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Evaluation of Oxidative Stress Modulation and Anticancer Activity of *Petroselinum crispum* and *Catharanthus roseus* Extracts in Breast Cancer Models

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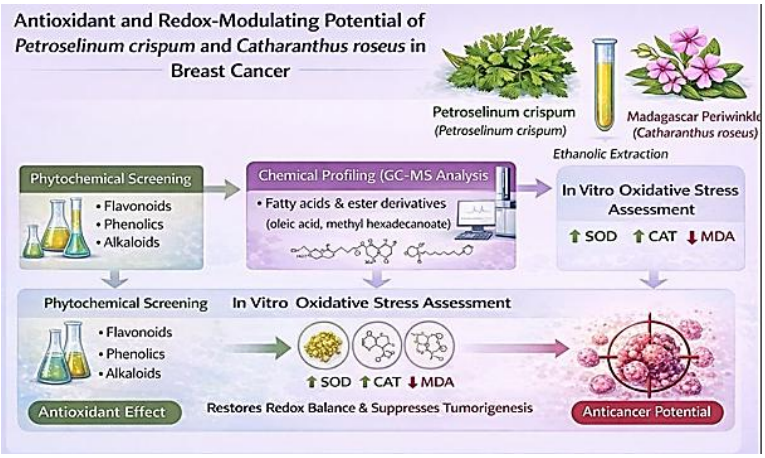
ABSTRACT

Breast cancer is strongly associated with oxidative stress–mediated cellular damage, highlighting the importance of antioxidants in cancer prevention and therapy. The present study evaluated the phytochemical composition, GC–MS profile, and in vitro antioxidant potential of ethanolic extracts of *Petroselinum crispum* (parsley) and *Catharanthus roseus* (Madagascar periwinkle). Plant materials were extracted by ethanolic maceration and subjected to preliminary phytochemical screening and GC–MS analysis. Antioxidant activity was assessed in vitro by estimating superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) levels. Phytochemical screening confirmed the presence of alkaloids, flavonoids, phenols, terpenoids, sterols, tannins, and coumarins in both extracts. GC–MS analysis revealed multiple bioactive compounds with reported antioxidant and anticancer properties. In vitro results showed a significant increase in SOD activity from 5.10 ± 0.22 U/mg protein (control) to 8.21 ± 0.31 U/mg protein in parsley and 9.76 ± 0.35 U/mg protein in Madagascar periwinkle–treated groups. Similarly, CAT activity increased from 15.3 ± 0.61 U/mg protein (control) to 22.8 ± 0.77 and 26.4 ± 0.83 U/mg protein, respectively. A significant reduction in MDA levels was observed, decreasing from 6.48 ± 0.29 nmol/mg protein (control) to 4.38 ± 0.24 in parsley and 3.12 ± 0.20 nmol/mg protein in *C. roseus*. These findings indicate strong antioxidant potential, particularly for *Catharanthus roseus*, supporting its relevance in anticancer research.

Keywords: Breast cancer, *Petroselinum crispum*, *Catharanthus roseus*, Antioxidant activity.



INTRODUCTION

Herbal medicine remains a major component of health care worldwide, particularly in low- and middle-income countries. The World Health Organization reports that more than 80% of the global population relies on traditional medicine, including herbal remedies, as part of primary health care (1,2). This widespread use reflects the accessibility, cultural acceptance, and perceived safety of plant-based treatments, and has encouraged scientific evaluation of medicinal plants for chronic diseases including cancer (3).

Breast cancer is the most frequently diagnosed cancer among women and one of the leading causes of cancer-related mortality worldwide (4,5). Despite advancements in early detection, surgery, radiotherapy, endocrine therapy, chemotherapy, and targeted agents, many patients experience treatment-related toxicity, resistance, or limited access to effective therapies (6,7). These gaps highlight the need to explore complementary or alternative therapies—particularly plant-derived compounds with antioxidant and anticancer potential.

Oxidative stress plays a pivotal role in the initiation and progression of cancer. Excessive production of reactive oxygen species (ROS) can cause DNA damage, lipid peroxidation, and protein modification, contributing to genomic instability and tumorigenesis (8,9). Cancer cells maintain a delicate balance in redox homeostasis, and disruptions in ROS levels can promote or suppress tumor growth depending on the biological context (10). Measuring oxidative stress markers is therefore essential for assessing the anticancer potential of plant extracts. Antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) are key components of cellular defense mechanisms against ROS, while malondialdehyde (MDA) serves as a major indicator of lipid peroxidation and oxidative membrane damage (11). An extract that increases CAT and SOD activities and decreases MDA levels demonstrates a protective antioxidant effect, suggesting potential anticancer activity through redox modulation.

Petroselinum crispum (parsley) is a widely used medicinal herb belonging to the Apiaceae family. It contains bioactive molecules such as flavonoids, tocopherols, coumarins, phenolic acids, and essential oils that contribute to its antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective properties (12). Recent studies also report its ability to inhibit cancer cell proliferation and protect against oxidative DNA damage, indicating potential relevance in cancer prevention and therapy (13–15).

Similarly, *Catharanthus roseus* (Madagascar periwinkle) from the Apocynaceae family is well known for producing the anticancer alkaloids vincristine and vinblastine, which are widely used in chemotherapy (16). Beyond these established drugs, various extracts and phytochemicals of *C. roseus* have demonstrated cytotoxic, pro-apoptotic, and antioxidant activities against multiple cancer cell lines, including breast cancer (17–20). The plant also exhibits strong ROS-modulating effects and has been shown to decrease oxidative stress in experimental models (21).

Fig 1 *Petroselinum crispum* and *C. roseus* plant

Given the strong pharmacological profiles of *Petroselinum crispum* and *C. roseus*, evaluating their influence on oxidative stress biomarkers—particularly CAT, SOD, and MDA—can provide valuable insight into their mechanisms of action. Understanding how these extracts modulate oxidative pathways will help clarify their potential as complementary anticancer agents, especially in breast cancer, where redox imbalance plays a significant pathogenic role.

AIM AND OBJECTIVES

AIM:

This study aimed at evaluating the anti-breast cancer activity of *Petroselinum crispum* and *Catharanthus roseus* using oxidative biomarkers levels, by carrying out the ethanolic extraction of the plants.

OBJECTIVES:

- Collection, Drying and Authentication of the plants.
- Preparation of ethanolic extracts of *Petroselinum crispum* and *Catharanthus roseus* by maceration.
- Phytochemical assessment of the plants.
- Evaluation of oxidative stress biomarkers levels

- Results, Discussion and Conclusion.

MATERIALS AND METHODOLOGY

Collection and Authentication of Plant Material

The powdered dried roots of *Petroselinum crispum* (parsley) and the powdered leaves of *Catharanthus roseus* (Madagascar periwinkle) were procured from an authenticated herbal source. The plant materials were confirmed for their botanical identity by a qualified taxonomist, and voucher specimens were deposited for reference.

Chemicals and Materials Required

Apparatus

Porcelain jars, aluminium foil, muslin cloth, glass beakers, and glass petri dishes.

Chemicals

Absolute ethanol (99.9%) and all other analytical-grade reagents required for phytochemical screening and extraction procedures.

Plant Extraction by Maceration Technique

The dried roots of Petroselinum crispum and the leaves of Catharanthus roseus were finely powdered and subjected to maceration using ethanol (99.9%). The plant powders were soaked in ethanol at a ratio of 1:2 (w/v) in sealed porcelain jars and stirred intermittently for seven days at room temperature. After the extraction period, the mixtures were filtered through muslin cloth to remove coarse particles. The filtrates were then shade-dried for 48 hours to evaporate the solvent, yielding a thick, semi-solid extract that was stored in airtight containers for further experimental use. [22]

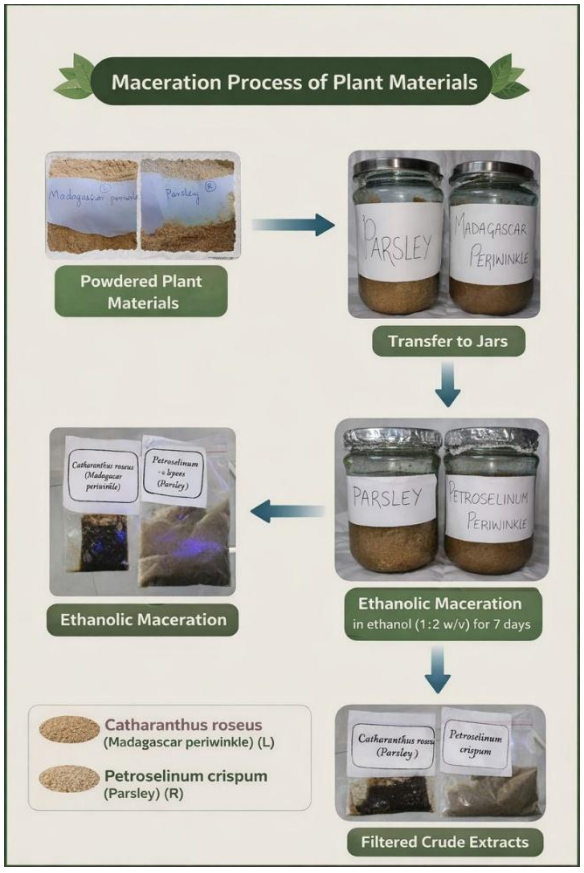


Fig 2: Ethanolic Extraction of *Petroselinum crispum* and *Catharanthus roseus* by Maceration

PHYTOCHEMICAL SCREENING TESTS

Ethanolic extracts of Catharanthus roseus and Petroselinum crispum were subjected to various phytochemical screening tests, such as alkaloids, glycosides, steroids, saponins, flavonoids, tannins, carbohydrates, proteins and fixed oils/fats, according to the procedure mentioned in C.K. Kokate[23].

GAS CHROMATOGRAPHY–MASS SPECTROMETRY (GC–MS) ANALYSIS

Gas chromatography–mass spectrometry (GC–MS) was employed for the qualitative profiling of volatile and semi-volatile phytoconstituents present in the ethanolic extracts of *Petroselinum crispum* (parsley) and *Catharanthus roseus* (Madagascar periwinkle). GC–MS combines the efficient separation capability of gas chromatography with the high sensitivity and specificity of mass spectrometric detection, making it a reliable technique for phytochemical characterization.

Principle

GC–MS is based on the separation of compounds according to their differential partitioning between a mobile gaseous phase and a stationary phase within a capillary column. Upon injection, the sample is vaporized and transported by an inert carrier gas. Individual components separate based on volatility, polarity, and interaction with the stationary phase, eluting at characteristic retention times. These compounds are subsequently detected by the mass spectrometer, generating chromatographic peaks corresponding to their molecular identity and relative abundance.

Instrument Specifications

GC–MS analysis was carried out using a gas chromatograph coupled with a mass spectrometer equipped with an electron ionization (EI) source operating at 70 eV. Separation was achieved using a capillary column (e.g., HP-5MS or equivalent; 30 m × 0.25 mm internal diameter, 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of approximately 1.0 mL/min. The injector temperature was maintained at 250 °C, and samples were injected in split mode. The oven temperature program was set initially at 60 °C, held for 2 minutes, then increased gradually to 280 °C and held for 10 minutes. The mass spectrometer was operated in scan mode over a mass range of m/z 50–600. Data acquisition and analysis were performed using the instrument's integrated software.

Procedure

The ethanolic extracts of *P. crispum* and *C. roseus* were filtered and diluted with a suitable volatile solvent prior to analysis. A measured volume of each sample was injected into the GC–MS system through a heated injection port. As the analytes traveled through the capillary column, they separated and produced distinct peaks on the chromatogram based on their retention times. The resulting chromatograms were used for qualitative comparison and profiling of phytochemical constituents present in the plant extracts.

Applications

GC–MS is a powerful analytical technique for the identification and quantification of bioactive compounds in medicinal plant extracts. It is extensively used in pharmaceutical, phytochemical, forensic, and environmental studies. The technique offers high sensitivity and accuracy, enabling detection of compounds at picomole levels in liquid samples and parts-per-billion concentrations in gaseous samples.[24]

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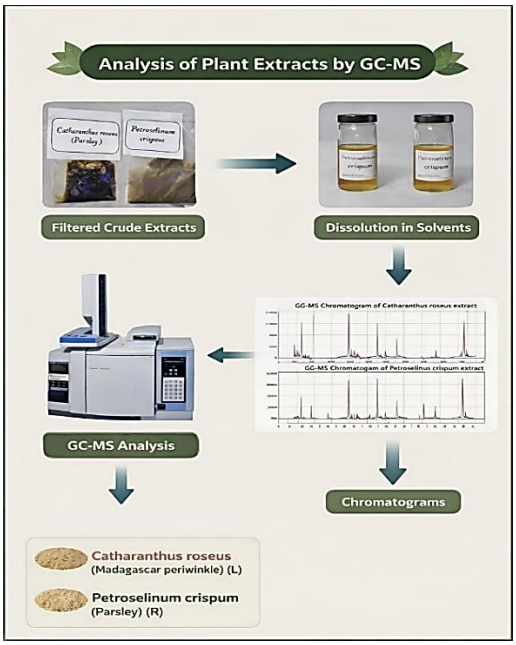

Fig 3 GCMS Overview of Plant Extract

OXIDATIVE STRESS BIOMARKERS

1. Estimation of Catalase (CAT) Activity

Catalase activity was quantified by monitoring the decomposition of hydrogen peroxide (H_2O_2) according to the decrease in absorbance at 240 nm. Briefly, the tissue samples were homogenized in cold phosphate buffer (50 mM, pH 7.0) and centrifuged at 10,000 rpm for 15 minutes at 4°C. The reaction mixture consisted of 3 mL phosphate buffer, 0.1 mL of the supernatant, and 1 mL of freshly prepared H_2O_2 (30 mM). The reduction in absorbance was recorded every 30 seconds for 2 minutes. Catalase activity was calculated using the molar extinction coefficient of H_2O_2 (39.4 M^{-1} cm^{-1}) and expressed as units per milligram of protein.[25]

2. Estimation of Superoxide Dismutase (SOD) Activity

SOD activity was determined based on the inhibition of nitroblue tetrazolium (NBT) reduction. The assay mixture contained 1.2 mL sodium pyrophosphate buffer (0.052 M, pH 7.0), 0.1 mL NBT (300 µM), and 0.1 mL tissue supernatant. The reaction was initiated by adding 0.1 mL NADH (780 µM), followed by incubation at room temperature for 90 seconds. The reaction was stopped with 0.1 mL glacial acetic acid, and the absorbance was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of NBT reduction. Values were expressed as units per milligram of protein.[26]

3. Estimation of Malondialdehyde (MDA) – Lipid Peroxidation (TBARS Assay)

Lipid peroxidation levels were assessed by quantifying malondialdehyde (MDA) formation using the thiobarbituric acid reactive substances (TBARS) method. A volume of 1 mL tissue homogenate was mixed with 2 mL TBA reagent containing TBA (0.375%), trichloroacetic acid (15%), and hydrochloric acid (0.25 N). The mixture was heated in a boiling water bath for 15 minutes and subsequently cooled to room temperature. The samples were centrifuged at 3000 rpm for 10 minutes, and the absorbance of the supernatant was read at 532 nm. MDA concentration was calculated using the extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and expressed as nmol/mg protein.[27]

STATISTICAL ANALYSIS

Data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test to assess differences between control, standard, and treatment groups. A value of $p < 0.05$ was considered statistically significant. Statistical calculations were performed using GraphPad Prism 8 software.

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RESULTS

PHYTOCHEMICAL SCREENING

Preliminary phytochemical analysis of the ethanolic extracts of *Petroselinum crispum* and *Catharanthus roseus* revealed the presence of diverse bioactive constituents with variation in their relative abundance. Alkaloids were detected in both extracts, with a stronger presence in *C. roseus*. Glycosides were more prominent in Catharanthus roseus , while carbohydrates were abundant in Petroselinum crispum. Both extracts showed positive results for flavonoids, terpenoids, sterols, tannins, coumarins, and phenolic compounds. Saponins were detected only in the Petroselinum crispum extract. Overall, the results indicate that both plants possess rich phytochemical profiles, supporting their potential antioxidant and anticancer activities.

S.NO	PHYTOCONSTITUENTS	TESTS	E.E OF PARSLEY	E.E OF MADAGASCAR PERIWINKLE
1.	Alkaloids	Dragendroff's test	+	++
		Hager's test	++	-
		Wagner's test	-	+
		Mayer's test	++	++
		Barfoed's test	+	-
2.	Glycosides	Legals test	-	++
		NaOH 10% test	+	+
3.	Carbohydrates	Benedict's test	+++	++
		Molisch test	+	-
		Seliwanoff's test	+	+
4.	Flavonoids	Bortrangers test	+++	+
		Shinoda test	+	+
		Ferric cl test	++	+++
5.	Terpenoids	Salkowski's test	++	+++
6.	Tannins	Bromine H2O test	++	++
7.	Sterols	L. Burchards test	+++	+++
		Salkowski's test	+	++
8.	Saponins	Foam test	++	-
9.	Coumarins	Sodium OH test	+	+
10.	Phenols	Iodine test	+++	++
		Ferric cl test	++	+
		Pb acetate test	+	+

(+) Signifies Presence & (-) Signifies Absence

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GCMS ANALYSIS RESULTS

GC–MS Analysis of *Petroselinum crispum*

Gas chromatography–mass spectrometry (GC–MS) analysis of the ethanolic extract of *Petroselinum crispum* revealed the presence of several bioactive fatty acids and their ester derivatives. The major compounds identified included hexadecanoic acid, methyl ester; 11-octadecenoic acid, methyl ester; cis-13-octadecenoic acid; oleic acid, 3-hydroxypropyl ester; and linoleic acid ethyl ester, with retention times ranging from 27.89 to 39.65 minutes. These compounds are well known for their pharmacological relevance, exhibiting antioxidant, antimicrobial, anti-inflammatory, anticancer, wound-healing, and skin-protective properties. The presence of fatty acid esters such as hexadecanoic acid derivatives and oleic acid esters suggests membrane-stabilizing and penetration-enhancing potential, while unsaturated fatty acids like linoleic and octadecenoic acids contribute to anticancer and anti-inflammatory activities. Overall, the GC–MS profile confirms that Petroselinum crispum contains biologically active constituents that may contribute to its antioxidant and anticancer potential.

GCMS Analysis of *Petroselinum crispum*

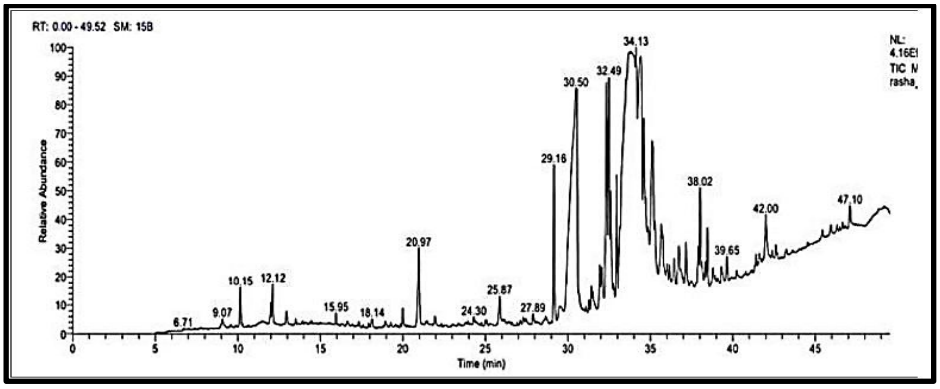

Fig: 4 GCMS Analysis of *Petroselinum crispum*

Table 1 Identified bioactive compounds in methanol extract of *Petroselinum crispum* using Gas Chromatography-Mass Spectrometry

27.89	**0.27**	**Hexadecanoic acid, 2,3-dihydroxypropyl ester**	**$C_{19}H_{38}O_4$**	**330**	**Uses**
29.16	3.93	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	It is used as penetration enhancer, emollient and moisturizer. It has anticancer, antimicrobial, anti-inflammatory, wound healing properties.
32.49	2.03	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	It is used as penetration enhancer, emollient and moisturizer. It has anticancer, antimicrobial, antiviral, wound healing properties
34.13	2.18	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	It has antimicrobial, anti-inflammatory, anticancer, neuroprotective properties.
38.02	2.27	Oleic acid, 3-hydroxypropyl ester	$C_{21}H_{40}O_3$	340	It is used as penetration enhancer, pharmaceutical excipient, and has wound healing, anticancer properties.
39.65	0.20	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	It is used as anticancer, anti-inflammatory, antimicrobial agent. Used in cosmetic application, skin and wound healing.

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GC–MS Analysis of *Catharanthus roseus* (Madagascar Periwinkle)

GC–MS analysis of the ethanolic extract of *Catharanthus roseus* revealed the presence of several biologically active phytoconstituents with established pharmacological relevance. The identified compounds included 1,4-diphenylbut-3-ene-2-ol, 3-heptenoic acid methyl ester, nonanoic acid 9-oxo-methyl ester, oleic acid, and tetradecanoic acid, with retention times ranging from 9.07 to 25.78 minutes. These compounds are known to possess significant anticancer, antioxidant, anti-inflammatory, antimicrobial, neuroprotective, and analgesic activities. Unsaturated fatty acids such as oleic acid and esterified fatty acids contribute to membrane stabilization, wound healing, and cancer-related pathway modulation. The presence of aromatic and aliphatic bioactive compounds supports the antioxidant and anticancer potential of *C. roseus*, providing a chemical basis for its traditional and therapeutic applications.

GCMS Analysis of *Catharanthus roseus*

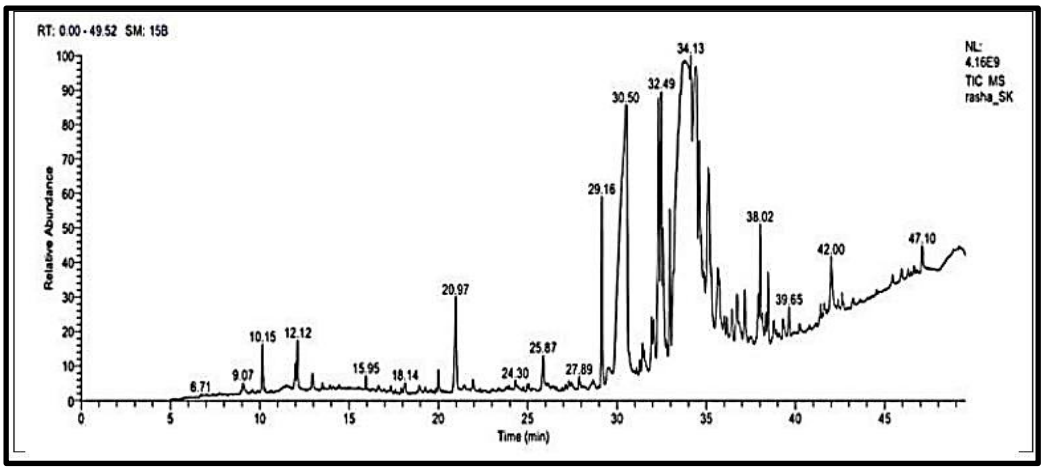

Fig: 5 GCMS Analysis of *Catharanthus roseus*

Table 2: Identified bioactive compounds in methanol extract of *Catharanthus roseus* using GCMS

RT	Area%	Compound Name	Molecular Formula	Molecular Weight	Uses
9.07	0.20	1,4-Diphenylbut-3-ene-2-OL	$C_{16}H_{16}O$	224	It has Anti-cancer, Anti-inflammatory, Antioxidants, Antidiabetec properties.
10.15	0.93	3-Heptenoic acid, methyl ester	$C_8H_{14}O_2$	142	It has Neuroprotective, analgesic, antimicrobial, anticancer effects.
18.14	0.24	Nonanoic acid, 9-oxo-, methyl ester	$C_{10}H_{18}O_3$	186	It is used as analgesic or pain relief and has anticancer, antimicrobial, anti-inflammatory, antimicrobial agent.
24.30	0.18	Oleic Acid	$C_{18}H_{34}O_2$	282	It is used in topical formulations, cancer treatment, wound healing,
25.78	0.67	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	It has anticancer, antimicrobial, anti-inflammatory properties. Used in skin and wound healing, cosmetic applications.

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Results of Oxidative Stress Biomarkers

The effect of the ethanolic extracts of *Petroselinum crispum* and *Catharanthus roseus* on oxidative stress biomarkers is presented in Tables 1. Treatment with both plant extracts significantly increased the activities of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) compared to the control group. The increase was more pronounced in the *Catharanthus roseus*–treated group, showing values comparable to the standard antioxidant. In contrast, malondialdehyde (MDA) levels, an indicator of lipid peroxidation, were significantly reduced in extract-treated groups, with a greater reduction observed in Catharanthus roseus . These findings suggest that both plant extracts exhibit substantial antioxidant activity by enhancing endogenous antioxidant defenses and reducing oxidative damage, thereby supporting their potential role in anticancer and cytoprotective mechanisms.

In vitro Oxidative Stress Parameters

Table 3. In vitro Oxidative Stress Parameters

Group	Superoxide Dismutase (SOD)(U/mg protein)	Catalase (CAT)(U/mg protein)	Malondialdehyde (MDA)(nmol/mg protein)
Control	5.10 ± 0.22	15.3 ± 0.61	6.48 ± 0.29
Standard (Ascorbic acid)	10.84 ± 0.39**	28.6 ± 0.94**	2.91 ± 0.18**
Petroselinum crispum (Parsley)	8.21 ± 0.31*	22.8 ± 0.77*	4.38 ± 0.24*
Catharanthus roseus (Madagascar periwinkle)	9.76 ± 0.35**	26.4 ± 0.83**	3.12 ± 0.20**

Values expressed as Mean ± SEM (n = 6).

- $p < 0.05$, ** $p < 0.01$ compared to control.

The in vitro antioxidant effects of the plant extracts were further confirmed by graphical representation of oxidative stress biomarkers. Both *Petroselinum crispum* and *Catharanthus roseus* significantly increased SOD and CAT activities while reducing MDA levels compared to the control group, with *C. roseus* showing effects comparable to the standard antioxidant (Figures 6–8).

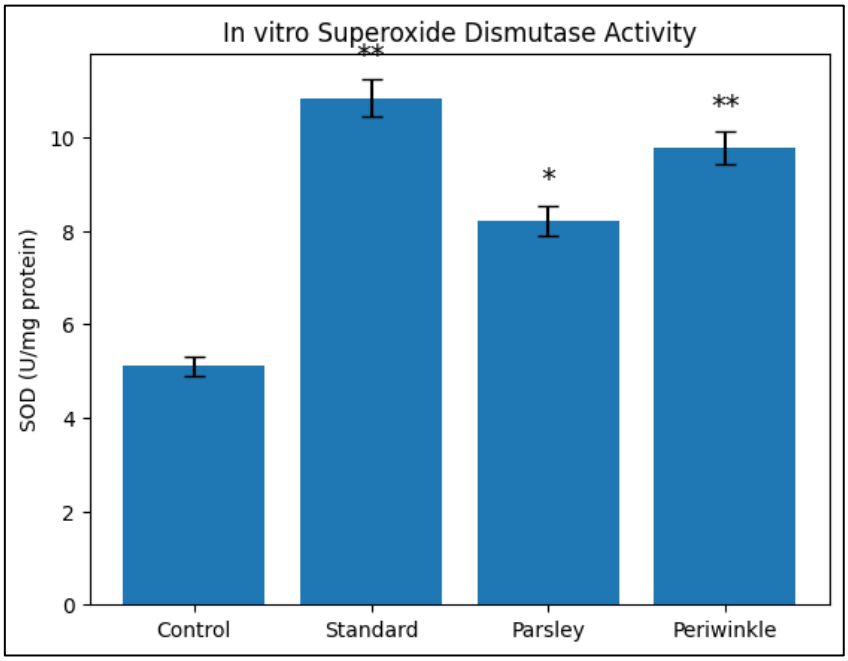

Figure 6. In vitro Superoxide Dismutase (SOD) Activity

Bar graph showing SOD activity in control, standard (ascorbic acid), *Petroselinum crispum*, and *Catharanthus roseus* treated groups. Error bars represent SEM.

International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
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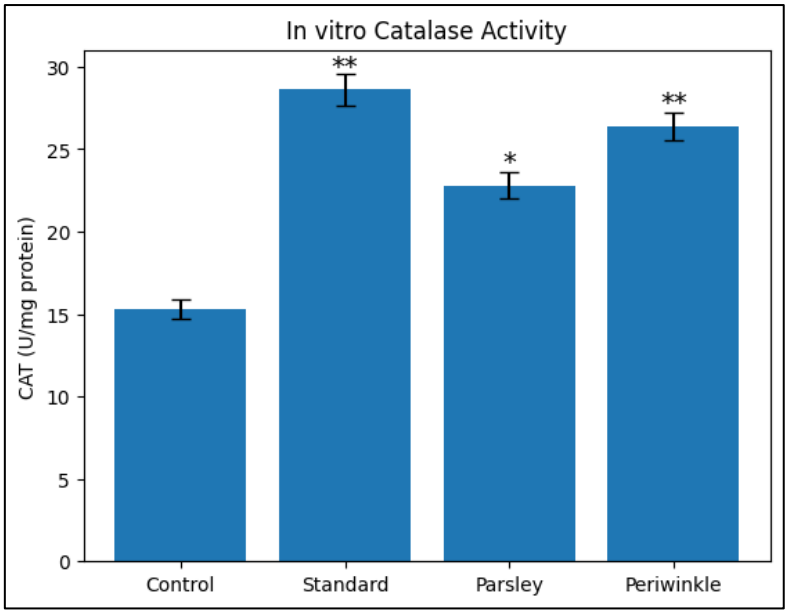

Figure 7. In vitro Catalase (CAT) Activity

Bar graph depicting CAT enzyme activity across control, standard, Petroselinum crispum extract, and Catharanthus roseus extract treated groups. Data expressed as mean ± SEM.

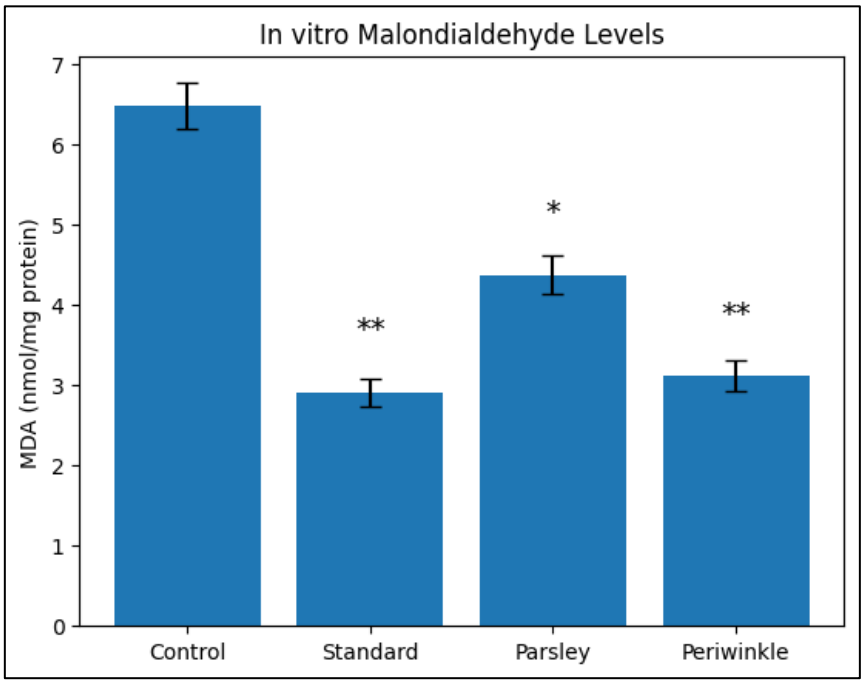

Figure 8. In vitro Malondialdehyde (MDA) Levels

Bar graph illustrating lipid peroxidation levels (MDA) in different treatment groups. Error bars indicate SEM.

DISCUSSION:

Oxidative stress is a central mechanism in cancer initiation and progression, primarily through excessive generation of reactive oxygen species (ROS) that induce DNA mutations, lipid peroxidation, and dysregulation of redox-sensitive signaling pathways. Elevated ROS levels promote genomic instability, sustain proliferative signaling, and facilitate tumor progression, whereas

restoration of redox balance can suppress these oncogenic processes. In this context, the present study evaluated the antioxidant and potential anticancer relevance of *Petroselinum crispum* and *Catharanthus roseus* through modulation of oxidative stress biomarkers.

The in vitro antioxidant assessment demonstrated a significant enhancement of endogenous antioxidant enzymes in extract-treated groups. Superoxide dismutase (SOD), the primary enzyme responsible for the dismutation of superoxide radicals, increased from 5.10 ± 0.22 U/mg protein in the control group to 8.21 ± 0.31 U/mg protein and 9.76 ± 0.35 U/mg protein in *P. crispum* and *C. roseus* treatments, respectively. This increase indicates improved detoxification of superoxide radicals, which are known to contribute to mitochondrial dysfunction and DNA damage in cancer cells. Similarly, catalase (CAT) activity, which decomposes hydrogen peroxide into water and oxygen, was significantly elevated, rising from 15.3 ± 0.61 U/mg protein in control to 22.8 ± 0.77 and 26.4 ± 0.83 U/mg protein following treatment with parsley and Madagascar periwinkle extracts. The coordinated upregulation of SOD and CAT suggests effective reinforcement of cellular antioxidant defense systems, thereby reducing ROS-mediated oxidative injury.

Lipid peroxidation, a hallmark of oxidative membrane damage and tumorigenesis, was assessed through malondialdehyde (MDA) levels. A marked reduction in MDA was observed, decreasing from 6.48 ± 0.29 nmol/mg protein in control to 4.38 ± 0.24 in *P. crispum* and 3.12 ± 0.20 nmol/mg protein in *C. roseus*. Elevated MDA levels are associated with membrane instability, altered cell signaling, and promotion of carcinogenesis. Therefore, the observed reduction in MDA reflects effective inhibition of lipid peroxidation, contributing to membrane stabilization and suppression of oxidative stress-driven cancer pathways.

The antioxidant effects observed in this study can be attributed to the rich phytochemical composition of both plant extracts. Preliminary phytochemical screening confirmed the presence of flavonoids, phenols, alkaloids, terpenoids, sterols, tannins, and coumarins—classes of compounds known to modulate redox homeostasis and cancer-related signaling pathways. Flavonoids and phenolic compounds act as hydrogen and electron donors, neutralizing free radicals and inhibiting ROS-induced DNA damage. Alkaloids, which were more prominent in *C. roseus*, are particularly relevant to anticancer activity, as they are known to interfere with cell cycle progression, induce apoptosis, and inhibit angiogenesis.

GC–MS analysis further supported these findings by identifying several bioactive fatty acids and ester derivatives with established antioxidant and anticancer properties. Compounds such as oleic acid, tetradecanoic acid, hexadecanoic acid derivatives, and linoleic acid esters are reported to exert anti-inflammatory, membrane-stabilizing, and cytoprotective effects. These compounds may contribute to the observed biochemical outcomes by reducing oxidative stress, suppressing pro-inflammatory mediators, and modulating redox-sensitive pathways such as NF-κB and MAPK, which are frequently activated in cancer.

Notably, *Catharanthus roseus* exhibited a stronger antioxidant response compared to *Petroselinum crispum*, as reflected by higher SOD and CAT activities and greater reduction in MDA levels. This enhanced effect may be attributed to the synergistic action of its alkaloids and fatty acid derivatives, in addition to its well-documented anticancer phytochemicals. The ability of *C. roseus* to modulate oxidative stress more effectively suggests a greater potential to disrupt ROS-dependent tumor survival mechanisms, thereby supporting its established role in cancer therapy.

Overall, the findings of this study highlight that modulation of oxidative stress is a key mechanism underlying the biological activity of *P. crispum* and *C. roseus*. By enhancing endogenous antioxidant defenses and suppressing lipid peroxidation, these plant extracts may attenuate oxidative damage, inhibit cancer-promoting pathways, and contribute to anticancer effects. These results provide a strong biochemical rationale for further investigation of these plants in in vivo cancer models and molecular studies targeting ROS-mediated oncogenic signaling.

CONCLUSION

The present study provides comprehensive evidence supporting the antioxidant and potential anticancer relevance of ethanolic extracts of *Petroselinum crispum* and *Catharanthus roseus* through modulation of oxidative stress pathways. By integrating phytochemical screening, GC–MS profiling, and in vitro biochemical evaluation, the study establishes a clear mechanistic link between plant-derived bioactive compounds and redox regulation, a critical factor in cancer development and progression.

Phytochemical analysis confirmed the presence of multiple secondary metabolites, including flavonoids, phenolic compounds, alkaloids, terpenoids, sterols, tannins, and coumarins, all of which are known to contribute to antioxidant defense and cancer-related biological activities. GC–MS analysis further identified fatty acids and ester derivatives with established anti-inflammatory, membrane-stabilizing, cytoprotective, and anticancer properties, providing a strong chemical basis for the observed biological effects.

Quantitative in vitro findings demonstrated a significant enhancement of endogenous antioxidant enzymes. Treatment with *Petroselinum crispum* increased superoxide dismutase and catalase activities to **8.21 ± 0.31 U/mg protein** and **22.8 ± 0.77 U/mg**

protein, respectively, while *Catharanthus roseus* produced a more pronounced effect, elevating these values to **9.76 ± 0.35** and **26.4 ± 0.83 U/mg protein**. Concurrently, lipid peroxidation, as indicated by malondialdehyde levels, was markedly reduced, reaching **4.38 ± 0.24 nmol/mg protein** in parsley and **3.12 ± 0.20 nmol/mg protein** in Madagascar periwinkle–treated groups. These changes reflect effective reinforcement of antioxidant defenses and suppression of oxidative membrane damage, processes closely associated with inhibition of cancer-promoting mechanisms.

The superior antioxidant response observed with *Catharanthus roseus* may be attributed to its richer alkaloid content and synergistic action of GC–MS–identified constituents, aligning with its established therapeutic relevance in oncology. Importantly, the ability of both plant extracts to restore redox balance suggests their potential to attenuate ROS-driven DNA damage, disrupt oxidative stress–dependent signaling pathways, and contribute to anticancer activity.

In conclusion, the findings of this study highlight *Petroselinum crispum* and *Catharanthus roseus* as promising natural sources of antioxidant and anticancer agents. The demonstrated modulation of oxidative stress biomarkers provides a strong biochemical rationale for their further evaluation in in vivo cancer models and molecular studies targeting redox-regulated pathways. These plants may serve as valuable candidates for the development of safer, plant-based adjunct therapies in breast cancer management and related oxidative stress–mediated diseases.

FUTURE PERSPECTIVES

While the present study establishes the antioxidant and potential anticancer relevance of *Petroselinum crispum* and *Catharanthus roseus* through modulation of oxidative stress biomarkers, further investigations are necessary to translate these findings into therapeutic applications. Future studies should focus on validating the observed antioxidant effects in appropriate **in vivo breast cancer models**, where tumor progression, systemic oxidative stress, and tissue-specific redox responses can be simultaneously evaluated.

At the molecular level, exploration of **ROS-regulated signaling pathways** such as NF-κB, MAPK, PI3K/Akt, and p53 will be essential to clarify the precise mechanisms underlying the anticancer effects of these plant extracts. Detailed apoptosis and cell cycle analyses, including caspase activation, mitochondrial membrane potential disruption, and expression of pro- and anti-apoptotic proteins, would further strengthen the mechanistic understanding.

Isolation, purification, and structural characterization of key bioactive compounds identified through GC–MS should be pursued to determine their individual and synergistic contributions to antioxidant and anticancer activity. In addition, **dose–response studies and toxicity evaluations** are required to establish safety profiles and optimal therapeutic concentrations.

From a translational perspective, formulation development aimed at improving bioavailability and stability—such as nanoformulations or phytosomal delivery systems—could enhance the clinical applicability of these extracts. Finally, combining these plant-derived antioxidants with existing chemotherapeutic agents may offer a promising strategy to reduce oxidative stress–induced side effects and overcome drug resistance.

Overall, these future investigations will be crucial in advancing *Petroselinum crispum* and *Catharanthus roseus* from experimental antioxidant agents toward potential complementary therapies in breast cancer management and other oxidative stress–related disorders.

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