ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun1163

In Vitro Investigation of Antioxidant-Mediated Cardio-Protective Effects of *Myristica fragrans* Extract

Aadil Ansari[1*]; Abhishek Nagar[2]; Rajkumari Thagele[2]

[1]Scholar, Career Point School of Pharmacy, Career Point University, Kota, Rajasthan
[2]Professor, Career Point School of Pharmacy, Career Point University, Kota, Rajasthan

Corresponding Author: Aadil Ansari*

Publication Date: 2026/06/22

Abstract: The present study investigates the in vitro antioxidant-mediated cardioprotective effects of *Myristica fragrans* seed extract. Cardiovascular diseases (CVDs) are major contributors to global morbidity and mortality, with oxidative stress playing a crucial role in their pathogenesis through the excessive generation of reactive oxygen species (ROS). The ethanolic extract of *Myristica fragrans* seeds was prepared using the maceration method, yielding 12.5% extractive value, indicating efficient extraction of bioactive constituents. Preliminary phytochemical screening revealed the presence of important secondary metabolites such as flavonoids, tannins, alkaloids, saponins, terpenoids, steroids, and glycosides, which are known for their antioxidant potential. Quantitative analysis showed significant total phenolic content (48.23 ± 0.75 mg GAE/g) and total flavonoid content (36.37 ± 0.75 mg QE/g), suggesting strong free radical scavenging capacity. In vitro antioxidant activity was evaluated using DPPH and hydrogen peroxide scavenging assays, where the extract demonstrated dose-dependent radical scavenging activity comparable to ascorbic acid. Further, cytoprotective effects were assessed using H9c2 cardiomyocyte cell lines. The extract significantly improved cell viability in oxidative stress-induced conditions (H_2O_2 exposure) as evidenced by MTT assay results. Additionally, intracellular ROS levels were markedly reduced in treated groups, confirming its antioxidant efficacy. Statistical analysis indicated significant reduction in ROS levels at higher concentrations ($p < 0.001$), highlighting the potent cardioprotective potential of the extract. Overall, the findings suggest that *Myristica fragrans* possesses substantial antioxidant properties that may contribute to its protective effects against oxidative stress-induced cardiac damage.

Keywords: *Myristica fragrans, Cardioprotective Activity, Oxidative Stress, Reactive Oxygen Species (ROS), Antioxidants, Flavonoids, Phenolic Compounds, DPPH Assay, H9c2 Cell Line.*

How to Cite: Aadil Ansari; Abhishek Nagar; Rajkumari Thagele (2026) In Vitro Investigation of Antioxidant-Mediated Cardio-Protective Effects of *Myristica fragrans* Extract. *International Journal of Innovative Science and Research Technology*, 11(6), 868-881. https://doi.org/10.38124/ijisrt/26jun1163

I. INTRODUCTION

The cardiovascular diseases (CVDs) are amongst the primary causes of mortality and morbidity globally and thus pose a significant health burden. The CVDs are caused by several factors, such as poor nutrition, lack of physical exercise, tobacco consumption, and psychological tension. One of the biological pathways implicated in the pathogenesis of the cardiovascular diseases is oxidative stress, which is a consequence of an imbalance between the formation of reactive oxygen species (ROS) and the antioxidant response of the body.

The reactive oxygen species are extremely unstable entities that cause cellular damage through the disruption of membranes, protein structures, and genetic material. Within the cardiovascular system, an abundance of reactive oxygen species is associated with dysfunctional endothelium, inflammatory responses, oxidation of fatty substances, and tissue injury to cardiac muscle cells. These detrimental processes have been shown to play a pivotal role in the development of cardiovascular pathologies, including atherosclerosis. Thus, the reduction of oxidative stress via the administration of antioxidants is one of the promising treatment approaches for CVDs.

Antioxidants are chemical compounds that help in the neutralization of free radicals. Antioxidants can either be produced by the body (endogenous) or consumed from food sources (exogenous). Recently, there has been growing interest in the use of natural antioxidants found in medicinal plants due to their efficacy, safety, and lack of side effects

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun1163

compared to synthetic antioxidants. Natural antioxidants like flavonoids, phenolic compounds, and essential oils have been shown to possess excellent free-radical scavenging abilities and anti-inflammatory properties and are thus helpful for heart disease prevention.

II. MATERIALS AND METHODS

➢ *Collection and Authentication of Plant Material:*

The seeds of *Myristica fragrans* will be collected from a reliable and authenticated source.

➢ *Preparation of Nutmeg Extract:*

V mechanical grinder, 300 gm of nutmeg seeds were powdered. Then, absolute ethanol(500 ml) was added; the powder was mixed and shaken for 72 h and the supernatant solvent was removed by using a rotary evaporator (in a vacuum, temperature under 45°C). For evaporating the solvent entirely, the extract was collected in a plastic bottle and then stored at −80°C to prevent oxidative damage (Ghorbanian et al 2019).

➢ *Determination of Extractive Value*

The determination of extractive value is an important parameter used to evaluate the efficiency and effectiveness of the extraction process. The percentage extractive value was calculated by comparing the weight of the dried extract with the initial weight of the powdered plant material taken for extraction. The calculation was performed using the following formula:

Extractive value (%) = (Weight of dried extract / Weight of initial plant material) × 100

➢ *Phytochemical Screening*

Phytochemical studies for preliminary identification of the presence or absence of important secondary metabolites like alkaloids, saponins, tannins, flavonoids, steroids, glycosides, terpenoids, and phlobotannins were conducted in the case of the M. fragrans plant extract. Qualitative tests based on color changes and precipitation reactions were employed in the study. These tests help in detecting the presence of bioactive ingredients.

- *Test for Alkaloids (Dragendorff's Test)*

The extract in the amount of about 5 g was diluted in 5 ml of dilute hydrochloric acid (1% HCl). After that, a few drops of Dragendorff's reagent were added to the solution obtained. Appearance of orange-brown or bluish-black precipitates means that the drug contains alkaloids. It is well known that alkaloids can exert various pharmacological effects such as analgesic and antimicrobial actions.

- *Test for Saponins (Frothing Test)*

About 5 g of the extract was added to distilled water and stirred thoroughly inside a test tube. A persistent foam that remains in the solution for several minutes is an indication that there are saponins present. Saponins have been known to have permeability and antioxidant effects.

- *Test for Tannins (Ferric Chloride Test)*

Approximately 5 grams of the extract was boiled with distilled water, then cooled and filtered. To the filtrate, a few drops of ferric chloride solution were added. Formation of a blue-black or greenish-black color indicates that the substance is a tannin. Tannins have potent antioxidant and astringent characteristics.

- *Test for Phlobotannins*

The solution prepared from the extract was titrated with dilute HCl. The presence of phlobotannins is confirmed by the precipitation of red color. Phlobotannins are condensed tannins having antioxidant and anti-inflammatory properties.

- *Test for Flavonoids (Alkaline Reagent Test)*

A small amount of extract was treated with a few drops of dilute ammonium solution followed by the addition of concentrated sulfuric acid (H_2SO_4). The development of a yellow coloration, which disappears on standing, confirms the presence of flavonoids. Flavonoids are potent antioxidants and contribute significantly to free radical scavenging.

➢ *Estimation of Total Phenolic and Flavonoid Content*

- *Estimation of Total Phenolic Content (TPC) – Folin–Ciocalteu Method Procedure*

✓ Prepare a suitable concentration of the plant extract (e.g., 1 mg/ml).
✓ Pipette 0.5 ml of the extract into a test tube.
✓ Add 2.5 ml of 10% Folin–Ciocalteu reagent and mix well.
✓ Allow the mixture to stand for 5 minutes at room temperature.
✓ Add 2 ml of 7.5% sodium carbonate solution to the mixture.
✓ Incubate the reaction mixture for 30 minutes in the dark at room temperature.
✓ Measure the absorbance at 765 nm using a UV–Visible spectrophotometer.

- *Standard Calibration Curve*

✓ Prepare gallic acid solutions of different concentrations (e.g., 10–100 µg/ml).
✓ Treat them using the same procedure as above.
✓ Plot a graph of absorbance vs concentration to obtain a standard curve.

- *Estimation of Total Flavonoid Content (TFC) – Aluminum Chloride Method Procedure*

✓ Prepare the extract solution at a suitable concentration (e.g., 1 mg/ml).
✓ Take 0.5 ml of the extract in a test tube.
✓ Add 1.5 ml of methanol to the extract.
✓ Add 0.1 ml of 10% aluminum chloride solution.
✓ Add 0.1 ml of potassium acetate solution.
✓ Add 2.8 ml of distilled water to make up the volume.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun1163

- ✓ Mix thoroughly and incubate at room temperature for 30 minutes.
- ✓ Measure the absorbance at 415 nm using a UV–Visible spectrophotometer.

➢ *In Vitro Antioxidant Assays*

- *DPPH Radical Scavenging Assay Procedure*

- ✓ Prepare 0.1 mM DPPH solution in methanol (freshly prepared and protected from light).
- ✓ Prepare different concentrations of the plant extract (e.g., 10, 20, 40, 60, 80, 100 µg/ml).
- ✓ Take 1 ml of DPPH solution in test tubes.
- ✓ Add 1 ml of each concentration of extract to the respective test tubes.
- ✓ For control, mix 1 ml DPPH + 1 ml methanol (without extract).
- ✓ For standard, use ascorbic acid instead of extract.
- ✓ Mix thoroughly and incubate the mixture in the dark for 30 minutes at room temperature**.**
- ✓ Measure the absorbance at 517 nm using a UV–Visible spectrophotometer.

- *Hydrogen Peroxide (H_2O_2) Scavenging Assay Procedure*

- ✓ Prepare hydrogen peroxide solution (40 mM) in phosphate buffer (pH 7.4).
- ✓ Prepare different concentrations of the plant extract (e.g., 10–100 µg/ml).
- ✓ Take 0.6 ml of H_2O_2 solution in test tubes.
- ✓ Add different concentrations of extract to each test tube.
- ✓ For control, use H_2O_2 solution without extract**.**
- ✓ Incubate the reaction mixture for 10 minutes at room temperature**.**
- ✓ Measure the absorbance at 230 nm using a UV–Visible spectrophotometer against a blank solution containing phosphate buffer only.

- *Cell Culture Studies*
  Procedure for Culturing H9c2 Cells (Cardiac Cells)

- ✓ H9c2 rat cardiomyoblast cells were obtained from a certified cell repository and stored under cryopreserved conditions until use.
- ✓ The frozen vial was rapidly thawed in a 37°C water bath under aseptic conditions.
- ✓ The thawed cell suspension was transferred into a sterile centrifuge tube containing pre-warmed complete medium.
- ✓ Cells were centrifuged to remove DMSO (cryoprotectant), and the supernatant was carefully discarded.
- ✓ The cell pellet was resuspended in fresh complete growth medium.
- ✓ The complete culture medium consisted of DMEM supplemented with 10% FBS**,** penicillin (100 U/mL), and streptomycin (100 µg/mL).
- ✓ The cell suspension was transferred into sterile culture flasks under laminar airflow conditions.
- ✓ The flasks were incubated at 37°C in a humidified atmosphere with 5% CO_2**.**
- ✓ Cells were allowed to grow until they reached 70–80% confluency**.**
- ✓ For experiments, cells were seeded at a density of 1×10^4 cells/well in multi-well plates.
- ✓ Plates were gently rocked to ensure uniform cell distribution.
- ✓ Cells were incubated for 24, 48, and 72 hours**.**
- ✓ At each time point, cells were observed under an inverted phase-contrast microscope**.**
- ✓ Morphological observations included:

  - ▪ Cell shape (elongated, spindle-shaped)
  - ▪ Attachment
  - ▪ Cell density

- ✓ Cell viability and confluency were assessed using standard methods (e.g., MTT assay)**.**
- ✓ Culture conditions such as pH, contamination, and nutrient status were regularly monitored.
- ✓ Spent medium was replaced with fresh pre-warmed medium when required.
- ✓ Observations including cell viability %, confluency, and morphological changes were recorded.
- ✓ Healthy H9c2 cells showed elongated, muscle-like morphology and uniform growth, indicating proper culture conditions.

➢ *Assessment of Cytoprotective Effect (MTT Assay) Procedure*

- *Cell Seeding:*

- ✓ Seed cells into a 96-well plate at a density of approximately 1×10^4 cells per well.
- ✓ Incubate overnight at 37°C in a CO_2 incubator (5% CO_2) to allow proper cell attachment.

- *Treatment with Extract:*

- ✓ Replace the medium with fresh medium containing different concentrations of *Myristica fragrans* extract.
- ✓ Incubate for a specific time period (e.g., 24 hours).

- *Induction of Oxidative Stress:*

- ✓ Add hydrogen peroxide (H_2O_2) to induce oxidative stress in the cells.
- ✓ Incubate for an additional 2–4 hours depending on the experimental design.

- *Addition of MTT Reagent:*

- ✓ Add MTT solution (0.5 mg/ml) to each well.
- ✓ Incubate for 3–4 hours at 37°C.
- ✓ During this period, viable cells convert MTT into purple formazan crystals.

- *Solubilization of Formazan Crystals:*

- ✓ Carefully remove the culture medium without disturbing the crystals.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun1163

- ✓ Add DMSO (100–150 µl per well) to dissolve the formazan crystals.
- ✓ Gently shake the plate to ensure complete dissolution.

- *Measurement of Absorbance:*

- ✓ Measure the absorbance at 570 nm using a microplate reader.

➤ *Measurement of Intracellular ROS Levels Procedure*

- *Cell Seeding:*

- ✓ Seed cells in a 6-well plate (1×10^5 cells/well) or 96-well plate.
- ✓ Incubate overnight at 37°C with 5% CO_2 to allow cell attachment.

- *Treatment with Extract:*

- ✓ Treat cells with different concentrations of *Myristica fragrans* extract.
- ✓ Incubate for 2 hours (pre-treatment).

- *Induction of Oxidative Stress:*

- ✓ Add oxidative stress inducer (e.g., hydrogen peroxide or rotenone).
- ✓ Incubate for 24 hours.

- *DCFH-DA Staining:*

- ✓ Remove the culture medium and wash cells gently with PBS.
- ✓ Add DCFH-DA solution (10 µM) to each well.
- ✓ Incubate for 30 minutes at 37°C in the dark.

- *Washing Step:*

- ✓ After incubation, wash the cells twice with PBS to remove excess dye.

- *Fluorescence Measurement:*

- ✓ Add fresh PBS to the wells.
- ✓ Measure fluorescence intensity using:
- ✓ Fluorescence microplate reader (Excitation: 485 nm, Emission: 530 nm)

➤ *Statistical Analysis*

All experiments were performed in triplicate, and results were expressed as mean ± standard error of mean (SEM). Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by appropriate post hoc tests such as Tukey's or Dunnett's test. A p-value of less than 0.05 was considered statistically significant, indicating meaningful differences between experimental groups.

III. RESULT AND DISCUSSION

➤ *Preparation of Plant Extract:*

Table 1 Extraction Yield and Physical Characteristics of Plant Extract

S. No.	Parameter	Observation / Value
1	Plant part used	Dried Seeds
2	Weight of powdered material (g)	100 g
3	Solvent used	Ethanol
4	Volume of solvent used	500 ml
5	Method of extraction	Maceration (with shaking)
6	Duration of extraction	72 hours
7	Temperature condition	Room temperature
8	Weight of dried extract obtained (g)	12.5 g
9	Percentage yield (%)	12.5%
10	Color of extract	Dark brown
11	Consistency	Semi-solid
12	Odor	Characteristic

Table 1 below shows observations and results observed when extracting the plant material with ethanol through the maceration technique. The factors shown in Table 1 have a significant influence on the effectiveness of the process.

Percentage yield (12.5%) is an essential variable that shows how effective the extraction method was. It is obtained by dividing the weight of the extract by the initial plant material. A high percentage yield means the process was efficient, and there was more extraction.

➤ *Determination of Extractive Value*

Table 2 Determination of Extractive Value of Plant Extract (Ethanolic Extract)

S. No.	Weight of Plant Material (g)	Weight of Dried Extract (g)	Extractive Value (%)
1	100	12.3	12.3%

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun1163

2	100	12.7	12.7%
3	100	12.5	12.5%
	Mean ± SD	—	**12.5 ± 0.20%**

The table reflects the estimation of the extractive value of the plant raw material with ethanol as the extraction solvent. The extractive value is an essential characteristic in pharmacognosy since it characterizes the quantity of the active ingredients extractable from a certain quantity of crude drug.

The weight of dried extracts (12.3 g, 12.7 g, 12.5 g) reflects the quantity of the solid fraction left over after full evaporation of the solvent used. The differences in the values observed can be explained by insignificant deviations that could occur throughout the experiment.

➢ *Phytochemical Screening:*

Table 3 Qualitative Phytochemical Screening of Plant Extract

S. No.	Phytochemical Constituent	Test Performed	Observation	Result
1	Alkaloids	Mayer's / Dragendorff's test	Cream/orange precipitate	+
2	Flavonoids	Shinoda test	Pink/red coloration	+
3	Tannins	Ferric chloride test	Blue-black coloration	+
4	Saponins	Foam test	Persistent froth formation	+
5	Terpenoids	Salkowski test	Reddish-brown coloration	+
6	Steroids	Liebermann–Burchard test	Green coloration	+
7	Glycosides	Keller–Killiani test	Brown ring formation	+
8	Proteins	Biuret test	Violet coloration	–
9	Carbohydrates	Molisch's test	Violet ring formation	+

The table above shows the findings of the initial qualitative phytochemical analysis of the plant extract. The process involves the identification of various classes of bioactive compounds whose presence or absence influences the medicinal and pharmacological properties of the herb.

➢ *Estimation of Total Phenolic and Flavonoid Content*

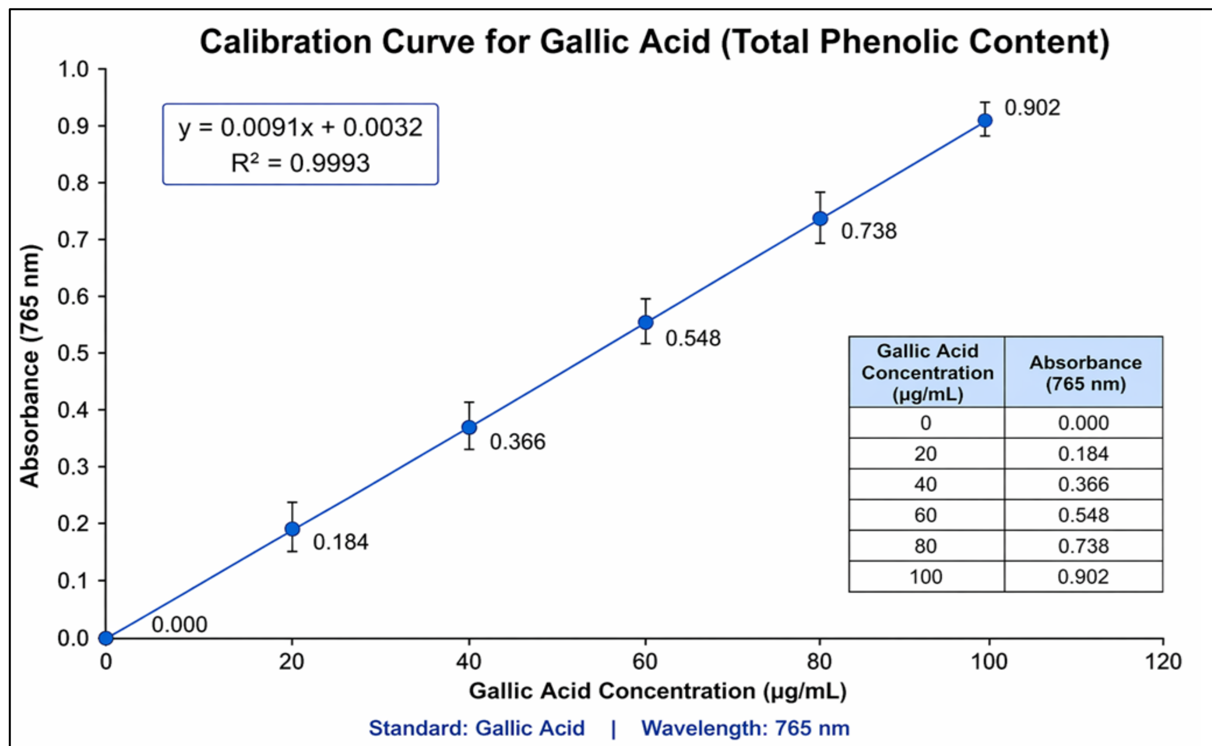
Table 4 Total Phenolic Content (TPC) of Plant Extract

S. No.	Absorbance (765 nm)	TPC (mg Gallic Acid Equivalent/g extract)
1	0.485	48.2
2	0.492	49.0
3	0.478	47.5
	Mean ± SD	**48.23 ± 0.75 mg GAE/g**

Table 5 Total Flavonoid Content (TFC) of Plant Extract

S. No.	Absorbance (415 nm)	TFC (mg Quercetin Equivalent/g extract)
1	0.365	36.4
2	0.372	37.1
3	0.358	35.6
	Mean ± SD	**36.37 ± 0.75 mg QE/g**

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun1163



Gallic Acid Concentration (µg/mL)	Absorbance (765 nm)
0	0.000
20	0.184
40	0.366
60	0.548
80	0.738
100	0.902

Fig 1 Calibration Curve of Gallic Acid showing the Relationship between Concentration (µg/mL) and Absorbance at 765 nm Used for the Estimation of Total Phenolic Content.

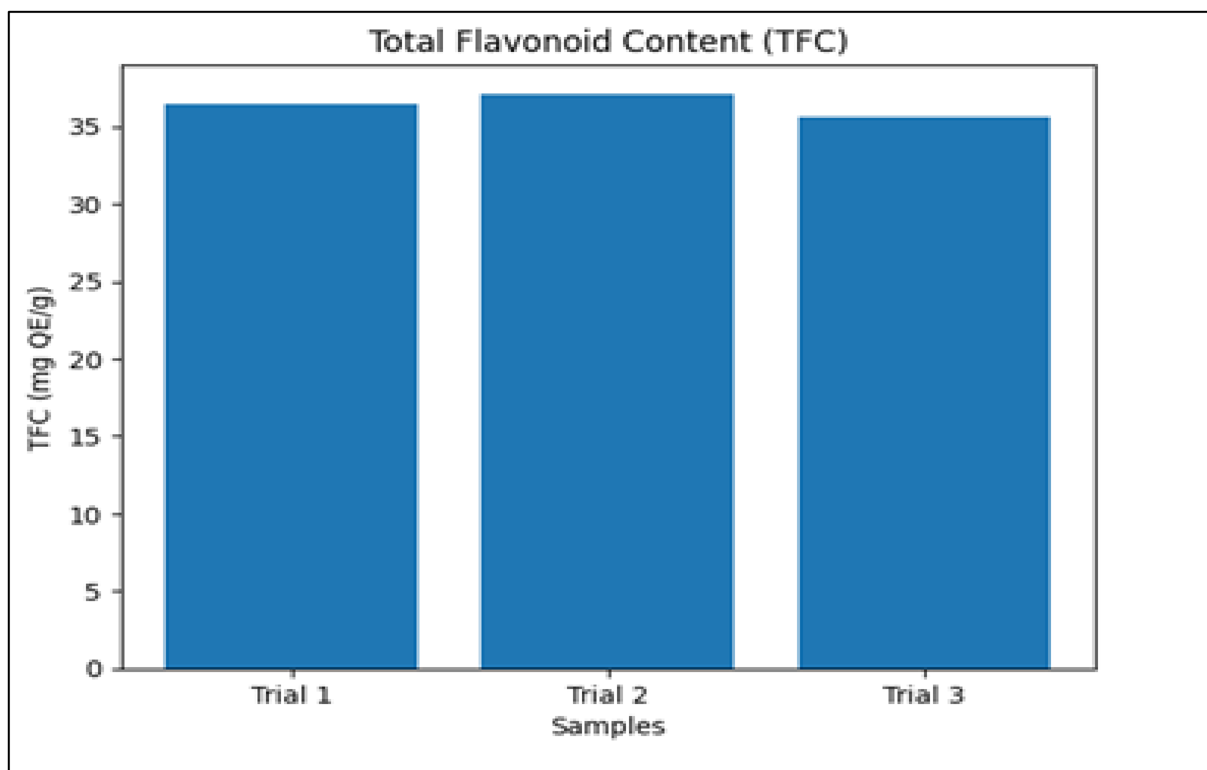


Fig 2 Bar Graph showing Total Flavonoid Content (TFC) of the Plant Extract (mg QE/g)

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun1163

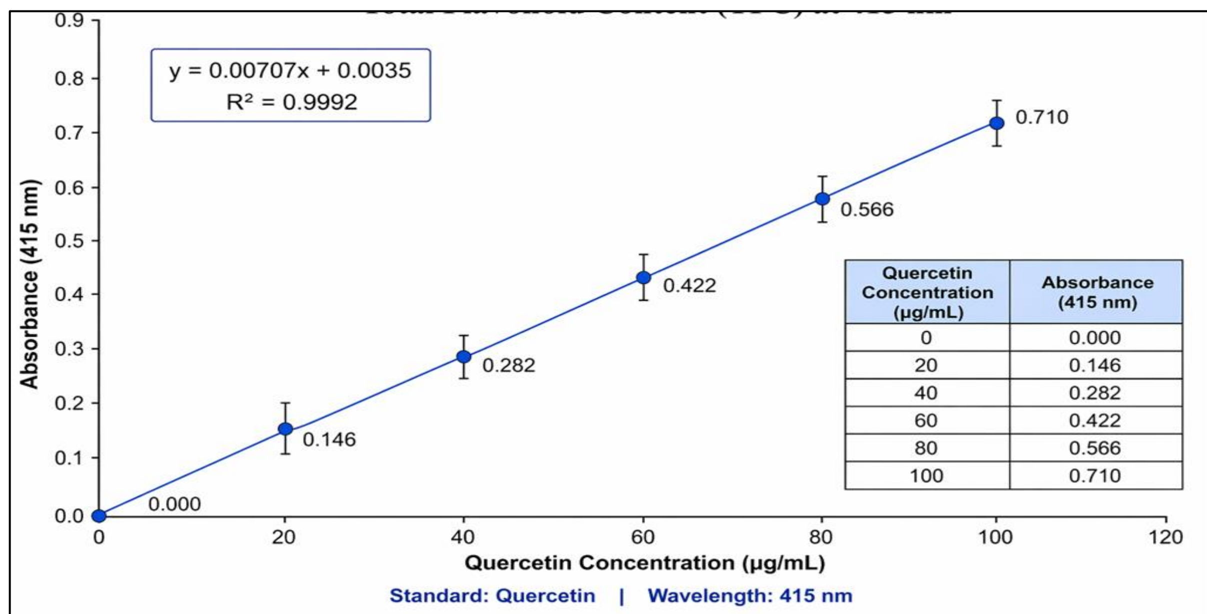


Fig 3 Calibration curve of Quercetin Showing the Relationship between Concentration (µg/mL) and Absorbance at 415 nm Used for the Estimation of Total Flavonoid Content

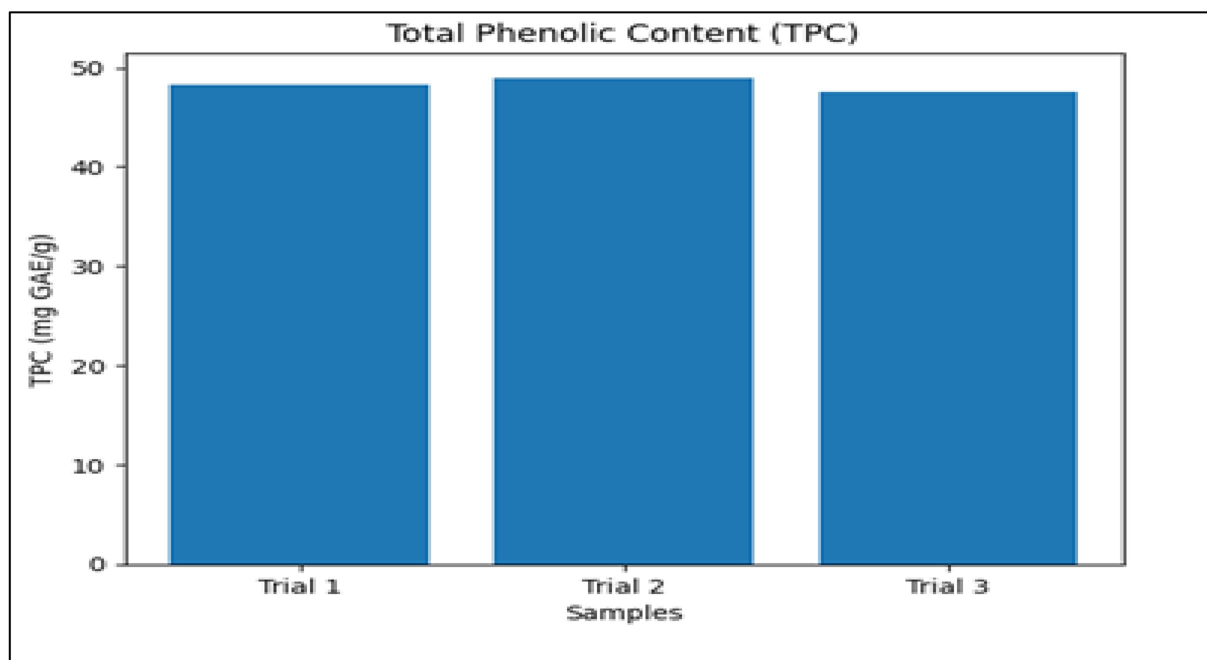


Fig 4 Bar Graph showing Total Phenolic Content (TPC) of the Plant Extract (mg GAE/g)

The above tables present data related to the calculation of total phenol content (TPC) and total flavonoid content (TFC) in the plant extract under study. Phenolic compounds and flavonoids represent an important class of antioxidants contained in plants.

➢ *In vitro Antioxidant Assays:*

Table 6 DPPH Radical Scavenging Activity of Plant Extract

S. No.	Concentration (µg/ml)	% Inhibition (Plant Extract)	% Inhibition (Ascorbic Acid)
1	10	15	22
2	20	28	40
3	40	45	60
4	60	58	72
5	80	68	82
6	100	78	92

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun1163

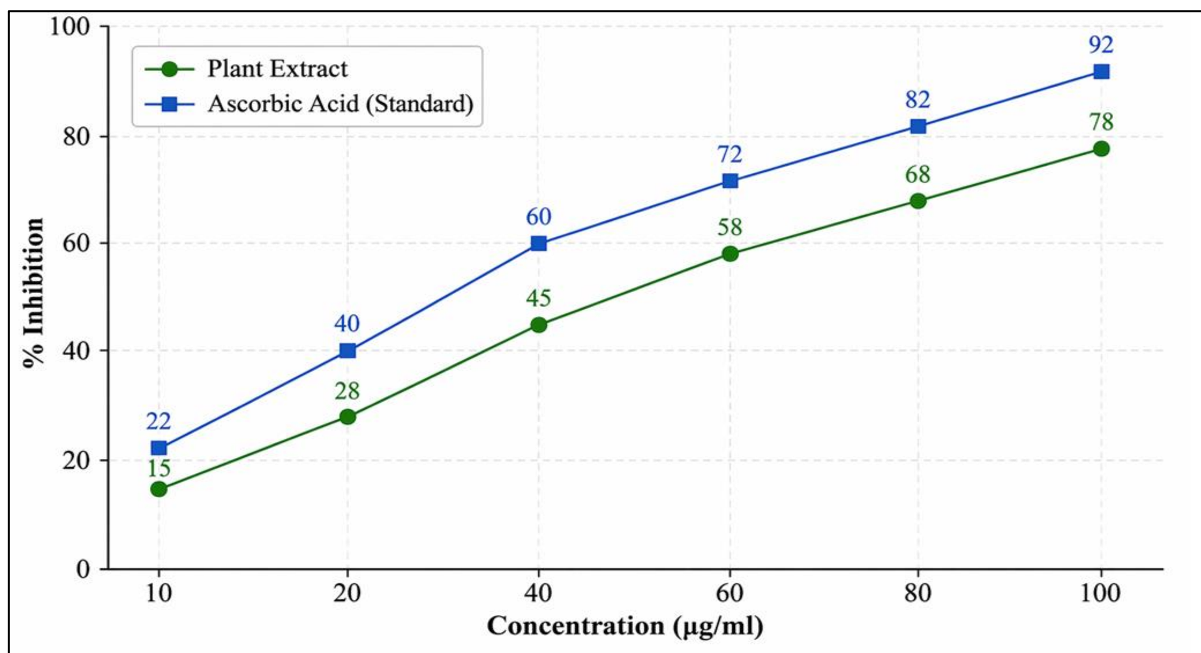


Fig 5 Line Graph showing DPPH Radical Scavenging Activity of Plant Extract and Standard (Ascorbic Acid)

Table 7 Hydrogen Peroxide (H_2O_2) Scavenging Activity

S. No.	Concentration (μg/ml)	% Inhibition (Plant Extract)	% Inhibition (Ascorbic Acid)
1	10	12	18
2	20	20	30
3	40	33	45
4	60	46	60
5	80	58	72
6	100	70	85

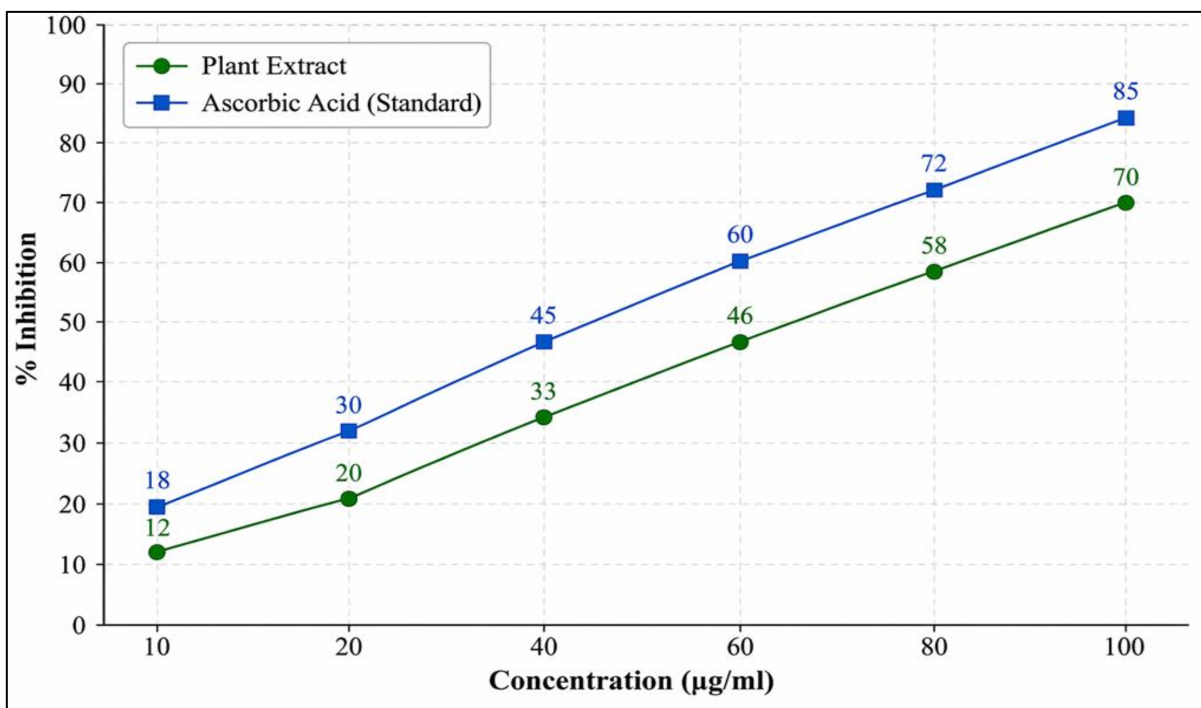


Fig 6 Line Graph showing Hydrogen Peroxide (H_2O_2) Scavenging Activity of Plant Extract and Standard (Ascorbic Acid)

The plots indicate the antioxidant properties of the plant extract based on in vitro analysis using two common techniques: DPPH radical scavenging test and hydrogen peroxide (H_2O_2) scavenging test. These tests are commonly employed for determining the free radical scavenging properties of plant extracts.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun1163

➤ *Cell Culture Studies:*

Table 8 Growth and Viability of H9c2 Cardiac Cells

S. No.	Seeding Density (cells/well)	Incubation Time (hrs)	Cell Viability (%)	Confluency (%)	Morphological Observation
1	1×10^4	24	90 ± 1.4	55	Round to slightly elongated cells
2	1×10^4	48	93 ± 1.2	75	Elongated, spindle-shaped cardiac cells
3	1×10^4	72	95 ± 1.0	90	Dense, elongated cells with uniform growth

The proliferation and vitality of cardiac cells were investigated by the H9c2 in vitro experiments. The cultivation of cells offers an appropriate milieu to analyze the activities, proliferation, and morphology of cells.

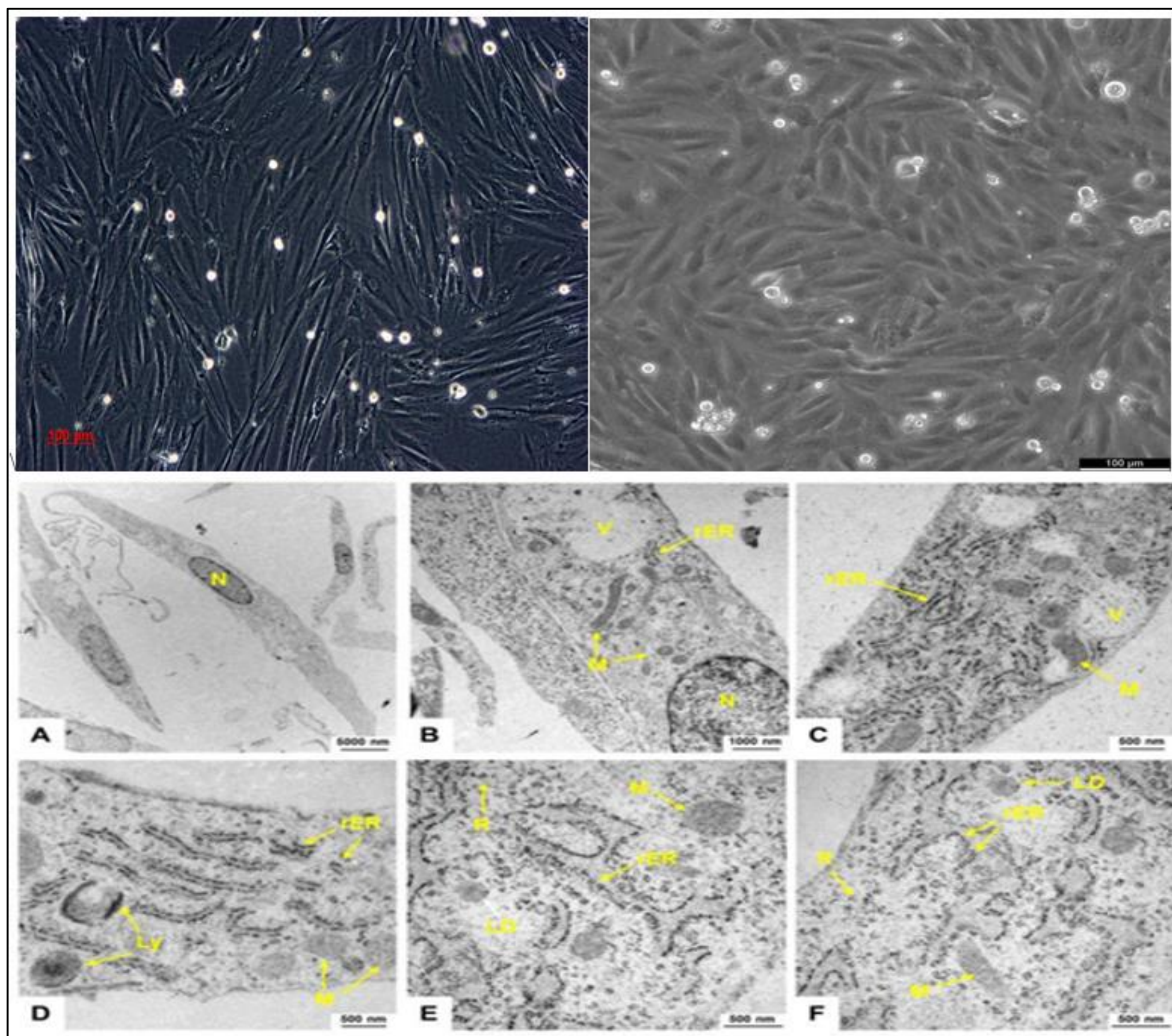


Fig 7 Growth, Viability and Morphological Changes of H9c2 Cardiac Cells Over Time (24–72 hrs)

➤ *Assessment of Cyto-protective Effect (MTT Assay):*

Table 9 Effect of Plant Extract on Cell Viability (MTT Assay)

S. No.	Group	Concentration (µg/ml)	Cell Viability (%)
1	Normal Control	—	100 ± 0.00
2	Toxic Control (H_2O_2 treated)	—	48 ± 1.5
3	Standard (Ascorbic acid)	100	88 ± 1.2
4	Test Group I	25	58 ± 1.3
5	Test Group II	50	68 ± 1.4
6	Test Group III	75	78 ± 1.1

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun1163

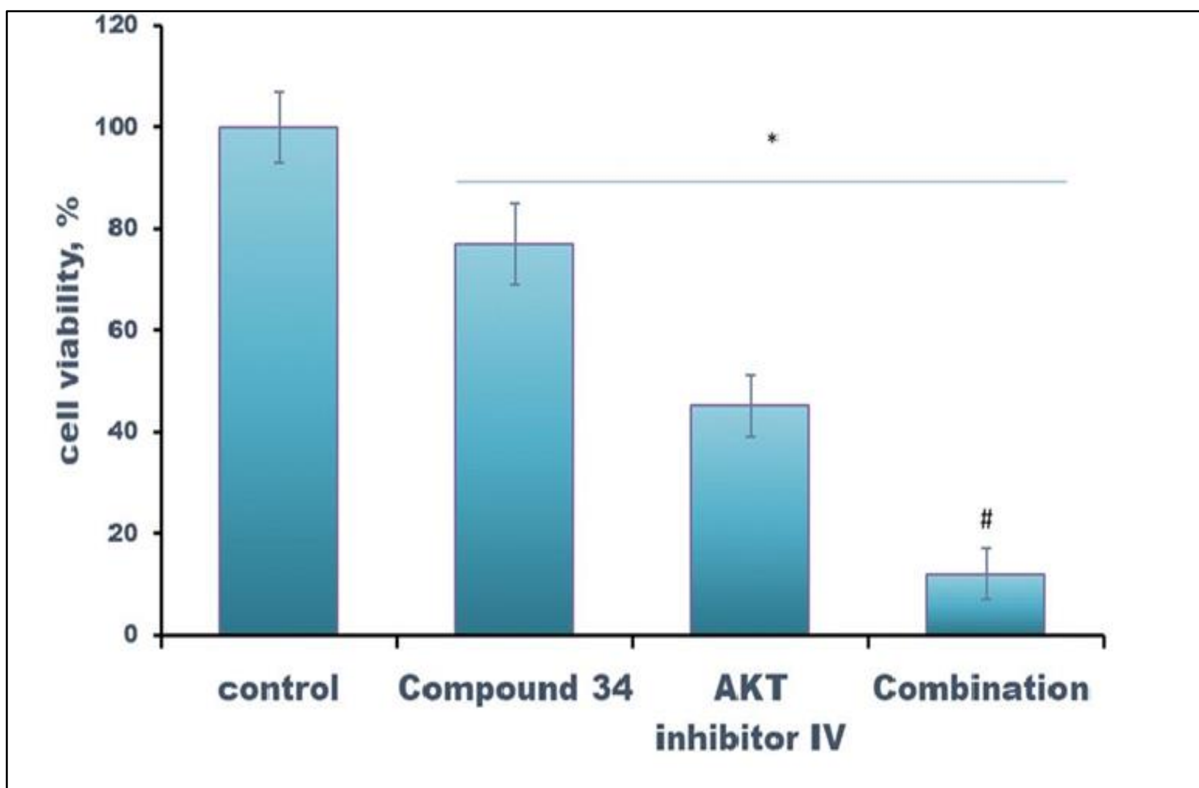


Fig 8 Effect of Plant Extract on Cell Viability in H9c2 Cells Determined by MTT Assay.

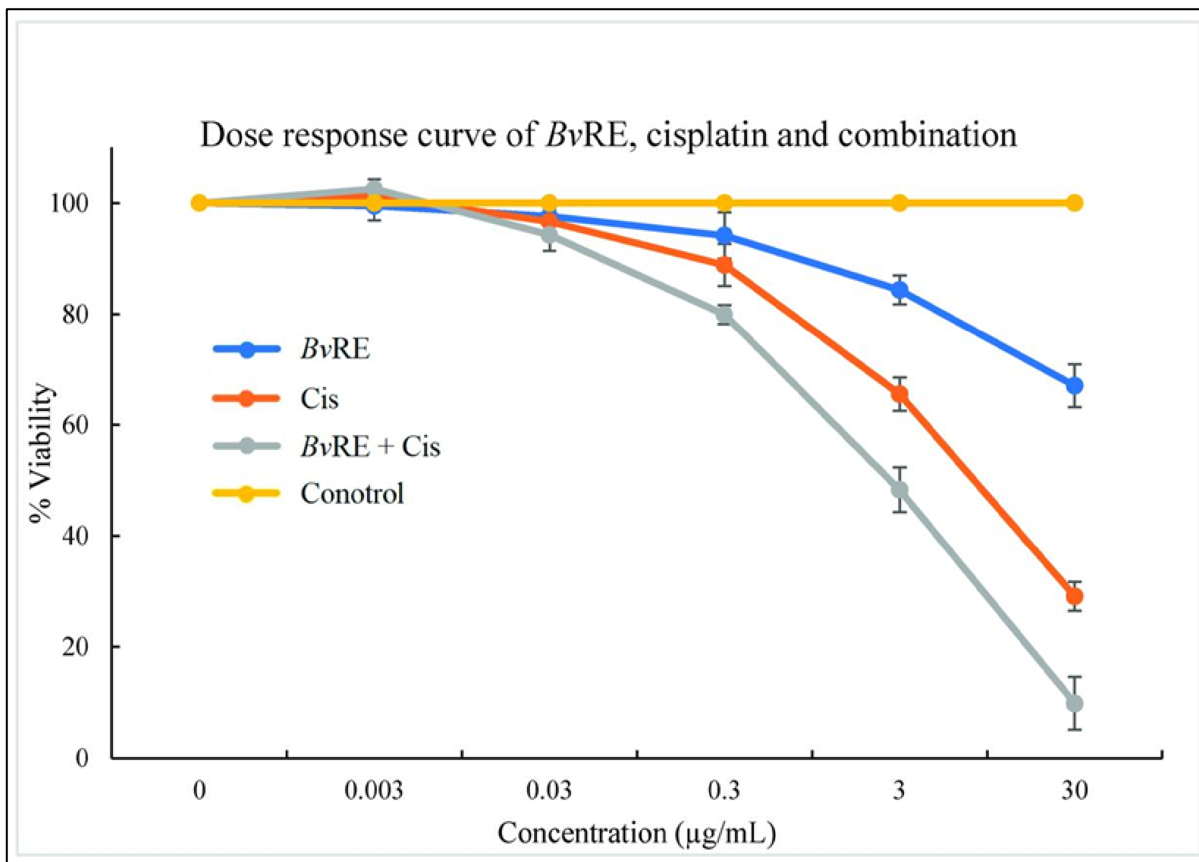


Fig 9 Dose–Response Curve Showing the Effect of BvRE, Cisplatin, and their Combination on Cell Viability Across Varying Concentrations

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun1163

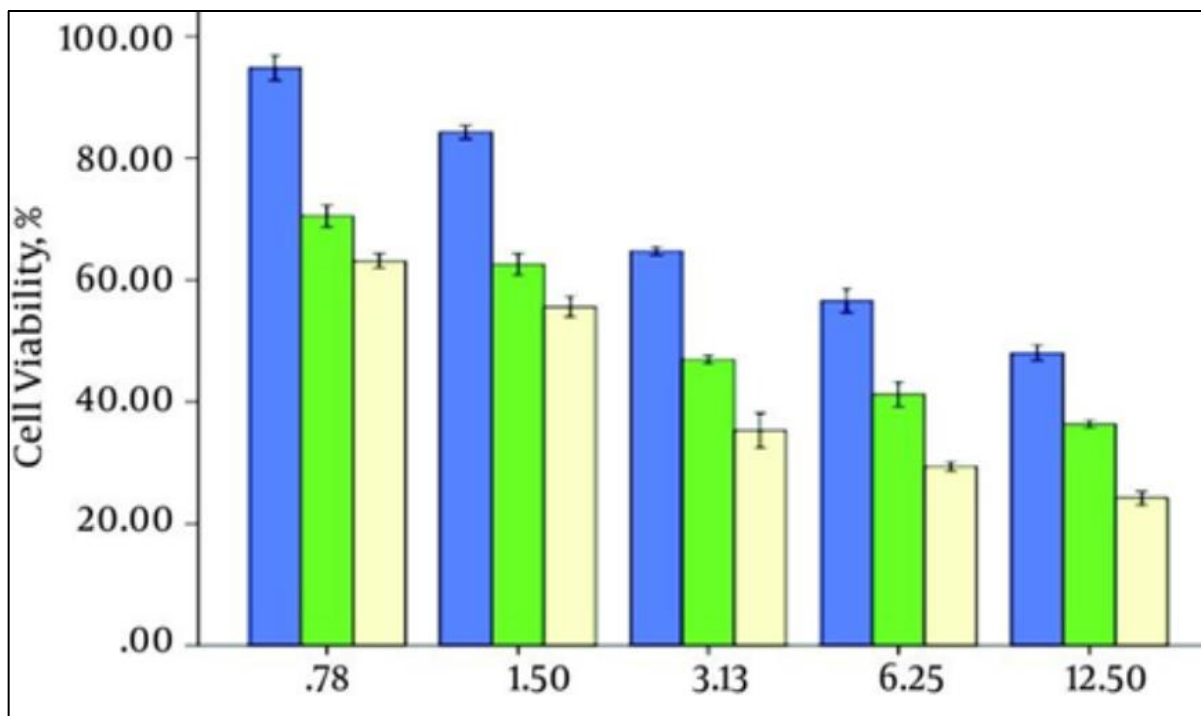


Fig 10 H_2O_2-Treated Cells Showed Reduced viability, while Treatment with Plant Extract (25–75 µg/ml) and Ascorbic Acid Improved Cell Viability in a Dose-Dependent Manner.

The table represents the evaluation of the cytoprotective effect of the plant extract using the MTT assay. This assay is based on the ability of viable (living) cells to convert the yellow-colored MTT reagent into purple formazan crystals by mitochondrial enzymes. Therefore, higher absorbance (or % viability) indicates a greater number of living cells.

Table 10 Effect of Plant Extract on Intracellular ROS Levels

S. No.	Group	Concentration (µg/ml)	Fluorescence Intensity (a.u.)	% ROS Level
1	Normal Control	—	100 ± 2.0	100%
2	Toxic Control (H_2O_2 treated)	—	220 ± 3.5	220%
3	Standard (Ascorbic acid)	100	120 ± 2.5	120%
4	Test Group I	25	190 ± 3.0	190%
5	Test Group II	50	160 ± 2.8	160%
6	Test Group III	75	130 ± 2.2	130%

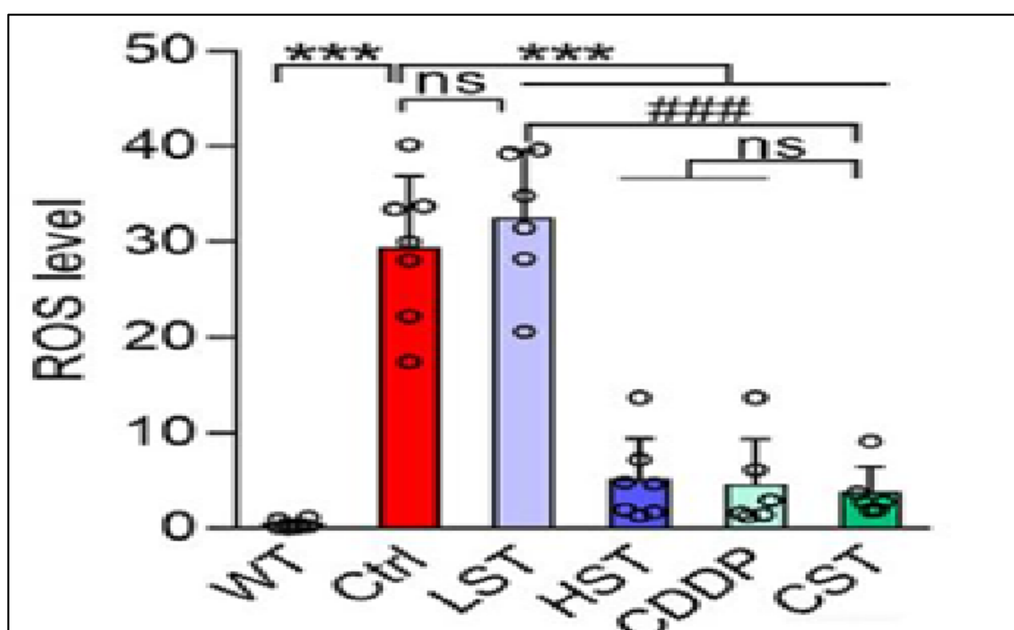


Fig 11 Effect of Plant Extract on Intracellular ROS Levels in H9c2 Cells

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun1163

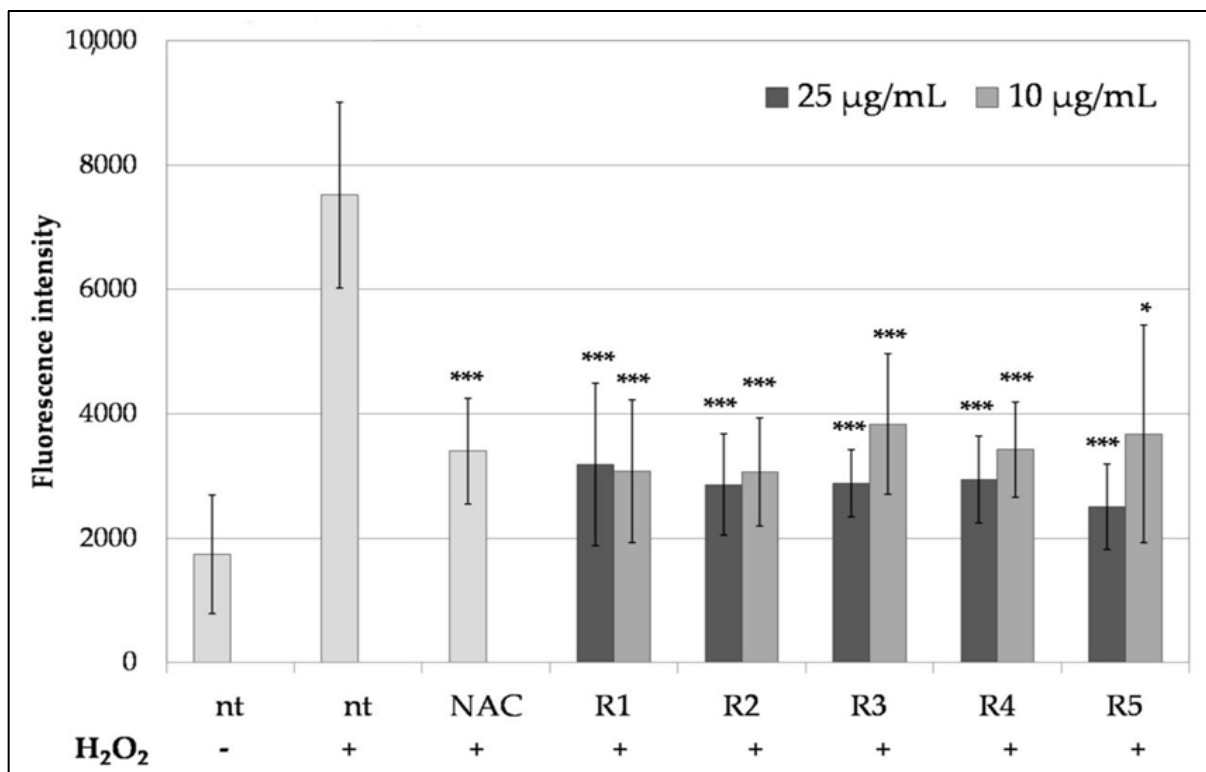


Fig 12 Effect of Plant Extract on Intracellular ROS Production Measured by Fluorescence Intensity

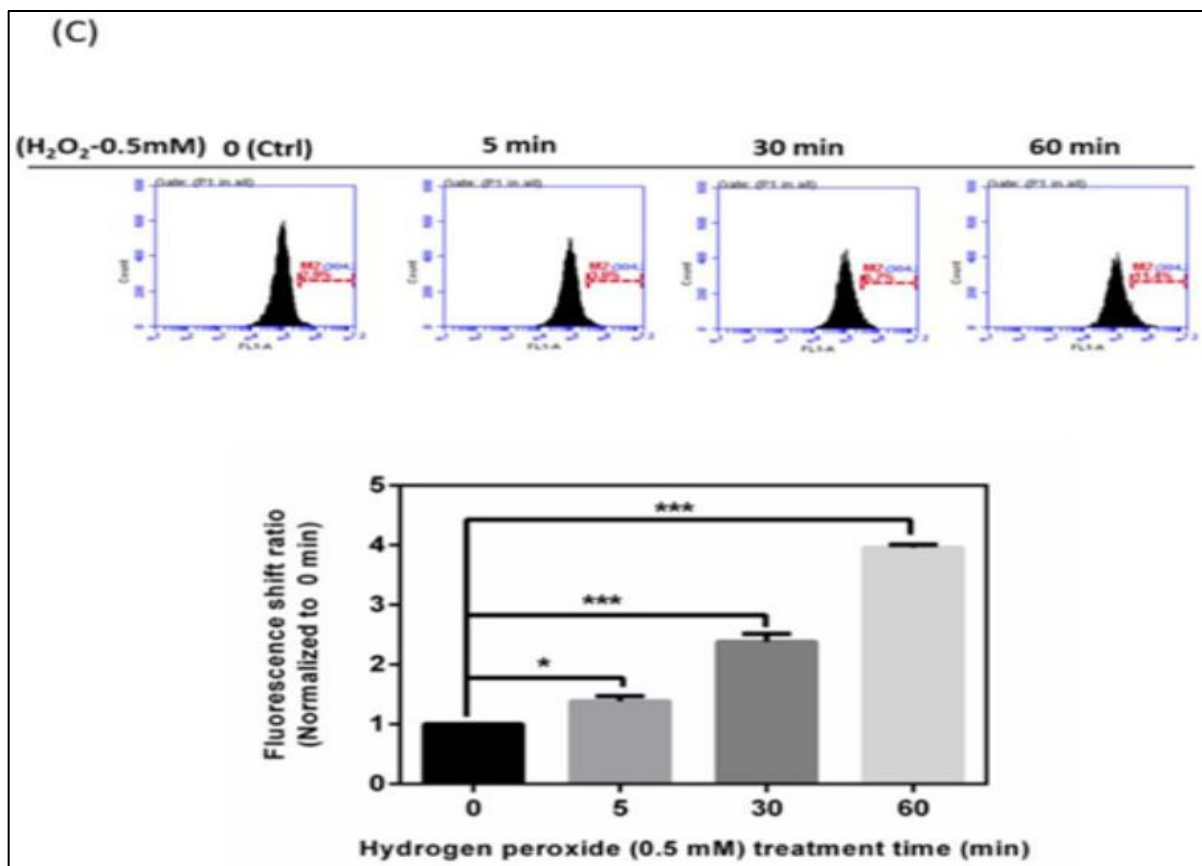


Fig 13 Intracellular ROS Levels in H9c2 Cells Following Treatment with Plant Extract and Ascorbic Acid, Expressed as Percentage Relative to Control.

Assessment of the level of intracellular reactive oxygen species (ROS) by the DCFH-DA fluorescence dye is an extensively employed in vitro method for the assessment of the antioxidative effects of plants extracts.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun1163

➢ *Statistical Analysis:*

Table 11 Statistical Analysis of Intracellular ROS Levels (DCFH-DA Assay) (One-way ANOVA followed by Dunnett's post hoc test vs Toxic Control)

S. No.	Group	Fluorescence Intensity (a.u.)	% ROS Level	Statistical Significance (vs Toxic Control)
1	Normal Control	100 ± 2.0	100%	***$p < 0.001$
2	Toxic Control (H_2O_2 treated)	220 ± 3.5	220%	—
3	Standard (Ascorbic acid, 100 µg/ml)	120 ± 2.5	120%	***$p < 0.001$
4	Test Group I (25 µg/ml)	190 ± 3.0	190%	ns (not significant) / $p > 0.05$
5	Test Group II (50 µg/ml)	160 ± 2.8	160%	**$p < 0.01$
6	Test Group III (75 µg/ml)	130 ± 2.2	130%	***$p < 0.001$

The data were analyzed using one-way Analysis of Variance (ANOVA) to determine whether significant differences existed among the experimental groups. This was followed by an appropriate post hoc test (e.g., Dunnett's test) to compare each treatment group with the toxic control group.

The results are expressed as mean ± standard error of mean (SEM), which indicates the precision and variability of the data. A p-value less than 0.05 ($p < 0.05$) was considered statistically significant.

Among the test groups, Group I showed no statistically significant protection, indicating a weak effect at lower concentration. However, Group II showed moderate significance ($p < 0.01$), and Group III exhibited highly significant reduction in ROS levels ($p < 0.001$), demonstrating strong dose-dependent antioxidant activity of the plant extract.

IV. CONCLUSION

The study demonstrates that the ethanolic extract of *Myristica fragrans* possesses significant antioxidant and cardioprotective potential. The presence of phenolic compounds and flavonoids contributes to its strong free radical scavenging activity, as confirmed by in vitro assays. The extract effectively improved cell viability and reduced intracellular ROS levels in H9c2 cardiac cells under oxidative stress conditions. Overall, these findings suggest that *Myristica fragrans* may serve as a promising natural therapeutic agent for the prevention of oxidative stress–induced cardiovascular diseases.

REFERENCES

[1]. Barbiero JK, Santiago RM, Lima MM, Ariza D, Morais LH, Andreatini R, Vital MA. Acute but not chronic administration of pioglitazone promoted behavioural and neurochemical protective effects in the MPTP model of Parkinson's disease. Behav Brain Res. 2011;216:186-92.
[2]. Barnham KJ, Masters CL, Bush AI. Neurodegenerative diseases and oxidative stress. Nat Rev Drug Discov. 2004;3:205-14.
[3]. Bashkatova V, Alam M, Vanin A, Schmidt WJ. Chronic administration of rotenone increases levels of nitric oxide and lipid peroxidation products in rat brain. Exp Neurol. 2004;186:235-41
[4]. Bassani TB, Gradowski RW, Zaminelli T, Barbiero JK, Santiago RM, Boschen SL, da Cunha C, Lima MM, Andreatini R, Vital MA. Neuroprotective and antidepressant-like effects of melatonin in a rotenone-induced Parkinson"s disease model in rats. Brain Res. 2014;1593:95-105.
[5]. Baydar T, Papp A, Aydin A, Nagymajtenyi L, Schulz H, Isimer A, Sahin G. Accumulation of aluminum in rat brain: does it lead to behavioral and electrophysiological changes?. Biol Trace Elem Res. 2003;92:231-44.
[6]. Bazzu G, Biosa A, Farina D, Spissu Y, Dedola S, Calia G, Puggioni G, Rocchitta G, Migheli R, Desole MS, Serra PA. Dual asymmetric-flow microdialysis for in vivo monitoring of brain neurochemicals. Talanta. 2011;85:1933-40.
[7]. Beal MF. Experimental models of Parkinson"s disease. Nat Rev Neurosci. 2001; 2:325-334.
[8]. Bender A, Krishnan K, Morris C, Taylor G, Reeve A, Perry R, Jaros E, Hersheson J, Betts J, Klopstock T, Taylor R, Turnbull D. High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson's disease. Nat Genet. 2006;38:515-517.
[9]. Berardelli A, Rothwell JC, Thompson PD, Hallett M. Pathophysiology of bradykinesia in Parkinson's disease. Brain. 2001;124:2131-2146.
[10]. Bernheimer H, Birkmayer W, Hornykiewicz O, Jellinger K, Seitelberger F. Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. J Neurological Sci. 1973;20:415-455.
[11]. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV and Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson"s disease. Nat. Neurosci. 2000;3:1301-1306.
[12]. Bezard E, Brotchie JM, Gross CE. Pathophysiology of L-dopa-induced dyskinesia: potential for new therapies. Nat. Rev. Neurosci. 2001;2:577-588.
[13]. Bezard E, Gross CE, Fournier MC, Dovero S, Bloch B, Jaber M. Absence of MPTP-induced neuronal death in mice lacking the dopamine transporter.Exp Neurol. 1999;155:268-273.
[14]. Bhattacharya A, Ghosal S, Bhattacharya SK. Antioxidant effect of Withania somnifera glycowithanolides in chronic foot-shock stress-induced perturbations of oxidative free radical

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jun1163

scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. J Ethnopharmacol. 2001;74:1-6.
[15]. Biedler JL, Roffler-Tarlov S, Schachner M, Freedman LS. Multiple neurotransmitter synthesis by human neuroblastoma cell lines and clones. Cancer Res. 1978;38:3751-3757.
[16]. Birkmayer W, Hornykiewicz O. The L-3, 4-dioxyphenylalanine (DOPA)-effect in Parkinson-akinesia. Wien Klin Wochenschr. 1961;73:787-8.
[17]. Bjorklund LM, Sanchez-Pernaute R, Chung S, Andersson T, Chen IY, McNaught KS, Brownell AL, Jenkins BG, Wahlestedt C, Kim KS, and Isacson O. Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model. Proc. Natl. Acad. Sci. 2002;99:2344-2349.
[18]. Blume SR, Cass DK, Tseng KY. Stepping test in mice: a reliable approach in determining forelimb akinesia in MPTP-induced Parkinsonism. Exp Neurol. 2009;219:208-11.
[19]. Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, Dekker MC, Squitieri F, et al., P. Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science. 2003;299:256-259.
[20]. Borek LL, Kohn R, Friedman JH. Phenomenology of dreams in Parkinson's disease. Mov Disord. 2007;22:198-202.
[21]. Bronte-Stewart HM, Minn AY, Rodrigues K, Buckley EL, Nashner LM. Postural instability in idiopathic Parkinson's disease: the role of medication and unilateral pallidotomy. Brain. 2002;125:2100-2114.
[22]. Brooks SP, Trueman RC, Dunnett SB. Assessment of motor coordination and balance in mice using the rotarod, elevated bridge, and footprint tests. Curr Protoc Mouse Biol. 2012;2:37-53
[23]. Broussolle E, Krack P, Thobois S, Xie-Brustolin J, Pollak P, Goetz CG. Contribution of Jules Froment to the study of parkinsonian rigidity. Mov Disord. 2007;22:909-914.
[24]. Brunelle JK, Letai A. Control of mitochondrial apoptosis by the Bcl-2 family. J Cell Sci. 2009;122:437-441. Burn DJ. Depression in Parkinson‟s disease. Eur J Neurol. 2002;3:44–54.