, protein carbonylation, formation of 8-hydroxydeoxyguanosine (8-OHdG) in the nuclear and mitochondrial DNA, depletion of cellular glutathione, and loss of mitochondrial membrane potential (Mao et al, 2014).

Plant polyphenols interrupt this cascade at several levels. Direct free radical scavenging stops lipid peroxidation chain reactions. Metal chelation prevents formation of hydroxyl radical. Transcriptional activation of the Nrf2 pathway increases phase II antioxidant enzymes. These all provide sustained cytoprotection after depletion of parent compound (Morariu-Briciu et al, 2026). Bax /Bcl-2 ratio shift inhibition, cytochrome c release suppression downregulation, and caspase-3 activity are some of the anti-apoptotic actions of flavonoids, shown to occur for flavonoids similar or related in structure to those found in *Ruellia* species (Yuvaraj et al., 2023; Chang et al., 2016). Luteolin that isolated from *Ruellia* species has a dose-dependent capacity to enhance the viability and inhibit apoptosis of H9C2 cells under H₂O₂ stress via modulation of intracellular ROS and apoptosis-regulatory proteins (Chang et al. 2016).

A crude hydroethanolic extract was the focus of the present study; the active principle(s) responsible for the observed cytoprotection is still unknown. Future studies utilizing activity-guided fractionation, high-performance liquid chromatography–mass spectrometry (HPLC-MS), and mechanistic assays for quantifying reactive oxygen species (ROS) using DCFH-DA fluorescent probe, measuring mitochondrial membrane potential using JC-1 staining, and assessing caspase-3/9 activity and Bcl-2/Bax protein expression would provide insights into the structural and mechanistic basis underlying cardioprotection. The translational value of these findings will also be further strengthened

by molecular docking of identified phytoconstituents in silico against validated cardiac targets.

Comparative context and significance

Rehmanniaglutinosa has cardioprotective effects. The research conducted on *prostrata* contributes to a growing body of evidence supporting phytochemical intervention against cardiac cytoprotection. Medicinal plant-derived compounds are being increasingly recognized by modern-day pharmacology as having multi-targets, being safer and more accessible to the underprivileged sections of society (Shah et al., 2019; Morariu-Briciu et al., 2026). The *R. prostrata* extract exhibited a clear dose-response relationship, a requirement for pharmacological relevance, and the highest tested dose (40 µg/mL) provided nearly additive protection, based on the extent of H₂O₂ injury reversed.

The MTT assay used to find the mitochondrial metabolic activity as an indicator of cell viability. Cells that preserve their mitochondrial reducing capacity are, by definition, maintaining sufficient bioenergetics. Adequate bioenergetics is critical for cardiomyocyte survival during ischaemia and oxidative stress. Cardiac cells are unique in that they contain around 30% mitochondrial volume and rely heavily on mitochondrial ATP production to provide the energy needed for contracting (Aon et al. 2015). Involvement of R. The ability of *prostrata* extract to preserve mitochondrial activity in H₂O₂-stressed H9C2 cells implies that there might be some degree of mitochondrial protection that would be beneficial under cardiac ischaemic conditions.

4. Conclusion

It has concentration-dependent cytoprotective effects on H9C2 rat cardiomyoblasts challenged by H₂O₂. The extract treatment from the concentrations of 2.5-40 µg/mL increased cell viability from (H₂O₂ control) 52.30% to a maximum of 74.50%. Thus, it provides an appreciable reduction of oxidative injury on cardiomyocytes. These finding were confirmed through phase-contrast microscopy that showed restored spindle shape and cellular attachment with reduced cell damage. The rich phytochemicals of *Rheum* species. The discovery that the phytochemical composition of *Prostrata* contains these phytochemicals flavonoids, tannins, phenolic acids, triterpenoids, saponins, and cardiac glycosides give a sound mechanistic justification as to why *Prostrata* showed cardioprotective activity. R's position is established by these results. *Prostrata* could be a good candidate for further phytopharmacological development. According to the researcher, the future studies should be focused on the bioactivity-guided fractionation, the identification of molecular targets, in vivo validation on rodent cardiac injury models, and toxicological

profiling to endorse its potential developments as natural cardioprotectant.

REFERENCES

- Alhashim, M., & Lombardo, J. (2021). Mechanism of action of dietary flavonoids as cardioprotective agents: An update. *Cardiology in Review*, 29(4), 207–215. <https://doi.org/10.1097/CRD.0000000000000304>
- Alley, M. C., Scudiere, D. A., Monks, A., Czerwinski, M., Shoemaker, R., & Boyd, M. R. (1986). Validation of an automated microculture tetrazolium assay (MTA) to assess growth and drug sensitivity of human tumour cell lines. *Proceedings of the American Association for Cancer Research*, 27(1), 389.
- Aon, M. A., Tocchetti, C. G., Bhatt, N., Paolocci, N., & Cortassa, S. (2015). Protective mechanisms of mitochondria and heart function in diabetes. *Antioxidants & Redox Signaling*, 22(17), 1563–1586. <https://doi.org/10.1089/ars.2014.6123>
- ATCC. (n.d.). H9c2(2-1) [CRL-1446]. American Type Culture Collection. Retrieved June 2025, from <https://www.atcc.org/products/crl-1446>
- Cao, P., Xue, Y., Guo, M., Jiang, X., Lei, Z., Gao, S., & Wu, H. (2022). The active ingredient (DSH-20) of *Salvia miltiorrhiza* flower reduces oxidative damage and apoptosis in cardiomyocytes by regulating miR-1. *Molecular Biology Reports*, 49(5), 3675–3684. <https://doi.org/10.1007/s11033-022-07175-6>
- Chang, H., Li, C., Huo, K., Wang, Q., Lu, L., Zhang, Q., & Wang, W. (2016). Luteolin prevents H₂O₂-induced apoptosis in H9C2 cells through modulating Akt-P53/Mdm2 signalling pathway. *BioMed Research International*, 2016, Article 5125836. <https://doi.org/10.1155/2016/5125836>
- Danz, E. D. B., Skramsted, J., Henry, N., Bennett, J. A., & Keller, R. S. (2009). Resveratrol prevents doxorubicin cardiotoxicity through mitochondrial stabilization and the Sirt1 pathway. *Free Radical Biology and Medicine*, 46(12), 1589–1597. <https://doi.org/10.1016/j.freeradbiomed.2009.03.011>
- Jin, S., & Kang, P. M. (2024). A systematic review on advances in management of oxidative stress-associated cardiovascular diseases. *Antioxidants*, 13(8), Article 923. <https://doi.org/10.3390/antiox13080923>
- Maiwulanjiang, M., Chen, J., Xin, G., Gong, A. G., Miernisha, A., Du, C. Y., & Tsim, K. W. (2014). The volatile oil of *Nardostachys Radix et Rhizoma* inhibits oxidative stress-

- induced cell injury via reactive oxygen species scavenging and Akt activation in H9c2 cardiomyocytes. *Journal of Ethnopharmacology*, 153(2), 491–498. <https://doi.org/10.1016/j.jep.2014.02.046>
- Mao, C. Y., Lu, H. B., Kong, N., Li, J. Y., Liu, M., Yang, C. Y., & Yang, P. (2014). Levocarnitine protects H9c2 rat cardiomyocytes from H₂O₂-induced mitochondrial dysfunction and apoptosis. *International Journal of Medical Sciences*, 11(11), 1107–1115. <https://doi.org/10.7150/ijms.9153>
- Mojarrab, M., Jamshidi, M., Ahmadi, F., Alizadeh, E., & Hosseinzadeh, L. (2013). Extracts of *Artemisia ciniformis* protect against cytotoxicity induced by hydrogen peroxide in H9c2 cardiac muscle cells through inhibition of reactive oxygen species. *Advances in Pharmacological and Pharmaceutical Sciences*, 2013, Article 141683. <https://doi.org/10.1155/2013/141683>
- Morariu-Briciu, D. M., Fijie, A. R., Bolintineanu, S. L., Pah, A. M., Chiriac, S. D., Chevereșan, A., Dumitrașcu, V., Prodan Bărbulescu, C., & Jipa, R. (2026). Medicinal plants and phytochemicals in cardioprotection—Mechanistic pathways and translational roadmap. *Life*, 16(1), Article 175. <https://doi.org/10.3390/life16010175>
- Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1–2), 55–63. [https://doi.org/10.1016/0022-1759\(83\)90303-4](https://doi.org/10.1016/0022-1759(83)90303-4)
- Niyonzima, F. N., Nkurunziza, T., Ahishakiye, E., & Ngabonziza, J. (2022). Ethnomedicinal uses, phytochemistry, and biological activities of the Acanthaceae family: A review. *Asian Pacific Journal of Tropical Biomedicine*, 12(6), 235–250. <https://doi.org/10.4103/2221-1691.346682>
- Peoples, J. N., Saraf, A., Ghazal, N., Pham, T. T., & Kwong, J. Q. (2019). Mitochondrial dysfunction and oxidative stress in heart disease. *Experimental and Molecular Medicine*, 51(12), 1–13. <https://doi.org/10.1038/s12276-019-0355-7>
- Ryter, S. W., Kim, H. P., Hoetzel, A., Park, J. W., Nakahira, K., Wang, X., & Choi, A. M. (2007). Mechanisms of cell death in oxidative stress. *Antioxidants & Redox Signaling*, 9(1), 49–89. <https://doi.org/10.1089/ars.2007.9.49>
- Shah, S. M. A., Akram, M., Riaz, M., Munir, N., & Rasool, G. (2019). Cardioprotective potential of plant-derived molecules: A scientific and medicinal approach. *Dose-Response*, 17(2), 1559325819852243. <https://doi.org/10.1177/1559325819852243>
- Shen, Y., Shen, Z., Li, P., Chen, Z., Wei, B., Liu, D., Si, X., Pan, J., Wu, D., & Li, W. (2022). Protective activity of *Malus doumeri* leaf extract on H₂O₂-induced oxidative injury in H9C2 rat cardiomyocytes. *Frontiers in Cardiovascular Medicine*, 9, Article 1005306. <https://doi.org/10.3389/fcvm.2022.1005306>
- Tan, K., Bhanu Priya, P., Namasivayam, E., & Arumugam, M. (2021). H9c2 cardiomyocytes as a cellular model for cardiovascular disease research: Advantages, limitations, and future perspectives. *Journal of Cardiovascular Development and Disease*, 8(12), Article 183.
- World Health Organization. (2023). Cardiovascular diseases (CVDs): Key facts. WHO. [https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-\(cvds\)](https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds))
- Yuvaraj, S., Ajeeth, A. K., Puhari, S. S. M., Abhishek, A., Ramprasath, T., Vasudevan, V., & Selvam, G. S. (2023). Chrysin protects cardiac H9c2 cells against H₂O₂-induced endoplasmic reticulum stress by up-regulating the Nrf2/PERK pathway. *Molecular and Cellular Biochemistry*, 478(3), 539–553. <https://doi.org/10.1007/s11010-022-04534-w>
- Zhang, F., Huang, B., Zhao, Y., Tang, S., Xu, H., Wang, L., & Yang, H. (2013). BNC protects H9c2 cardiomyoblasts from H₂O₂-induced oxidative injury through the ERK1/2 signalling pathway. *Evidence-Based Complementary and Alternative Medicine*, 2013, Article 802784. <https://doi.org/10.1155/2013/802784>
- Zhang, L., Liu, Y., Li, J. Y., Li, L. Z., Zhang, Y. L., Gong, H. Y., & Cui, Y. (2018). Protective effect of rosamultin against H₂O₂-induced oxidative stress and apoptosis in H9c2 cardiomyocytes. *Oxidative Medicine and Cellular Longevity*, 2018, Article 8415610. <https://doi.org/10.1155/2018/8415610>