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Phytochemical Screening and Antioxidant Capacity of Banana Peel Ethanol Extract of Ketip (*Musa paradisiaca* Forma Typiaca) on Sperm Concentration and Motility of Mice (*Mus musculus*)

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Abstract. *This study examines the effect of ethanol extract of banana peel ketip (Musa paradisiaca Forma Typiaca) on sperm concentration and motility of mice (Mus musculus) following cigarette smoke exposure. This study aims to determine the phytochemical content and antioxidant capacity of the extract after cigarette smoke exposure. This study began with extracting chemical compounds from the sample, followed by phytochemical screening to determine their content. The antioxidant capacity of the extract was examined using the DPPH method with a UV-Vis spectrophotometer and an in vivo test using adult male mice. Sperm concentration and motility data were analyzed by one-way ANOVA and Tukey's HSD test. The phytochemical screening results showed that the ketip banana peel ethanol extract contains terpenoids, alkaloids, flavonoids, tannins, and saponins. The DPPH test results showed that the IC_{50} value of the extract was 554.84 ppm. Statistical analysis of the in vivo data showed that the ethanol extract of ketip banana peel significantly increases sperm concentration (p value = 0.005486; F value 4.197422; F critical= 2.510158) and motility (P value= 0.0000; F value =41.59092; F critical= 2.510158) following cigarette smoke exposure. Tukey's HSD analysis of sperm concentration (HSD score = 2.271) showed that the 5000 ppm extract significantly increases sperm concentration without cigarette smoke exposure and sperm concentration after 5 minutes of cigarette smoke exposure. Nevertheless, this treatment failed in restoring sperm concentration following 30 minutes of cigarette smoke exposure. This indicates that longer duration of cigarette smoke exposure causes severe damage to sperm production (spermatogenesis) that is difficult to restore, even with high-dose treatment. Tukey's HSD analysis of sperm motility (HSD score = 0.207) showed that treatment with the ethanol extract of Ketip banana peel alone insignificantly increases sperm motility. The decrease in sperm motility after cigarette smoke exposure was significantly restored with high doses of the peel extract. These findings suggest that the sperm maturation stage, where the sperm motility is determined, was protected by antioxidant substances contained in the ethanol extract of Ketip banana peel.*

Keywords: *ethanol extract; ketip; phytochemical screening; sperm concentration; sperm motility.*

Type of the Paper: Regular Article.

1. Introduction

Around 6 million active smokers and 600 thousand passive smokers die each year due to cigarette smoke exposure [1]. This is thought to result from toxins contained in cigarette smoke. Cigarette smoke contains more than 4,000 chemical compounds, most of which have been proven harmful to health. These compounds include nicotine, heavy metals, and tar [2]. Nicotine can reduce sperm number, their motility, and functionality [3]. Heavy metals such as Cadmium are

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also proven to reduce sperm cell numbers and sperm motility while increasing DNA fragmentation and sperm abnormality [4], whereas tar acts as a mutagen and carcinogen [5]. Carcinogen compounds in cigarettes accelerate the apoptotic rate of spermatogonia, which develop into sperm stem cells. This apoptotic rate is inversely linked to testosterone level [4]. Testosterone is the androgen in the testis required to initiate and maintain spermatogenesis, and the production of mature sperm is highly dependent on androgen action within the testis [6]. Low testosterone levels can lead to reduced sperm count and the production of sperm with abnormal shapes and structures, making them less effective at fertilizing an egg. This condition is associated with abnormal sperm morphology [7].

In men, Leydig cells produce testosterone after being stimulated by luteinizing hormone (LH), a glycoprotein hormone secreted by the pituitary gland in response to the pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus [8]. This indicates that cigarettes reduce sperm quality by reducing sperm quantity and motility, increasing the apoptotic rate of spermatogonia cells by inhibiting testosterone synthesis in Leydig cells.

Reduction in fertility parameters appears connected to a high concentration of free radicals and oxidants in cigarette smoke. If free radicals overwhelm the body's regulatory capacity, an imbalance between oxidation and antioxidant potential occurs, leading to oxidative stress. The application of external antioxidant sources can help mitigate oxidative stress; however, synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole have recently been reported to be dangerous for human health [9]. Thus, the search for effective, nontoxic natural compounds with antioxidative activity should be intensified.

Some efforts to mitigate the negative health impacts of cigarette smoke exposure, maintaining a healthy lifestyle, consuming sufficient fruits and vegetables, and implementing a regular sleep pattern. In addition, consuming antioxidant supplements from herbal products can be an alternative solution to improve clinical conditions after exposure to cigarette smoke.

One tropical fruit rich in antioxidants is bananas. Bananas have been reported to contain approximately 40 different types of phenolic compounds, with a total amount of 47 mg gallic acid equivalents (GAE) per g dry matter (DM) [10]. The high antioxidant activity of the banana is associated with their high phenolic compound content [11]. Many variables influence the concentration of phenolic compounds, including genetics, cultivation region, growth condition, level of ripeness, handling methods, and post-harvest processing [10].

Several studies have shown that banana peels contain secondary metabolite compounds with medical properties. Phytochemical test results reported by Bahri et al. [12] showed that the ethanol extract of Kepok banana peel contains tannin, flavonoid, steroid, and saponin. These compounds have been shown to have antioxidant effects [13]. By using the DPPH method, ethanol extracts of

Kepok, Mas, and Nangka banana peels showed IC_{50} values of 9.702 ppm, 13.322 ppm, and 10.747 ppm, respectively [14]. According to Yuniarti et al. [15], these IC_{50} values fall within a very strong category. Using the same method, Heriani et al. [16] also reported the antioxidant capacity of Uli banana peel, which was classified as moderate, with an IC_{50} of 114.86 µg/ml. Therefore, banana peels that are usually discarded and become environmentally polluting organic waste, can be utilized by being transformed into a more valuable product.

Musa paradisiaca Forma Typiaca, locally known as pisang ketip, is a banana cultivar that is abundant in Lombok and readily available for sale, particularly in Lombok traditional markets. Due to limited information regarding the chemical content and bioactivity of Ketip peels, local residents typically discard them or use them as animal food. Therefore, phytochemical screening is necessary to examine the secondary metabolite content of this material and to investigate its antioxidant capacity. This research is expected to show the antioxidant performance of substances contained in the Ketip peel tissue**.** Hence, banana peels that are usually discarded as domestic waste can be used as raw materials for producing natural antioxidants, thereby increasing their economic value and reducing the accumulation of organic waste in the environment. Based on the previous explanation, we hypothesized that the ethanol extract of Ketip banana peel contains substances capable of restoring sperm damage caused by the negative effects of cigarette smoke. Hence, this study aims to determine the kind of antioxidant in Ketip banana peels ethanol extract and to evaluate its capability in restoring sperm quality following cigarette smoke exposure. The antioxidant performances of the extract were examined using chemical methods and *in vivo* tests.

2. Materials and Methods

2.1. Time and place of research

This research was conducted at the Immunology Laboratory of the University of Mataram and the Chemistry Laboratory of the FKIP University of Mataram for 5 months, from April to October 2024. This study employed laboratory experimental methods for phytochemical screening and antioxidant capacity testing. This research includes three stages. The ethanol extract of the ketip banana peel is obtained in the first stage and the content of secondary metabolite compounds is tested using phytochemical tests in the second stage. In the final stage, the antioxidant activity of the ethanol extract of the ketip banana peel is assessed using the DPPH test and an *in vivo* test using adult male mice.

2.2. Sample preparation and extraction procedure

2.2.1. Sample preparation

The research sample consisted of ripe Ketip banana peels purchased from traditional markets in Mataram. The banana peel, which had been removed from the fruit flesh, was then cut into small

pieces and air-dried indoors. After drying, the samples were ground into powder using a blender and sieved.

2.2.2. Extraction Procedure

Banana peel extraction was performed using 96% ethanol solvent with the maceration method. The extraction process began by mixing ethanol solvent with banana peel powder at a ratio of 1:2. After 72 hours of soaking, the mixture was filtered to obtain the extract. This extract is then concentrated using a rotary evaporator.

2.2.3. Phytochemical Screening

The concentrated extract was then tested for its chemical compound content through phytochemical screening. The compounds tested for presence in the extract included phenol, flavonoid, tannin, saponin, and steroid. The alkaloid test was performed using Dragendorf's reagent by adding 3 drops of the Dragendorf reagent to a test tube containing 1 mL of the sample. A positive alkaloid result was indicated by the formation of an orange-red precipitate at the bottom of the tube [17]. The flavonoid test was conducted by adding concentrated HCl and magnesium reagents, followed by H_2SO_4, and NaOH. The development of a pink or dark red hue signifies a positive result. The tannin test was performed by treating the sample with 3% $FeCl_3$ reagent; the presence of tannin was indicated by a color change to green, red, purple, blue, or black. The saponin test was conducted by adding 1 ml of Aquadest to the sample. A stable foam after shaking indicated a positive result for saponins. The terpenoid test was conducted by adding 3 drops of Lieberman-Burchard reagent; the formation of a blue, red–orange, or purple color indicated the presence of terpenoids.

2.2.4. DPPH Test

A stock solution is made from a 1000 ppm ethanol extract of banana peel. The stock solution was then diluted to acquire various concentrations, namely 100 ppm, 150 ppm, 250 ppm, 350 ppm, and 500 ppm. Ten milliliters of 1000 µg/mL DPPH solution were added to each test tube, along with five milliliters of each concentration. The absorbance of each solution is measured at a wavelength of 517 nm.

2.3. In vivo antioxidant capacity test

2.3.1. Experimental animal

Twenty-seven adult male mice (*Mus musculus* L) as experimental animals and randomly divided equally into 9 groups. Each group consisted of 3 animals weighing approximately 22-25 g and aged 2 to 2.5 months. To allow adaptation to the research environment, the animals were acclimatized for a week before treatment. During acclimatization and treatment, the animals were housed in a plastic cage. All animals were provided *ad libitum* access to drinking water and standard commercial food (CP551). The treatment comprised two factors: the duration of cigarette

smoke exposure (R Factor) and the dose of banana peel extract (P Factor) (Table 1). The R Factor comprised 3 durations: no exposure (R0), exposure to cigarette smoke for 5 minutes (R1), and exposure to cigarette smoke for 30 minutes. Smoke exposure was conducted by placing the animals in a plastic container measuring 50 (L) x 30 (W) x 20 cm (H), and draining smoke into the container using an aerator. A preliminary study showed that most experimental animals died after exposure to cigarette smoke for more than 30 minutes. The P Factor comprised 3 doses: 0 ppm (P0), 500 ppm (P1), and 5000 ppm (P2). These doses were determined by considering the low IC_{50} value. Based on the DPPH test, the IC_{50} value was 554.84 ppm, indicating that a concentration of 554.84 ppm of peel extract is required to reduce 50% of pro-oxidants.

Table 1. Combination of treatments

Exposure Time	Dosage		
	P0	P1	P2
R0	R0P0	R0P1	R0P2
R1	R1P0	R1P1	R1P2
R2	R2P0	R2P1	R2P2

Note: R0P0 = No exposure to cigarette smoke with banana peel extract 0 ppm
R0P1 = No exposure to cigarette smoke with banana peel extract 500 ppm
R0P2 = No exposure to cigarette smoke with banana peel extract 5000 ppm
R1P0 = Exposure to cigarette smoke for 5 minutes with banana peel extract 0 ppm
R1P1 = Exposure to cigarette smoke for 5 minutes with banana peel extract 500 ppm
R1P2 = Exposure to cigarette smoke for 5 minutes with banana peel extract 5000 ppm
R2P0 = Exposure to cigarette smoke for 30 minutes with banana peel extract 0 ppm
R2P1 = Exposure to cigarette smoke for 30 minutes with banana peel extract 500 ppm
R2P2 = Exposure to cigarette smoke for 30 minutes with banana peel extract 5000 ppm
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The experimental animals were exposed to cigarette smoke by placing them in a plastic box connected to an aerator that allowed cigarette smoke to flow into the box. Two hours after cigarette smoke exposure, banana peel extract was administered orally using a gavage needle. Each treatment combination was applied daily for 15 consecutive days. At the end of the treatment period, the animals were dissected to assess the cauda epididymis's sperm quality. The components of sperm quality observed included sperm concentration and sperm motility.

2.3.2. Sperm Concentration

Semen was collected from the cauda epididymis in a 3.5 cm Petri dish (NuncTM Thermo Scientific) containing 1 ml of 0.9% NaCl and gently stirred to ensure homogeneity. A microscope with a 100x magnification was used to count the spermatozoa [18].

2.3.3. Sperm motility

A glass slide containing the spermatozoa suspension was covered with a cover glass. A Nikon Eclipse E100 light microscope with 400x magnification was used to make the observations. Sperm motility was determined based on the percentage of sperm exhibiting progressive movement out of 100 observed sperm [18].

2.3.4. Data Analysis

Sperm concentration and motility were analyzed using one-way ANOVA to compare group means and determine statistically significant differences among treatment combinations. When a significant difference was indicated by a p-value < 0.05, further analysis was performed using Tukey's HSD test.

3. Results and Discussion

3.1. Result

3.1.1. Phytochemical screening

The results of the phytochemical test, presented in Table 2, showed that the ethanol extract of Ketip banana peel positively contains flavonoids, alkaloids, saponins, tannins, and terpenoids.

Table 2. Results of phytochemical screening of the ethanol extract of the Ketip banana peel

Chemical content	Test method	Results	Conclusion
Alkaloid	Dragendorff	orange-red sediment	+
Flavonoid	Wikstater Cyanidin	dark red	+
Tannin	$FeCl_3$ 3%	blackish blue	+
Saponin	Froth	Foamy	+
Terpenoid	Lieberman-Burchard	orange red	+

3.1.2. 1,1-diphenyl-2-picrylhydrazyl Test (DPPH Test)

One chemical technique frequently used to assess a sample's antioxidant capacity is the DPPH method due to its simplicity and low cost. Using UV-Vis spectrophotometry, the inhibitory activity on DPPH radicals can be measured. The IC_{50} represents the concentration needed to stabilize 50% of DPPH. Antioxidant capacity is inversely proportional to IC_{50}.

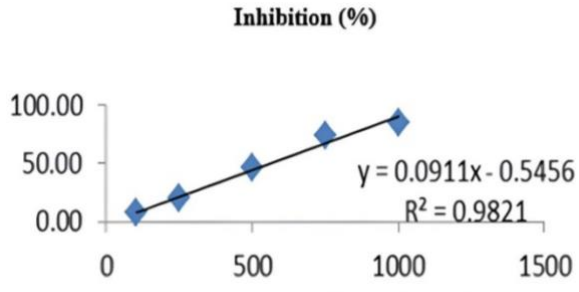

Fig.1. Linear regression curve of Ketip banana peel extract (*Musa paradisiaca* Forma typica) using DPPH test

The regression curve, as presented in Fig. 1, yielded the equation y = 0.0911x - 0.5456, with R^2 = 0.9821. The antioxidant capacity of the ketip banana peel ethanol extract was categorized as very weak based on the regression equation, as the IC_{50} value was 554.84 ppm. This indicates that a concentration of 554.84 ppm of the extract is required to neutralize 50% of DPPH radicals. An antioxidant molecule is considered to have very strong antioxidant activity if its IC_{50} value is less than 50 ppm. If the IC_{50} falls between 50 and 100 ppm, the antioxidant activity is classified as

high; if it falls between 100 and 150 ppm, it is classified as moderate; if it falls between 150 and 200 ppm, it is classified as weak; and if it exceeds 200 ppm, it is classified as very weak [19].

3.1.3. Sperm concentration

The effect of ethanol extract of the Ketip banana peel on sperm concentration is presented in Fig. 2. The sperm concentration in mice treated only with the ethanol extract of Ketip banana peel (R0P1 & R0P2) was higher than control (R0P0). In contrast, the sperm concentration in mice only exposed to cigarette smoke (R1P0 & R2P0) was lower than sperm concentration in the control group (R0P0). If exposure to cigarette smoke for 5 minutes was followed by administration of the ethanol extract of the Ketip banana peel 500 ppm (R1P1) and 5000 ppm (R1P2), sperm concentration increased.

Variance analysis showed that administration of ethanol extract of the Ketip banana peel considerably raised sperm concentration (p value = 0.005486; F value 4.197422; F critical 2.510158). Tukey's HSD analysis on sperm concentration (HSD score = 2.271) showed that 5000 ppm extract significantly increases sperm concentration without cigarette smoke exposure and concentration after 5 minutes of cigarette smoke exposure. Nevertheless, this treatment failed in restoring sperm concentration following 30 minutes of cigarette smoke exposure. This indicates that prolonged exposure to cigarette smoke causes severe damage to sperm production (spermatogenesis) that is difficult to restore, even with high-dose treatment.

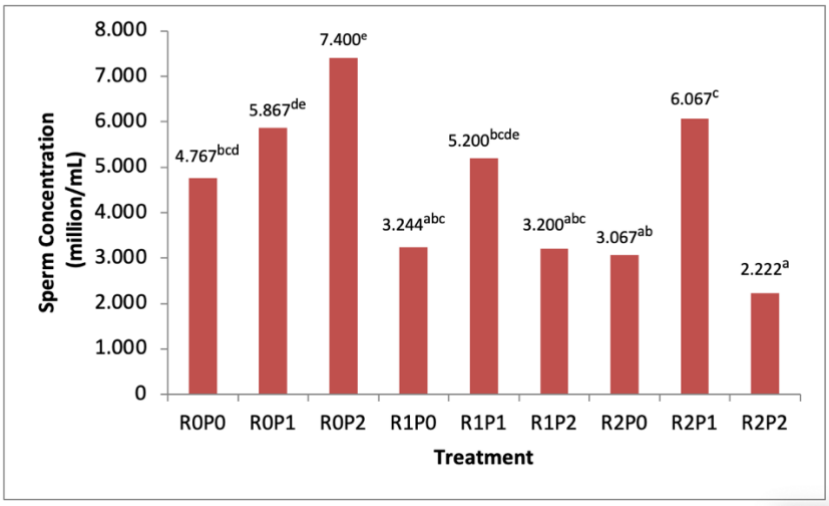

Fig. 2. Effect of ethanol extract of Ketip banana peel on the sperm concentration (10^6/ mL) of mice exposed to cigarette smoke. The mean value at the top graph bar, followed by different letter is statistically different according to Tukey (HSD) Test at the 0.05 significant level; HSD score (0.05) = 2.271, s = 0.629130999

3.1.4. Sperm motility

The highest sperm motility was observed in mice administered the ethanol extract of Ketip banana peel without exposure to cigarette smoke (R0P1, R0P2). Sperm motility increased followed this increase in the dose of extract. This means that sperm motility is increased in a dose-

dependent manner with increasing extract concentrations, indicating that administration of the ketip banana peel ethanol extract enhances sperm motility. In contrast, mice exposed to cigarette smoke without extract administration (R1P0 and R2P0) exhibited sperm motility to be lower than the control (R0P0). Sperm motility rises again when the extract is administered after cigarette smoke exposure. The R2P0 group, which was exposed to cigarette smoke for 30 minutes without receiving the ethanol extract of Ketip banana peel, had the lowest sperm motility.

The treatment with the ethanol extract of banana peel significantly enhanced sperm motility in mice (P value 0.0000; F value 41.59092; F critical 2.510158). Exposure to cigarette smoke for 5 minutes, as seen in the R1P0 treatment, tended to decrease sperm motility. When the duration of cigarette smoke exposure increases to 30 minutes, sperm motility decreases significantly. This is evident from the results of Tukey's HSD Test, whose values are listed at the top of the bar in Fig. 3. The decrease in sperm motility caused by 5 or 30 minutes of cigarette smoke exposure was significantly restored by administration of 5000 ppm ethanol extract.

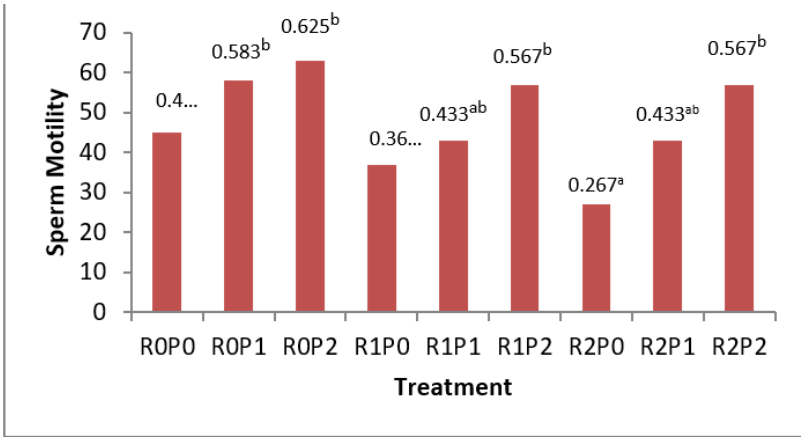

Fig. 3. Graph of the effect of ethanol extract of the Ketip banana peel on the percent sperm motility of mice exposed to cigarette smoke. The mean value at the top graph bar, followed by a different letter, is statistically different according to the Tukey (HSD) Test at the 0.05 significance level; HSD score (0.05) = 0.207

3.2. Discussion

Phytochemical screening results showed that the ethanol extract of Ketip banana peel positively contains alkaloids, flavonoids, tannins, saponins, and terpenoids. The formation of precipitate in the alkaloid test using the Dragendorff method is caused by the reaction between potassium bismuth iodide of Dragendorff reagent and alkaloid, forming a Bi-I-alkaloid complex. This complex produces an orange-red sediment, indicating the presence of alkaloid compounds in the extract. This phytocchemical screening also showed that this extract contains flavonoids. Dark red color is formed by the interaction of flavonoid compounds, especially anthocyanins and flavonols, with cyanide, one of the components of the Wikstater reagent. The blackish-blue color indicating the presence of tannin results from the formation of a ferric–tannin complex following

the reaction between tannins and $FeCl_3$. The formation of foam in a sample containing saponin using the Froth method occurs because saponin reduces the surface tension of water, allowing air to be trapped in the solution. The Lieberman-Burchard reagent used to identify the presence of terpenoids in the extract is a mixture of sulfuric acid (H_2SO_4) and acetic anhydride (Ac_2O). The reaction between H_2SO_4 and terpenoids forms an orange-red color.

Studies on other banana cultivars also detected the presence of those compounds. Heriani et al. [16] found that Uli Banana Peel Extract (*Musa x Paradisiaca* L. AAB) contains flavonoid and tannin, while Aboul-Enein et al. [20] also detected those compounds in *Musa paradisiaca* peel extract. Although flavonoid, triterpenoid, and alkaloid were detected in *Musa acuminata* peel extract, tannin and phenolic substances were not detected [21]. Meanwhile, Baskar et al. [22] reported the presence of phenolic substances in *Musa sapientum* peel extract. This condition shows that different banana cultivars can contain different secondary metabolites.

Many factors are thought to influence the type and concentration of secondary metabolites contained in different banana cultivars. In addition to genetics, cultivation region, growth condition, and level of ripeness, the extraction method may also influence metabolite composition.

Based on statistical analysis of *in vivo* data, cigarette smoke exposure significantly reduced sperm concentration and motility. This condition is thought to result from harmful chemical substances contained in cigarette smoke. Decreased sperm motility due to smoking has been reported by Sharma et al., Horak et al., and Gerhard et al. [23-25], while decreased sperm concentration in smokers have been reported by Chia et al. and Mitra et al. [26,27]. Although the pathogenesis mechanism remains under investigation, oxidative stress induced by cigarette smoke occurs through excessive ROS production is believed to be the main molecular event leading to decline in fertility parameters, including sperm concentration and motility.

An imbalance between oxidation and antioxidant potential leads to oxidative stress. This imbalance involves excessive production of prooxidants and/or dysfunction of the antioxidant system [28]. Prooxidants include ROS, free radicals, and other oxidizing agents [29]. Prooxidants, ROS, and free radicals are highly reactive and act as oxidizing agents in redox reactions. ROS, the most dominant endogenous prooxidant, is a derivative of molecular oxygen (O2) that shows greater reactivity than O2 [30]. Oxidative stress becomes more important when the body's natural antioxidant capacity is insufficient to counteract ROS formation (ROS) [31]. The inability of high doses of extract to improve sperm concentration after prolonged cigarette smoke exposure suggests that cigarette smoke may damage the nucleic acid of sperm stem cells, thereby inhibiting the division and proliferation of spermatogonia into spermatocytes. Reduced spermatocyte formation consequently leads to a lower number of spermatozoon. A prolonged cigarette smoke exposure is thought to lead to excessive ROS formation. The higher ROS level induced by tobacco smoking

may result in DNA damage. This condition causes failure of the animal's endogenous antioxidant system to neutralize ROS, leading to oxidative stress in sperm. According to Yam et al., this damage can result in genomic instability, causing diminished cellular fitness, unbridled proliferation, or cell death [32]. Long-term cigarette smoke exposure can also disrupt androgen production in Leydig cells, which plays a crucial role in spermatogenesis. A lack of androgen, such as testosterone, leads to lower sperm counts or absence of sperm production [33]. Cui et al. [34] reported that nicotine exposure, one of the substances contained in cigarette smoke, reduced the proportion of somatic cells and terminally elongating spermatids, inhibited meiosis, and impeded histone to protamine transition.

In contrast to sperm concentration, which cannot be restored by high-dose extract treatment following long-term cigarette smoke exposure, sperm motility increased significantly following long-term cigarette smoke exposure by administering high doses of extract. This suggests that the sperm maturation stage (spermiogenesis), which determines sperm motility, can be protected by antioxidant substances contained in the banana peel ethanol extract.

The decrease in sperm motility by cigarette smoke is caused by a negative effect on sperm mitochondrial activity in producing ATP. Cui et al. [34] reported that nicotine exposure, one of the substances of cigarette smoke, disrupted energy metabolism by interfering with the tricarboxylic acid cycle and promoting anaerobic respiration, leading to decreased ATP levels in the testes. This disruption also reduced the proportion of somatic cells and terminal elongating spermatids, inhibited meiosis, and impeded the histone to protamine transition.

Our result showed that spermatogonia are more susceptible to damage than spermatozoa, as indicated by the limited restorative capacity of the extract following longer duration of cigarette smoke exposure. This condition is likely due to the presence of highly unsaturated fatty acids in the spermatogonia cell membrane, combined with a high rate of cell division. According to Kaur & Bansal [35], another factor contributing to the vulnerability of spermatogonia to free radical damage is the location of germ cells adjacent to the phagocytic cell type.

Tukey's HSD analysis of sperm motility (HSD score = 0.207) showed that administration of 500 ppm and 5000 ppm extract without cigarette smoke exposure increased sperm motility, although not significantly. When 5 minutes of cigarette smoke exposure preceded administration of the 500 ppm extract, sperm motility also increased insignificantly; however, a significant increase was observed at the 5000 ppm dose. A similar phenomenon was observed following 30 minutes of cigarette smoke exposure: administration of 500 ppm extract resulted in an insignificant increase in sperm motility, whereas a significant increase occurred with the 5000 ppm dose. This indicates that cigarette smoke damages sperm stem cells or interferes with sperm production. The damage seems to be difficult to restore, even with high-dose treatment.

Sperms have a unique membrane structure characterized by a high content of unsaturated fatty acids that increase membrane flexibility and are essential for penetration of the egg membrane during fertilization. However, this high unsaturated fatty acid content also makes it extremely vulnerable to ROS-induced damage [36,37]. During the acrosome response and capacitation phase, ROS are required for successful fertilization. Nevertheless, excessive ROS generation and/or reduced ROS neutralization lead to oxidative stress, resulting in sperm DNA damage, decreased motility [38] and damage to membrane integrity [39].

Exposure to cigarette smoke is thought to induce oxidative stress by raising the production of prooxidants such as ROS and free radicals, which in turn reduces sperm concentration and motility. High prooxidant levels exceed the capacity of the endogenous antioxidant system. Despite the body having natural antioxidants, including glutathione, thioredoxin, superoxide dismutase, and vitamins C and E that can counteract free radical activity and shield sperm from ROS-induced damages [40], their effectiveness is restricted. The decrease in sperm concentration and motility observed in this study following cigarette smoke exposure is therefore thought to result from an imbalance between prooxidant levels and the body's natural antioxidant capacity. Excessive levels of prooxidants harm sperm, including through decreased testosterone levels [41], a key enzyme in the spermatogenesis process. This condition reduces gonadotropin concentration, accompanied by changes in testicular antioxidant status and spermatogenesis dysfunction, ultimately reducing the number of sperm stem cells in the seminiferous tubules of the testes [42].

Sperm concentration and motility were positively impacted by administration of the ethanol extract from the Ketip banana peel. Statistical analysis showed that the quality of both reproductive parameters was considerably restored by the Ketip banana peel extract. Phytochemical screening results presented in Table 2 showed that the ethanol extract of Ketip banana peel positively contains alkaloids, flavonoids, saponins, tannins, and terpenoids. These compounds have antioxidant effects that can delay, inhibit, or reduce oxidative damage in cells [13]. Antioxidants help maintain the balance of ROS production by neutralizing them, thereby improving sperm quality. The results of previous studies showed that men who consumed more antioxidants showed a lower frequency of sperm aneuploidy compared to men who consumed lower antioxidants [31,43]. It is therefore believed that the extract's secondary metabolites can either speed up spermatogenesis and spermiogenesis or restore sperm quality that has declined due to cigarette smoke exposure.

Secondary metabolite substances are the source of antioxidants found in plants. Antioxidants function as peroxide decomposers, hydrogen or electron donors, radical scavengers, and singlet oxygen neutralizers. Antioxidants found in cells and the extracellular environment detoxify ROS [44]. Alkaloids function as antioxidants by neutralizing free radicals and preventing cell damage

through hydrogen donation from their hydroxyl or amino groups. This explains why alkaloids can eliminate ROS, such as hydroxyl and superoxide radicals [45]. Derbak et al. [46] reported that alkaloids found in *Peganum harmala* significantly improved several fertility parameters, including sperm concentration. Martin-Hidalgo et al. [47] also explored the potential benefits of alkaloids on sperm health, showing that certain alkaloids can improve sperm motility, concentration, and morphology, possibly by reducing oxidative stress and inflammation. Secondary metabolite groups that can act as antioxidants by reducing free radicals include flavonoids and terpenoids [48,49], which were also found in this study and are classified as very strong antioxidants [50,51]. Flavonoids, specifically quercetin, have shown potential in restoring sperm parameters. Research suggests that flavonoid quercetin protects sperm from damage and improves fertility by reducing oxidative stress [9]. Tannins, such as carnosic acid, lycopene, and geraniol have also shown potential in restoring sperm parameters by improving sperm motility, concentration, and morphology. In a study on boar sperm, supplementing the thawing medium with a tannin-rich plant extract improved sperm motility and viability, leading to increased fertility rates [52].

Administration of the ethanol extract of banana peel at a dose of 500 ppm significantly increased sperm concentration, and this increase remained significantly different when the dose was increased to 5000 ppm. Administration of the ethanol extract of banana peel also increases sperm motility significantly. Similar to sperm concentration, the increase in sperm motility also follows the treatment dose. Hence, exposure to cigarette smoke was the cause of the decline in fertility quality observed in this study. However, the antioxidants in the banana peel ethanol extract can reverse the decline in sperm quality. The *in vivo* test result showed that the ethanol extract of the Ketip banana peel significantly increased sperm concentration and motility, despite the DPPH test results showing an IC_{50} value of 554.84 ppm. The relatively high IC_{50} value observed in the DPPH test is due to the high level of polysaccharide follicular gel present in the extract. This viscous and sticky substance is believed to inhibit the binding of DPPH to antioxidant compounds, thereby increasing the IC_{50} value. The presence of these substances in the extract can interfere with the binding of DPPH with antioxidants. This is because some extracts may contain compounds that are not antioxidants but still react with DPPH, leading to inaccurate results [53]. In addition, sticky substances or impurities in the extract may bind to DPPH, preventing its reaction with antioxidant compounds and resulting in an underestimation of the antioxidant activity [54]. To minimize this interference, it is essential to properly prepare and purify the extract before performing the DPPH assay. Techniques such as filtration, centrifugation, or solvent extraction can help remove impurities and sticky substances [55]. Heriani et al. prepared their sample using n-hexane to remove this substance before soaking the sample in ethanol. The IC_{50} score of Uli banana peel extract (*Musa x Paradisiaca* L. AAB) reported in their research was 114.86 µg/ml.

[16]. Therefore, more investigation is required to determine which components in the extract of the Ketip banana peel extract are most useful in restoring sperm quality.

4. Conclusions

Phytochemical screening results showed that the ethanol extract of the banana peel of Ketip positively contains terpenoids, alkaloids, flavonoids, tannins, and saponins. Using the DPPH method with a UV-Vis spectrophotometer, the IC_{50} value of the ethanol extract of the banana peel of Ketip was 554.84 ppm. Results from an *in vivo* test using adult male mice showed that the ethanol extract of Ketip banana peel has natural antioxidant capacity, as indicated by its capability in increasing sperm concentration and motility significantly following cigarette smoke exposure. Therefore, the banana peel of Ketip has the potential to act as a natural antioxidant in restoring sperm quality, especially in men who are frequently exposed to cigarette smoke. By using an appropriate solvent and proper sample preparation, the natural antioxidants contained within can be maximally extracted. The maceration process using ethanol, preceded by soaking the sample powder in n-hexane to remove the viscous sticky substance, is also an important aspect to be further investigated. This additional stage is expected to reduce the IC_{50} value. It is suggested that cigarette smoke interferes with the spermiogenesis stage in the epididymis and affects spermatogonia cells and testosterone-producing cells in the seminiferous tubules. Therefore, it is important to examine the effects of cigarette exposure on spermatogonia cells, spermatocyte cells, Leydig cells, Sertoli cells, and testosterone levels after cigarette smoke exposure, to gain a deeper understanding of how the banana peel of Ketip ethanol extract restores sperm parameters following cigarette smoke exposure. The use of the banana peels of Ketip will increase its economic value and reduce the accumulation of organic waste in the environment.

Abbreviations

DPPH	1,1-diphenyl-2-picrylhydrazyl
DNA	deoxyribonucleic acid
DM	dry matter
$FeCl_3$	iron (III) chloride
GAE	gallic acid equivalents
HCl	hydrochloric acid
H_2SO_4	sulfuric acid
IC_{50}	inhibitory concentration 50%
NaOH	sodium hydroxide
O2	oxygen gas
ROS	reactive oxygen species

Data Availability Statement

Readers can request access to the data.

CRediT Authorship Contribution Statement

Syamsul Bahri: Introduction, Methodology, Research project, Discussion, Original draft. **Dadi Setiadi**: Editing, Source, Writing. **Tri Ayu Lestari**: Data, Investigation, Software.

Declaration of Competing Interest

The authors of this manuscript declare no conflict of interest or competing interest.

Declaration of Use of AI in the Writing Process

Nothing to disclose.

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