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Research Article

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Evaluation of Antioxidant Enzyme Activities and Lipid Peroxidation Markers in *Maresia nana* Methanolic Extract

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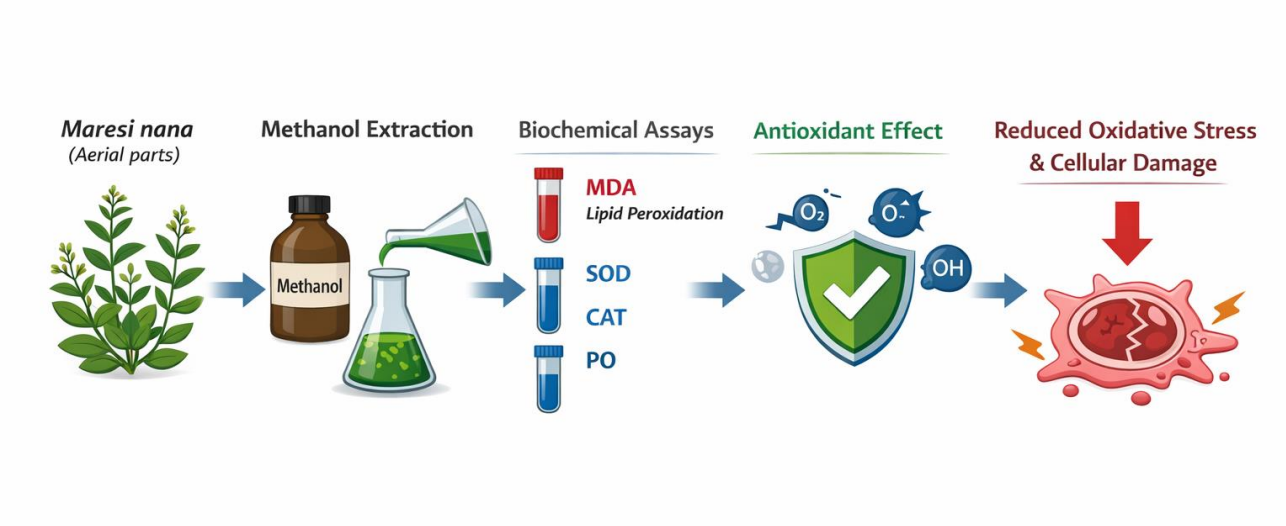
Highlights

- *Maresi nana* aerial parts were extracted using methanol.
- Lipid peroxidation was evaluated via malondialdehyde levels.
- The extract showed notable superoxide dismutase activity.
- Catalase and phenol oxidase activities were markedly increased.
- Results indicate strong antioxidant potential against oxidative stress.

Graphical Abstract



Abstract
This study investigates the lipid peroxidation and antioxidant enzyme activities of *Maresi nan*a extract. The plant's aerial parts were extracted using methanol, and the extract was concentrated for further analysis. Lipid peroxidation was assessed by measuring malondialdehyde levels, revealing a concentration of 15.0 ± 1.41 nmol/mL. Antioxidant enzyme activities, including superoxide dismutase, catalase, and phenol oxidase, were also evaluated. The extract exhibited superoxide dismutase activity of 25.42 ± 1.1 U/mL, catalase activity of 43.17 ± 1.54 U/mL, and phenol oxidase activity of 13.7 ± 0.49 U/mL. These findings suggest that *M. nana* extract may be used in the treatment of diseases associated with oxidative stress.

Keywords: Catalase; malondialdehyde; *Maresi nana*; phenol oxidase; superoxide dismutase

1. Introduction

Reactive oxygen species (ROS) are highly reactive molecules formed because of oxygen metabolism. At low levels, they play roles in essential physiological functions; however, excessive production leads to tissue damage. ROS cause oxidative stress by damaging DNA, lipids, proteins, and enzymes. Oxidative stress arises from an imbalance between oxidant and antioxidant systems and plays a significant role in the pathogenesis of various chronic diseases, including neurodegenerative disorders, cardiovascular diseases, diabetes, and certain types of cancer (Hong et al., 2024). Lipid peroxidation (LPO), which occurs through ROS activity, is one of the primary mechanisms of cellular damage, disrupting the integrity and function of cell membranes. One of the key biomarkers used to monitor this process is malondialdehyde (MDA). Antioxidants protect cells by neutralizing free radicals. The antioxidant system consists of different types of components, including enzymatic and non-enzymatic elements. Antioxidant enzymes include superoxide dismutase (SOD) and catalase (CAT) (Chandimali et al., 2025).

Maresia nana (DC.) Batt. (commonly known as "Yanal ot") is an annual plant belonging to the genus *Maresia* within the family Brassicaceae. It typically grows to a height of 5 to 15 cm. The plant has a whitish appearance due to its prominent stellate hairs. The stem is shortly pubescent and branched. The leaves are oblong-ovate with entire to wavy-dentate margins and appear whitish. Basal leaves are petiolate, while the stem leaves are nearly sessile. Flowers may be either pedicellate or sessile (Figure 1). The native range of this species is Medit. to Afghanistan. It is an annual and grows primarily in the subtropical biome (POWO, 2025).

To the best of our knowledge, it has been reported that studies on the biological activities of *M. nana* extract are limited in the literature. A review of the literature indicates that only the study conducted by Gamze et al. stands out. In the study conducted by Demiral et al. (2025), the extract of *M. nana* was reported to exhibit antioxidant and anticancer activities.

The aim of this study is to evaluate the capacity of *M. nana* extract to neutralize free radicals and its suppressive effects against these radicals. In this context, the antioxidant enzyme activities and lipid peroxidation levels of the plant will be investigated to assess its potential protective effects against oxidative stress.

Figure 1. Representation of *Maresia nana* (source: POWO, 2025).

2. Materials and Methods

2.1. Plant extraction

The above-ground parts of *M. nana* were washed, oven-dried at 40 °C for two days, and ground into powder. A total of 100 g of dried material was macerated in 1 L of methanol for 72 hours in the dark. After filtration, the extracts were concentrated using a rotary evaporator at 40 °C and stored at 4 °C for further use (Aytar and Aydın, 2025; Aytar et al., 2025).

2.2. Enzyme activity assays

2.2.1. Malondialdehyde (MDA) activity

Lipid peroxidase activity was determined using a spectrophotometric method. A sample of 0.5 mL was added to 2.5 mL of 10% TCA and vortexed, and the sample was kept in a 90 °C water bath for 15 minutes. The tubes taken from the water bath were kept in ice for 15 minutes and then allowed to reach room temperature. The supernatant was obtained by centrifuging at 3000 rpm for 10 minutes. 2 mL of supernatant was taken and transferred to another tube, and 1 mL of 0.675% TBA was added and kept in a 90 °C water bath for 15 minutes again. The samples were kept in a container full of ice for 15 minutes again, and after they reached room temperature, their absorbances were read against the blank tube at 532 nm in a spectrophotometer. MDA levels were calculated in nmol/mL using the extinction coefficient of the MDA-TBA complex at 532 nm ($\varepsilon = 1.56 \times 10^5$ $cm^{-1}M^{-1}$) (Draper and Hadley, 1990; Durmaz et al., 2025).

2.*2.2. Superoxide dismutase (SOD) activity*

Superoxide dismutase (SOD) activity was determined using a modified spectrophotometric method based on the inhibition of cytochrome C reduction by superoxide

radicals generated via the xanthine/xanthine oxidase system. SOD activity was determined with nitroblue tetrazolium (NBT); the activity was measured in the mixture containing a 75 µM NBT (4-nitro blue tetrazolium chloride) solution, 0.1 mM EDTA, 4 µM riboflavin, and 50 µL enzyme extract. Absorbance was measured at 550 nm, and one unit of SOD activity was defined as the enzyme amount causing 50% inhibition of cytochrome C reduction (Flohé, 1984; McCord and Fridovich, 1969).

2.2.3. Catalase (CAT) activity

The 1/15 M $Na_2HPO_4.H_2O$-KH_2PO_4 buffer was prepared at pH 7 for catalase activity determination. 160 µL of H_2O_2 was added to 100 mL of this buffer (solution A). A blank tube was prepared by adding 0.15 µL of sample to 3 mL of distilled water. For activity determination, absorbance was read at 240 nm at 2-minute and 30-second intervals after adding a 0.15 µL sample to 3 mL of solution A.

Enzyme unit number in mL, C ΔODx2 (minute) x 1000 / 0.036 x µL supernatant / Lowry µmol/mg total protein, was calculated with the formula. One unit of catalase activity was defined as the amount of enzyme that catalyses the breakdown of one µmol of H_2O_2 per minute (Lück, 1965).

2.2.4. Phenol oxidase (PPO) activity

Phenol oxidase activity was determined using a spectrophotometric method. 50 µL of homogenate was rapidly added to 950 µL of phosphate buffer (pH=7) containing 20 mM L-DOPA. Absorbance changes at 420 nm were recorded every 10 seconds for 2 minutes. PO activity was calculated from the slope of the linear phase of the graph obtained from 12 readings (Sezgin, 2025).

3. Results

The *M. nana* extract exhibited lipid peroxidation and antioxidant enzyme activities with an MDA concentration of 15.0 ± 1.41 nmol/mL, SOD activity of 25.42 ± 1.1 U/mL, CAT activity of 43.17 ± 1.54 U/mL, and PO activity of 13.7 ± 0.49 U/mL (Table 1). These results indicate the potential antioxidant properties of the extract, reflecting its ability to mitigate oxidative stress.

Table 1 *Maresi nana* extract of lipid peroxidation and antioxidant enzyme activities (n=3, mean ± SD)

	MDA (nmol/mL)	**SOD (U/mL)**	**CAT (U/mL)**	**PO (U/mL)**
Maresi nana	15.0 ± 1.41	25.42 ± 1.1	43.17 ± 1.54	13.7 ± 0.49

4. Discussion

Lipid peroxidation in biological membranes causes changes in membrane fluidity, a decrease in membrane potential, increased permeability to H^+ and other ions, and membrane

ruptures leading to the release of cellular and organelle contents. Cytotoxic aldehydes formed as a result of lipid peroxidation inhibit protein synthesis, inactivate enzymes, and trigger thrombin formation. Therefore, lipid peroxidation plays a critical role in the development of inflammation, cancer, and cardiovascular diseases (Kedlaya and Vasudevan, 2004).

Antioxidant supplementation constitutes an important defense mechanism against various diseases associated with oxidative stress. Individuals consuming diets rich in fruits and vegetables are reported to have a longer life expectancy due to high intake of polyphenolic antioxidants. Flavonoids are strong metal chelators that inhibit lipid peroxidation and oxidative modification of LDL (Demirel et al., 2025). The presence of conjugated ring structures and hydroxyl groups enables phenolic compounds to effectively scavenge free radicals.

Plants are used in traditional medicine as therapeutic agents for the prevention of cardiovascular diseases, inflammatory conditions, and the reduction of cancer risk (Cuevas-Cianca et al., 2023). Additionally, plants hold significant value in the food and cosmetic industries due to the protective effects of antioxidant compounds (Michalak, 2022). In this study, *M. nana* was investigated in terms of its antioxidant enzymatic potential to support its traditional medicinal use. The antioxidant enzyme activity results of *Rumex obtusifolius* were evaluated for CAT, SOD, and PPO activities. *R. obtusifolius* extract showed good enzyme activities for CAT (38.65 ± 0.00026 U/mL), SOD (11.59 ± 0.01600 U/mL), and PPO (12.84 ± 0.00025 U/mL). In our study, *M. nana* extract showed better antioxidant activity than *R. obtusifolius*, with CAT (43.17 ± 1.54 U/mL), SOD (25.42 ± 1.1 U/mL), and PPO (13.7 ± 0.49 U/mL) activities (Alici et al., 2016). Therefore, it can be concluded that the antioxidant enzymes present in *M. nana* can be effectively extracted under suitable conditions. In a study by Rani et al. (2004), SOD activity was reported as 21.02 U/g for blueberry, 6.58 U/g for grape, 30.45 U/g for orange, and 6.91 U/g for tomato.

M. nana demonstrated much higher SOD activity than these examples. These findings suggest that *M. nana* extract exhibits cellular oxidative damage-preventing and free radical scavenging properties, and therefore, it can be considered a potential antioxidant agent.

5. Conclusions

In this study, four different antioxidant-related parameters (CAT, MDA, SOD, and PO) were determined in *M. nana*. Based on the results, it can be concluded that this plant may have regulatory effects on the antioxidant enzyme system and could potentially be used in the treatment of diseases associated with oxidative stress.

Author Contribution Statement

Emine Incilay Torunoğlu: Experimentation, writing the original manuscript, supervisor work Betül Aydın: contribution to the writing of the original manuscript; Alper Durmaz: plant material.

Conflict of interest

The authors declare no competing conflict of interest.

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