Nutmeg (*Myristica fragrans*) Extracts Accelerate Oral Mucosal Wound Healing *In Vivo*

Dewi Rosmalia 1, Minarni 2, Mhd Riza Marjoni 2*

Abstract

Background: Oral mucosal pain and sores are common concerns in dental care, often resulting from surgeries, infections, trauma, or autoimmune disorders. Conventional treatments like NSAIDs offer relief but carry adverse side effects. Nutmeg (Myristica fragrans) has been traditionally used for its analgesic, anti-inflammatory, and antimicrobial properties. However, its potential in oral mucosal wound healing remains underexplored. Methods: This study utilized male Wistar rats (Rattus norvegicus) to assess the wound healing effects of nutmeg extracts and fractions. Nutmeg was extracted using maceration, and the fractions were obtained through liquid-liquid extraction. Rats were divided into control and treatment groups, and topical nutmeg treatments were applied daily to incised gingival mucosa. Wound closure time was monitored daily, and healing rates were analyzed. Results: The ethyl acetate fraction of nutmeg exhibited the fastest wound healing, achieving an average healing rate of 88.79%, closely matching the positive control (94.65%). Other fractions also demonstrated efficacy, with the aqueous fraction achieving 82.46% and the ethanol fraction 75.54%. The hexane fraction showed the slowest healing at 63.67%. Statistical analysis revealed significant differences between the groups ($p < 0.05$). Conclusion: Nutmeg extracts, particularly the ethyl acetate fraction, significantly accelerated wound healing in the oral mucosa of Wistar rats, highlighting its potential as a natural wound-healing agent. Further research is needed to isolate the bioactive compounds responsible for these effects and to explore their clinical applications in dental care.

Keywords: Nutmeg, Wound healing, Oral mucosa, Bioactive compounds, Dental care

Significance | This study demonstrates Nutmeg's potential in enhancing oral mucosal wound healing, providing safer, effective alternatives for dental care.

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Introduction

Oral mucosal pain and sores represent a significant concern in dental care, affecting a substantial number of patients. These issues often arise due to various factors, including surgical interventions, infections, trauma, or autoimmune diseases (Bhatnagar et al., 2018). The discomfort caused by oral mucosal wounds not only leads to significant pain but also reduces the quality of life and heightens the risk of secondary complications such as infections (Akintoye & Greenberg, 2014). Managing this pain effectively and promoting wound healing are essential for enhancing patient comfort and preventing further complications.

Traditionally, the management of oral mucosal pain relies on the use of analgesics and non-steroidal anti-inflammatory drugs (NSAIDs). While these medications are effective, they are often accompanied by undesirable side effects such as gastric ulcers, indigestion, and kidney complications (Aoki et al., 2019). Consequently, there is growing interest in finding safer and more effective therapeutic options. Natural products are increasingly being recognized as potential alternatives due to their therapeutic properties and lower side effect profiles. Among the promising

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candidates is Nutmeg (Myristica fragrans), a plant known for its medicinal benefits. Nutmeg has long been used in traditional medicine to treat conditions involving pain and inflammation. The plant contains various bioactive compounds, such as myristin, eugenol, and terpenoids, which have been demonstrated to possess analgesic, anti-inflammatory, and antibacterial properties (Mbabi et al., 2021; Kizil et al., 2017; Zhang et al., 2020). Studies on both in vitro and in vivo models have supported the potential of Nutmeg extracts in mitigating pain and inflammation (Ozaki et al., 2019; Thakur et al., 2021). Furthermore, Nutmeg has shown antibacterial activity against bacteria involved in dental plaque formation (Rosmalia D et al., 2022). Despite these findings, the clinical application of Nutmeg in oral mucosal wound care and pain management remains underexplored.

Given this gap, the current study aims to investigate the effectiveness of Nutmeg extracts and fractions in promoting wound healing in the gingival mucosa of Wistar rats. The findings of this research may pave the way for developing safer and more effective alternatives for treating common dental conditions. By focusing on Nutmeg's bioactive compounds, this study has the potential to offer insights into the broader implications of natural products in dental and pharmaceutical fields.

Materials and Methods

This experimental laboratory study employed a posttest-only control group design. The subjects consisted of male Wistar strain rats (Rattus norvegicus), selected based on inclusion and exclusion criteria. The inclusion criteria were male Wistar rats aged 2 to 3 months, with a body weight of 200-250 grams. Rats were excluded if they showed signs of poor health, characterized by reduced movement activity, cloudy or reddish eyes, slimy nose and mouth, excessive salivation, or diarrhea.

Extraction of Nutmeg (Myristica fragrans)

Nutmeg extraction was performed using the maceration method. A total of 1000 grams of Nutmeg (*Myristica fragrans*) was subjected to maceration with 70% ethanol for 72 hours (3 x 24 hours) in a dark-colored container that was tightly sealed to minimize light exposure. The resulting macerate was filtered and concentrated using a rotary evaporator at a temperature of 40-50°C to yield a thick extract. This extract was used for further fractionation.

Fractionation (Liquid-Liquid Extraction)

The thick Nutmeg extract was weighed and dissolved in a small amount of ethanol before adding 500 mL of distilled water. The resulting solution was poured into a separatory funnel, and 500 mL of n-hexane solvent was added at a 1:1 volume ratio. The mixture was fractionated by vigorous shaking, and the n-hexane fraction was collected. The process was repeated three times until the n-hexane fraction was clear. The aqueous fraction was then partitioned with 500 mL of ethyl acetate, also at a 1:1 ratio, using the separatory funnel. After repeating the partitioning process three times, the ethyl acetate and water fractions were collected. Each fraction was concentrated using a rotary evaporator to obtain a thick fraction.

Anti-Wound Activity Test

The study variables were the Nutmeg extracts and fractions, as well as the wound closure time of incisions made on the oral mucosa of Wistar rats. Wound closure time was observed daily. Rats that met the inclusion criteria were anesthetized using pure ether by inhalation. Once anesthetized, an incision was made in the mesial gingiva of the lower left and right first incisor teeth. Distilled water was used to irrigate the incision wounds.

The treatment group received topical applications of Nutmeg extract or fractions daily after the incision was made. Treatment was administered once a day until the wounds fully healed. The incision wound healing process in Wistar rats was monitored and recorded through direct visual observation daily, without the aid of special tools.

The control groups consisted of a positive control (likely treated with a standard wound healing agent) and a negative control (without treatment). The healing time in these groups was compared with that in the Nutmeg extract treatment groups.

Results and Discussion

The process of wound closure in the oral mucosa of Wistar rats treated with nutmeg (*Myristica fragrans*) extracts and fractions was observed daily. This allowed for the assessment of the wound healing effects of different treatments, including the positive and negative control groups. The results of this study, summarized in Table 1, demonstrated that topical application of nutmeg extracts significantly affected the wound healing time (Figure 1). Among the treatment groups, the ethyl acetate fraction exhibited the fastest healing rate, while the hexane fraction showed the slowest.

The statistical analysis confirmed that the differences in wound healing time among the groups were statistically significant, as indicated by the p-value (> 0.05) from the Shapiro-Wilk normality test (Table 2). The results of the study provide substantial evidence that nutmeg extracts and fractions can accelerate the healing process of oral mucosal wounds, with some fractions being more effective than others.

Effect of Nutmeg Fractions on Wound Healing

The healing rate for the positive control group, which was treated with a synthetic wound-healing agent, was the highest at 94.65%. This group served as the benchmark for comparing the wound healing efficacy of the nutmeg fractions. Among the nutmeg fractions, the ethyl acetate fraction had the most significant wound healing activity, with an average healing rate of 88.79%, comparable to the positive control. This was followed by the aqueous fraction (82.46%), ethanol fraction (75.54%), and hexane fraction (63.67%).

Table 1. Wound healing rates in the oral mucosa of Wistar rats treated with Nutmeg (*Myristica fragrans*) extracts and fractions.

Test group	Wound healing rate (%)	Average cure (%)
Negative Control (Na-CMC)	10,23	10,56
	7,99	
	13,47	
Positive Control	93,71	94,65
	94,67	
	95,58	
Hexane Fraction	62,20	63,67
	64,83	
	63,97	
Ethanol fraction	75,56	75,54
	75,36	
	75,71	
Ethyl acetate fraction	89,61	88,79
	87,57	
	89,18	
Water Fraction	81,88	82,46
	82,39	
	83,13	

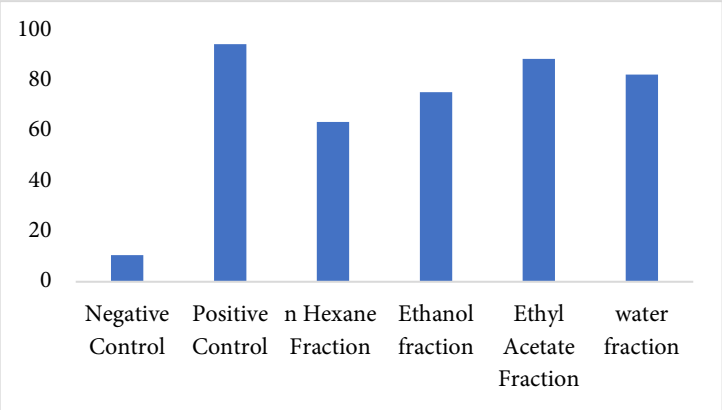

Figure 1. Comparative wound healing times of oral mucosal incisions in Wistar rats treated with Nutmeg (*Myristica fragrans*) extracts and fractions.

Table 2. Normality test results using the Shapiro-Wilk test

Statistics	Value
n	6
Mean	69,27
Standard Deviation	30,72
W statistic (b2/SS)	0,808
Sig.	0,072

The negative control group, which did not receive any treatment, had the lowest healing rate at 10.56%.

The ethyl acetate fraction's superior performance can be attributed to its high content of bioactive compounds such as lignans and neolignans. According to Kotlia and Singh (2015), these compounds possess potent anti-inflammatory, antimicrobial, and antioxidant properties, which play critical roles in promoting wound healing. This fraction's effectiveness in accelerating the healing process aligns with previous studies that have identified nutmeg as a potent natural remedy for wound healing.

Mechanisms of Action of Nutmeg Fractions

The bioactive compounds present in the ethyl acetate fraction of nutmeg, particularly lignans and neolignans, are known for their diverse biological activities that contribute to the wound healing process. These compounds exhibit anti-inflammatory, antimicrobial, and antioxidant properties, which are essential for wound closure and tissue repair.

Anti-inflammatory Activity: Inflammation is a crucial phase in wound healing, but prolonged inflammation can delay healing. The ethyl acetate fraction likely reduced inflammation by inhibiting the production of pro-inflammatory mediators such as prostaglandins and cytokines. Amin et al. (2022) reported that nutmeg-derived lignans like dehydrodiisoeugenol possess strong anti-inflammatory effects, making them valuable for modulating the inflammatory response during wound healing.

Antioxidant Properties: Oxidative stress can damage cells involved in tissue repair, slowing the healing process. The antioxidant activity of lignans and neolignans in the ethyl acetate fraction helps neutralize reactive oxygen species (ROS) and prevents oxidative damage. Gupta et al. (2021) emphasized that nutmeg's antioxidant compounds protect cells from oxidative stress, which is critical during the wound healing process.

Antimicrobial Activity: Preventing infection is essential for successful wound healing. Nutmeg lignans and neolignans possess antimicrobial properties, which help create a sterile environment for wound closure. Chatterjee et al. (2020) found that these compounds were effective against various pathogens responsible for wound infections, further supporting the use of nutmeg fractions as a natural wound healing agent.

Cell Proliferation and Collagen Production: Nutmeg extracts may also stimulate cellular processes involved in wound healing. Takzaree et al. (2016) highlighted the role of nutmeg in enhancing the expression of collagen and insulin-like growth factor-1 (IGF-1), both of which are crucial for tissue regeneration. Collagen provides structural support to the healing tissue, while IGF-1 promotes cell proliferation, which is necessary for closing wounds.

The combined effect of these mechanisms likely explains the superior wound healing observed in the ethyl acetate fraction group compared to other fractions. While the hexane fraction showed the least wound healing activity, it still performed better than the negative control. This suggests that even less polar compounds in nutmeg have some potential to aid wound healing, though their effects may be weaker than the more polar compounds found in the ethyl acetate fraction.

Clinical Relevance and Potential Applications

The findings of this study highlight the potential of nutmeg as a natural wound healing agent, particularly in dental and oral health contexts. The ethyl acetate fraction's remarkable wound healing properties could be applied in the development of topical formulations for treating oral mucosal wounds or other soft tissue injuries.

Natural products, such as nutmeg, have been increasingly recognized for their therapeutic potential. Salehi et al. (2019) described how traditional remedies, including nutmeg, have been used for centuries to treat various ailments, including wounds. The bioactive compounds in nutmeg offer an alternative to synthetic drugs, which may have side effects or lead to antimicrobial resistance. The results of this study provide further support for the incorporation of nutmeg extracts into modern pharmaceutical and dental health practices.

Future Directions

Although this study has demonstrated the effectiveness of nutmeg extracts and fractions in wound healing, further research is needed to identify the specific bioactive compounds responsible for these effects. Advanced analytical techniques, such as high-performance liquid chromatography (HPLC) and mass spectrometry (MS), could be used to isolate and characterize the individual compounds present in each fraction. This would allow for a more detailed understanding of their specific roles in the wound healing process.

Additionally, future studies could investigate the molecular pathways influenced by nutmeg compounds during wound healing. This would help elucidate the precise mechanisms by which nutmeg extracts promote tissue repair and offer insights into how these compounds could be optimized for therapeutic use.

Conclusion

In conclusion, nutmeg (*Myristica fragrans*) extracts and fractions have significant potential to accelerate the healing of oral mucosal incision wounds in Wistar rats. The ethyl acetate fraction exhibited the strongest wound healing effect, likely due to its rich content of bioactive compounds such as lignans and neolignans, which possess anti-inflammatory, antimicrobial, and antioxidant properties. These findings support the potential use of nutmeg as a natural wound healing agent, particularly in dental and pharmaceutical applications. Further research is warranted to explore the specific bioactive compounds responsible for these effects and their mechanisms of action.

Author contributions

D.R. and M. conceptualized the study and designed the methodology. M.R.M. contributed to data analysis, manuscript drafting, and provided critical revisions. All authors reviewed and approved the final version of the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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