BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY
Volume 15, Number 1, April 2026 | Pages: 391-399 | DOI: 10.14421/biomedich.2026.151.391-399
ISSN 2089-6514 (paper)
ISSN 2540-9328 (online)

Protective Effect of Ethanolic Mistletoe Leaves (*Scurula atropurpurea* (Bl.) Dans) Extract Against Protein Denaturation In Vitro

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Manuscript received: 16 December 2025. Revision accepted: 08 April 2026, Published: 04 May 2026.

Abstract

Inflammation is a complex biological response that eliminates harmful stimuli, clears damaged tissues, and initiates healing. Protein denaturation is known to trigger inflammatory reactions and is associated with chronic disorders such as rheumatoid arthritis, lupus erythematosus, and serum sickness. Although steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) are widely used, their long-term use may cause adverse effects. Herbal plants represent potential alternative therapies with fewer side effects. This study examined the anti-inflammatory potential of *Scurrula atropurpurea*, a hemiparasitic plant traditionally used for various medicinal purposes. The ethanolic leaf extract was evaluated for its phytochemical composition, antioxidant properties, and ability to inhibit egg albumin denaturation in vitro. Fresh leaves were collected from tea plants in Sapuran, Wonosobo, and extracted using Soxhlet with 96% ethanol. Phytochemical screening indicated the presence of alkaloids, flavonoids, tannins, phenolics, saponins, steroids, and terpenoids. Quantitative analysis showed a high total phenolic content (126.10 ± 1.5 mg GAE/g extract), moderate tannins, and relatively low flavonoids. The extract demonstrated weak antioxidant activity (IC50 = 140.4 ± 4.9 µg/mL) and minimal protein denaturation inhibition (maximum 1.51% at 100 ppm), significantly lower than sodium diclofenac as a positive control. Overall, the ethanolic extract of *S. atropurpurea* leaves exhibited low in vitro anti-inflammatory activity, suggesting the need for further investigation using alternative extraction methods, plant parts, or host species.

Keywords: antioxidant; anti-inflammatory; phytochemical; protein denaturation; tea mistletoe.

INTRODUCTION

Inflammation is an essential physiological response activated when tissues encounter harmful stimuli such as pathogens, physical injury, or toxic compounds. Although initially beneficial, uncontrolled or chronic inflammation contributes to a wide range of diseases, such as rheumatoid arthritis, asthma, dermatitis, diabetes mellitus, cardiovascular diseases, and several autoimmune disorders. In Indonesia, several diseases associated with inflammatory mechanisms remain highly prevalent, for example, the national Basic Health Research (2013) reported that acute respiratory infections accounted for 25% of cases, dermatitis for 6.8%, asthma for 4.5%, and rheumatoid arthritis for 7.3% (Ifmaily et al., 2021). These statistics underscore the significant public health burden posed by inflammation-driven diseases.

One important molecular event contributing to inflammation is protein denaturation, a process in which proteins lose their native conformation due to physical or chemical stress. When proteins lose their native structure, their altered conformation may trigger immunogenic responses that contribute to chronic inflammatory diseases. Protein denaturation is closely associated with the pathophysiology of rheumatoid arthritis, lupus erythematosus, and serum sickness (Aidoo et al., 2021). Therefore, inhibition of protein denaturation is widely used as an in vitro model to evaluate potential anti-inflammatory agents.

NSAIDs, including diclofenac sodium, remain the primary pharmacological treatment for inflammatory disorders. These drugs act by inhibiting COX-1 and COX-2 enzymes and blocking prostaglandin synthesis, thereby reducing inflammation and pain (Gan, 2010). However, excessive use of NSAIDs is associated with significant adverse effects, including gastrointestinal irritation, hepatotoxicity, nephrotoxicity, hypertension, fluid retention, and increased cardiovascular risk (Apridamayanti et al., 2018; Teslim et al., 2014). The global emphasis on safer, more sustainable therapeutics has intensified scientific efforts to explore plant-derived anti-inflammatory agents with lower toxicity profiles.

In this context, medicinal plants rich in secondary metabolites such as phenolics, flavonoids, tannins,

alkaloids, saponins, terpenoids, and steroids, are of great interest due to their natural anti-inflammatory, antioxidant, and immunomodulatory properties. Indonesia, with its extensive biodiversity, provides abundant opportunities for discovering new bioactive compounds. One such plant is *Scurrula atropurpurea* (tea mistletoe), a hemiparasitic species traditionally used in Indonesian herbal medicine. Previous studies have demonstrated that *S. atropurpurea* exhibits antioxidant, anticancer, antimicrobial, and immunomodulatory properties, likely due to its rich secondary metabolite profile (Aditiyarini et al., 2022; Marvibaigi et al., 2014).

Despite its widespread traditional use, the anti-inflammatory potential of *S. atropurpurea*, particularly its ability to inhibit protein denaturation, has not been thoroughly investigated. Moreover, as a hemiparasite, *S. atropurpurea* absorbs nutrients and secondary metabolites from its host plant through a haustorial connection, resulting in significant chemical variability depending on host species, altitude, soil nutrient composition, temperature, and environmental stress. Given these considerations, the present study aims to investigate the anti-inflammatory activity of ethanolic extracts of *S. atropurpurea* leaves collected from tea host plants in Sapuran, Wonosobo. The study evaluates phytochemical content, antioxidant capacity, and in vitro inhibition of protein denaturation to determine whether this mistletoe species possesses measurable anti-inflammatory properties. This research provides valuable insights into the pharmacological potential of *S. atropurpurea*, contributes to the growing interest in natural anti-inflammatory agents, and lays the scientific foundation for future studies exploring host-dependent variations in bioactivity.

MATERIALS AND METHODS

Study area

Fresh *S. atropurpurea* leaves were collected from tea plants in Sapuran, Wonosobo, Central Java. Species identity was confirmed at the Plant Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada. Figure 1 shows the appearance of fresh (A) and dried (B) *S. atropurpurea* leaves. Environmental parameters—including soil pH, temperature, humidity, elevation, and soil nutrient content—were recorded and analyzed. Soil samples were analyzed at the Laboratory of the Faculty of Agriculture INSTIPER Yogyakarta.

(A) (B)

Figure 1. (A) Fresh *S. atropurpurea* leaves and (B) Dried *S. atropurpurea* leaves.

Procedures

Extraction Procedure

A total of 952 g of fresh leaves were cleaned, air-dried for two days, and oven-dried at 40°C for two hours. The dried material was pulverized and sieved (40 mesh). Soxhlet extraction was performed using 15 g of powder and 150 mL of 96% ethanol (1:10 ratio), with the solvent replaced every three cycles. The extract was concentrated with a rotary evaporator and oven-dried to obtain a constant-weight crude extract (Aditiyarini et al., 2022).

Phytochemical Screening

The phytochemical of S.atropurpurea was conducted in colometry method for alkaloid, flavonoid, saponin, tannin, steroid and terpenoid, In alkaloid assay, 0.1 g of mistletoe leaf extract was added to 1 mL of 2N HCl, then 9 mL of distilled water was added and heated for 2 minutes. The mixture was left at room temperature until cool, then filtered. The solution was divided into 3 tubes, each filled with 3 drops of Mayer's, Wagner's, and Dragendorff's reagents, respectively. A positive result is indicated by the formation of a reddish precipitate (Auwal et al., 2014). For flavonoid assay, 0.05 g of extract was homogenized in 2 mL ethanol 96%. Then magnesium ribbon 2 cm and 1 mL of concentrated HCl were added and homogenized. Positive results are indicated by a change in the color of the solution to brick red or orange (Oktavia & Sutoyo, 2021; Wahid & Safwan, 2020). For saponin, 0.05 g extract was dissolved into 10 mL of hot distilled water. Then the solution was cooled in room temperature and shaken vigorously for 10 seconds. When 1 cm of froth is formed and allowed to stand for 10 minutes then 1 mL of 2N HCl is added. If the foam remains constant for ± 10 minutes after being

dripped with 2N HCl, the test results are positive for saponin compounds (Kopon et al., 2020). In tannin assay, S. atropurpurea mistletoe leaf extract as much as 0.05 g was added to 2 mL of 96% ethanol in a tube and then homogenized. Then the solution was added as much as 3 drops of $FeCl_3$ 10%. Positive results of tannin testing are indicated by a change in color to blue-black or green-black and there is a precipitate (Aditiyarini et al., 2022; Kopon et al., 2020). For steroid and terpenoid assay, 0.05 g extract was dissolved into 1 mL of chloroform. Then 5 drops of 98% anhydrous acetate and 3 drops of conc. H_2SO_4 was added through the tube wall. Positive results of terpenoids are indicated by a change in color to a brownish color or purple ring at the end of the solution, and positive results of steroids produce a blue-green ring (Aditiyarini et al., 2022; Padmasari et al., 2013). In phenolic assay, 0.05 g extract was dissolved into 10 drops of methanol. Then 3-4 drops of 10% $FeCl_3$ solution was added. Positive results of phenolic testing are indicated by a change in color to blue, green, purple, or reddish, indicating that there are phenolic compounds in the sample (Oktavia & Sutoyo, 2021).

Total Phenolics Content

The measurement of phenolic content was performed using a colorimetric method with gallic acid as a standard, based on modified methods from Auad et al. (2019) and Megawati et al. (2021). A standard curve was prepared using gallic acid concentrations ranging from 20 to 220 ppm. For both the standard and sample solutions, 1 mL of each concentration was mixed with 0.3 mL of 10% Folin-Ciocalteu reagent, allowed to stand for 4-8 minutes, then combined with 0.3 mL of 20% Na_2CO_3. The mixture was vortexed, diluted to 10 mL with distilled water, and left to stand at room temperature in dark conditions for 2 hours. The sample preparation involved dissolving 10 mg of extract in 10 mL of 96% ethanol, with subsequent 3-fold dilutions. Absorbance measurements for both standard and sample solutions were taken at 765 nm using a UV-Vis spectrophotometer, allowing for the determination of total phenolic content in the extract.

Total Flavonoid Content

Flavonoid content testing was conducted using a colorimetric method with quercetin as a standard, based on a modified method from Lindawati & Ma'ruf (2020). A standard curve was prepared using quercetin concentrations ranging from 10 to 100 ppm. For both standard and sample solutions, 1 mL of each concentration was mixed with 3 mL of methanol (absolute for standard, 96% for sample), 0.2 mL of 10% $AlCl_3$, and 0.2 mL of 1 N potassium acetate, with 3-6 minutes standing period between $AlCl_3$ and potassium acetate additions. The mixtures were diluted to 10 mL with distilled water and left to stand at room temperature in dark conditions (30 minutes for standard, 2 hours for sample). The sample preparation involved dissolving 10 mg of extract in 10 mL of absolute methanol, with subsequent 3-fold dilutions. Absorbance measurements were taken using a UV-Vis spectrophotometer at 430 nm for the standard curve and 431 nm for the sample, allowing for the determination of total flavonoid content in the extract.

Total Tannin Content

Total tannin content was determined using a colorimetric method with tannic acid as a standard, based on modified methods from Auad et al. (2019) and Megawati et al. (2021). A standard curve was prepared using tannic acid concentrations ranging from 10 to 100 ppm. For both standard and sample solutions, 1 mL of each concentration was mixed with 1 mL of 10% Folin-Ciocalteu reagent, allowed to stand for 4-8 minutes, then combined with 3 mL of 20% Na_2CO_3. The mixtures were vortexed, diluted to 10 mL with distilled water, and left to stand at room temperature for 2 hours. Sample preparation involved dissolving 10 mg of extract in diethyl ether, incubating for 20 hours, evaporating the solvent, and reconstituting in 10 mL of absolute methanol, followed by 3-fold dilutions. Absorbance measurements for both standard and sample solutions were taken at 760 nm using a UV-Vis spectrophotometer, allowing for the determination of total tannin content in the extract.

Determination of Antioxidant Activities

The antioxidant activity of *S. atropurpurea* extracts was determined using a modified ABTS method based on Marraskuranto et al. (2021), Oktavia & Sutoyo (2021) and Saputri et al. (2020). The process involved preparing an ABTS stock solution 7.1 mg/25 mL, a vitamin C (quercetin) as control solution, and ethanol extract solutions of *S. atropurpurea* leaves at various concentrations. The concentration of vitamin C was 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ppm, whereas the concentration of extract was 20, 80, 140, 200, 260, 320, 380, 440, 500, and 560 ppm. Antioxidant activity measurements were conducted using a 96-well microplate, comparing ABTS reagent with samples, positive controls, negative controls, and blanks. After 30 minutes of incubation at room temperature in dark conditions, absorbance was measured at 734 nm using a microplate reader. The percentage inhibition was calculated, and IC50 values were determined using a linear regression equation. The antioxidant activity of the *S. atropurpurea* extract was then compared to that of quercetin by evaluating their respective IC50 values.

$$\% \, inhibition = \frac{A_{Control} - A_{sample}}{A_{Control}} \times 100\%$$
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Determination of Protein Denaturation Inhibition

The anti-inflammatory activity of *S. atropurpurea* extract was assessed using a modified protein denaturation inhibition method based on Fadlilaturrahmah et al., (2022). The process involved preparing Tris Buffer Saline (TBS) 605 mg/500 mL, 4% fresh egg albumin in TBS, negative control (methanol), positive control (diclofenac sodium), and *S. atropurpurea* extract solutions at various concentrations (25, 50, and 100 ppm). The test solutions were prepared by mixing 0.1 mL of each concentration with 4% egg albumin solution to a total volume of 10 mL. After initial absorbance measurement, solutions were heat-treated at 70°C for 10 minutes, cooled, and re-measured at 660 nm to determine turbidity. The percentage of inhibition was calculated, with values >20% considered to indicate anti-inflammatory activity (Novika et al., 2021). This method allows for the comparison of the extract's anti-inflammatory potential with that of the positive control, diclofenac sodium.

$$\% \, Protein \, Inhibition = \frac{\boldsymbol{A_{Control} - A_{sample}}}{\boldsymbol{A_{Control}}} \times \mathbf{100\%}$$

LC-HRMS Analysis

LC-HRMS analysis was performed in Biotek Rekayasa Indonesia using Thermo Scientific™ Vanquish™ UHPLC Binary Pump) coupled to Orbitrap high-resolution mass spectrometry (Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ High Resolution Mass Spectrometer). The column was ThermoScientific™ Accucore™ Phenyl-Hexyl 100 mm × 2.1 mm ID × 2.6 µm. Flow rate was 0.3 mL/min with MS-grade water containing 0.1% formic acid as eluent A and MS-grade methanol containing 0.1% formic acid as eluent B. Injection volume was 3 µL. The column oven temperature was 40°C. Mass Spectrometer was Electrospray Ionization (ESI) with the scan range 66.7-1000 m/z and resolution 70000 for full MS and 17600 for dd-MS2.

Data analysis

The sample data was quantitative test of total phenolic content, total flavonoid content, total tannin content, antioxidant activity, and protein denaturation inhibition with three repetitions of each treatment. Descriptive analysis was conducted using Microsoft Excel. Protein denaturation inhibition data will be further analyzed, namely statistical analysis using SPSS One Way Anova method, if the P value <0.05 states that there is a significant difference in the average, a post hoc Tukey test will be carried out. If the normality and homogeneity data are found to be unqualified with a significant $p < 0.05$, the Kruskall-wallis test will be performed.

RESULTS AND DISCUSSION

Phytochemical of Ethanolic *S.atropurpurea* Leaves Extract

Phytochemical screening showed the presence of alkaloid, phenolic, flavonoid, saponin, steroid, tannin, and terpenoid in the ethanolic extract of *S.atropurpurea* leaves as shown in Table 1.

Table 1. Phytochemical of Ethanolic *S.atropurpurea* Leaves Extract.

Phytochemical	Result	Description
Alkaloid	+	Mayer (-): no white precipitate; Wagner (+): there is a brown precipitate; Dragendroff (+): there is a red-orange precipitate
Flavonoid	+	Red-orange
Fenolic	+	Blackish green
Saponin	+	Constant foam 1cm > 10 minutes
Steroid & Terpenoid	+	Brownish gold colored ring
Tanin	+	Blackish green

LC-HRMS data of *S. atropurpurea* leaf extract, as shown in Table 4, confirmed the presence of caffeine (4.74%), theobromine (1.48%), fatty acids (including octadec-12-en-8,10-diynoic acid, octadeca-9,12,15-trienoic acid, α-linolenic acid, 8,10-octadecadienoic acid, 9,12-octadecadienoic acid, and oleic acid), catechin and epicatechin, acariside group compounds, quercetin derivatives, aviculin, rutin, and quercetin 3-rutinoside.

Table 4. LC-HRMS Data of *S.atropurpurea* Leaves Extract.

No	Phytochemical	Molecular Mass	Retention Time (min)	% Area
	Xanthines			
1	Theobromine	181.0719	2.01	1.38
2	Caffein	195.0882	4.16	4.74
	Fatty acids			
3	Octadeca-9,12,15-trienoic acid	273.1855	10.01	0.11

No	Phytochemical	Molecular Mass	Retention Time (min)	% Area
4	Octadec-12-en-8,10-diynoic acid	275.2006	12.50	1.52
5	α-Linolenic acid	279.2317	12.60	0.76
6	8,10-octadecadienoic acid; 9,12-octadecadienoic acid	281.2474	15.04	0.03
7	Oleic acid	283.2631	15.62	0.05
	Flavanes			
8	Catechin; Epicatechin	291.0842	0.84	0.003
9	Catechin; Epicatechin	291.0863	1.88	0.01
	Monoterpene glucoside			
10	Icariside Group*	387.2050	3.00	0.00
11	Icariside Group*	387.2012	4.66	0.03
	Flavonol glycosides			
12	Quercetin Group**	449.1078	6.34	0.59
13	Rutin; Quercetin 3-rutinoside	611.1605	5.59	0.01
	Lignan glycoside			
14	Aviculin	507.2276	13.12	0.00

*Icariside Group : Icariside B2; 4-(2,2,6-trimethyl-4-{[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-7-oxabicyclo[4.1.0]heptan-1-yl)but-3-en-2-one

**Quercetin Group: Quercetin 3-O-alpha-L-rhamnoside; Quercitrin; 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-{[(2s,3s,4r,5r,6s)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxy}chromen-4-one.

The quantitative analysis of phytochemical is shown in Table 3. The total phenolic, flavonoid, and tannin content of extract was 126.10 mg GAE/g ext., 43.01 mg QE/g ext., and 73.54 mg TAE/g respectively.

Table 3. Total Phytochemical Content of *S.atropurpurea* Leaves Extract.

Phytochemical	Amount ± STD
Phenolic	126.10 ± 1.5 mg GAE/g ext.
Flavonoid	43.01 ± 1.3 mg QE/g ext.
Tannins	73.54 ± 4.2 mg TAE/g

Description: QE: Quercetin Equivalent; GAE: Gallic Acid Equivalent; TAE: Tannic Acid Equivalent.

Antioxidant Activity

The antioxidant activity assessed using the ABTS method revealed an IC50 of 153.40 µg/mL for the extract using three repetitions (Figure 2). In comparison, quercetin which is a strong antioxidant, exhibited an IC50 of 7.1 ± 1.2 µg/mL.

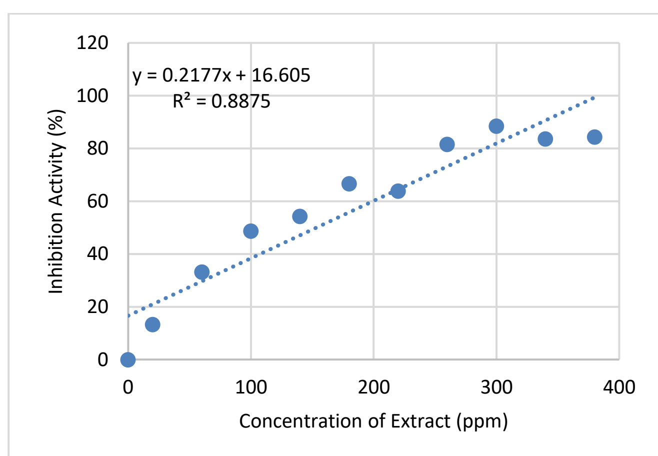

Figure 2. Antioxidant Activity of *S. atropurpurea* Leaves Extract.

Protein Denaturation Inhibition Activity

The extract displayed low inhibitory activity, with inhibition percentages of –2.80%, 1.08%, and 2.59% at concentrations of 25, 50, and 100 ppm, respectively (Figure 3).

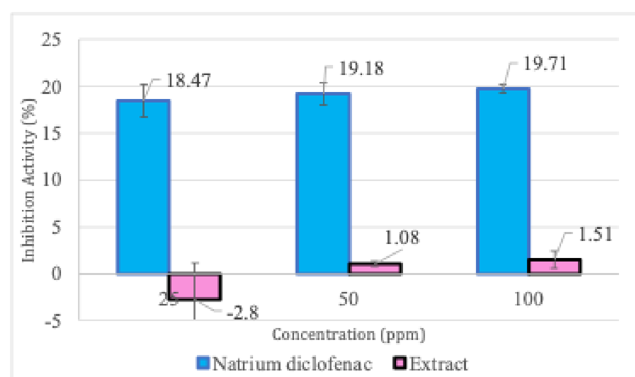

Figure 3. Protein denaturation inhibition activity of sodium diclofenac and *Scurrula atropurpurea* leaves extract.

Discussion

The ethanolic extraction of *Scurrula atropurpurea* leaves using the Soxhlet method produced a 41.72% yield. This relatively high yield indicates that ethanol 96% effectively extracted polar and semi-polar secondary metabolites. The extraction efficiency is strongly associated with the solvent-to-sample ratio, temperature, and duration of extraction. A prolonged extraction cycle increases the interaction time between solvent and plant material, allowing more extensive cell wall degradation and diffusion of metabolites into the solvent (Winata & Yunianta, 2015). The yield obtained in this study meets the recommended minimum standard of ≥10% for herbal extracts (Ministry of Health of Republic of Indonesia, 2022) suggesting good extractability of bioactive compounds from tea mistletoe leaves.

Phytochemical screening confirmed the presence of major secondary metabolite groups, consistent with previous studies on *S. atropurpurea* from different host plants (Aditiyarini et al., 2022; Mustarichie & Ramdhani, 2022; Wirasti, 2019). However, the detection of alkaloids in this study differed from some earlier reports, highlighting the influence of environmental variability on metabolite biosynthesis. As a hemiparasitic species, mistletoe absorbs water, minerals, and even secondary metabolites from its host via haustorial connections. Consequently, differences in host species, soil nutrient availability, altitude, and microclimatic conditions contribute to the variability of phytochemical profiles across locations (Tiwari & Cummins, 2013). These factors emphasize the importance of site-specific and host-specific investigations to better understand the chemical diversity of mistletoe.

The tea mistletoe and its host plant grow in high-altitude environments (>700 m above sea level), where environmental conditions strongly influence secondary metabolite production. Elevation and temperature are known to affect phenolic accumulation in tea plants (Wang et al., 2022). Although tea grows optimally at 13–25°C (Haq & Mastur, 2016), the measured air temperature at the sampling site reached 27°C, a level that can negatively affect growth and metabolite formation (Duncan et al., 2016). Temperature fluctuations alter plant metabolic pathways and secondary metabolite biosynthesis, including phenolics and flavonoids (Yadav, 2010; Zargoosh et al., 2019). Furthermore, the soil moisture recorded in this study (5%) was far below the optimal range of 50–70%) (Meilianto et al., 2022), ndicating water stress that can further modify plant defense responses and metabolite synthesis. Both temperature stress and water deficiency can trigger changes in plant defense systems and metabolic processes.

Soil nutrient conditions also contribute to variations in secondary metabolite levels. Tea plants parasitized by mistletoe have been reported to exhibit low nitrogen (0.426%), phosphorus (0.112%), and potassium (0.154%) levels. Low soil pH can reduce phosphorus availability, while nutrient factors such as soil aeration, temperature, and organic matter also influence P uptake. Excess nitrogen tends to promote vegetative growth but reduce flavonoid content (Sakr & Husein, 2012). Conversely, adequate potassium and phosphorus can enhance phenolic and flavonoid synthesis (Ahmad et al., 2018). Fertilizer composition and soil nutrient balance therefore directly affect the biosynthesis of secondary metabolites (Ibrahim et al., 2013). Under environmental stress, activation of phenylalanine ammonia-lyase (PAL) promotes phenolic and flavonoid formation. However, excessively high phosphorus may suppress phenolic production, whereas surplus potassium may shift plant metabolism toward storage rather than secondary metabolite synthesis (Afifaturrosydah et al., 2022).

As a hemiparasitic plant, *S. atropurpurea* depends heavily on its host through a haustorial connection that penetrates the xylem and phloem. This allows not only the transfer of water and minerals but also the bidirectional movement of primary and secondary metabolites. As a result, compounds typically found in tea leaves can also appear in mistletoe. LC-HRMS analysis showed that some compounds in mistletoe extract is similar with the compounds in tea, such catechin, theobromine, and caffeine. Catechin, a primary phytochemical with a flavan-3-ol backbone, is key compounds contributing to the antioxidant quality of tea. Several types of catechins found in tea leaves include catechin, epicatechin, gallocatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, and gallocatechin gallate. Generally, catechins constitute approximately 42% of the polyphenol compounds in tea and are responsible for its antioxidant activity. Similar catechin-rich profiles have been identified in a study by Ohashi et al. (2003) in leaf and stem extracts of *S. atropurpurea* (Bl.) Danser parasitizing *Thea sinensis* L. plants. Another study by Sudiwati et al., (2015) identified additional phytochemicals in various fractions of *S. atropurpurea* extract from tea plants in Wonosari Lawang, Malang, East Java. The n-hexane fraction contained flavanones, dihydroflavonols, and flavones; the chloroform fraction contained flavanones, dihydroflavonols, and catechins; and the ethanol fraction contained epigallocatechin gallate (EGCG), flavonols, and flavones. This collective evidence reinforces that the host plant substantially shapes the chemical profile of *S. atropurpurea*, contributing to the diversity and biological activity of its secondary metabolites.

The quantitative analysis was conducted to determine the number of compounds per mass of extract. Total phenolic content (TPC) was higher than flavonoid and tannin content, measuring 126.10 ± 1.5 mg GAE/g extract. This value was lower than the TPC of *Dendrophthoe pentandra* (L.) Miq. from the same location, which was 48.584 mg QE/g extract (Haryono et al., 2024). This difference suggests that phytochemical content can be influenced by mistletoe species. Phenolic compounds and their derivatives have the potential to inhibit pro-inflammatory mediators such as COX and LOX, reduce the formation of reactive oxygen species, modulate the regulation of transcription elements involved in the antioxidant pathway, regulate eicosanoid synthesis, inhibit nitric oxide and prostaglandin activity, and prevent platelet aggregation. The phenolic compounds are thermolabile, meaning they can undergo hydrolysis when exposed to heat, leading to a decrease in total phenolic content. The higher total phenolic content observed in mistletoe extract can be attributed to the fact that phenolic compounds are widely produced in plant leaves, where they act as signalling molecules in symbiotic interactions and serve as plant defense agents. This explains why the mistletoe extract, primarily

derived from leaves, exhibits a higher phenolic content compared to other plant parts.

Compared to Aditiyarini et al. (2022), total flavonoid content (TPC) in this study was higher but total tannin content (TTC) was lower than *S. atropurpurea* from Nglinggo, Yogyakarta. The difference can be influenced by the location where the host plant grows and environmental factors obtained from the growth location. Secondary metabolite compounds synthesized by host plants are an adaptive response of host plants when experiencing stress and genetic factors from the host plant (Setyorini & Yusnawan, 2016). The high phenolic content in this study reflects the natural physiological role of phenolics in plant stress responses. Since mistletoe leaves serve as the primary site for photosynthesis and defense, the accumulation of phenolic compounds is consistent with their function in scavenging reactive oxygen species (ROS), regulating plant–host interactions, and protecting against environmental stressors.

The antioxidant activity of mistletoe extract was classified as weak antioxidant capacity. The considerable difference indicates that despite the presence of phenolics and tannins, the extract's radical-scavenging ability is limited. A key factor influencing this low antioxidant activity is the relatively low flavonoid content. Flavonoids contribute significantly to antioxidant mechanisms due to their structural features, such as multiple hydroxyl groups capable of donating hydrogen or electrons. The thermolabile nature of flavonoids may also account for their reduced levels, as high extraction temperatures can degrade or oxidize these compounds (Andarwulan et al., 1996; Syafrida et al., 2018). Additionally, environmental stresses and host variability may result in altered metabolite distribution, further affecting antioxidant outcomes.

Nonetheless, the presence of moderate phenolic and tannin levels suggests that the extract retains some antioxidant components, even though they are not potent enough to exert strong radical-scavenging effects. This finding becomes particularly relevant when interpreting the extract's anti-inflammatory activity, as antioxidant potential is often correlated with anti-inflammatory mechanisms.

The anti-inflammatory activity of *S. atropurpurea* extract was evaluated through inhibition of heat-induced albumin denaturation. The value of protein denaturation inhibitory activity of mistletoe extract was low that fall significantly below the ≥20% threshold generally used to indicate meaningful anti-inflammatory potential (Novika et al., 2021). In contrast, the positive control, sodium diclofenac, exhibited consistently high inhibition (18.47–19.71%), demonstrating the validity of the assay. The poor inhibition performance of extract contrasts sharply with medicinal plants known for strong anti-inflammatory effects, such as *Morus mesozygia*, which exhibited a protein denaturation IC50 of 11.89 μg/mL due to its high phenolic and flavonoid content (Elisha et al., 2016). This comparison highlights the importance of flavonoid concentration in influencing anti-inflammatory activity.

Although phytochemical screening confirmed the presence of anti-inflammatory constituents such as phenolics, tannins, and flavonoids, their concentrations, particularly flavonoids, were insufficient to exert meaningful protein protection. Flavonoids are known to inhibit inflammatory mediators including COX-1, COX-2, LOX, nitric oxide, and prostaglandins, and to modulate signaling pathways such as MAPK and Nrf2 (Al-Khayri et al., 2022). However, these mechanisms require adequate concentrations of active compounds, which may not have been present in the extract.

Moreover, weak antioxidant activity may also contribute to poor anti-inflammatory performance. Since oxidative stress promotes protein unfolding and inflammatory responses, limited antioxidant protection would reduce the extract's ability to prevent protein denaturation. Statistical analysis using the Kruskal–Wallis test showed a significant difference ($p < 0.05$) between the inhibitory effects of the extract and sodium diclofenac, confirming that the extract's activity was significantly lower across all tested concentrations. These results consistently demonstrate that *S. atropurpurea* ethanolic extract exhibits low anti-inflammatory activity under the conditions evaluated.

CONCLUSIONS

The ethanolic extract of *Scurrula atropurpurea* leaves contains diverse bioactive secondary metabolites but demonstrates weak antioxidant and anti-inflammatory activities in vitro. Protein denaturation inhibition remained far below than the positive control, indicating limited ability to prevent protein structural degradation under the tested conditions. These findings suggest that while *S. atropurpurea* contains pharmacologically relevant compounds, its anti-inflammatory potential at the ethanolic leaf extract appears low. Further studies are recommended using different solvents, extraction temperatures, or other plant parts, as well as evaluation on various host plants to better understand metabolite variability.

Acknowledgements: We extend our sincere gratitude to the entire research team for their dedication and invaluable contributions throughout this study. Special thanks to the laboratory staff for their technical support. We thank the local communities in Sapuran Wonosobo for their cooperation in plant sample collection. Finally, we are grateful to our colleagues and peer reviewers for their constructive feedback, which significantly improved the quality of this work.

Authors' Contributions: In this study, the authors contributed as follows: Dwi Aditiyarini conceptualized the research, developed the methodology, and supervised the overall project. She also played a primary role in writing and editing the manuscript. Christini Bernaindah Nadapdap conducted the laboratory experiments, performed data analysis, and created the figures and tables. She also contributed to the writing of the methods and results sections. Ratih Restiani was responsible for data curation, statistical analysis, and interpretation of results. She also assisted in the literature review and contributed to the discussion section of the manuscript. All authors were involved in the review and editing process and have approved the final version of the manuscript for publication.

Competing Interests: The authors declare that there are no competing interests.

Funding: We appreciate the financial assistance provided by LPPM UKDW (Grant No. 84/D.01/LPPM/2024) which made this research possible.

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