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Antioxidant and Anti-Inflammatory Activities of *Myristica fragrans* Houtt. Seed Extract-Loaded Gel: An *In Vitro* Study for Therapeutic Applications

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ABSTRACT

Myristica fragrans Houtt. (nutmeg) seeds are rich in bioactive compounds, including phenolics, flavonoids, and fatty acids, which are widely recognized for their potent antioxidant and anti-inflammatory effects. These pharmacological properties make nutmeg seed extract a promising candidate for developing natural therapeutic agents targeting oxidative stress and inflammation. This study aimed to assess the antioxidant and anti-inflammatory activities of topical herbal gels formulated with the ethanol extract of nutmeg seeds. The phytochemical profile of the extract revealed significant levels of phenolics (19.29 ± 1.94 mg GAE/g) and flavonoids (11.73 ± 0.05 mg QE/g), with major bioactive compounds identified through GC–MS analysis, including 9-octadecenamide, palmitoleamide, and myristic acid. Herbal gels containing 0.05%, 0.10%, and 0.20% w/w of the extract were formulated using Carbopol® Ultrez 21 as the gelling agent, and were evaluated for their physicochemical stability. Antioxidant activity was measured using ABTS and DPPH assays, while anti-inflammatory activity was assessed by inhibition of nitric oxide production *in vitro*. Among the formulations, the 0.20% w/w extract-loaded gel exhibited the highest antioxidant activity (ABTS: 90.65 ± 0.54%; DPPH: 84.06 ± 0.48%) and nitric oxide inhibition (82.42 ± 4.03%), significantly outperforming the standard 1% diclofenac gel. These findings highlight the potent antioxidant and anti-inflammatory effects of nutmeg seed extract-loaded gel, supporting its potential as a natural, plant-derived therapeutic agent for topical applications. Further *in vivo* and clinical studies are needed to confirm its clinical efficacy and safety, paving the way for its future use in treating inflammatory conditions.

***Keywords*:** *Myristica fragrans*, Nutmeg seed, Antioxidant activity, Anti-inflammatory activity, Nitric oxide inhibition, Herbal gel.

Introduction

Myofascial pain syndrome (MPS) is a common musculoskeletal condition, particularly among the working-age population, and is often associated with repetitive motion, prolonged static posture, and occupational stress.[1] Inflammatory responses play a central role in its pathogenesis. Vasodilation, induced by inflammatory mediators such as nitric oxide released from leukocytes, promotes immune cell infiltration into affected tissues**.** Concurrently, these events generate reactive oxygen species (ROS), which in turn promote oxidative damage to nucleic acids, lipids, proteins, and carbohydrates. Such molecular alterations can impair cellular function and reduce muscle contractility, ultimately contributing to fatigue.[2-4] Conventional therapies for musculoskeletal disorders, such as pharmacological agents, physical therapy, and complementary approaches, are often constrained by limited efficacy, adverse effects, and restricted accessibility. Among these, non-steroidal anti-inflammatory drugs (NSAIDs) are widely used but carry significant risks, including gastrointestinal ulceration, thrombotic events, stroke, and myocardial infarction.[5]

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These limitations have prompted interest in plant-derived compounds with antioxidant and anti-inflammatory activities as potential therapeutic alternatives. In particular, agents capable of modulating oxidative stress and nitric oxide-mediated pathways may offer therapeutic potential for the management of MPS.

Myristica fragrans Houtt. (nutmeg), a member of the Myristicaceae family, has been widely employed in traditional medicine, with various plant parts utilized to alleviate a wide range of ailments.[6] Among them, the seed is traditionally used to treat stomach cramps, dyspepsia, diarrhea, anxiety, tendon inflammation, muscle pain, and homeostatic imbalances.[7] Nutmeg has demonstrated various pharmacological activities, notably antimicrobial,[8] antioxidant,[8,9] anti-inflammatory,[8,10] and analgesic effects.[11] The essential oil derived from nutmeg seed is particularly rich in bioactive constituents such as sabinene (20-53%), α-pinene (13-22%), and myristicin (10-20%).[12] Recognizing its therapeutic value, nutmeg seed was added to Thailand's National List of Essential Medicines in 2023 for the treatment of bone and muscle pain.[13] Given its well-established analgesic and anti-inflammatory properties, the extract of nutmeg emerges as a promising candidate for the development of topical anti-inflammatory gels targeting muscle pain, with potential applications in musculoskeletal therapeutics. Previous studies have explored the topical application of nutmeg seed extract, with one such study demonstrating that a gel containing 12% ethanolic extract exhibited potent anti-inflammatory activity in the carrageenan-induced rat paw edema model, achieving efficacy comparable to a 1% diclofenac sodium gel.[14] Although previous studies have shown favorable outcomes with high-concentration formulations, such approaches may present limitations in terms of formulation stability, safety, and production cost. However, the development of nutmeg-based gels using lower extract concentrations has not been

systematically investigated. The novelty of the present study lies in optimizing low-concentration formulations (0.05-0.20% w/w) to achieve antioxidant and anti-inflammatory efficacy comparable to standard NSAID gels.

The development of a topical gel containing nutmeg seed extract is of interest due to its potential application in managing muscle pain, which is often associated with oxidative stress and localized inflammation. Topical gels are favored for their ability to deliver active compounds directly to the affected site, while minimizing systemic exposure. Expanding on previous reports on the antioxidant and anti-inflammatory properties of nutmeg, this study aims to evaluate the antioxidant activity of the nutmeg seed extract-loaded gel using ABTS and DPPH assays, as well as examining its ability to inhibit nitric oxide production *in vitro*. The results are expected to provide preliminary insights into the biological activity of the gel formulation and its possible application in managing musculoskeletal inflammatory conditions.

Materials and Methods

Chemicals

Ascorbic acid, 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), quercetin, sodium nitroprusside, and sulfanilamide were obtained from Sigma-Aldrich Chemical (St. Louis, Missouri, USA). Absolute ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent, gallic acid, and n-)1-naphthyl(ethylenediamine dihydrochloride)NED(were purchased from Merck)Darmstadt, Germany(. Aluminum chloride and sodium acetate were obtained from Univar Ajax Finechem (Australia). Carbopol® Ultrez 21, phenoxyethanol, polyethylene glycol (PEG400), polypropylene glycol (PPG), and triethanolamine were supplied by Chemipan Corporation Co., Ltd.)Bangkok, Thailand(. A 1% w/w diclofenac gel was obtained from Thai Nakorn Patana Co., Ltd.)Difelene®, Nonthaburi, Thailand(. All other laboratory chemicals were of analytical grade and obtained from chemical suppliers.

Plant material and extraction

Nutmeg seeds were purchased from a herbal medicine store in Thap Khlo district, Phichit province, Thailand, in July 2022)Figure 1(. The specimen was identified as MF2022-YS002 by a certified botanist at the Faculty of Natural Resources, Rajamangala University of Technology Isan Sakon Nakhon Campus, Sakon Nakhon, Thailand. A voucher specimen was deposited in the Herbarium of the Thai Traditional Medicine Program, Faculty of Natural Resources, Rajamangala University of Technology Isan, Sakon Nakhon Campus.

The seeds were dried at 50 ˚C and ground into a fine powder. The powdered material was macerated with 95% ethanol for 5 days. The ethanol extract was filtered and concentrated under reduced pressure using a rotary evaporator (Rotavapor® R-300, Buchi, Flawil, Switzerland(. The extraction yield was 1.93% w/w, calculated based on the weight of the dried starting material. The nutmeg seed extract was kept at 4 ˚C prior to further analysis to minimize degradation and maintain compound stability.

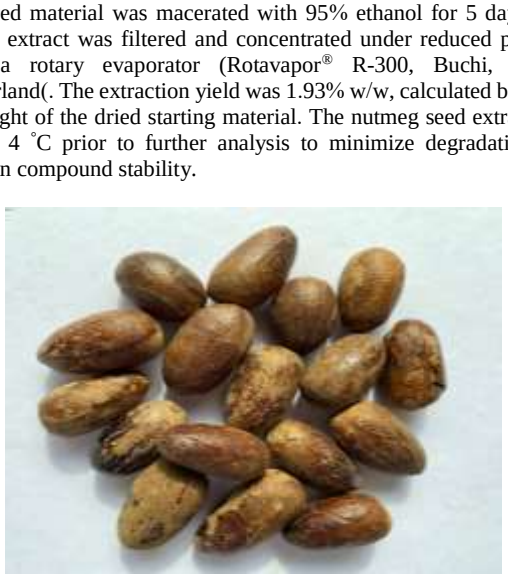

Figure 1: *Myristica fragrans*)nutmeg(seeds.

Total phenolic content of nutmeg seed extract

The total phenolic content of the nutmeg seed extract was determined using the Folin-Ciocalteu method as described by Sriset *et al.*[15] Briefly, a solution of crude extract)50 mg/mL(was mixed with Folin-Ciocalteu reagent and allowed to stand in the dark for 30 minutes at room temperature. Absorbance was measured at 700 nm using a UV-Vis microplate reader)Infinite® 200 pro, Tecan, Männedorf, Switzerland). Total phenolic content was calculated from a calibration curve for the standard gallic acid (0-400 µg/mL(and is expressed as mg of gallic acid equivalent per g of dry weight extract)mg GAE/g dry extract(.

Total flavonoid content of nutmeg seed extract

The total flavonoid content of the nutmeg seed extract was determined using the aluminum chloride)$AlCl_3$(colorimetric assay as described by Sriset *et al.*[15] A solution of crude extract)50 mg/mL(was added to a reaction mixture containing 10% w/v $AlCl_3$ and 1 M sodium acetate (1:1), followed by incubation for 30 minutes at room temperature in the dark. Absorbance was measured at 405 nm using a UV-Vis microplate reader. Total flavonoid content was quantified from a calibration curve for the standard quercetin)0-400 µg/mL(and is expressed as mg of quercetin equivalent per g of dry weight extract)mg QE/g dry extract(.

Gas chromatography-mass spectrometry (GC-MS) analysis of nutmeg seed extract

Phytochemical analysis of the ethanol extract of nutmeg seeds was performed using a GC-MS-QP2020 instrument)Shimadzu, Kyoto, Japan(, equipped with a Rtx-5MS capillary column)30 m × 0.25 mm × 0.25 µm(. The GC oven was programmed as follows: initial temperature of 60 ˚C held for 1 min, increased to 100 ˚C at a rate of 3 ˚C/min and held for 5 min, then raised to 120 ˚C at a rate of 5 ˚C/min and held for 5 min, and followed by an increase to 260 ˚C at a rate of 3 ˚C/min. The injection volume was 1 µL with a split ratio of 5:1. Helium was used as the carrier gas at a flow rate of 1.36 mL/min. The mass unit was set at an ion source temperature of 280 ˚C and an ionization voltage of 70 eV. The relative percentage amount of each compound was calculated from its average peak area relative to total area, as determined using the peak area data from the total ionic chromatogram)TIC(. Each compound was identified by searching the National Institute of Standards and Technology)NIST(Library database.[16]

Preparation of herbal gels containing the ethanol extract of nutmeg seeds

Herbal gels were formulated according to the compositions provided in Table 1, following the method described by Khan *et al.*,[17] with slight modifications. The formulations incorporated the ethanol extract of nutmeg seeds at three concentrations: 0.05%, 0.10%, and 0.20% w/w. The concentration range was selected based on the researchers' previous study,[18] in which the ethanolic extract of nutmeg seeds demonstrated antioxidant (ABTS and DPPH) and anti-inflammatory (nitric oxide inhibition) activities with IC_{50} values ranging from 0.11 to 0.40 mg/ml. Accordingly, extract concentrations slightly higher than the IC_{50} values—0.5, 1, and 2 mg/ml—were used for gel formulation, corresponding to 0.05%, 0.10%, and 0.20% w/w in the final products. Carbopol® Ultrez 21 was employed as the gelling agent. To prepare the gel base)mixture A(, Carbopol® Ultrez 21 was gradually dispersed in distilled water under continuous magnetic stirring until a homogeneous dispersion was achieved. In a separate step, PEG 400, PPG, and phenoxyethanol were added to mixture A and stirred continuously until fully homogenized, forming mixture B. The ethanolic extract of nutmeg seeds was then incorporated into mixture B, and the final volume was adjusted to 100 mL with distilled water, yielding mixture C. Finally, triethanolamine was added dropwise to mixture C to adjust the pH to a physiologically acceptable skin pH range)pH 4-6(.

Evaluation of the accelerated stability of the nutmeg seed extract gels

The accelerated stability of the herbal gels was assessed using the heating-cooling method for 4 cycles, as described in a previous study.[19] Herbal gels were stored at 4 ± 1 ˚C for 24 h, followed by 45 ± 1 ˚C for 24 h, accounting for 1 cycle. After each cycle, the physical appearance

of the gels was then observed visually. The pH value of the herbal gels was measured using a pH meter)ST3100F, OHAUS, Shanghai, China(, and the viscosity was measured using a Brookfield Viscometer)DV2T, AMETEK Brookfield, Massachusetts, USA(.

Table 1: Composition of herbal gel formulations with the ethanol extract of nutmeg seeds.

Compositions	% w/w in the formulations (F)				Function
	F1	F2	F3	F4	
Carbopol® Ultrez 21	1.00	1.00	1.00	1.00	Gelling agent
PEG400	4.00	4.00	4.00	4.00	Emulsifier
PPG	8.00	8.00	8.00	8.00	Humectant
Phenoxyethanol	0.30	0.30	0.30	0.30	Preservative
Nutmeg seed extract	-	0.05	0.10	0.20	Active ingredient
Distilled water (q.s. to)	100	100	100	100	Vehicle
Triethanolamine	q.s.	q.s.	q.s.	q.s.	pH adjusting agent (pH 4-6)

F1 represents the gel base; F2, F3, and F4 correspond to gels containing 0.05%, 0.10%, and 0.20% w/w nutmeg seed extract, respectively.
q.s.: quantity sufficient

Evaluation of ABTS$^{\bullet+}$ radical scavenging activity of the nutmeg seed extract gels

The antioxidant activity of herbal gels was evaluated using the ABTS$^{\bullet+}$ cation assay, as described by Sriset *et al.*[15] A 7 mM ABTS solution was mixed with 140 mM potassium persulfate)1:0.02(and allowed to stand in the dark at room temperature for 16 hours to produce ABTS$^{\bullet+}$ radicals. The absorbance of this mixture was adjusted to 0.70 ± 0.02 at 700 nm before use. Herbal gels containing 0.05%, 0.10%, and 0.20% w/w extract were mixed with the ABTS solution at a ratio of 1:20 and incubated in darkness at room temperature for 6 minutes. The absorbance was then measured at 700 nm using a UV-Vis microplate reader. Antioxidant activity, expressed as %inhibition, was calculated relative to a standard 1% w/w diclofenac gel.

Evaluation of DPPH$^{\bullet}$ radical scavenging activity of the nutmeg seed extract gels

The free radical scavenging activity of herbal gels against stable DPPH$^{\bullet}$ radicals was performed as described by Uaraksakul *et al.*[20] Herbal gels at concentrations of 0.05%, 0.10%, and 0.20% w/w were mixed with 0.2 mM DPPH solution and incubated in darkness at room temperature for 30 minutes. Absorbance was measured at 517 nm using a UV-Vis microplate reader. Antioxidant activity, expressed as %inhibition, was calculated relative to a standard 1% w/w diclofenac gel.

Evaluation of anti-inflammatory activity of the nutmeg seed extract gels via inhibition of nitric oxide production

Nitric oxide (NO) was automatically generated from sodium nitroprusside in an aqueous solution at physiological pH 7.4, where it reacted with oxygen to produce nitrite ions)NO_2^-(.[21] Therefore, the accumulation of NO_2^- was determined as an indicator of NO using the Griess reagent, as described by Sriset *et al.*[15] Briefly, a solution of 10 mM sodium nitroprusside in phosphate buffered saline)pH 7.4(was mixed with the different concentrations of herbal gels)0.05%, 0.10%, and 0.20% w/w(and incubated at room temperature for 150 minutes. The Griess reagent)1% w/v sulfanilamide and 0.1% w/v NED in 20% v/v glacial acetic acid; 1:1(was then added and the mixture incubated at room temperature for 30 min. Absorbance was measured at 540 nm using a UV-Vis microplate reader. The anti-inflammatory activity, expressed as %inhibition, was calculated relative to a standard 1% w/w diclofenac gel.

Statistical analysis

The results were expressed as mean ± standard deviation)SD((n=3). All data were analyzed using one-way analysis of variance)ANOVA(followed by Tukey's *post hoc* test to determine statistical significance)$p < 0.05$(using the Statistical Product and Service Solutions)SPSS(IBM version 29.0)Armonk, USA(.

Results and Discussion

Total phenolic and flavonoid contents of ethanol nutmeg seed extract

Phenolic compounds are a major class of plant-derived secondary metabolites, renowned for their broad spectrum of biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties.[22,23] Among these, flavonoids represent a prominent subclass, distinguished by their polyphenolic structures and extensively documented pharmacological effects. In the present study, the ethanol extract of nutmeg seeds was analyzed for its antioxidant constituents, particularly total phenolic and flavonoid contents. As summarized in Figure 2, the extract exhibited a total phenolic content of 19.29 ± 1.94 mg GAE/g dry weight and a total flavonoid content of 11.73 ± 0.05 mg QE/g dry weight. Similarly, Assa *et al.*[24] reported total phenolic content of 24.35 mg GAE/g dry weight in the methanol nutmeg seed extract.[24] Furthermore, Tan *et al.*[25] reported total phenolic and total flavonoid contents of 18.80 mg GAE/g dry weight and 1,345.75 mg RE/g dry weight, respectively, in the methanol extract of nutmeg seeds.[25] Conversely, Pashapoor *et al.*[26] reported lower total phenolic)1.12 mg GAE/g dry weight(and total flavonoid)0.26 mg QE/g dry weight(contents in the petroleum ether extract of nutmeg seeds.[26] Such variations, compared with the present findings, may arise from genetic, environmental, and methodological differences.

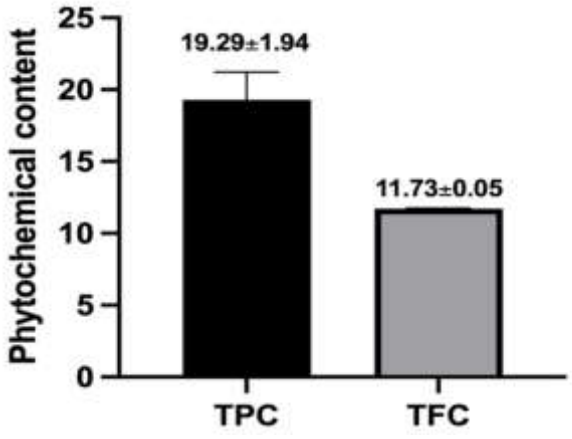

Figure 2: Phytochemical content of the nutmeg seed extract. Results are expressed as mean ± SD (n=3). TPC: total phenolic content)mg GAE/g dry weight(; TFC: Total flavonoid content)mg QE/g dry weight(.

The bioactivity of phenolics and flavonoids is strongly influenced by the hydroxyl groups on their aromatic rings, which enhance electron-donating capacity, facilitate radical scavenging, and enable metal ion chelation, all of which are active during both the initiation and

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propagation of oxidative processes.[22] Hydroxyl group number and position also modulate anti-inflammatory pathways, underscoring their dual role in oxidative stress mitigation and inflammation control.[23] These structural features may explain inter-study differences in measured activities and further support the pharmacological relevance of hydroxyl group patterns in nutmeg seed constituents.

Although the colorimetric assays employed in this study are widely accepted for preliminary screening of total phenolic and flavonoid contents, they present certain limitations, including non-specific reactions with other reducing agents, matrix interferences, and the inability to identify individual compounds. Additionally, variations in extraction conditions may influence the measured values.[27,28] Therefore, in this study, chemical composition analysis was further performed using GC-MS to confirm the presence of specific bioactive constituents in the nutmeg seed extract.

Phytocomponent identification of the ethanol nutmeg seed extract by GC-MS

Sixteen compounds were identified in the ethanol extract of nutmeg seeds using GC-MS analysis (Figure 3). The identified compounds, along with their retention times)RT), peak areas, molecular formulas, molecular weights)MW(, and reported pharmacological activities, are summarized in Table 2.

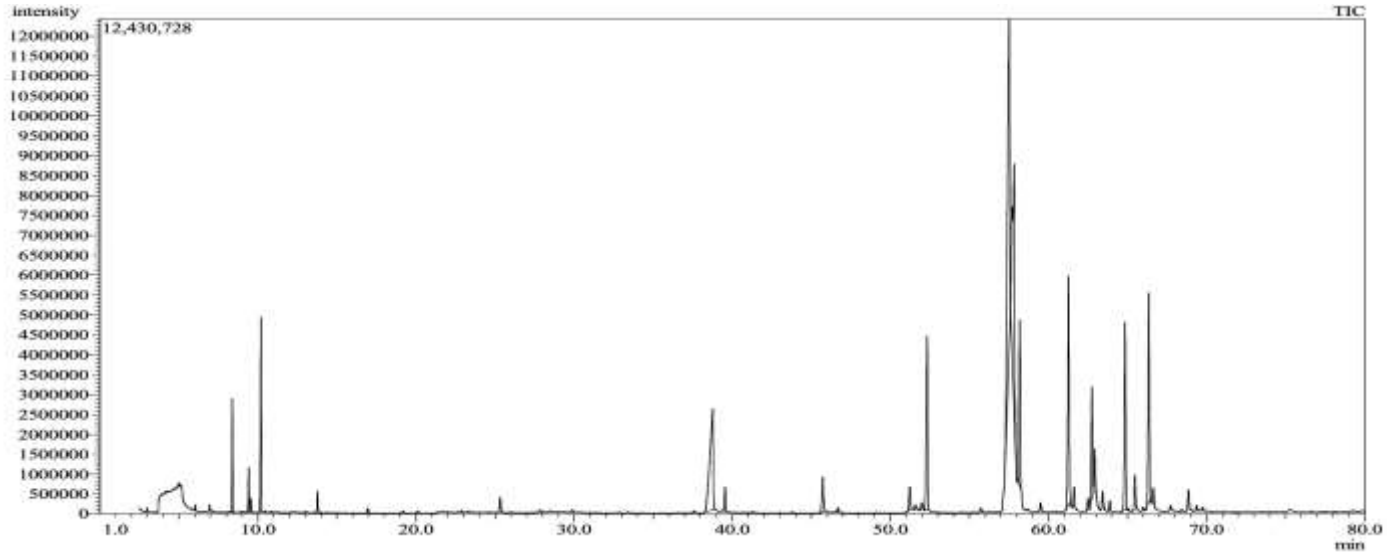

Figure 3: GC-MS chromatogram of the ethanol nutmeg seed extract.

Table 2: Phytochemical constituents identified in the ethanol extract of nutmeg seeds by GC-MS analysis.

No.	RT	Peak area (%)	Name of the compound	Molecular formula	MW (g/mol)	Compound nature	Pharmacological activity
1	8.38	2.36	(+)-2-Bornanone (camphor)	$C_{10}H_{16}O$	152.23	Monoterpene ketone	Antibacterial,[33] Antioxidant[34]
2	9.42	0.94	dl-Menthol	$C_{10}H_{20}O$	156.26	Monoterpene alcohols	Antioxidant[35]
3	9.56	0.28	Terpinen-4-ol	$C_{10}H_{18}O$	154.25	Monoterpene alcohols	Anti-inflammatory,[36] Anticancer[37,38]
4	10.19	4.31	o-Aminobenzohydroxamic acid	$C_7H_8N_2O_2$	152.15	Hydroxamic acid derivatives	Antioxidant[39]
5	13.77	0.51	Safrole	$C_{10}H_{10}O_2$	162.18	Phenylpropanoids	Antibacterial[40]
6	38.77	9.27	Myristic acid	$C_{14}H_{28}O_2$	228.37	Saturated fatty acids	Anti-inflammatory,[41] Antinociceptive[42]
7	45.73	1.46	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Saturated fatty acids	Anti-inflammatory,[43] Antioxidant[44]
8	51.23	0.93	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.50	Monounsaturated fatty acids	Antioxidant[45]
9	52.33	6.86	Hexadecanamide	$C_{16}H_{33}NO$	255.44	Fatty acid amides	Anti-inflammatory,[46] Antibacterial[46]
10	57.52	22.74	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281.50	Fatty acid amides	Anti-inflammatory,[47] Antioxidant[48]
11	57.82	16.26	Palmitoleamide	$C_{16}H_{31}NO$	253.42	Fatty acid amides	Antioxidant[49]
12	58.19	5.22	Octadecanamide	$C_{18}H_{37}NO$	283.50	Fatty acid amides	Antioxidant,[48] Antibacterial[50]
13	61.28	8.29	Glycerol 1-palmitate	$C_{19}H_{38}O_4$	330.50	Monoglycerides	Antitumor[51]
14	62.93	1.33	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337.60	Fatty acid amides	Antifungal[52]
15	64.85	6.72	4-((2S,3R)-4-(Benzo[d][1,3]dioxol-5-yl)-2,3-dimethylbutyl)-2-methoxyphenol	$C_{20}H_{24}O_4$	328.40	Phenolics	Anticholinesterase[53]
16	66.36	7.72	Myristic acid glycidyl ester	$C_{17}H_{32}O_3$	284.43	Glycidyl esters	Not reported

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The most abundant constituents in the extract were 9-octadecenamide,)Z(-)22.74%(, palmitoleamide)16.26%(, myristic acid)9.27%(, glycerol 1-palmitate)8.29%(, myristic acid glycidyl ester)7.72%(, hexadecanamide)6.86%(, 4-))2S,3R(-4-)Benzo]d[]1,3[dioxol-5-yl(-2,3-dimethylbutyl)-2-methoxyphenol (6.72%), octadecanamide (5.22%), o-aminobenzohydroxamic acid (4.31%),)+(-2-bornanone (2.36%), n-hexadecanoic acid (1.46%), and 13-docosenamide,)Z(-)1.33%). The remaining compounds were present in lower amounts, including dl-menthol)0.94%(, cis-vaccenic acid)0.93%), safrole)0.51%), and terpinen-4-ol (0.28%). Many of these phytochemical constituents have previously been reported to possess various pharmacological properties, including anti-inflammatory, antioxidant, antibacterial, antinociceptive, and antitumor activities)Table 2(.

GC-MS analysis revealed that fatty acid derivatives represented the major class of compounds in the extract. Among the sixteen identified compounds, eight were fatty acids, with 9-octadecenamide)Figure 4A(, palmitoleamide)Figure 4B(, and myristic acid)Figure 4C(showing the highest relative abundances. This observation is consistent with the findings of Chacko and Nag[29] and Oo *et al.*,[30] who also reported the presence of various fatty acids and hydrocarbons, such as myristic acid, undecanoic acid, oleic alcohol, and octadecanoic acid, in the crude extract and essential oil of nutmeg seeds.

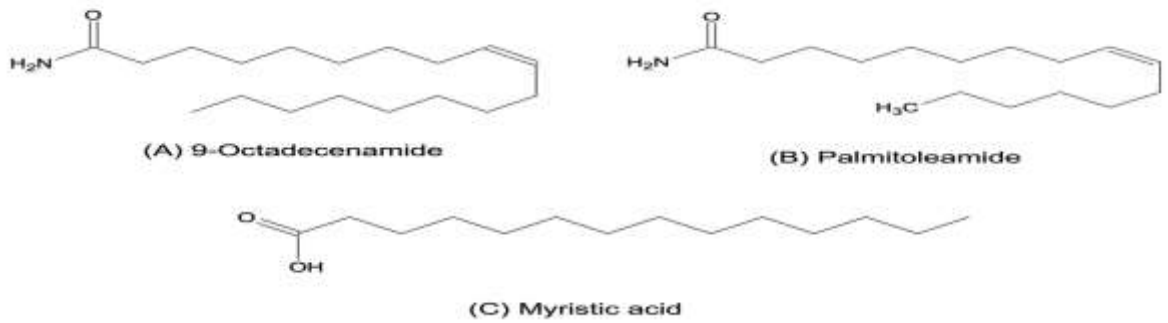

Figure 4: Chemical structures of the predominant compounds identified in the ethanol extract of nutmeg seeds by GC-MS:)A(9-Octadecenamide,)B(Palmitoleamide, and)C(Myristic acid. All structures are illustrated using ChemDraw version 16.0.

Physicochemical stability of the nutmeg seed extract gels under accelerated stability conditions

Accelerated stability testing confirmed that all formulated gels maintained their physicochemical characteristics throughout the testing cycles)Table 3(. No changes were observed in visual appearance, pH, or viscosity after each cycle. The pH values of the gel base)F1(and herbal gel formulations)F2, F3, and F4(ranged from 4.04 to 4.81, which are values suitable for topical application as they closely match the natural acidity of human skin)pH 4-6).[31] The viscosity of the gels remained within a high and acceptable range, between 20,840 and 23,550 cP, aligning with established standards for semisolid gel formulations.[32] These findings indicate that all formulations demonstrated excellent physicochemical stability under accelerated storage conditions. Therefore, it can be concluded that alternating temperature stress and variations in nutmeg extract concentration had no adverse effects on the physical appearance, viscosity, or pH of the gel formulations.

Table 3: Physicochemical properties of nutmeg seed extract gel formulations under accelerated stability conditions.

Formulations)F(	Physical/Chemical parameters	0 cycle	1st cycle	2nd cycle	3rd cycle	4th cycle
F1	Appearance	Homogenous, clear, and translucent	Homogenous, clear, and translucent	Homogenous, clear, and translucent	Homogenous, clear, and translucent	Homogenous, clear, and translucent
	pH	4.25 ± 0.05[a]	4.20 ± 0.03[a]	4.04 ± 0.03[a]	4.75 ± 0.04[a]	4.46 ± 0.06[a]
	Viscosity)cP(	23,550.00 ± 544.04[b]	22,804.60 ± 577.29[b]	22,805.80 ± 921.81[b]	22,924.80 ± 609.21[b]	22,900.60 ± 903.59[b]
F2	Appearance	Homogenous, clear, and translucent	Homogenous, clear, and translucent	Homogenous, clear, and translucent	Homogenous, clear, and translucent	Homogenous, clear, and translucent
	pH	4.80 ± 0.06[a]	4.81 ± 0.05[a]	4.47 ± 0.08[a]	4.55 ± 0.02[a]	4.33 ± 0.41[a]
	Viscosity)cP(	21,914 ± 864.32[b]	21,114 ± 242.61[b]	21,132 ± 253.09[b]	21,970 ± 131.15[b]	20,948 ± 127.49[b]
F3	Appearance	Homogenous, clear, and translucent	Homogenous, clear, and translucent	Homogenous, clear, and translucent	Homogenous, clear, and translucent	Homogenous, clear, and translucent
	pH	4.18 ± 0.15[a]	4.54 ± 0.20[a]	4.28 ± 0.12[a]	4.31 ± 0.05[a]	4.77 ± 0.04[a]
	Viscosity)cP(	21,546 ± 390.36[b]	21,772 ± 157.91[b]	21,028 ± 267.53[b]	21,278 ± 387.164[b]	21,132 ± 256.70[b]
F4	Appearance	Homogenous,	Homogenous,	Homogenous,	Homogenous,	Homogenous, clear, and translucent

	clear, and	clear, and	clear, and	clear, and	
pH	translucent	translucent	translucent	translucent	4.57 ± 0.02[a]
Viscosity)cP(	4.44 ± 0.29[a]	4.56 ± 0.04[a]	4.13 ± 0.07[a]	4.18 ± 0.00[a]	21,156 ± 400.13[b]
	21,464 ± 533.98[b]	20,840 ± 134.46[b]	21,078 ± 291.30[b]	21,282 ± 485.11[b]	

F1 represents the gel base; F2, F3, and F4 correspond to gels containing 0.05%, 0.10%, and 0.20% w/w nutmeg seed extract, respectively.
All values are expressed as mean ± SD (n=3).
Mean values within the same row sharing the same superscript letters (a or b) are not significantly different ($p < 0.05$).

Antioxidant and NO production inhibitory effects of the herbal gels

The *in vitro* antioxidant activities of the herbal gels formulated with different concentrations of the ethanol extract of nutmeg seeds were evaluated based on their ability to scavenge non-biological free radicals)$ABTS^{\bullet+}$ and $DPPH^{\bullet}$(.[54] The free radical scavenging capacities of the gels are summarized in Table 4. Significant differences)$p < 0.05$(in free radical inhibition were observed among the formulations containing 0.05%, 0.10%, and 0.20% w/w of the extract. The 0.20% w/w formulation exhibited the highest free radical scavenging activity, with inhibition values of 90.65 ± 0.54% for $ABTS^{\bullet+}$ and 84.06 ± 0.48% for $DPPH^{\bullet}$. The 0.10% w/w formulation showed moderate activity)80.58 ± 0.67% for $ABTS^{\bullet+}$ and 72.13 ± 0.46% for $DPPH^{\bullet}$(, while the 0.05% w/w formulation demonstrated the lowest activity)68.53 ± 1.43% for $ABTS^{\bullet+}$ and 62.77 ± 1.61% for $DPPH^{\bullet}$(, comparable to the activity of 1% w/w diclofenac gel)67.42 ± 1.36% and 66.23 ± 3.25% for $ABTS^{\bullet+}$ and $DPPH^{\bullet}$, respectively( )$p < 0.05$(. Notably, both the 0.10% and 0.20% w/w gels showed significantly greater scavenging activities than the diclofenac gel)$p < 0.05$(. These findings indicate a concentration-dependent increase in the antioxidant potential of the nutmeg seed extract gels.

Table 4: Percentage inhibition of antioxidant and anti-inflammatory activities of nutmeg seed extract-loaded gels.

Samples	**%Inhibition**		
	Antioxidant activity		**Anti-inflammatory activity**
	$ABTS^{\bullet+}$	**$DPPH^{\bullet}$**	**NO**
0.05**%** w/w nutmeg seed extract gel	68.53 ± 1.43[a]	62.77 ± 1.61[a]	65.32 ± 1.32[a]
0.10**%** w/w nutmeg seed extract gel	80.58 ± 0.67[b]	72.13 ± 0.46[b]	75.30 ± 0.75[b]
0.20**%** w/w nutmeg seed extract gel	90.65 ± 0.54[c]	84.06 ± 0.48[c]	82.42 ± 4.03[c]
1**%** w/w diclofenac gel	67.42 ± 1.36[a]	66.23 ± 3.25[a]	75.76 ± 2.46[b]

Values are presented as mean ± SD (n=3).
Mean values within the same column followed by different superscript letters)a, b, or c(are significantly different at $p < 0.05$.

NO is a biologically active free radical that serves as a major pro-inflammatory mediator. It is enzymatically synthesized from L-arginine and molecular oxygen via nitric oxide synthase.[55] Excessive NO production contributes to inflammation, primarily through its reaction with superoxide anions to form peroxynitrite, a highly reactive and cytotoxic oxidant capable of exacerbating tissue damage. Therefore, inhibition of NO production is widely employed as an *in vitro* indicator of anti-inflammatory activity.[21,55] The NO inhibitory activities of the nutmeg seed extract gels are presented in Table 4. The 0.20% w/w gel exhibited the highest NO inhibition)82.42 ± 4.03%(, which was significantly greater than that of 1% w/w diclofenac gel)75.76 ± 2.46%( )$p < 0.05$(. The 0.10% w/w formulation showed comparable NO inhibition)75.30 ± 0.75%(to the diclofenac gel, while the 0.05% w/w gel showed the lowest NO inhibitory activity)65.32 ± 1.32%(, which was significantly lower than the standard)$p < 0.05$(. These results indicate a clear concentration-dependent inhibitory effect of the nutmeg extract gels on NO production. Mechanistically, the suppression of NO production by the nutmeg seed extract-loaded gel may be attributed to the downregulation of inducible nitric oxide synthase)iNOS(expression or activity in inflammatory cells. This effect is consistent with known pathways in which bioactive fatty acids, such as 9-octadecenamide, palmitoleamide, and myristic acid, modulate NF-κB signaling, thereby reducing the transcription of pro-inflammatory mediators.[41,42,47-49] Furthermore, phenolic and flavonoid compounds in the extract can scavenge ROS, indirectly lowering the oxidative triggers that upregulate iNOS and cyclooxygenase-2 expression.[2,55]

Among all formulations, the 0.20% w/w nutmeg seed extract gel exhibited the most potent antioxidant and anti-inflammatory activities, exceeding the performance of 1% w/w diclofenac gel, a standard topical NSAID commonly used for the relief of muscle pain and inflammation. These findings underscore the potential of nutmeg seed extract as a functional active ingredient in topical herbal gel formulations. The results are in line with a previous study in which ethanol extracts of nutmeg seeds were incorporated into anti-inflammatory gels at concentrations ranging from 2% to 12% w/w. These formulations significantly reduced inflammation and paw edema in rat models, with inhibition rates ranging from 11.69% to 44.92%, depending on the extract concentration.[14] The increasing anti-inflammatory activity with higher extract concentrations observed in the current study is consistent with these earlier findings.

The free radical scavenging and NO inhibitory effects observed in this study are likely attributable to the synergistic actions of phytochemicals naturally present in nutmeg seeds. These effects are mediated through hydrogen or electron donation, which neutralizes free radicals, and through the suppression of NO production, which mitigates inflammatory responses. Nutmeg seeds are known to contain several bioactive compounds, including phenolics, flavonoids, 9-octadecenamide, palmitoleamide, and myristic acid, that have been reported to possess both antioxidant and anti-inflammatory properties.[22,23,41,42,47-49] From a biochemical perspective, the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging activities suggest hydrogen atom donation and single electron transfer mechanisms, which neutralize reactive species before they propagate lipid peroxidation. The high abundance of 9-octadecenamide may further contribute to membrane stabilization, thereby reducing oxidative stress at the cellular level. Together, these synergistic effects between lipid-soluble amides and polyphenolic antioxidants may explain the superior efficacy observed in comparison to diclofenac gel, which primarily targets cyclooxygenase pathways.

Evaluation in comparison to other herbal topical formulations provides context for positioning the antioxidant and anti-inflammatory efficacy of the nutmeg seed extract gel developed in this study. Arif *et al.*[56] reported that a *Phyllanthus niruri* gel (0.05-0.10% w/w) exhibited DPPH- and ABTS-scavenging activities of 61.31% and 17.73%, respectively.[56] Likewise, Kabilan *et al.*[57] formulated a polyherbal gel (0.10% w/w) containing *Cichorium intybus* and *Sida cordata*, which showed 74.59% DPPH and 76.56% nitric oxide inhibition.[57] In addition,

Thiraphatthanavong *et al.*[58] developed a herbal ultrasound gel from *Zingiber cassumunar*, *Curcuma longa*, and *Zingiber officinale* extracts, reporting IC_{50} values of 0.82 mg/ml for DPPH, 0.98 mg/ml for ABTS, and 53.38% NO inhibition.[58] In comparison, the 0.20% w/w nutmeg seed gel in the present study exhibited DPPH, ABTS, and NO inhibition values of 84.06%, 90.65%, and 82.42%, respectively, placing its efficacy within the higher range of these reported herbal formulations. Collectively, these results suggest that the nutmeg seed extract gel possesses comparable antioxidant and anti-inflammatory potential to other herbal and commercial topical gels beyond diclofenac, even when formulated at relatively low extract concentrations.

Although the present findings provide valuable evidence of the antioxidant and anti-inflammatory activities of nutmeg seed extract gel, this study was limited to *in vitro* evaluations. Additional investigations involving cytotoxicity assessment, skin permeability testing, and *in vivo* models are required to confirm the safety, bioavailability, and therapeutic potential of the formulation for clinical applications.

Conclusion

The ethanol extract of nutmeg seeds exhibited measurable antioxidant and anti-inflammatory activities, likely attributable to its diverse phytochemical constituents, including phenolics, flavonoids, terpenes, and fatty acid derivatives. These bioactivities supported the successful development of stable tropical herbal gel formulations containing 0.05%, 0.10%, and 0.20% w/w of the extract. Among the tested formulations, the gel containing 0.20% w/w of the extract showed higher *in vitro* antioxidant and anti-inflammatory responses compared to the standard 1% w/w diclofenac gel. Overall, the findings suggest that the nutmeg seed extract-loaded gel has potential as a plant-derived topical formulation for managing oxidative stress and inflammation associated with muscle discomfort. This study provides preliminary evidence supporting the pharmacological potential of the extract, and further optimization and validation of the formulation under practical and clinical settings are recommended.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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