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Article

Prevention of Oxidative Stress and Inflammation by Ashitaba (*Angelica Keiskei*)'s Ethanol Extract

Muhamad Khafid [1], Dyah Ika Krisnawati [1*], Erika Martining Wardani [1], Dwi Rahayu [2], Eppy Setiyowati [1], Rusdianingseh [1]

[1] Department of Nursing, Faculty of Nursing and Midwifery, Universitas Nahdlatul Ulama Surabaya, Surabaya 60237, Indonesia

[2] Department of Nursing STIKES Pamenang, Bendo Pare, Kediri Regency, East Java 64225, Indonesia

[*] Correspondence: dyahika@unusa.ac.id

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Abstract: Cigarette smoke (CS) contains a complex combination of thousands of different chemicals that increase oxidative stress. Inhaling CS causes an inflammatory response, which leads to several diseases linked to tobacco. Ashitaba has various derivatives that serve as intermediates in the biosynthesis of one type of bioactive flavonoid antioxidant, which is thought to neutralize oxidative stress events. Ashitaba has also been shown to inhibit platelet aggregation, exhibit vasorelaxant effects, and suppress the differentiation of preadipocytes. Safety studies indicate that administering ashitaba chalcone orally to male Wistar rats for 28 days showed no signs of toxicity. Similarly, gavage administration of ashitaba extract in male ICR mice demonstrated safe absorption and metabolism of 4-hydroxyderricin and xanthoangelol without adverse effects. Additionally, a related compound, xanthohumol, a prenylated chalcone found in hops, was given to female BALB/c mice at approximately 1000 mg/kg daily for three weeks without toxic effects. This study proves that Ashitaba contains antioxidants that can help prevent oxidative stress and inflammation induced by CS. Flavonoids isolated from ashitaba were found to have a flavonoid content of 38.115 ± 0.124 ppm, which was then implemented in *in-vivo* studies The ability of these antioxidants to decrease the level of pro-inflammatory cytokines in rat blood serum and lung tissue after exposure to CS was investigated. In this study, we isolated flavonoids from ashitaba and obtained a flavonoid, then implemented it for *in vivo* studies, and the ability of antioxidants in *Angelica keiskei* to prevent inflammatory events was proved.

Keywords: Ashitaba; Cigarette Smoke; Biomarkers; Oxidative Stress; Inflammation

1. Introduction

Tobacco smoke contains a complex mixture of thousands of chemicals, including reactive oxygen/nitrogen species (ROS/RNS), quinones, aldehydes, ketones, and metals, all of which contribute significantly to increased oxidative stress. A single puff of cigarette smoke (CS) can deliver over 10^{15} oxidants or free radicals, found in both the gas and tar components. The gas phase carries various ROS along with compounds like epoxides, peroxynitrite, and nitric oxide (NO), while the tar phase includes substances such as semiquinones, peroxides, hydroxyl radicals, hydrogen peroxide, and other organic molecules that participate in redox reactions and generate ROS, including the superoxide anion [1].

These reactive compounds can trigger oxidative damage either directly or indirectly by promoting the production of pro-inflammatory substances (like chemokines, cytokines, prostaglandins, leukotrienes, and isoprostanes)

and by activating intracellular pathways involved in inflammation (MAPK, NF-kB, AP-1, Keapl-Nrf2-ARE) [2–5]. As a result, inhalation of cigarette smoke contributes to the onset of an inflammatory response, resulting in several tobacco-related diseases (respiratory and cardiovascular diseases and tumors).

Whether inhaled actively or passively, CS quickly dissolves into the fluids lining the oral and respiratory epithelium and is absorbed into the bloodstream. The combustion process is critical because it generates ROS that are not present in unburned tobacco or its ash. The byproducts of combustion include both gaseous and particulate matter, with most harmful substances found in the particulate phase. CS has widespread effects on the immune system, affecting innate immune responses in the oral, nasal, and respiratory mucosa, as well as altering the adaptive immunity systemically. Many of CS's toxic effects, especially those linked to cancer development, arise from genetic and epigenetic changes that disrupt normal gene function, such as those involved in the cell cycle, DNA repair, and tumor suppression. It is important to note that CS contributes to cancer and other diseases through a variety of mechanisms.

The immune system first encounters the gaseous and particulate components of CS at mucosal surfaces such as those in the mouth, sinuses, and respiratory tract. Cigarette combustion generates thousands of ROS, which are not effectively filtered out by cigarette filters. The ROS in the gas phase are typically short-lived and primarily impact the upper respiratory tract. In contrast, ROS in the particulate phase—especially semiquinone radicals—can generate additional free radicals. These ROS harm airway epithelial cells by triggering lipid peroxidation, damaging other membrane structures, activating redox-sensitive cellular signaling pathways, and causing DNA damage. Additionally, these compounds stimulate intracellular pathways in epithelial cells, resulting in the expression of pro-inflammatory genes, such as interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF-α). This, in turn, promotes the ongoing recruitment of immune cells and contributes to the development of chronic inflammation.

Human beings have always involved themselves in various activities to ensure their well-being and survival. In doing so, the human body has directed the release of different free radicals or reactive substances, which are either inhaled or consumed [6]. RNS/ROS play a dual role, both toxic and beneficialto the organism's system. At lower concentrations, they have beneficial effects and participate in different physiological processes, including redox regulation, mitogenic responses, cellular signaling pathways, and immune function. However, at higher levels, these reactive species generate nitrosative and oxidative stress [7]. To reduce or prevent free radical-directed oxidative damage, the human body has developed an antioxidant defense mechanism that involves free radical scavenging, metal chelation, and enzymatic activities to neutralize ROS: just after they have formed.

Antioxidant supplements are substances derived either from natural food sources or produced synthetically. However, they differ in composition from the natural antioxidants found in food. As a result, there is ongoing debate about whether these supplements provide the same health benefits as those obtained from food-based antioxidants. While antioxidant supplements are gaining popularity, especially in industrialized countries, the scientific evidence supporting their benefits remains unclear. Although some epidemiological studies suggest that antioxidants may help in preventing chronic illnesses, their routine use is limited by the lack of long-term studies, unclear dosage guidelines, and uncertain long-term effects. Additionally, if taken in doses much higher than the recommended daily intake (RDI), antioxidant supplements may act as pro-oxidants and cause oxidative stress. Like traditional medicines, these supplements can cause side effects or interact negatively with other drugs or supplements, potentially worsening health conditions. Nevertheless, supplements might be beneficial in certain situations—for instance, for soldiers, sailors, individuals with digestive issues, or people with limited access to a variety of healthy foods. In such cases, a daily multivitamin and fish oil supplement at recommended doses can support overall health. However, high doses can be harmful, and it is essential to seek medical advice before combining supplements with prescribed medications. Whenever possible, it is preferable to obtain antioxidants through a diet rich in fruits and vegetables, rather than relying on supplements [8–12].

In addition, the consumption of dietary antioxidants can maintain an adequate level of antioxidants in the organism's body [13]. The level of reactive species in the cellular system may be reduced by antioxidants either by restricting the expression or activities of free radical-producing enzymes such as xanthine oxidase (XO) and NAD(P)H oxidase, or by enhancing the expression and activities of antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) [14]. The growing interest in antioxidants among the public, health professionals, and food scientists is due to their protective function in food items against oxidative deterioration and in the body against oxidative stress-induced abnormal processes. These potent natural antioxi-

dants are in huge demand for pharmaceuticals/nutraceuticals and as food preservatives. Effective search for new sources of naturally occurring antioxidants and the formulation of new antioxidant compounds need reliable methods for evaluating their antioxidant activity. Many biological models, food models, and chemical assays have been developed to measure the reducing power, radical scavenging activity, and other related attributes, as well as overall oxidation inhibition, in more complex biological systems and food items. These processes vary in terms of ease of operation, result expression, oxidation initiator, substrate type, and antioxidant mechanism. The selection of a specific method or a combination of methods is required for the proper assessment of antioxidant potential as a health-enhancing agent or as a food preservative [15,16].

Studies proved the effectiveness of antioxidants against oxidative stress and inflammation in several kinds of disease, such as a study about phenolic compound p-Hydroxybenzaldehyde (HD) from Nostoc commune, which effectively mitigates intestinal inflammation, the protective effects and underlying mechanisms of the action of Achyranthes aspera water-soluble extract (AAW) on a murine model of cisplatin-induced acute kidney injury AKI. Additionally, AAW was found to enhance protective signaling pathways, including the cGMP/PKG-, cAMP-, AMPK-, and mTOR-dependent activation of autophagy and mitophagy pathways. Other studies mention that the utilization of antioxidant agents that act on the central nervous system may serve as a supplementary approach in the secondary prevention of epilepsy, both in laboratory animals and potentially in humans. Chlorogenic acid (CGA) is a significant compound widely prevalent in numerous medicinal and food plants, exhibiting an extensive spectrum of biological activities, including neuroprotection, antioxidant, anti-inflammatory, and analgesic effects, among others. A study on 22 new D-ring modified isosteviol derivatives was conducted and their cardioprotective effects were evaluated *in vivo* using the zebrafish cardiomyopathy model. The findings revealed that derivative 4e exhibited the most potent cardioprotective effect, surpassing its parent compound, isosteviol, and the positive drug levosimendan. The protective effects and cellular mechanisms of H7E, a novel small molecule that inhibits HDAC8, have also been investigated using *in vitro* and *in vivo* glaucoma-like models. It involves the ERK and JNK MAPK pathways prevent retinal cell death and reduce extracellular glutamate released from stressed Müller glia. In a mouse model of NMDA-induced retinal degeneration, This study newly identified compound H7E protects against glaucoma damage by specifically targeting HDAC8 activity in the retina. This protective effect is attributed to the inhibition of Müller glial activation and the prevention of retinal cell death caused by oxidative stress. Also, Chronic atrophic gastritis (CAG) research has indicated that Costunolide (COS), the primary active compound found in Aucklandiae Radix, a traditional herb, exhibits antioxidant properties [17,18].

Angelica keiskei, commonly referred to in Indonesia as Ashitaba or Japanese celery, has long been utilized in Asia for centuries to manage conditions such as anemia, diabetes, hypertension, aging, and cancer. Phytochemical research on *A. keiskei* has uncovered more than 100 bioactive compounds, including various flavonoids, coumarins, phenolics, acetylenes, sesquiterpenes, diterpenes, and triterpenes [19]. Its constituents, such as coumarins, flavanones, and chalcones, have therapeutic effects [20]. Chalcones, which are also allegedly contained in Ashitaba, have various derivatives as intermediaries in the biosynthesis of one type of bioactive flavonoid antioxidant, which is thought to be able to neutralize oxidative stress events [21]. Extensive studies have been carried out to discover the pharmacological potential of chalcones, as well as molecular mechanism of action; however, these studies are not sufficient to explain the therapeutic potential of chalcones contained in Ashitaba.

Additional sources report that compounds isolated from *Angelica keiskei* exhibit a wide range of biological activities. In particular, two primary chalcones—xanthoangelol and 4-hydroxyderricin—have been widely researched and shown to possess various beneficial effects, including anti-tumor, anti-inflammatory, antidepressant, anti-obesity, anti-thrombotic, anti-diabetic, anti-hyperlipidemic, anti-hypertensive, and anti-ulcer properties [19].

Ashitaba has also been shown to inhibit platelet aggregation, exhibit vasorelaxant effects, and suppress the differentiation of preadipocytes. Ashitaba green tea is commonly consumed as a health-promoting beverage in China, Japan, and India. In Korea, it is used as an ingredient in vegetable juice, and on Japan's Izu Islands, the leaves have long been consumed both as food and medicine.

Safety studies indicate that administering ashitaba chalcone orally to male Wistar rats for 28 days at doses of 17, 170, and 1700 mg/kg body weight showed no signs of toxicity. Similarly, gavage administration of ashitaba extract at doses of 50, 100, 200, and 500 mg/kg body weight in male ICR mice demonstrated safe absorption and metabolism of 4-hydroxyderricin and xanthoangelol without adverse effects. Additionally, a related compound, xanthohumol, a prenylated chalcone found in hops, was given to female BALB/c mice at approximately 1000 mg/kg

daily for three weeks without toxic effects [22].

In this study, we isolated flavonoids from ashitaba and obtained a flavonoid content of 38.115 ± 0.124 ppm then implemented it for *in vivo* studies, and the ability of antioxidants in AK to prevent inflammatory events was proved.

2. Materials and Methods

2.1. Plant Materials

We got Ashitaba powder from the Ashitaba plantation in the Mount Welirang area, Trawas, Mojokerto. Ashitaba was dried at room temperature, avoiding direct sunshine. After drying, it is then ground to get the Ashitaba powder. We then sent the material to the Analyst Laboratory of Ahmad Dahlan University Yogyakarta.

2.2. Extraction

The extraction process was conducted at the Analyst Laboratory of Ahmad Dahlan University, Yogyakarta, to isolate flavonoid compounds. The flavonoid content was measured at 38.115 ± 0.124 ppm, as recorded in certificate number 11/LHU-LAMDA/VII/2023. The leaves were first shade-dried and then ground into coarse powder. This powdered material was initially defatted using petroleum ether (60–80°C) in a Soxhlet apparatus, followed by methanol extraction. The resulting extract was then concentrated using a rotary evaporator at 45°C until a semisolid mass was obtained, which was subsequently stored in airtight containers in a refrigerator at temperatures below 10°C.

2.3. Animal

Wistar male rats, weighing about 150250 g, were used in the study. Animals were maintained under standard environmental conditions, i.e. ambient temperature of (22 ± 2)°C and at 45%–55% relative humidity for 12 h under dark and light cycles. They were fed with a standard rat pellet diet, and water was supplied ad libitum. We used 30 rats, divided into 6 groups, with 5 rats in each group.

2.4. Experimental Design

All animals were randomly divided into six groups with five animals in each group. They were treated once a day for 21 days as follows:

Group I (Normal healthy control): given only normal saline. Group II served as a positive control and received only Ashitaba extract. Group III served as the Inflammation control and received only CS. Group IV Inflammation rats received CS and Ashitaba extract 0.5 gr/Kg.b.w. Group V Inflammation rat received CS and Ashitaba extracts 1 gr/Kg.b.w. Group VI Inflammation rat received CS and Ashitaba extract 2 gr/Kg.b.w.

Blood was collected for TNF-α and malondialdehyde detection by ELISA, while the lung tissue was collected for Inflammation detection by Immunohistochemistry.

2.5. Smoking and Ashitaba Extract Treatment

Mice were treated with 2 cigarettes smoked twice a day in the morning and evening in a smoking chamber. Meanwhile, ashitaba extract was administered twice daily, with different doses in each treatment group, as explained in the Experimental Design section.

2.6. Serum Preparation

Serums are separated and prepared by using a conventional method as described elsewhere https://www.thermofisher.com/id/en/home/references/protocols/cell-and-tissue-analysis/elisa-protocol/elisa-sample-preparation-protocols/plasma-and-serum-preparation.html.

2.7. Reagen and Abs

The recombinant human cytokine TNF-α was purchased from PeproTech (Rocky Hill, NJ). Other reagents used in this study were obtained from Sigma-Aldrich (St Louis, MO). The antibodies used included those generated in rabbits and directed against mouse TNF-α.

2.8. ELISA

A sandwich ELISA method was employed to detect TNF-α and malondialdehyde (MDA) using a commercial mouse TNF-α and MDA sandwich ELISA kit (Medikbio), with modifications made according to the manufacturer's guidelines. In short, microwells were coated with capture antibodies specific to TNF-α and MDA, then blocked using 0.5% bovine serum albumin (BSA) in PBS containing Tween 20. Afterwards, test serum samples were added, and the detection was carried out using HRP-conjugated antibodies specific for mouse IgG.

2.9. Cytokine Detection

The concentration of TNF-α and Manlone de Aldehide in cell-conditioned culture medium and serum is determined using ELISA kits (Medikbio) according to the manufacturer's instructions.

2.10. IHC

A polyvalent DAB staining kit (Scytech, Canada) was used for immunohistochemical staining according to the manufacturer's protocol. The paraffin-embedded section slides were deparaffinized and washed with deionised water (ddH_2O) several times. Slides were further subjected to heat (heat-induced epitope retrieval) to recover antigenicity. Tissue nonspecific binding was blocked with a blocking buffer (Protein Block) for 15 min. Then, the slides were washed with PBS three times and stained at 4°C overnight with primary antibodies against active TNF-α (1:250, SC-23900) (Santa Cruz, USA), which were diluted in Scytech antibody diluent. After one night, the slides were stained using a Scytek Canada Polymer Detection System, and the signal was visualized with the Scytek Canada diaminobenzidine substrate buffer. Then, counterstaining with hematoxylin was performed for nuclear staining for 3 min. The levels of TNF-α were photographed under a conventional microscope (Accu Scope, Tokyo, Japan).

2.11. Statistical Analyses

Values are expressed in mean $\pm$ standard deviation (SD). Groups are compared by using Student's two-tailed unpaired t-test or one-way ANOVA analysis followed by Dunnet post-hoch test, as appropriate. These analyses are performed by using GraphPad Prism 5 software (GraphPad Software, La Jolla, CA). Statistical significance is set at $p < 0.05$.

3. Results

The results of the flavonoid content test in the Ashitaba extract showed that the flavonoid content was 38.115 $\pm$ 0.124 ppm. This indicates that Ashitaba (**Figure 1**) contains antioxidants that are believed to inhibit oxidative stress and inflammation.

3.1. Sandwich ELISA to Detect TNF-α and MDA Levels

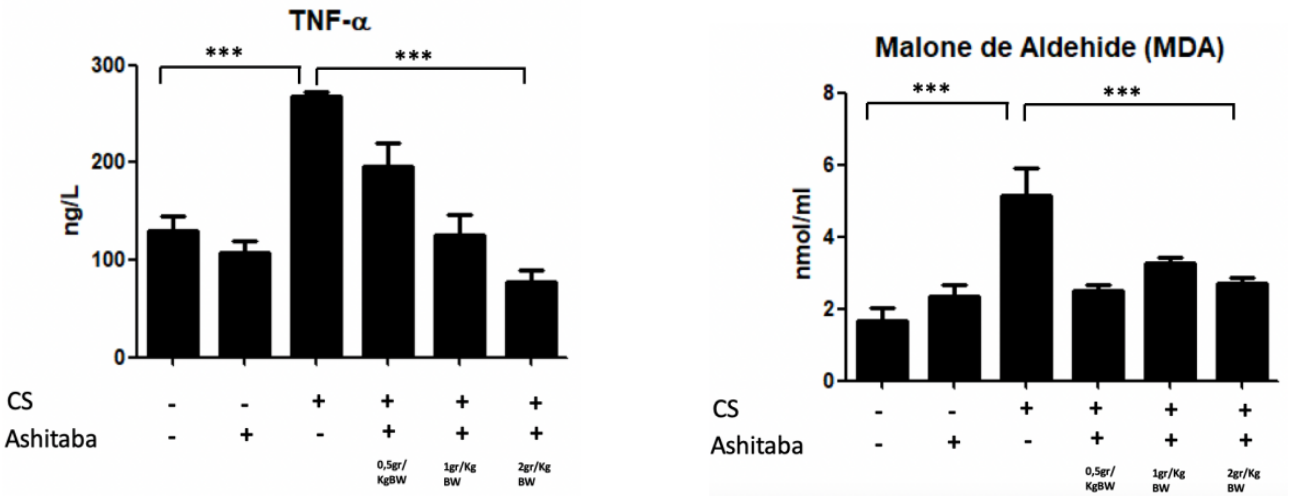
The assay of TNF-α and MDA is one of the biomarkers for inflammation; however, the methods are not used on a routine basis. In this study, we utilized a sandwich ELISA developed using a conventional and commercially available TNF-α and MDA sandwich ELISA kit to measure TNF-α and MDA in the serum of Wistar male rats after different treatments in each group. The collection and preparation of the tested sera are described in the Materials and Methods section. The protocol for the modified sandwich ELISA is summarized in the Materials and Methods section. Compared with groups 1 and 2, which served as a control group where the samples had not received CS, group 3, where the samples were given CS, showed significantly increased production of TNF-α and MDA ($p < 0.001$). On the other hand, the treatment of giving Ashitaba extract with different doses in groups 4, 5, and 6 showed a significant decrease in the production of TNF-α and MDA ($p < 0.001$) [**Figures 2(a)** and **2(b)**]. This result indicates that Ashitana extract could reduce inflammation caused by CS exposure.

3.2. TNF-α Levels on the Rat's Lung Tissue for Inflammation Biomarker

The Wistar rat sample's lung was taken up after the treatment was finished, then it was thinly cut with a microtome, and then IHC was carried out using the protocol as mentioned in the materials and methods section. Similar results to those obtained with ELISA were observed in the detection of TNF-α in the lung tissue of these mice. The

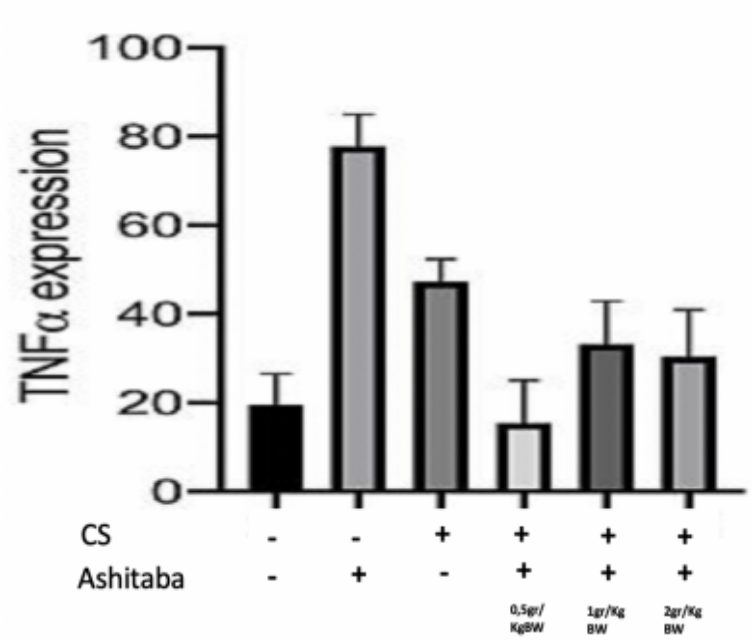
treatment group, which received Ashitaba in conjunction with CS, showed a reduction in TNF-α production compared to the group without Ashitaba and CS [**Figures 3(a)** and **3(b)**].

Figure 1. Ashitaba.

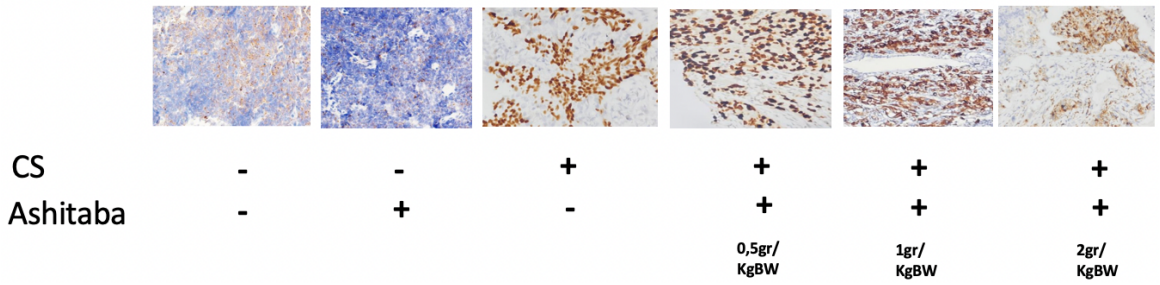


(a) **(b)**

Figure 2. Ashitaba extract inhibits the production of **(a)** TNF-α and **(b)** Malondialdehyde (MDA). ELISA showed high production of TNF-α and MDA in group 3 as the positive control group which received CS treatment, compared to groups 1 and 2 as the negative control group, then the production of TNF-α and MDA decreased again in groups 4, 5 and 6 which received ashitaba extract treatment, which showed inhibition of the production of oxidative stress originating from CS.



(a)



(b)

Figure 3. Immunohistochemistry showed that Ashitaba extract inhibited the production of TNF-α in the rat's lung tissue. The results of determining the expression of TNF-α in the lung by using AccuView imaging analyses showed a significant reduction in TNF-α in the group treated with ashitaba with CS. **(a)** supported by the results of tissue staining **(b)**.

4. Discussion

CS is a rich source of known and suspected carcinogens, ROS, and RNS that can damage macromolecules, including nucleic acids, proteins, and lipids. Its carcinogenic components include, among others, polycyclic aromatic hydrocarbons, aromatic amines, tobacco-specific nitrosamines, and phenolic compounds [23]. The gas phase of CS produces ROS during the combustion of the tobacco and is inhaled during smoking [24,25]. Particulates in CS can also accumulate in the lungs as a layer of tar, and, in aqueous solution, can produce oxidative agents via redox cycling reactions. The role of smoking-induced oxidative stress in inflammation is now widely acknowledged, with inflammatory reactions themselves further generating ROS.

The importance of ROS for macrophage-mediated immunity is unquestioned. Their functions comprise direct antimicrobial activity against bacteria and parasites, as well as redox regulation of immune signaling and induction of inflammasome activation [26].

Macrophages are present in almost all tissues of the body [27]. In the lung, they are the most abundant immune cells present under homeostatic conditions, representing over 90% of the alveolar immune cells. As sentinel cells, AMs play an important gate-keeping role in innate immunity within the respiratory tract. AMs classically exert regulatory effects via non-specific immune-defense mechanisms such as phagocytosis, the production of inflammatory

mediators such as ROS, and the expression of inflammatory cytokines such as interleukin (IL)-1, IL-2, IL-4, IL-6, IL-8, tumor necrosis factor-Alpha (TNF-α) and interferon-gamma (IFN-γ) [28].

During inflammation, oxidative stress generated by polymorphonuclear neutrophils (PMNs) disrupts inter-endothelial junctions, facilitating the movement of inflammatory cells through the endothelial barrier. While these migrating cells aid in eliminating pathogens and foreign substances, they can also contribute to tissue damage [29].

Smokers may benefit from consuming antioxidant supplements or a diet rich in fruits and vegetables, which can help reduce CS-related oxidative stress. This study reveals a vegetable named Ashitaba which is rich in flavonoids and can reduce the inflammation process in the lungs caused by CS, in some modified dozes. In this study, flavonoids inside the Ashitaba could reduce the TNF-α as well as malonaldehyde level as biomarkers of inflammation in the rat serum significantly. Another confirmation of data showed on the rat lung tissue that this antioxidant could also reduce TNF-α levels by using Immunohistochemistry. Further action, this product could probably be patented as one of the products that could reduce the inflammation caused by any smoke, including CS, and prevent more people from having lung tissue damage.

Consuming antioxidants from fruits and vegetables is recognized as an effective way to support the treatment of cardiovascular diseases. These antioxidants also play a preventive role against neurodegenerative disorders such as Parkinson's and Alzheimer's disease. Overproduction of reactive species can contribute to a range of pathological conditions, including ulcers, depression, cardiovascular problems, and rheumatoid arthritis. Antioxidants are believed to play a crucial role in preventing these diseases. Furthermore, they have shown promising potential in addressing issues related to sexual maturity, male infertility, and kidney stones (nephrolithiasis) [30].

Natural antioxidants work through various mechanisms and can help prevent diseases without causing side effects. However, the use of antioxidants should be guided by healthcare professionals. Consumers should be informed about the health benefits of antioxidants and encouraged to consume foods rich in antioxidants, such as vegetable oils, nuts, seeds, leafy greens, and fresh fruits, which are primary sources. A major limitation in therapy is the excessive intake of antioxidant supplements, which can lead to side effects since antioxidants may behave as pro-oxidants at high doses. Additionally, there is a notable difference between obtaining antioxidants from whole foods versus isolated supplements. Many compounds that show positive effects in laboratory studies fail to produce similar results in the human body, often due to poor bioavailability. For example, the concentration of antioxidants, such as polyphenols in the bloodstream is sometimes too low to have a meaningful effect [31].

On the other hand, Phytochemicals are valuable assets from plants that can be developed into health, food, and industrial products. Through the proper isolation, extraction, and formulation processes, these compounds can be utilized to improve human health naturally and sustainably. Phytochemicals are natural chemical compounds produced by plants to help them survive, protect themselves from pests, pathogens, and environmental stress. These compounds are not essential nutrients for humans, but have important biological activities. This process occurs naturally through the biosynthesis of secondary metabolites, which are different from primary metabolites (such as glucose or amino acids). The main methods include: Biosynthesis through Metabolic Pathways and Extraction of Phytochemical Compounds. Biosynthesis through Metabolic Pathways involves the Shikimate Pathway, which produces flavonoids, tannins, lignins, and phenols; the Mevalonate and MEP/DOXP Pathways, which produce terpenoids and steroids; and the Polyketide Pathway, which produces anthocyanins and isoflavones.

In **Table 1**, Biosynthesis through the extraction of phytochemical compounds involves the process of Maceration, Soxhlet, or Reflux, using solvents such as ethanol, methanol, and water, followed by concentration and purification methods like chromatography.

Table 1. Types of phytochemical compounds.

Category	Examples of Compounds	Biological Activity
Flavonoids	Quercetin, Rutin	Antioxidants, anti-inflammatory
Alkaloids	Caffeine, morphine	Analgesics, nerve stimulation
Terpenoids	Limonene, menthol	Antimicrobial, anticancer
Phenolics	Gallic acid, ferulic acid	Strong antioxidants
Saponins	Saponins from ginseng	Immunomodulators, antihyperglycemic
Tannins	Catechins, proanthocyanidins	Antimicrobial, astringent

Utilization of Phytochemical Products in the Health Sector can be used for the development of herbal/traditional

medicines, such as to separate active components from herbal ingredients, such as temulawak containing curcumin, meniran containing flavonoids, or in this study, we separate the active components of Flavonoids from Ashitaba [32–37].

This writing was made out of concern for the rising number of illnesses caused by smoking, including harm to passive smokers, who may experience equal or even greater negative effects than active smokers. Anti-inflammatory research on CS which could later be expanded to include other types of smoke such as air pollution from motor vehicles and industrial emissions should be conducted more frequently to prevent smoke-related damage better. Both *in vitro* and *in vivo* studies can be conducted to identify agents that counteract the harmful effects of smoke exposure. This research could eventually lead to the development of a product made from Ashitaba as a herbal ingredient to prevent smoke-induced inflammation.

5. Conclusion

Tobacco smoke is a complex mixture of thousands of different types of chemicals that significantly contribute to the increased incidence of oxidative stress. Direct or indirect oxidative damage can be induced by these reactive chemical compounds through the formation of pro-inflammatory compounds. As a result, inhalation of CS contributes to the onset of an inflammatory response, resulting in several tobacco-related diseases. *Angelica keiskei* in Indonesia, better known as Ashitaba or Japanese celery, has various derivatives as an intermediary in the biosynthesis of one type of bioactive flavonoid antioxidant, which is thought to be able to neutralize oxidative stress. In this study, flavonoids inside the Ashitaba could reduce the TNF-α as well as malonaldehyde level as biomarkers of inflammation in the rat serum significantly. Another confirmation of data showed on the rat lung tissue that this antioxidant could also reduce TNF-α levels by using Immunohistochemistry.

Free radical-directed oxidative stress is known to be harmful to human health. Various experimental studies have determined that free radicals contribute to the progression and inhibition of various pathologies, ranging from cardiovascular disease to cancer. Antioxidants can counteract oxidative stress and mitigate all the effects on human health. These compounds gained a lot of attention from the field of biomedical research, as they have demonstrateda higher degree of efficacy in the treatment and prevention of various diseases. Synthetic antioxidants are found to be detrimental to the health of an organism. Therefore, the search for a non-toxic and natural compound with greater antioxidant activity has increased in recent years. Through the literature survey, we can conclude that oxidative stress should be exploited as a therapeutic tool when and if we can understand the fine-tuning of this phenomenon within a living body. Newer approaches that utilize modern technology and collaborative research, in combination with established conventional health practices, can be employed shortly to improve health status, especially among individuals who do not have access to more expensive therapeutic drugs.

Author Contributions

Conceptualization: D.I.K. and M.K.; Methodology: E.M.W., R., and E.S.; Software: D.I.K., M.K., and D.R.; Validation: M.K., D.I.K., E.M.W., R., and E.S.; Formal Analysis: D.I.K.; Investigation: M.K.; Resources: M.K.; Data Curation: E.M.W., E.S., and R.; Writing—Original Draft: M.K. and D.I.K.; Writing—Review & Editing: M.K., D.I.K., and D.R.; Visualization: D.I.K.; Supervision: D.I.K.; Project Administration: M.K.; Funding Acquisition: M.K. All authors reviewed and approved the final version of the manuscript.

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Institutional Review Board Statement

All the studies were conducted by the Animal Ethical Committee of the University of Institute Ilmu Kesehatan Surya Mitra Husada Kediri with certificate number: 000460/EC/KEPK/I/10/2023.

Informed Consent Statement

Not applicable.

Data Availability Statement

The data supporting the findings of this study are available from public database e.g Pubmed and Scopus.

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Conflicts of Interest

The authors declare no conflict of interest.

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