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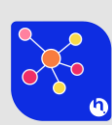

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Investigating Scabies Pathogenesis and Therapeutic Potential of Nutmeg Extract in Experimental Animals

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Abstract

Nutmeg (*Myristica fragrans*) is one of Indonesia's agricultural commodities with recognized health benefits as a traditional medicine. In addition, nutmeg has potential as a natural treatment for scabies. This study aimed to evaluate the effectiveness of nutmeg fruit bioactive compounds in scabies treatment. Nutmeg extraction was conducted using three different solvents: ethanol, ethyl acetate, and n-hexane. The extract with the most dominant phytochemical composition was further analyzed for bioactive compounds using GC-MS and antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Subsequently, the extract was tested against *Sarcoptes scabiei* mites obtained from 15 stray cats. The effectiveness of nutmeg extract was evaluated in a spray formulation by observing mite mortality and lesion reduction. The results demonstrated that the ethanol extract of nutmeg fruit contained the most abundant phytochemicals, with 3-Methyl-2,5-Furandione (21.26%) and Maleic Anhydride (14.21%) as the dominant compounds. The ethanol extract also exhibited strong antioxidant activity with an IC_{50} value of 21.41 ppm. In vitro testing showed 100% mite mortality at a 25% extract concentration within 24 hours, while in vivo testing on scabies-infected cats treated with the nutmeg spray extract revealed a significant reduction in scab lesions compared to the control group. These findings indicate that nutmeg extract possesses potent acaricidal and antioxidant properties, making it a promising alternative treatment for scabies. Further studies are required to refine the formulation and explore its clinical applications.

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1. Introduction

Scabies is a highly contagious skin disease caused by *Sarcoptes scabiei* var. *hominis*, affecting millions of people worldwide, particularly in densely populated and resource-limited regions. Classified by the World Health Organization (WHO) as an infestation, the mite burrows into the skin, leading to intense itching, rashes, and secondary infections [1]. Approximately 300 million cases occur annually, with the majority affecting children and adolescents due to close contact in schools [2]. The disease is prevalent in regions such as Africa, Southeast Asia, and Latin America, where poverty and limited healthcare access perpetuate its spread [3]. In Indonesia, prevalence declined from 5.60%–12.96% in 2018 to 3.9%–6% in 2020; however, scabies remains a significant

concern in Islamic boarding schools and orphanages [4]. Transmission occurs mainly through direct contact, although contaminated clothing and bedding can also serve as sources [5]. The WHO defines scabies as an infectious skin disease caused by infestation and sensitization by the female mite *Sarcoptes scabiei* var. *hominis*, which belongs to the class Arachnida [2].

Scabies mites (*Sarcoptes scabiei*) are ectoparasites commonly found in cats. These mites can infect cats of all ages, from as early as three weeks old to adulthood. Infestation with *S. scabiei* in cats is highly contagious, transmitting easily from one cat to another [6]. Infected cats typically exhibit hair loss, dermatitis, anemia, hypersensitivity, and discomfort, and the disease can also be transmitted to humans [7]. Treatment of scabies can be administered both orally and topically, including agents such as permethrin, benzoic acid, lindane, and precipitated sulfur.

According to Alhaffar et al. [8], the majority of cases are treated with topical 5% permethrin ointment due to its faster efficacy, while oral ivermectin has also been reported to reduce prevalence rates by up to 94%. However, effective scabies management requires educational interventions, since commonly used chemical treatments are often associated with side effects and relatively high costs [9]. Infected animals typically experience deteriorating body condition, which, due to the zoonotic nature of scabies, poses negative implications not only for the affected animals but also for their owners and surrounding environments [10].

Nutmeg (*Myristica fragrans*) has long been utilized by local communities worldwide for various purposes. In traditional practices, nutmeg fruit has been used to relieve common colds, alleviate insomnia, and stimulate appetite. Its consumption is also associated with improved blood circulation and the regulation of blood pressure. Pharmacological studies have demonstrated the antimicrobial potential of nutmeg, primarily attributed to compounds such as myristicin terpene, hydrocarbons, and phenylpropane derivatives. These compounds are capable of disrupting bacterial cell walls, ultimately leading to bacterial cell death upon exposure [11]. Furthermore, nutmeg contains essential oil components that exhibit a wide range of biological activities, including antioxidant, antimicrobial, anti-inflammatory, anticancer, antimalarial, anticonvulsant, hepatoprotective, antiparasitic, insecticidal, and nematocidal effects [12]. The essential oils in nutmeg, therefore, represent a promising source of alternative acaricides. Acaricidal activity of various plant-derived essential oils and their chemical constituents has been demonstrated against several mite species, including *S. scabiei*. Given the presence of these bioactive compounds, nutmeg fruit holds potential as an alternative treatment for scabies. Accordingly, this study was conducted to evaluate the effectiveness of nutmeg-derived bioactive compounds in the treatment of scabies.

2. Materials and Methods

This study was conducted at the Chemistry Research Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, and the Parasitology Laboratory, Faculty of Veterinary Medicine, Universitas Syiah Kuala. The samples used in this research were nutmeg fruits (*Myristica fragrans*) obtained from South Aceh, Aceh Province.

2.1. Sample Preparation and Extraction Process

The nutmeg fruit samples (*Myristica fragrans*) were thoroughly washed, and the flesh was cut into small pieces weighing approximately 3 kg. The material was dried in a shaded area to avoid direct exposure to sunlight. The dried simplicia were ground into powder and prepared for extraction. A total of 50 g of powdered nutmeg flesh simplicia was macerated for three (3) days using 500 mL of each solvent: 96% ethanol, ethyl acetate, and n-hexane, with stirring performed once every 24 hours. After the three-day maceration process, the extracts were filtered using filter paper and concentrated with a rotary evaporator to obtain viscous ethanol, ethyl acetate, and n-hexane extracts from the nutmeg flesh simplicia. The extraction yield percentage was calculated to compare the amount of dry sample before processing with the extract obtained.

2.2. Phytochemical Analysis

2.2.1. Alkaloid Screening

A total of 0.5 g of the ethanol, ethyl acetate, and n-hexane extracts was added with three drops of chloroform and three drops of Mayer's reagent. The formation of a white precipitate indicated the presence of alkaloid compounds [13].

2.2.2. Flavonoid Screening

A total of 0.5 g of the ethanol, ethyl acetate, and n-hexane extracts was added with a small amount of magnesium powder and three drops of concentrated hydrochloric acid. The appearance of a yellowish-red color indicated a positive result for the presence of flavonoid compounds [13].

2.2.3. Tannin Screening

A total of 0.5 g of the ethanol, ethyl acetate, and n-hexane extracts was added with three drops of 5% $FeCl_3$ solution.

The formation of a blue or blackish-green color indicated the presence of tannin compounds [13].

2.2.4. Saponin Screening

A total of 0.5 g of the ethanol, ethyl acetate, and n-hexane extracts was placed into a test tube, followed by the addition of 10 mL of distilled water and vigorous shaking. The formation of foam that persisted for up to 5 minutes indicated the presence of saponin compounds [13].

2.2.5. Steroid/Triterpenoid Screening

A total of 0.5 g of the ethanol, ethyl acetate, and n-hexane extracts was added with three drops of Liebermann–Burchard reagent. The appearance of a green color indicated the presence of steroids, while a reddish-purple color indicated the presence of triterpenoids [13].

2.3. Determination of Total Phenolic Content (TPC)

The determination of total phenolic content (TPC) was carried out using a modified Folin–Ciocalteu method [14]. The total phenolic content of the nutmeg flesh extract was determined following the method of Wulandari et al. [15]. A total of 20 mg of nutmeg flesh extract was dissolved in ethanol to a final volume of 10 mL, with the procedure performed in triplicate. From this solution, 100 µL was pipetted and mixed with 500 µL of Folin–Ciocalteu reagent (diluted 1:10 v/v with water), left to stand for 6 minutes, and then 400 µL of Na_2CO_3 solution (7.5% w/v in water) was added. The absorbance was measured at a wavelength of 759 nm using a UV–Vis spectrophotometer. The absorbance values of the samples were calculated based on the regression equation of the gallic acid standard curve to determine the total phenolic content, expressed as gallic acid equivalent percentage (% w/w GAE) [15].

2.4. Determination of Total Flavonoid Content (TFC)

The determination of total flavonoid content was carried out using a modified aluminum chloride colorimetric method [14]. A total of 20 mg of nutmeg flesh extract was dissolved in ethanol to a final volume of 10 mL, with the procedure performed in triplicate. From this solution, 1 mL of extract was mixed with 4 mL of distilled water and 0.3 mL of 10% $NaNO_2$ solution, allowed to stand for 6 minutes, followed by the addition of 4 mL of 10% $AlCl_3$ solution, and the volume was adjusted to 10 mL. The absorbance of the extract was measured using a UV–Vis spectrophotometer at a wavelength of 495 nm. The total flavonoid content was expressed as milligrams of quercetin equivalent per milliliter of extract (mg QE/mL). The overall flavonoid content in the sample was expressed as grams of quercetin equivalent per 100 grams of sample (% w/w QE) [16].

2.5. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC–MS analysis was conducted at the Analytical Chemistry Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala. The instrument used aTRACE 1310 coupled with an iSQ 7000 single quadrupole MS (Thermo Fisher, USA). The column employed was a TG-5MS (30 m × 0.25 mm × 0.25 µm) containing 95% diphenylpolysiloxane and 5% dimethylpolysiloxane [17, 18]. The instrument conditions were set with an injector temperature of 250°C and an ion source temperature of 250°C. The column temperature was programmed from 60 to 280°C at a rate of 10°C/min, with a helium gas flow rate of 1 µL/min. Mass spectra were recorded at 75 eV within a range of 400–500 amu. Compound identification was performed using the Wiley 7 LIB library.

2.6. Antioxidant Analysis

The antioxidant activity analysis began with the preparation of the sample solution. A total of 2.5 g of nutmeg ethanol extract was dissolved in ethanol p.a. to a final volume of 5 mL (stock solution). Serial dilutions were then prepared to obtain extract concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 ppm. Each solution was homogenized using a vortex mixer and incubated for 30 minutes at 30°C. Subsequently, 1 mL of 0.4 mM DPPH solution was diluted with ethanol p.a. to a final volume of 5 mL in a test tube, homogenized by vortex, and incubated for 30 minutes at 30°C. Absorbance was measured using a UV–Vis spectrophotometer at a wavelength of 517 nm. The absorbance values of each concentration were recorded and used to calculate the percentage of inhibition and the IC_{50} value [19]. All assays were performed in triplicate [20, 21].

The absorbance value of the DPPH solution on the sample solution was calculated as inhibition percentage (% inhibition) using Equation 1:

$$Inhibition\ (\%) = \left(1 - \frac{A_s}{A_b}\right) \times 100 \qquad (1)$$

where (A_s) is the absorbance of the sample and (A_b) is the absorbance of the blank.

2.7. Sampling and PCR Analysis of Sarcoptes scabiei Mites

Sarcoptes scabiei mites were isolated from cats infected with scabies. A total of 15 stray cats were used in this study. Samples were collected directly from affected skin areas, such as the ears, head, or tail. Skin scrapings were obtained using a blade (scalpel) until slight bleeding occurred to ensure the collection of *S. scabiei* mites residing in the stratum corneum of the epidermis [22].

Macroscopic examination of the skin scrapings was performed. The samples were analyzed directly using the native method, in which the skin scrapings from lesions were placed on a glass slide, followed by the addition of 10% KOH solution, serving as a keratolytic agent to lyse keratin in the skin scrapings, and then covered with a cover slip [23].

Collected mite samples were fixed in 70% ethanol and stored at 4°C prior to DNA extraction [24, 25]. *S. scabiei* mites were placed in sample tubes, and Deoxyribonucleic Acid (DNA) was extracted using ultrasonication for 10 minutes. DNA extraction was then performed using the Qiagen Tissue Kit. In this study, *S. scabiei* DNA amplification was carried out using Sarms 15 F/R primers with the following sequences: forward 5'-ATTAAATCATTGCACAATAGAGCG-3' and reverse 5'-CTACCATTAATTTTTTCCACCCTC-3'. PCR products were electrophoresed on a 2% agarose gel and visualized using a *geldoc transilluminator* system.

2.8. Evaluation of the Efficacy of Nutmeg (Myristica fragrans) Extract Against Sarcoptes scabiei

2.8.1. Preparation of Myristica fragrans Fruit Extract

The ethanol extract of nutmeg was prepared at various concentrations, namely 6.25%, 12.5%, and 25% in distilled water. Each extract concentration was used to observe mite growth. The samples to be tested were divided into several groups as follows:

- G-0: negative control group (Aquadest)
- G-1: positif control group (Ivermectin/marker)
- G-2: extract of nutmeg 6,25%
- G-3: extract of nutmeg 12,5%
- G-4: extract of nutmeg 25%

For each group, three Petri dishes were prepared for replication, with each dish containing five *S. scabiei* mites. Each group was subjected to spraying (three sprays) on the dishes containing the mites. The mortality of *S. scabiei* was then observed under a microscope at intervals of 1, 2, 3, 4, 8, and 24 hours. Mite mortality was identified by the absence of movement in the observed *S. scabiei* [26].

2.8.2. Production of Nutmeg Fruit (Myristica fragrans) Extract Spray

The effectiveness of nutmeg flesh ethanol extract in spray formulation as a *scabicidal* agent against *S. scabiei* mites was evaluated. The analysis was carried out by preparing a 25% ethanol extract of nutmeg flesh, which was then diluted with distilled water [27]. The extract was placed in a spray bottle and applied to experimental animals, followed by observation of the scabies crust width. Observations of scabies crusts were conducted over a 21-day treatment period, specifically on days 3, 6, 9, 12, 15, 18, and 21. The crust width was measured using a caliper.

2.9. Data Analysis

Data were analyzed using one-way ANOVA, followed by Duncan's test at a 95% confidence level. This study was approved by the Veterinary Ethics Committee, Faculty of Veterinary Medicine, Syiah Kuala University (Ref. No. 303/KEPH/VII/2024, July 29, 2024).

3. Results and Discussion

3.1. Extraction Results of Nutmeg Fruit (Myristica fragrans)

The extraction process began with drying the nutmeg flesh using an indirect sunlight drying method. This process aims to reduce mold growth and reduce enzymatic reactions that can damage the simplicia condition. The results of the drying process were presented in Table 1. The findings showed that the drying loss rate of the simplicia after 48 hours was 90.56%. This result complies with the Indonesian National Standard (SNI 0006:2015), which stipulates that the drying loss of nutmeg extract should be less than 10% [28].

The dried nutmeg flesh was extracted using several types of solvents, namely 98% ethanol, ethyl acetate, and n-hexane. The extraction process yielded different percentages of extract recovery, as shown in Table 2. The use of ethanol as a solvent produced the highest yield, as more compounds possess solubility characteristics similar to ethanol compared to ethyl acetate and n-hexane [29].

3.2. Phytochemical Profile of nutmeg (Myristica fragrans)

Every plant contains chemical compounds with biological activity (bioactive substances). These bioactive substances are derived from secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and terpenoids/steroids. In general, secondary metabolites can be qualitatively identified through phytochemical screening [30]. The phytochemical content of nutmeg fruit analyzed in this study is presented in Table 3.

Based on the results of the phytochemical analysis, the ethanol extract showed the presence of alkaloids, steroids, saponins, and flavonoids. This finding was consistent with a previous study reporting that the ethanol extract of nutmeg contains the secondary metabolites alkaloids, saponins, and flavonoids [31]. Similarly, Erizal et al. [27] also reported that the ethanol

Table 1. The results of drying nutmeg pulp.

Wet Sample Weight (gram)	Drying Time (hours)	Dry Sample Weight (gram)	Depreciation Percentage
3162.29	48	298.53	90.56%

Table 2. The extraction yield of nutmeg from different solvents.

Extraction Method	Solvent	Solvent Volume (mL)	Simple Weight (gram)	Extract Weight (gram)	Yield Weight (%)
Maceration	Ethanol 98%	500	50.33	9.24	18.35
	Ethyl acetate	500	50.17	2.39	4.76
	n-hexane	500	50.26	1.68	3.34

Table 3. The active compounds of nutmeg extract with different solvents.

Test Parameters	Results of Phytochemical Screening of Nutmeg Flesh Simplicia			Positive Results
	Ethanol 98%	Ethyl Acetate	n-hexane	
Alkaloid:				Orange precipitate
- Dragendorf	+	-	-	
- Mayer	+	-	-	
- Wagner	+	-	-	
Triterpenoid	-	+	+	Purplish red
Steroid	+	-	-	Green
Saponin	+	-	-	There is foam
Flavonoid	+	+	-	Yellow precipitate
Tanin	-	-	-	Blue, blackish green

extract of nutmeg flesh contains flavonoid-type secondary metabolites, which function as antibacterial and antioxidant agents. In addition, phytochemical screening of the ethyl acetate extract of nutmeg revealed the presence of triterpenoid and flavonoid secondary metabolites, whereas the n-hexane extract contained only triterpenoid secondary metabolites. In general, triterpenoid compounds are classified as semipolar–nonpolar, thus they can be effectively extracted using nonpolar solvents such as n-hexane. This finding is consistent with the work of Ginting et al. [32], who reported that the n-hexane extract of nutmeg contained only triterpenoid secondary metabolites, which act as antioxidants [32].

3.3. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Based on the results of the phytochemical screening, the ethanol extract exhibited a more complete profile of secondary metabolites compared to the ethyl acetate and n-hexane extracts; therefore, further testing was focused solely on the ethanol extract. The total phenolic and flavonoid contents were analyzed spectrophotometrically at wavelengths of 759 nm and 495 nm, respectively [33]. The purpose of this quantitative analysis of secondary metabolites was to determine the number of phytochemical compounds present in the investigated extracts. The TPC and TFC values of the ethanol extract of nutmeg were presented in Table 4. The results were calculated based on the linear regression equations of the calibration standards and expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g) and milligrams of quercetin equivalent per gram of extract (mg QE/g).

Phenolic and flavonoid compounds are valuable plant metabolites that exhibit diverse biological activities, including antimicrobial, antifungal, and anticancer properties [34]. The presence of phenolic and flavonoid compounds in the ethanol extract of nutmeg indicates the abundance of polar compounds [35]. The total phenolic and flavonoid contents in the ethanol extract of nutmeg analyzed in this study differed from those reported in previous studies, which may be attributed to geographical, climatic, and genetic factors [36].

3.4. Gas Chromatography-Mass Spectrometry (GC–MS) Analysis

GC–MS analysis identified 25 compounds present in the ethanol extract of nutmeg, of which only six compounds had concentrations ≥5%, namely 3-Methyl-2,5-Furandione, 3-Acetoxy-3-Hydroxypropionic Acid (Methyl Ester), 2-Hydroxypropane-1,2,3-tricarboxylic Acid (Dimethyl Ester), Maleic Anhydride, 2,6-Dimethoxy-4-(2-propenyl)-Phenol, and Myricetin, as presented in Table 5.

The compounds 3-Methyl-2,5-Furandione and Maleic Anhydride were found in high percentages in the ethanol extract of nutmeg, accounting for 21.26% and 14.21%, respectively. These compounds belong to the furan group, classified as volatile compounds, and are described as bioactive molecules naturally produced by

Table 4. Total phenolic (TPC) and total flavonoid (TFC) values in the ethanol extract of nutmeg.

Parameters	Sample weight (mg)	Content ± SD
TPC	20.2	76.972 ± 0.97 mg GAE/g
TFC	20.1	15.625 ± 0.26 mg QE/g

Table 5. Results of GC–MS analysis of ethanol extract of nutmeg simplicia.

No	Retention Time (minutes)	Structure/Compound Name	Peak Area (%)
1.	7.541	*3-Methyl-2,5-Furandione*	21.26
2.	12.656	*3-Acetoxy-3-Hydroxypropionic Acid, Methyl Ester*	14.87
3.	5.412	*Maleic Anhydride*	14.21
4.	25.074	*2,6-dimethoxy-4-(2-propenyl)-Phenol*	9.27
5.	24.302	*2-Hydroxypropane-1,2,3-tricarboxylic acid, dimethyl ester*	8.50
6.	23.142	*Myricitin*	5.35

various plants with insecticidal, antimicrobial, and efficient biofumigant activities [37–39]. Meanwhile, 3-Acetoxy-3-Hydroxypropionic Acid, Methyl Ester, and 2-Hydroxypropane-1,2,3-tricarboxylic acid, Dimethyl Ester were also identified in the ethanol extract of nutmeg. These compounds are derivatives of the saponin group, which are commonly found in plants and act as antifungal agents [40]. The ethanol extract of nutmeg contained high levels of 3-Methyl-2,5-Furandione (21.26%) and Maleic Anhydride (14.21%), both furan derivatives known for insecticidal, antimicrobial, and biofumigant activity [37–39]. Similar compounds have been reported in Citrus limon and lime extracts with strong antimicrobial effects [37, 41].

The compound 2,6-dimethoxy-4-(2-propenyl)-Phenol was detected in the ethanol extract of nutmeg at a percentage of 9.27%. This compound belongs to the polyphenol group (pseudotannin), is recognized as a potent antioxidant bioactive, and exhibits antimicrobial activity [42, 43]. It has also been identified in nutmeg oil extracts at 4.01% [44] and in acetone extracts of nutmeg leaves, though in smaller amounts (0.4%) [45]. Another important compound was Myricitin, which is a flavonoid known for its strong antibacterial properties and is commonly found in nutmeg essential oil [46]. In this study, Myricitin was detected in the ethanol extract of nutmeg at 5.35%. Previous findings by Wahyuni et al. [47] also reported the presence of Myricitin in the ethanol

Table 6. Antioxidant activity of nutmeg extract.

Concentration (ppm)	Absorbance	% Inhibition	IC_{50} (ppm)
50	0.119	75.96	
40	0.166	66.46	
30	0.201	59.39	21.41
20	0.253	48.89	
10	0.303	38.79	

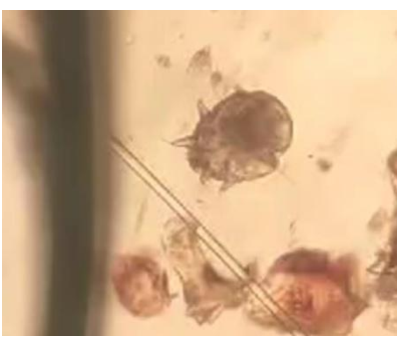
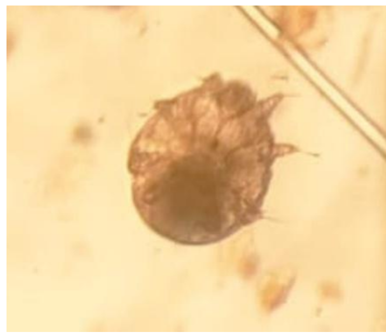

Figure 1. Morphology of the *Sarcoptes scabiei* mite was found.

extract of wild nutmeg seeds (*Myristica schefferi* Warb.) from South Aceh Province, with a concentration of 2.47%.

3.5. Antioxidant Content of Nutmeg (Myristica fragrans)

The antioxidant activity of the ethanol extract of nutmeg was determined based on the IC_{50} value, which represents the concentration required to inhibit 50% of DPPH free radicals [16]. A lower IC_{50} value indicates a stronger antioxidant activity of the ethanol extract of nutmeg flesh.

The antioxidant activity of the nutmeg ethanol extract is summarized in Table 6. The ethanolic extract of nutmeg exhibited antioxidant activity with an IC_{50} value of 21.41 ppm. This value was lower than that reported by Ginting et al. [32] for the n-hexane extract of nutmeg, which showed an IC_{50} value of 43.99 ppm. These findings indicated that the ethanolic extract possesses stronger antioxidant potential, attributed to the presence of multiple secondary metabolites such as alkaloids, saponins, and flavonoids [31], whereas the n-hexane extract only contains terpenoid compounds [32]. Consistent with Tang et al. [48], the greater the diversity of secondary metabolites within an extract, the stronger its antioxidant activity.

3.6. PCR Analysis Result of Sarcoptes scabiei Mites

Examination of 15 skin scraping samples from wild cats revealed that 10 samples were positive for infection with *Sarcoptes scabiei* type *Notoedres cati*. The microscopic findings of the collected mites are presented in Figure 1. This mite species is commonly associated with feline scabies, consistent with the findings by Fraser et al. [22], who reported that 54 of 88 examined samples confirmed the presence of *S. scabiei* type *N. cati*, further supporting its role as a prevalent ectoparasite in wild cats.

The visualization of the PCR products confirmed successful amplification of *S. scabiei* DNA, as indicated by the appearance of a DNA band at approximately 178 bp (base pairs). This band size corresponds to the expected target fragment, thereby confirming the presence of S. scabiei DNA in the tested samples. The visualization of the PCR products is presented in Figure 2.

Figure 2 presents the results of electrophoretic analysis of PCR products from scabies samples, showing a single specific band of approximately 178 bp amplified with Sarms 15 F/R primers on 2% agarose gel. This finding was consistent with the study of Shumaila et al. [25], which reported that the specific DNA band at approximately 178 bp using Sarms 15 F/R primers represents a microsatellite marker considered as a standard for the identification of *Sarcoptes* mites in human samples worldwide. The DNA bands observed in lanes 1 to 7 indicate that PCR amplification using Sarms 15 F/R primers from *S. scabiei* DNA yielded positive results for *S. scabiei* var. *hominis*, with an expected size between 100 and 200 bp (±178 bp).

3.7. Effectiveness of Nutmeg (Myristica fragrans) Extract Against Sarcoptes scabiei Mites

The effectiveness of the ethanol extract of nutmeg (*Myristica fragrans*) was determined by assessing the number of *Sarcoptes scabiei* mites that died at each observation interval, following the established procedure. The results of mite mortality at different treatment times are presented in Table 7.

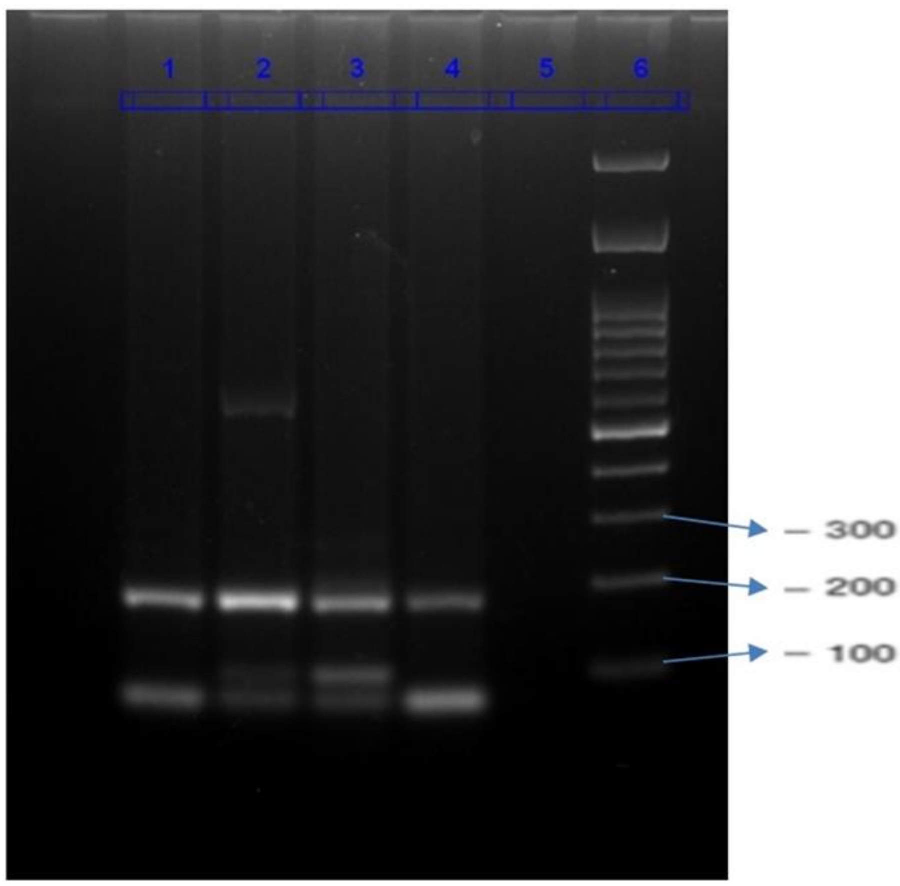

Figure 2. PCR amplification results of *Sarcoptes scabiei* var. hominis from skin samples of cats infected with scabies. 1; 2; 3; 4: cat sample, 5: negative control, 6: positive control/marker 100 bp.

Table 7. Average percent of *S. scabiei* mites' mortality.

Group	Concentration / Extract	*Average Mite Death (Hour To)						Total
		1	2	3	4	8	24	
Group 0 (G-0)	Control - (Aquadest)	0%	0%	0%	0%	0%	0%	0%
Group 1 (G-1)	Control + (Ivermectin)	93%	7%	0%	0%	0%	0%	100%
Group 2 (G-2)	6.25%	0%	0%	0%	0%	7%	20%	27%
Group 3 (G-3)	12.50%	0%	0%	0%	0%	20%	33%	53%
Group 4 (G-4)	25%	0%	0%	13%	20%	20%	47%	100%

*Each replication used 5 *Sarcoptes scabei* mites

These observations demonstrated a comparative percentage of mite mortality between nutmeg extract and ivermectin as the positive control. In the positive control group, mortality reached 100% within 2 hours of treatment. In contrast, treatment with the ethanol extract of nutmeg exhibited variable mortality rates. Higher extract concentrations corresponded to increased mite mortality. At a concentration of 25%, the ethanol extract of nutmeg achieved 100% mortality, albeit in a gradual manner. These findings indicated that nutmeg extract holds potential as a natural alternative for the control of *S. scabiei* infestations.

The ethanol extract of nutmeg (*Myristica fragrans*) in spray form was proven effective as an anti-scabies agent against *S. scabiei*, as indicated by the reduction in lesion size across all treatment groups except the negative control. The in vivo assay employed a 25% concentration, selected based on in vitro findings that demonstrated higher mite mortality compared to lower extract concentrations. The efficacy of nutmeg extract against scabies lesions was evaluated in a spray formulation as a scabicidal agent for *S. scabiei*. Statistical analysis confirmed a highly significant difference among treatments ($p < 0.05$), as presented in Table 8 and Figure 3. Clinical observations further demonstrated that the 25% ethanol extract of nutmeg in spray form significantly reduced scabies lesion size over the 21-day treatment period.

Table 8. Average value of scab width transformation.

Day to	Group		
	K – (aquadest)	K + (Ivermectin)	Nutmeg Extract (25%)
0	10.4 ± 0.54^{a}	15.4 ± 0.54^{a}	12.6 ± 1.81^{a}
3	12.2 ± 0.83^{a}	11.6 ± 0.55^{b}	11.0 ± 1.22^{ab}
6	13.0 ± 0.70^{a}	10.4 ± 0.54^{c}	10.2 ± 0.44^{ab}
9	14.4 ± 0.89^{a}	5.2 ± 0.83^{d}	9.0 ± 1.00^{bc}
12	13.8 ± 0.84^{a}	0^{e}	6.8 ± 1.64^{cd}
15	14.2 ± 0.83^{a}	0^{e}	4.4 ± 1.28^{de}
18	14.4 ± 0.89^{a}	0^{e}	4.0 ± 1.14^{e}
21	13.8 ± 0.44^{a}	0^{e}	2.4 ± 0.92^{e}

^{a,b} Superscript indicates significant difference (p<0.05)

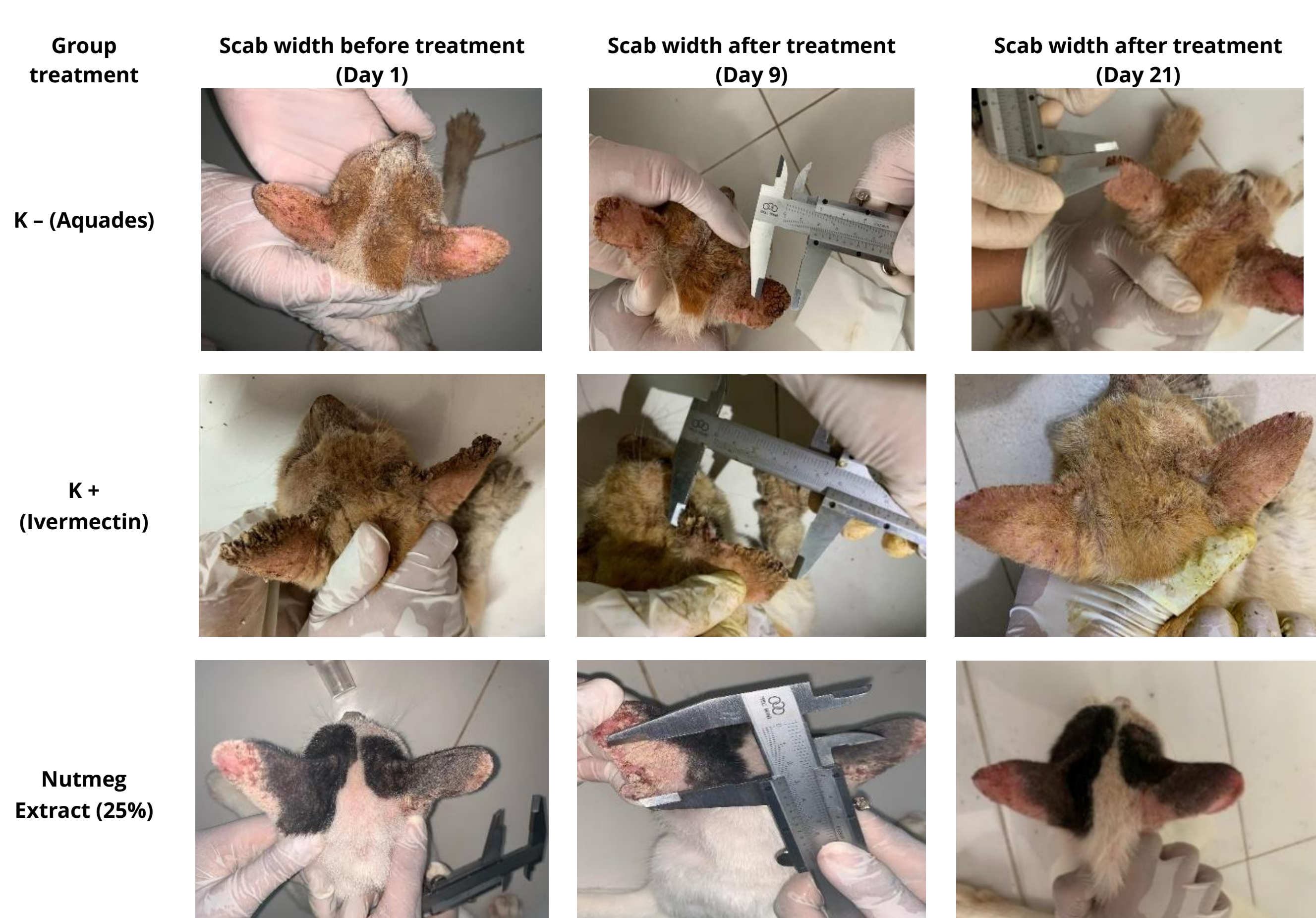

Figure 3. Reduction in the width of guinea pig scabs before and after treatment.

Animals suffering from scabies exhibited clinical symptoms such as alopecia, hyperkeratosis on the ear skin, persistent scratching behavior, the presence of self-inflicted lesions on the neck, forelimbs, and hind limbs, as well as reduced appetite. Scabies is one of the most common diseases infecting cats. Previous studies have reported that the healing of scabies is attributed to the presence of active compounds, including essential oils, saponins, tannins, and flavonoids. These bioactive compounds play a role in eliminating *S. scabiei*, thereby suppressing mite activity, preventing further lesion progression, and contributing positively to the reduction of scab lesion size.

4. Conclusions

This study demonstrated that the ethanol extract contained a more diverse profile of secondary metabolites compared to ethyl acetate and n-hexane extracts. The ethanol extract of nutmeg also exhibited strong antioxidant activity, with an IC_{50} value of 21.41 ppm, attributed to its high phenolic and flavonoid content. GC–MS analysis identified the presence of 3-Methyl-2,5-Furandione and Maleic Anhydride, compounds known for their insecticidal and antimicrobial activities. Furthermore, this study successfully identified *Sarcoptes scabiei* var. *hominis* using the Sarms 15 F/R primer, confirming the presence of *S.*

scabiei DNA in the tested samples. The ethanol extract of nutmeg proved highly effective in killing mites, achieving 100% mortality at a concentration of 25% within 24 hours. The 25% ethanol extract was subsequently formulated as a spray preparation for treating scabies in cats. Results indicated that the 25% ethanol extract significantly reduced scabies lesion size over a 21-day treatment period. These findings suggest that the ethanol extract of nutmeg has strong potential as a natural alternative treatment for scabies.

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Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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