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Evaluation of Nutmeg Flesh Extract as a Natural Anti-Acne Agent Against *Cutibacterium acnes*

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Abstract. Acne vulgaris is a common chronic inflammatory skin disorder primarily caused by *Cutibacterium acnes* infection. This study aims to investigate the phytochemical composition, total flavonoid content, and antibacterial activity of the ethanolic extract of *Myristica fragrans* fruit flesh against *C. acnes* and *Staphylococcus aureus*. The dried fruit flesh was extracted using ultrasonic-assisted ethanol extraction, followed by qualitative phytochemical screening and total flavonoid quantification via the aluminum chloride colorimetric method. Antibacterial activity was evaluated using the disc diffusion method at extract concentrations of 10%, 20%, and 30%, with standard antibiotics as positive controls. The extract contained key bioactive compounds including flavonoids, terpenoids, tannins, saponins, and alkaloids. Total flavonoid content averaged 0.15820 ± 0.00440 mg quercetin equivalent per gram of dry extract. The extract exhibited dose-dependent antibacterial effects, with more potent inhibition against *C. acnes* than *S. aureus*. Inhibition zones at 30% concentration reached notable diameters, confirming significant antibacterial activity based on CLSI standards. These findings highlight the potential of *M. fragrans* fruit flesh as a promising natural source of multifunctional bioactive compounds for anti-acne cosmeceutical development. Further studies involving compound isolation and in vivo efficacy testing are recommended to support its clinical application.

Keywords: *Myristica fragrans, flavonoids, phytochemicals, antibacterial activity, Cutibacterium acnes, acne, natural cosmetics, disc diffusion*

1. Introduction

Acne vulgaris represents a widespread chronic inflammatory disorder primarily affecting adolescents and young adults, with its multifactorial etiology encompassing microbial colonization and dysregulated innate immunity [1,2]. Clinically, acne is characterized by the appearance of comedones, papules, pustules, and, in more severe cases, nodules or cysts that can result in permanent scarring and significant psychological distress [3,4]. *Cutibacterium*

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acnes (*C. acnes*) plays a crucial role in acne pathogenesis by colonizing pilosebaceous units and triggering inflammatory cascades through pro-inflammatory mediators [5]. Although conventional treatments such as topical retinoids, antibiotics, and benzoyl peroxide provide therapeutic benefits, their associated adverse effects, potential for antibiotic resistance, and risk of skin barrier disruption underscore the need for alternative, safer, and more sustainable treatment options [6,7].

Responding to the growing consumer demand for long-term safety and environmental sustainability, the cosmetic industry has increasingly focused on natural bioactives as replacements for synthetic ingredients[8]. Plant-derived flavonoids have attracted considerable attention in this context due to their multifunctional properties, including antioxidant, anti-inflammatory, and antimicrobial activities [9,10,11]. These polyphenolic compounds benefit by interfering with bacterial cell wall integrity, scavenging reactive oxygen species, and modulating inflammatory signaling pathways, making them promising candidates in managing acne vulgaris [12,13].

Myristica fragrans, commonly known as nutmeg, is a tropical plant native to Indonesia and has long been utilized in culinary and traditional medicinal practices [14,15]. Its seeds possess abundant essential oils, tannins, phenolic compounds, and notably, flavonoids, several of which have demonstrated broad-spectrum antimicrobial activity [16,17]. While previous studies have predominantly focused on the antibacterial and antioxidant properties of nutmeg seed extracts, the phytochemical profile and bioactivity of the nutmeg fruit flesh have received limited attention in the literature. Moreover, there is a scarcity of studies evaluating the antimicrobial effects of nutmeg fruit flesh extracts specifically against *C. acnes*. This limited exploration constrains a comprehensive understanding of the plant's full therapeutic potential, particularly in acne management.

Flavonoids and other polyphenolic compounds in *Myristica fragrans* exert significant antibacterial effects through multiple mechanisms, including disrupting bacterial cell wall integrity, inhibiting key microbial enzymes, and interfering with quorum sensing pathways [14,18,19,20]. Additionally, their potent antioxidant properties help to mitigate oxidative stress-induced inflammation, a key contributor to acne pathogenesis [17,21]. These bioactivities collectively support the therapeutic potential of flavonoid-rich extracts in managing *C. acnes* proliferation and associated skin inflammation, thereby reinforcing the rationale for exploring nutmeg fruit flesh as a novel cosmeceutical agent.

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Accordingly, the present study centers on the nutmeg fruit flesh, undertaking detailed phytochemical characterization, flavonoid quantification, and evaluation of its antibacterial efficacy against *C. acnes*. The research aims to expand current knowledge and explore novel bioactive sources for cosmeceutical applications by focusing on this less-investigated plant part.

2. Materials and Methods

2.1 Materials

Dried flesh of *Myristica fragrans* was obtained from a traditional market in Lampung, Indonesia, and authenticated by a botanist at the Biology Laboratory, Institut Teknologi Sumatera. Analytical-grade ethanol (96%), aluminum chloride ($AlCl_3$), acetic acid, sodium chloride (NaCl), Dragendorff's reagent, and other reagents were purchased from certified distributors. *Cutibacterium acnes* and *Staphylococcus aureus* were obtained from clinical isolates and maintained under standard laboratory conditions.

2.2 Equipment

The leading equipment used in this study included an ultrasonic extraction bath and a rotary evaporator (IKA® RV 10, Germany) to remove ethanol under reduced pressure. A UV-Vis spectrophotometer (Shimadzu UV-1800, Japan) was employed to determine total flavonoid content. Sample mixing and homogenization were done using a magnetic stirrer and vortex mixer. An analytical balance (Ohaus®, USA) was used for accurate weighing, and an incubator was utilized to provide optimal conditions for microbial growth. Additional laboratory equipment included an autoclave for sterilization and standard microbiological glassware such as test tubes, Petri dishes, micropipettes, and sterile swabs.

2.3 Methods

2.3.1 Preparation of Dried Nutmeg Flesh

Myristica fragrans' flesh was manually peeled and cut into small pieces for uniform drying. The prepared aril pieces were dried in a laboratory oven at 40 °C until a constant weight was achieved, indicating sufficient dehydration. After drying, the aril was ground into powder using a standard laboratory grinder. The resulting powder was used without particle size fractionation, and no mesh specification was applied.

2.3.2 Extraction of *Myristica fragrans*

An amount of 70 g of dried nutmeg flesh powder (*Myristica fragrans*) was immersed in 350 mL of 96% ethanol and subjected to ultrasonic-assisted extraction at 50 °C for 90 minutes. This method has been previously utilized due to its efficiency in extracting bioactive compounds from plant materials, indicating that ultrasonic extraction improves yield by enhancing the penetration of solvents into plant tissues [22]. The resulting mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator under reduced pressure at 40 °C, as characterized by similar methodologies in effective phytochemical extractions [23,24]. The obtained viscous extract was stored at 4 °C in an amber glass container until further use.

2.3.3 Phytochemical Screening

Phytochemical screening of the concentrated extract was performed using standard qualitative tests to detect alkaloids, flavonoids, tannins, saponins, and terpenoids. Alkaloids were identified by treating 1 mL of the extract with NH_3 and H_2SO_4, followed by Mayer's reagent, where a white to yellowish precipitate indicated the presence. Flavonoids were detected by mixing the extract with absolute ethanol, magnesium powder, and concentrated HCl, resulting in yellow to red coloration. Tannins were confirmed by adding $FeCl_3$ to the extract, producing a blue-green color. Saponins were tested by diluting the extract in distilled water, heating, cooling, and vigorously shaking to observe stable foam formation. Terpenoids were identified by mixing the extract with chloroform and Salkowski reagent, where a reddish-brown layer indicated a positive result.

2.3.4 Preparation of Microbial Suspensions

A 24-hour culture of *C. acnes* was suspended in 5 mL sterile 0.9% NaCl solution. The suspension was vortexed for approximately 15 seconds to ensure homogeneity. Then, 750 µL was transferred to a cuvette and adjusted to an optical density of 0.08 – 0.1 at 625 nm using a UV-Vis spectrophotometer, corresponding to $1 – 2 \times 10^8$ CFU/mL, a standard procedure for microbial quantification [11]. Additional bacteria or NaCl solution was added to adjust if the turbidity was outside this range. *C. albicans* was cultured in potato dextrose broth (PDB) for 48 hours, and suspensions were prepared similarly, consistent with methods used in antimicrobial susceptibility testing [25].

2.3.5 Disc Diffusion Antibacterial and Antifungal Assay

The antimicrobial activity was tested using the disc diffusion method. Cultures were grown in Mueller Hinton Broth (MHB) and adjusted to the 0.5 McFarland standard. A total of 100 µL of microbial suspension was spread evenly on Mueller-Hinton Agar (MHA) using a sterile swab. Sterile filter paper discs (6 mm) were impregnated with ethanolic extract at 10%, 20%, 30%, 40%, and 50%, and placed on the agar surface. The methodology for this assay is widely acknowledged for evaluating antimicrobial efficacy and has been successfully employed in studies involving *Myristica fragrans* [25,26]. Plates were incubated at 37 °C for 24 hours for bacteria and 48 hours for fungi. Zones of inhibition were measured in millimeters using a digital caliper, with positive controls of 5% amoxicillin (for bacteria) and 2% ketoconazole (for fungi), while 10% DMSO served as the negative control.

2.3.6 Determination of Total Flavonoid Content

A total of 25 mg of the ethanolic extract was dissolved in 10 mL of ethanol and stirred using a magnetic stirrer at 300 rpm. The solution was diluted with ethanol to 50 mL to achieve a 500 ppm concentration. An amount of 1 mL of this solution was mixed with 1 mL of 2% aluminum chloride and 8 mL of 5% acetic acid. The mixture was incubated at room temperature for 30 minutes, and the absorbance was read at 410 nm using a UV-Vis spectrophotometer, following standard protocols to ensure the consistency of results [23,9]. Quercetin was utilized as the standard, and the total flavonoid content was expressed as mg quercetin equivalent per gram of extract (mg QE/g), with all measurements conducted in triplicate to ensure accuracy and reliability.

3. Result and Discussion

3.1 Phytochemical Constituents of *Myristica fragrans* Extract

Phytochemical screening of *Myristica fragrans* flesh's ethanolic extract revealed several key secondary metabolites, including terpenoids, flavonoids, saponins, tannins, and alkaloids. These findings were obtained through qualitative assays commonly used in natural product analysis. A reddish-brown coloration observed in the Salkowski test indicated the presence of terpenoids, while the Shinoda test produced a pink to red coloration, confirming the presence of flavonoids. The formation of persistent froth in the foam test confirmed the presence of saponins. A dark green to blue-black color in the ferric chloride test signified the existence of

tannins. In contrast, an orange precipitate in the Dragendorff's reagent test confirmed the presence of alkaloids [14,27].

Table 1. Phytochemical Profile of the Ethanolic Extract of *Myristica fragrans* Flesh

Test	Result
Terpenoid	+
Flavonoid	+
Saponin	+
Tannin	+
Alkaloid	+

The presence of these compounds is noteworthy as each contributes to antibacterial activity through distinct biochemical mechanisms. Flavonoids interfere with bacterial cell wall synthesis and disrupt membrane integrity, leading to increased permeability and leakage of vital intracellular contents. They can also inhibit nucleic acid synthesis, which impairs bacterial replication [28]. Due to their lipophilic nature, terpenoids can integrate into bacterial membranes, destabilizing the lipid bilayer and compromising cell viability [29]. Saponins interact with sterols in the cell membrane to form pores, causing leakage of cellular components and lysis [16]. Tannins exert their effects by binding to microbial proteins and enzymes, disrupting cell wall synthesis, and inhibiting essential metabolic processes [27]. Lastly, alkaloids have been shown to intercalate with microbial DNA or inhibit key enzymatic pathways, thereby interfering with microbial growth and survival [28].

Compared to previous studies primarily focusing on seed or essential oil extracts *of M. fragrans*, which are rich in monoterpenes and phenylpropanoids [21,22,30,31], this study provides novel insight into the phytochemical diversity present in the fruit flesh, a relatively underexplored part of the plant. Detecting these five phytochemical classes in the flesh broadens the understanding of *M. fragrans*' therapeutic potential and highlights the flesh as a promising source of bioactive compounds for cosmeceutical applications [32].

Furthermore, comparisons with other medicinal plants used in acne treatment, such as *Azadirachta indica* (neem) [33] and *Camellia sinensis* (green tea) [34], suggest that *M. fragrans* shares a similar phytochemical profile, particularly concerning the presence of flavonoids, tannins, and alkaloids. This indicates that nutmeg flesh extract may offer comparable therapeutic potential to more widely studied herbal agents. Altogether, the detection of these five phytochemical classes implies that the antimicrobial activity of *Myristica fragrans* flesh

extract is likely not attributed to a single compound, but rather a synergistic interaction among multiple bioactive constituents. These findings provide a strong phytochemical basis for the subsequent antibacterial assays and reinforce the potential of nutmeg extract, particularly from the underutilized flesh part, as a source of multifunctional agents for anti-acne cosmetic formulation [19].

3.2 Total Flavonoid Content

The total flavonoid content (TFC) of the ethanolic extract of *Myristica fragrans* flesh was quantified using the aluminium chloride colorimetric method. This method used quercetin as a standard to generate a calibration curve, and absorbance was measured at 410 nm using a UV-Vis spectrophotometer. The extract was tested in triplicate, and the results are presented in Table 1.

Table 1. Total Flavonoid Content (TFC) of Ethanolic Extract of *Myristica fragrans* Flesh (Triplicate Results)

Replicate	Absorbance	Quercetin Conc. (ppm)	Flavonoid Ratio (g/g extract)	Extract Yield (g/g)	TFC (mg QE/g dry extract)
1	0.544	6.62	0.00132	0.1187	0.1573
2	0.537	6.50	0.00130	0.1187	0.1544
R3	0.558	6.87	0.00137	0.1187	0.1630
Mean±SD	–	–	–	–	**0.1582 ± 0.0044**

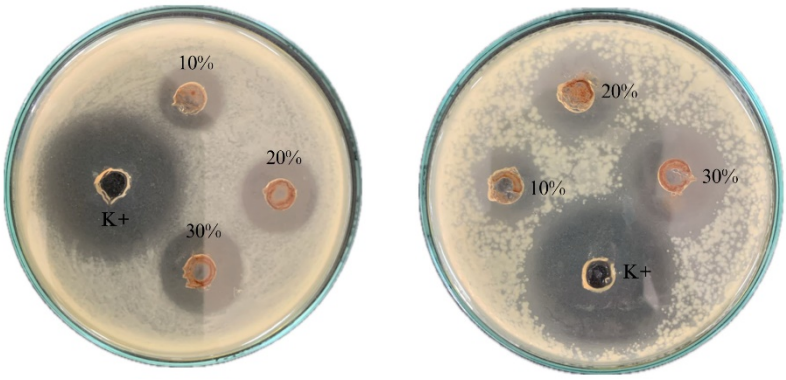
The triplicate measurements yielded consistent total flavonoid content (TFC) values ranging from 0.1544 to 0.1630 mg quercetin equivalent (QE) per gram of dry extract, with a mean of 0.1582 ± 0.0044 mg QE/g. This level of flavonoid content suggests a moderate concentration of polyphenolic compounds, which may contribute to the biological activity of the extract, including its antimicrobial potential [35]. Compared to previous studies that primarily focused on the seeds or essential oil of *Myristica fragrans*, this finding offers a novel contribution by characterizing the flavonoid content specifically from the flesh. While flavonoid values from nutmeg seed extracts are generally higher due to denser phytochemical storage in seeds, the significant flavonoids present in the flesh part, which has been previously underexplored, demonstrate their potential as an alternative source of active compounds [36].

The measured TFC is comparable to or slightly lower than reported for other medicinal plants used in acne treatment, such as *Azadirachta indica* (neem) and *Camellia sinensis* (green tea) [17]. Nevertheless, the relatively consistent flavonoid concentration obtained here and the extract's confirmed antibacterial activity support its suitability for further development in natural cosmetic formulations targeting acne-prone skin [37]. The synergistic effects of these

bioactive constituents within the flavonoid profile not only enhance the antimicrobial efficacy but also provide a phytochemical basis for their roles in skin health, reinforcing the therapeutic relevance of *Myristica fragrans* flesh in dermatological applications [38].

3.3 Antibacterial Activity

The antibacterial activity of the ethanolic extract of *Myristica fragrans* flesh was assessed against two acne-associated bacteria*: Staphylococcus aureus* and *Cutibacterium acnes*, using the disc diffusion method. The extract was tested at concentrations of 10%, 20%, and 30%, while a standard antibiotic served as the positive control. The results are visually presented in Figure 1.



(a) (b)

Figure 1. Antibacterial Activity of *Myristica fragrans* extract against *Staphylococcus aureus* (a) and *Cutibacterium acnes* (b)

As shown in Figure 1, the extract exhibited clear inhibition zones against both *S. aureus* and *C. acnes*. The inhibition zones increased proportionally with extract concentration, indicating a dose-dependent antibacterial response. The highest inhibition was observed at 30%, and minimal or no activity was noted at 10% for *S. aureus*. The positive control displayed the largest inhibition zones, validating the method's reliability. According to Clinical and Laboratory Standards Institute (CLSI) guidelines, inhibition zones with a diameter of ≥14 mm indicate significant antibacterial activity for the tested organisms [39].

Interestingly, *C. acnes* appeared more sensitive to the extract than *S. aureus*, as demonstrated by visibly larger inhibition zones at each concentration level. This finding is particularly relevant since *C. acnes* plays a central role in the pathogenesis of acne. Bioactive compounds such as flavonoids, terpenoids, and tannins, likely contributing to the observed

antibacterial effect, have been noted to disrupt bacterial cell walls and interfere with bacterial enzyme systems.

Compared to previous reports primarily focusing on nutmeg seed extract [30,31], this study uniquely demonstrates that the flesh of *Myristica fragrans*, an underutilized part of the plant, also possesses notable antimicrobial activity. For instance, Noviyandri et al. reported that extracts from nutmeg flesh effectively inhibit Gram-positive bacteria, reinforcing the potential of *Myristica fragrans* as a valuable source of antimicrobial agents [14].

When compared with other medicinal plants used in acne treatment, such as *Excoecaria cochinchinensis* and *Salvia officinalis*, which have minimum inhibitory concentrations (MIC) ranging from 1.56 to 6.25 mg/mL against *S. aureus* and *C. acnes* [40], the antibacterial activity of nutmeg fruit flesh extract presents a competitive profile. Meanwhile, *Strychnos ligustrina* leaf extract, although active against *P. acnes*, showed significantly smaller inhibition zones than standard antibiotics [41]. Although the nutmeg fruit flesh extract did not surpass the efficacy of conventional antibiotics, its substantial antibacterial activity supports its potential as a natural cosmeceutical ingredient for managing acne-prone skin.

These results highlight the promise of nutmeg flesh extract as a functional and underexplored ingredient in natural cosmetic formulations, offering a novel alternative source of bioactive compounds for acne treatment.

4. Conclusions

This study demonstrates that the ethanolic extract of *Myristica fragrans* fruit flesh contains diverse bioactive phytochemicals, including flavonoids, terpenoids, tannins, saponins, and alkaloids, which collectively contribute to its significant antibacterial activity against *Cutibacterium acnes* and *Staphylococcus aureus*. The extract exhibited dose-dependent inhibition, with notable effectiveness against *C. acnes*, a key pathogen in acne pathogenesis. These findings position nutmeg fruit flesh as a promising, underutilized natural resource with potential applications in developing safe and effective anti-acne cosmeceutical products.

From an industrial perspective, identifying the fruit flesh as a valuable bioactive source expands opportunities for the cosmetic and pharmaceutical industries to innovate natural formulations that meet growing consumer demand for environmentally sustainable and health-conscious products. The extract's moderate flavonoid content and broad antimicrobial spectrum suggest its viability for incorporation into commercial anti-acne skincare lines.

However, further research is necessary to fully realize nutmeg fruit flesh extract's clinical and commercial potential. Future studies should focus on isolating and characterizing specific active compounds responsible for the observed bioactivities and comprehensive in vivo efficacy and safety assessments. Such investigations will be essential to validate its dermatological effectiveness and support regulatory approval, facilitating its transition from experimental extract to marketable cosmeceutical ingredient.

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