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Original article

APUS BAMBOO SHOOT EXTRACT FORMULATION ACCELERATES THE HEALING OF MYASIS-INFECTED WOUNDS IN WISTAR RATS

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Summary

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Myiasis, a type of tissue infection caused by fly larvae, can lead to significant tissue damage and delayed wound healing. Conventional treatment with antibiotics and larvicides often poses risks of resistance and side effects. This study aimed to evaluate the effectiveness of apus bamboo shoot *(Gigantochloa apus)* extract in accelerating wound healing caused by myiasis infection in Wistar rats. Twenty-five Wistar rats were divided into five groups: a negative control (CN) group, a positive control (CP) with ivermectin, and three groups treated with 1%, 3%, and 5% apus bamboo shoot extract. Compared with the positive control (ivermectin), 5% apus bamboo shoot extract (P3) had the greatest effect on reducing wound length on days 7 and 11. Histopathological analysis revealed improved granulation tissue formation, increased fibroblast number, and reduced inflammation. Compared to the negative control group, the inflammatory cell counts were lower in all treatment groups, with significant decreases observed in groups treated with %, and 5% apus bamboo shoot extract. The apus bamboo shoot extract has potential as an effective natural treatment for myiasis wound healing, with bioactive compounds contributing to its anti-inflammatory, antibacterial, and larvicidal properties. However, further clinical studies are required to confirm its applicability.

Key words: Apus bamboo shoot extract, histopathology, myiasis, wound healing

INTRODUCTION

Myiasis is a parasitic disease caused by fly larvae that affects all vertebrates, including humans. The fly larvae survive by feeding on living, dead, or necrotic tissue (Fahma *et al.*, 2020). Several species of flies cause myiasis, such as *Cochliomya hominivorax,* which is found in the Americas; *Wohlfahrtia magnifica,* found in Europe and China; and *Chrysomya bezziana*, which is widespread in Africa, India, Southeast Asia, and Indonesia (Kaswardjono *et al.*, 2019).

Chrysomya bezziana is the most common cause of myiasis in Indonesia. This species is an obligate parasite that primarily manifests in the host tissue, especially in the skin. To date, myiasis remains a significant concern, particularly in several regions of Indonesia such as East Sumba, South Sulawesi, and parts of Java (Fahma *et al.*, 2020; Hosni *et al.*, 2020).

Globally, myiasis is most commonly observed in tropical climates. In humans, fly larval infestation sites include the mouth, thigh, perineal area, inguinal region, eyes, ears, nose, and other vital organs (Calvopina *et al.*, 2020; Pires *et al.*, 2018; Zhou *et al.*, 2019). This larval infestation destroys living tissues, causing deep, painful ulcerative lesions, bleeding, and purulent discharge. Recurrent infestations can result in tissue destruction and wound enlargement. Aggressive infections can lead to blindness and death in severe cases (Zhou *et al.*, 2019).

Currently, the most important and effective therapy for treating myiasis is the rapid removal of *C. bezziana* larvae from the infected areas (Lubis *et al.*, 2019; Serafim *et al.*, 2020). The treatment includes antibiotics and a single dose of 200 µg/kg ivermectin administered after the larvae were removed. Ivermectin is a broad-spectrum antiparasitic drug used to treat parasitic, nematode, arthropod, and dermatological infections (Serafim *et al.*, 2020). However, long-term use may lead to side effects, including muscle and joint pain, rash, fever, and Stevens-Johnson syndrome (Lee *et al.*, 2017). Therefore, alternative treatments, such as the use of biolarvicides are needed, with one promising option being natural biolarvicides derived from the apus bamboo shoots (*Gigantochloa apus*).

Apus bamboo *(G. apus)* is a member of the *Poaceae* family and is commonly found in Indonesia (Widiarso *et al.*, 2018). Studies have shown that apus bamboo is resistant to termite attack because of its biochemical properties. However, bamboo shoots are rarely used because of their full potential. Research has shown that the extract of apus bamboo shoots contains several bioactive compounds, including HCN, alkaloids, flavonoids, and other active ingredients (Mudaliana, 2021). These compounds also exhibit possibly antiparasitic properties. In addition, apus bamboo shoots contain high levels of antioxidants, reaching 72.32% when extracted with methanol (Soesanto, 2018).

Collagen plays a crucial role in wound healing by interacting with platelets and binding to cellular components to promote epidermal proliferation. Collagen forms a matrix to isolate and repair damaged tissues, maintaining the strength of the skin, bones, ligaments, and tendons (Calvopina *et al.*, 2020). In addition to collagen, capillaries are essential for the wound regeneration process, including in myiasis-infected wounds.

Thus, an alternative treatment for myiasis directly targets the infected area. The extract of apus bamboo shoots showed promising potential as a topical biolarvicide for treating myiasis. In this study, a gel spray formulation was used for practical applications, enabling the direct treatment of the infected site and minimising contamination risks. This study aimed to analyse the effects of an apus bamboo shoot extract formulation on wound reduction and histopathological changes in Wistar rats with myiasis.

MATERIALS AND METHODS

Ethical approval

This study was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Diponegoro (no. 31/EC/H/FK-UNDIP/IV/2022). This study involved only Wistar rats, with no human participants. The study followed the institutional and ARRIVE guidelines, using an appropriate number of animals with randomisation and good environmental conditions to ensure their welfare throughout the study.

Extraction of apus bamboo shoots

Apus bamboo shoots were extracted by drying the bamboo shoots in an oven at 40 °C. Dried bamboo shoots were ground into a fine powder and macerated with 96% ethanol at a 1:10 ratio. The resulting extract was concentrated using a rotary vacuum evaporator and evaporated at 40 °C at a speed of 50 rpm (Artanti and Mujahidah, 2021). The extract was analysed qualitatively using the phytochemical screening tube method; quantitative content analysis was also performed (Wani *et al.*, 2019).

Animal treatment and wound length reduction measurement

This true experimental research with a post-test-only control group design employed 25 male Wistar rats, selected on the basis of the following inclusion criteria: body weight between 180 and 200 g and age 2–3 months. The animals underwent a 7-day acclimatisation period with *ad-libitum* access to food and water in their respective cages. A random sampling technique was applied to select the experimental animal groups, minimising bias related to age, body weight, and other physiological factors.

After acclimatisation for 7 days, the dorsal fur of each rat was shaved, standardized square-shape wounds approximately 2×2 cm of size were made. Right after the wound sites were made, they were infested with 10 first-instar larvae. During the larval infestation procedure, rats were anesthetised using a combination of chetamine, xylazine, and bidistilled water in a 1:1:1 ratio, with an administered dose of 0.3 mL per 25 g body weight. Subsequently, ten first-instar larvae (L1) were introduced into each wound using tweezers. To simulate myiasis conditions, the wound was covered with moist gauze to allow the larvae to penetrate the skin layers. The rats were housed individually for 48 h. After two days, the gauze was removed, and the rats were categorised into five treatment groups: the negative control (CN) group, which had untreated wounds; the positive control (CP) group, which received standard treatment with ivermectin; treatment group 1 (P1), which received a 1% apus bamboo shoot extract spray; treatment group 2 (P2), which received a 3% extract; and treatment group 3 (P3), which received a 5% extract.

The number of larvae within the wound was recorded and the wound diameter was measured. Each group was then subjected to treatment with *G. apus* bamboo shoot extract via spraying twice daily in the morning and evening. The wound length was measured for each sample on days 1, 3, 7, 11, and 14. The data were compared by calculating the average wound length for each treatment group.

Histopathological analysis

On the 14th day, euthanasia was performed to collect wound tissue from all samples. Tissue samples were carefully selected to ensure that they were representative and free from contamination. After tissue collection, the samples were labelled, and histopathological staining was performed using haematoxylin and eosin (H&E) staining. Skin histopathology was examined under microscope at 400× in three different fields of view (Wibowo *et al.*, 2023). The number of collagen fibres, inflammatory cells, fibroblasts, and capillaries was counted.

Statistical analysis

All data were statistically analysed using the Kruskal-Wallis test to compare groups, followed by the Mann-Whitney test for post hoc analysis. Results are presented as the mean ± standard deviation (SD), and statistical significance was considered at $P<0.05$.

RESULTS

Apus bamboo shoots composition

Content analysis began with phytochemical screening using the tube method and proceeded with quantitative content analysis. Based on the results of the extract analysis (Table 1), the extract contained varying levels of hydrogen cyanide (HCN), tannins, and phenols.

Analysis of wound length reduction

As shown in Fig. 1, wound length in all groups gradually decreased over time, but with different dynamics. The negative control group (CN) exhibited the slowest reduction, with wounds remaining relatively large until day 14. In contrast, the positive control group (CP) and treatment groups (P1-P3) demonstrated a more rapid reduction, particularly during the first week.

As shown in Fig. 2, there were no significant differences between the treatment groups on day 1 ($P>0.05$). By day 3, significant differences emerged between the negative control (CN) and both the P2 and P3 groups ($P<0.05$), while other comparisons remained non-significant. On day 7, significant differences were observed between the CN and all other groups (CP, P1, P2, and P3), with no significant differences among the remaining group comparisons.

On day 11, CN differed significantly from CP, P1, P2, and P3 ($P<0.05$), while

Table 1. Results of extract content analysis

Compounds	Phytochemical screening results	Quantitative analysis		Content level (Mean ± SD)
		Method	Reagent	
HCN (ppm)	+ (Brown precipitate)	Argentometry	$AgNO_3$	33.84±3.30
Tannin (%)	+ (Yellow colour)	Spectrophotometry	Folin	0.75±0.00
Phenol (%)	+ (Dark green colour)	Spectrophotometry	Sulfanilic acid	1.71±0.02

Note: (+) indicates positive content of the tested compound.

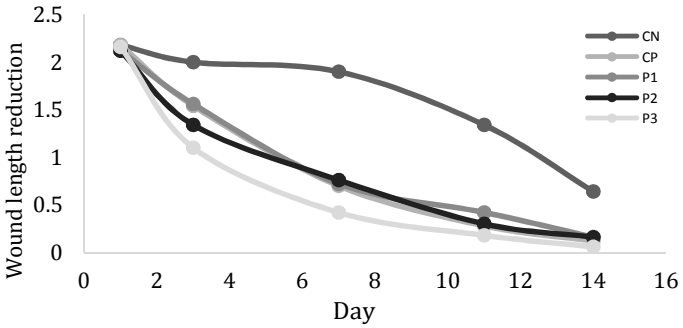

Fig. 1. Average wound length reduction for each treatment: CP: positive control group (ivermectin); CN: negative control group (untreated), P1: group with 1% apus bamboo shoot extract; P2: group with 3% apus bamboo shoot extract, and P3: group with 5% apus bamboo shoot extract. Data are presented as the mean of each measurement day for each treatment.

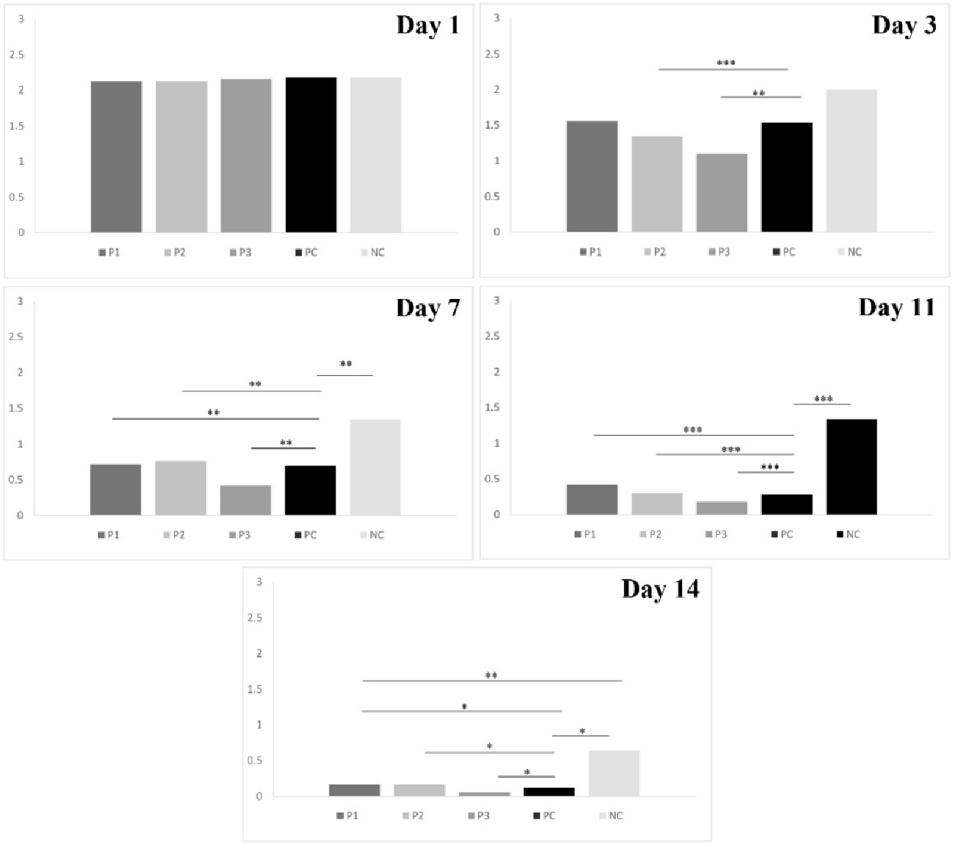

Fig. 2. Average reduction in wound length in each group at different observation days. Data are expressed as the mean of each repetition of the dependent experiments; *P<0.05, **P<0.01, ***P<0.001 (Mann–Whitney analysis).

the differences among the other group were not significant. By day 14, significant differences were noted between CN and all other groups (CP, P1, P2, and P3), as well as between CP and P1 ($P<0.05$).

Histopathological findings

Tissue samples collected on day 14 after treatment, were stained with haematoxylin and eosin (H&E), and examined under microscope (Fig. 3). Fibroblast counts differed significantly across the groups (Table 2; $P=0.011$), with CP and P3 showing higher values than the negative control (CN). Inflammatory cell counts were significantly lower in all treatment groups in relation to the CN group ($P=0.001$), with the strongest reductions observed in CP

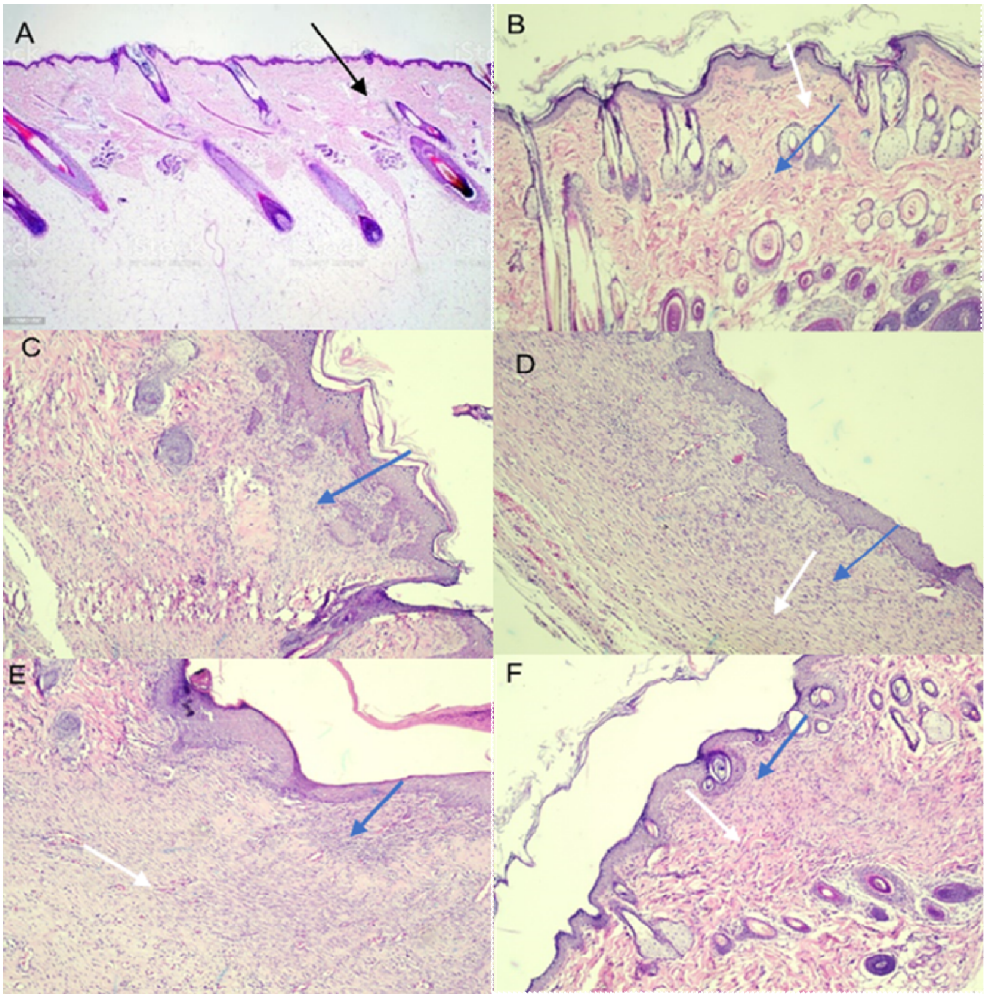

Fig. 3. Histopathological images at 400× magnification. **(A)** normal group; **(B)** positive control group (ivermectin); **(C)** negative control group (untreated); **(D)** group with 1% apus bamboo shoot extract; **(E)** group with 3% apus bamboo shoot extract; **(F)** group with 5% apus bamboo shoot extract. Black arrow: normal tissue; blue arrow: inflammatory tissue; white arrow: fibroblast tissue.

Table 2. Histopathological analysis by measuring the number of capillaries, fibroblasts, and inflammatory cells, and the percentage of collagen from myiasis wound tissue in experimental mice. CP: positive control group (ivermectin); CN: negative control group (untreated), P1: group with 1% apus bamboo shoot extract; P2: group with 3% apus bamboo shoot extract, and P3: group with 5% apus bamboo shoot extract. Data are presented as the mean ± SDs.

Treatment group	Total number of capillaries	Total number of fibroblasts	Total of number inflammatory cells	Percentage of collagen
CN	6.00 ± 2.345^{a}	67.80 ± 10.756^{a}	135.40 ± 29.788^{a}	50.00 ± 14.967^{a}
CP	6.80 ± 2.049^{a}	95.00 ± 18.960^{b}	79.40 ± 71.800^{b}	$60.00 \pm 21.190^{a,b}$
P1	7.60 ± 1.817^{a}	73.40 ± 7.635^{a}	110.20 ± 25.420^{a}	68.80 ± 6.140^{b}
P2	8.80 ± 2.387^{a}	$84.80 \pm 12.174^{a,b}$	105.60 ± 10.286^{b}	84.40 ± 10.280^{c}
P3	7.60 ± 1.517^{a}	96.60 ± 16.134^{b}	79.20 ± 6.834^{b}	71.40 ± 8.649^{b}

Note: ^{a,b,c} significant differences between groups within a column ($P<0.05$).

and P3 (P=0.000). The collagen percentage also showed significant differences between CN and all treatment groups (CP, P1, P2, and P3), while capillary counts did not differ significantly.

DISCUSSION

Gigantochloa apus is a type of bamboo that is commonly found in tropical and subtropical areas, including Indonesia. Bamboo shoots (*G. apus*) have been shown to contain secondary metabolites (Frihantini *et al.*, 2015; Setiawan *et al.*, 2018). These secondary metabolites such as alkaloids, flavonoids, tannin and saponins have been widely used as anthelmintic agents, (Ridwan *et al.*, 2020). For wound healing, the antioxidant levels of alkaloids and flavonoids are utilised. Previous studies have shown that the antioxidant activity of bamboo shoots can reach 72.32% (Frihantini *et al.*, 2015; Soesanto, 2018). In this study, the targeted compounds were alkaloids, flavonoids, saponins, and tannins, which explains the use of bamboo shoot extract as a biolarvicide against myiasis. These four compounds act as anti-metastasis and wound healing agents (Soesanto, 2018).

The wound length reduction data across the observation days demonstrated a consistent pattern of improved healing in all treatment groups compared with the negative control. The differences in the mean wound length for each treatment group indicated that the active compounds contained in the bamboo shoot extract, such as alkaloids, flavonoids, tannins, and saponins, helped in wound healing, as observed from the reduction in wound length in animal models (Ibrahim *et al.*, 2018; Zulkefli *et al.*, 2023).

The 5% apus extract (P3) showed the most effective reduction on day 3, whereas P2 outperformed the others on day 11, and P1 showed the best outcome on day 14. These findings suggest that the formulation had a dose-dependent effect that may vary across different phases of healing, possibly influenced by the dynamics of bioactive compound absorption, inflammation modulation, and tissue remodelling. The comparable effectiveness of all treatment groups to the standard ivermectin by day 7 onward supports the therapeutic potential of the apus bamboo shoot extract as a natural alternative. Giv-

en the increasing global concern regarding antibiotic resistance, plant-based therapies offer a promising alternative (Alsheikh *et al.*, 2020). Apus bamboo shoot extract, with its anti-inflammatory and antibacterial effects, may reduce reliance on synthetic drugs while providing an affordable and locally accessible treatment, especially in areas where myiasis is still endemic.

These phytochemicals exert their therapeutic effects via multiple mechanisms, particularly by modulating inflammation and stimulating tissue repair. One of the main mechanisms activated is reduction in inflammation, which is the early stage of the wound healing process. Uncontrolled inflammation can delay healing and increase tissue damage. Apus bamboo shoot extracts contain flavonoids, alkaloids, terpenoids, and tannins, which function as anti-inflammatory agents by inhibiting the synthesis of pro-inflammatory mediators such as tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and prostaglandin E2 (PGE2) (da Silva *et al.*, 2023; Zulkefli *et al.*, 2023). The reported reduction in these inflammatory mediators contributes to decreased swelling and pain while accelerating the transition from the inflammatory phase to the proliferative and maturation phases of wound healing.

The histopathological results obtained in this study revealed that bamboo shoot extract effectively accelerated wound healing in rats with myiasis. In the treated wound tissue, there was an increase in granulation tissue formation, decreased tissue necrosis, and a significant improvement in vascularisation. The increased collagen formation and the reduction in swelling and inflammation clearly indicate that the apus bamboo shoot extract promoted the formation of more stable and stronger skin structures (Tarigan *et al.*, 2024). This histopathology also reflects a faster recovery in the dermal and epidermal layers, allowing the wound to close more quickly and new tissue to form with better quality.

The histopathological results was evaluated based on the number of fibroblasts in the tissue samples, as their presence marks the beginning of the proliferative phase of the wound healing. In the statistical tests conducted, a significant difference in fibroblast count was observed between the negative control (CN) group, the positive control (CP) group (P=0.005), and P3 treatment group 3 (P=0.003). This showed that the 5% apus extract applied to group P3 had an effect comparable to that of the positive control group (CP), which received ivermectin spray as standard treatment for myiasis infection. Administration of ivermectin to the positive control group resulted in significant improvements, although it did not have regenerative effects. This may be attributed to its larvicidal effect, which quickly removes larvae from the lesion area, thus accelerating physiological processes in regenerating the wound area. In contrast, the treatment applied to group P3 also had a significant effect on the fibroblast count, according to the histopathological image. Flavonoids, alkaloids, tannins, and cyanogenic acid are larvicidal compounds that are toxic to pathogens, microorganisms, and parasites (Widiarso *et al.,* 2018; Opeña *et al.,* 2023).

The analysis of the number of fibroblasts with significant increase in group P3 was supported by the presence of immune modulators that stimulated the healing process. Fibroblasts play a crucial role in extracellular matrix production and wound contraction, whereas collagen is a key structural component in the remodeling phase (Moretti *et al.*, 2022). Saponins

can promote the release of IL-1β, and together with tannins, activate TGF-β, which further stimulates fibroblast formation (Ibrahim *et al.*, 2018). The stimulation of growth factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) is also influenced by the presence of saponins (Tarigan *et al.*, 2024). Re-epithelialisation and activation of myofibroblasts are assisted by the presence of flavonoids in bamboo shoot extract (Qiu *et al.*, 2018). The significantly lower inflammatory cell counts, particularly in P3 and CP, indicated the anti-inflammatory activity of the extract, which likely contributed to the accelerated healing response.

This study was also focused on the number of inflammatory cells in the histopathological images of the samples to understand the effects of the application of the apus bamboo shoot extract in spray gel form on the degree of inflammation in the lesions. The presence of inflammatory cells indicates an inflammatory phase, e.g. the first phase after a wound is formed. The number of inflammatory cells indicates the transition from the inflammatory to the proliferative phase during wound regeneration. The administration of ivermectin to the positive control (CP) group and apus bamboo shoot extract spray gel in the treatment group resulted in a significant reduction in the number of inflammatory cells. The ivermectin spray in the positive control group and the 5% apus bamboo shoot extract in the group P3 had similar effects. This may be attributed to the optimal action of the larvicidal agents in each group, which quickly evacuated the parasites and reduced inflammation through physiological responses.

The significant reduction in inflammatory cells in the treatment group can be explained by the presence of active compounds that act as antioxidants and anti-inflammatory agents. Flavonoids, alkaloids, saponins, and tannins inhibit phospholipase function and affect the cyclooxygenase process during the synthesis of leukotrienes and prostaglandins (Fitriyani and Fatahillah, 2022). The production of pro-inflammatory mediators, such as leukotrienes and prostaglandins, is inhibited, thus reducing the number of inflammatory cells (Abdulkhaleq *et al.*, 2018).

Histological images of normal skin are shown in Fig. 3A, while the positive control group (ivermectin) is shown on Fig. 3B. The histopathological images of the treatment groups revealed signs of inflammation, such as the spread of inflammatory cells and new tissue formation as evidence of the regenerative process, as shown in Fig. 3. These findings support the anti-inflammatory and pro-healing properties of bamboo shoot extract. These observations are in line with previous studies demonstrating that phytochemical compounds e.g. flavonoids and saponins modulate inflammatory responses and promote fibroblast activity (Vitale *et al.*, 2022).

Collagen appears during the remodelling phase of wound healing. Statistical tests revealed that the collagen percentage in the negative control (CN) group was significantly different from that in treatment groups P1 ($P=0.038$), P2 ($P=0.001$), and P3 ($P=0.020$). These results indicated that the apus bamboo shoot extract significantly improved collagen production, accelerating the healing process through physiological responses and effectively completing the healing process. The active compounds in the apus bamboo shoot extract are toxic and can damage the nervous system and enzymes of pathogens, parasites and other microorganisms, causing infection (Widiarso *et al.*, 2018).

The results showed absence of significant differences in capillary counts between groups that may be due to the study endpoint at day 7, as neovascularisation usually peaks around the second week post-injury. This suggests that longer observation periods may be required to fully evaluate the angiogenic effects of these treatments. Angiogenesis, or the growth of new blood vessels, is a normal part of wound healing and starts during the proliferative phase, gradually decreasing as the tissue matures (Everts *et al.*, 2023). Capillaries are the blood vessels involved in this process. These findings are consistent with previous studies showing that phytochemical constituents, such as flavonoids, tannins, and phenols, in bamboo shoot extracts exhibit both anti-inflammatory and pro-healing effects (Ibrahim *et al.*, 2018; Zulkefli *et al.*, 2023). The dose-dependent efficacy of 5% apus extract (group P3), which showed comparable results to ivermectin, further highlights its therapeutic potential in treating myiasis-infected wounds.

This result could be attributed to several factors. The study was terminated on day 7, which corresponds to the proliferative phase and is characterised by a reduction in inflammation. When inflammation decreases, the dilated capillaries in the inflammatory phase, which provide rapid nutrition to the tissue, begin to decrease as the tissue enters the maturation phase (Shukla *et al.,* 2019; Abdul-Nasir-Deen *et al.,* 2020). The capillaries become more clearly visible on day 14 as the tissue matured, organising into a scar tissue structure for healing. Overall, similar results were reported by Sadeghi *et al.* (2020), who investigated the effects of *Ferula assa-foetida* loaded chitosan nanoparticle biofilms on full-thickness wounds infected with methicillin-resistant *Staphylococcus aureus*. Their study demonstrated that the combination of phytochemicals and a biopolymeric carrier significantly enhanced healing outcomes, including fibroblast proliferation and collagen deposition, and improved histological architecture. Although the infection models differed, the principle of using bioactive plant compounds to treat infected wounds demonstrated comparable effectiveness, supporting the broader application of botanical therapeutics in managing resistant wound infections and supporting the findings of this study.

Apus bamboo shoot extract shows significant therapeutic potential in supporting wound healing, particularly in myiasis infections caused by fly larvae, which often result in tissue necrosis, severe inflammation, and secondary infections (Bambaradeniya *et al.*, 2019). Conventional treatments such as antibiotics and intensive wound care can lead to complications and contribute to antibiotic resistance. In contrast, plant-based therapies, such as apus extract, which has anti-inflammatory and antibacterial properties, offer a safer and more sustainable alternative. These natural agents not only reduce pathogen load, but also support faster and more effective healing (Laksono *et al.*, 2022). In addition, plant-based therapies may be more affordable and accessible, especially in regions with limited access to conventional medical care. In many tropical areas, myiasis remains a serious health issue, particularly in areas with poor hygiene standards and high exposure to blood-sucking flies (Rana *et al.*, 2020). Apus bamboo shoot extract, which is relatively easy to obtain and can be processed locally, offers a potential solution for reducing the social and economic impact of myiasis. Therefore, further research on the safety and efficacy of the apus extract in

humans is necessary to explore its clinical applications in the field.

The success of the apus bamboo shoot extract in reducing inflammation and improving tissue regeneration suggests that this plant has broad therapeutic potential, both as a wound-healing agent and as a preventive measure against secondary infections in myiasis-infected wounds. Altogether, these findings provide strong preclinical evidence that Apus bamboo shoot extract is not only effective in accelerating wound closure but also in modulating the inflammatory and regenerative phases of healing. This finding supports its advancement in clinical trials.

CONCLUSIONS

This study demonstrated that the extract of apus bamboo shoots *(G. apus)* is effective in accelerating the healing of wounds caused by myiasis infections in Wistar rats. Treatment with 5% extract (yielded the greatest reduction in wound length on days 7 and 11, with effects comparable to those of the standard treatment with ivermectin. The histopathological analysis showed that bamboo shoot extract enhanced granulation tissue formation, reduced inflammation, and accelerated proliferation and re-epithelialisation. The increase in fibroblast counts and decrease in inflammatory cells indicated that this extract supports an effective wound healing process. However, further human studies are required to confirm its clinical application.

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