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Evaluation of the Analgesic Effect of the Combined Aqueous Extracts of Zingiber officinale Rhizome and Myristica fragrans Seeds

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ABSTRACT

Background: *Zingiber officinale* rhizomes (ZOR) and *Myristica fragrans* seeds (MFS) are among the most important and widely used spices in cooking and in folk medicine for the management of pain.
Objectives: This research study aimed to evaluate the central and peripheral analgesic activities of ZOR alone and in combination with MFS.
Methods: Doses of 150, 300 and 400 mg/kg of the aqueous extracts of either ZOR or a combination of ZOR and MFS were administered orally to groups of albino mice of both gender and specific weight range. The analgesic activity of the extracts was investigated using Acetic acid-induced mouse writhing, Formalin-induced pain and Tail immersion test models. Negative control received distilled water orally for all models while aspirin 100 mg/kg (oral) and pentazocine 10 mg/kg (I.P) were used as standard reference drugs.
Results: ZOR aqueous extract showed significant ($p<0.05$) inhibitory effect in tail immersion test, acetic acid-induced writhing and the inflammatory phase of a formalin-induced pain test in comparison with the control. Meanwhile the combined aqueous extracts (150, 300 and 400 mg/kg) showed significant ($p<0.01$) decrease in the number of writhes, significant ($p<0.01$) decrease in the amount of time each mouse spent licking the injected paw for both neurogenic and inflammatory pain phases, but the increase in pain latency was insignificant for the tail immersion test model in comparison with the control.
Conclusion: In conclusion, our study showed that combined use of the aqueous extracts of ZOR and MFS seeds possesses a non-dose dependent analgesic effect, however better response was observed when ZOR was used alone, hence there may be no need to combine with MFS in pain management. This is a novel research study thus the findings will trigger numerous breakthroughs in the scientific community.

INTRODUCTION

The International Association for the Study of Pain (IASP) defined pain as "An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage[1]. This definition emphasizes the subjective nature of pain; meaning it is inherently personal and cannot be directly observed by others[2]. This subjectivity presents significant challenges for both clinical assessment and treatment of pain[3]. Analgesia refers to the absence of pain in response to stimuli that would normally be painful[4]. It is a crucial aspect of medical treatment, particularly in the management of acute and chronic pain[5]. Analgesia can be achieved through various methods, including pharmacological interventions and non-pharmacological interventions

(such as physical therapies and psychological approaches)[6]. Pharmacological approach to achieving analgesia involves the use of analgesic agents which are chemical agents/medications designed to alleviate pain without altering or causing loss of consciousness [7]. Herbal medicine has been an integral part of Nigerian traditional medicine since ancient times deeply rooted in the cultural practices of over 300 ethnic communities[8]. The use of herbal remedies is based on the active phytochemicals found in plants, which have been utilized for treating various ailments [9]. Ginger (*Zingiber officinale*) rhizomes as shown in Figure 1, a member of the Zingiberaceae family is an herbaceous perennial plant widely cultivated for its aromatic rhizome, which is used as a spice and in traditional medicine [10]. The analgesic effect of ginger makes it useful for managing pain from various sources such as migraines or menstrual cramps (dysmenorrhea) [11]. Studies suggest that consuming ginger can reduce pain intensity comparable to some non-steroidal anti-inflammatory drugs (NSAIDs)[11].

Nutmeg (*Myristica fragrans*) seeds (figure 2) are a tropical evergreen species that exhibits distinct morphological characteristics belonging to the Myristicaceae family. It has been traditionally used as a natural analgesic for relieving pain associated with headaches, toothaches, menstrual cramps, or muscular injuries. The presence of volatile oils such as myristicin enhances its ability to reduce pain perception by acting on the central nervous system[12]. As a result of their widespread use in ethno-pharmacy as antidiarrhea, anti-inflammatory and analgesic agents, crude extracts of these plants have been extensively evaluated for their active phytochemical constituents and therapeutic activities individually[12] and findings from these studies have served as a bedrock on which several other research studies are predicated. However, until now, the therapeutic activity (especially the analgesic property) of the combined crude extracts has not been scientifically demonstrated. The aim of this study is therefore to evaluate the combined analgesic effect of aqueous rhizome extract of Ginger rhizomes (*Zingiber officinale*) and Nutmeg seeds *(Myristica fragrans*) in comparison with the use of ginger rhizomes alone using mice models.

MATERIALS AND METHODS

Collection & preparation of plant material

The fresh plant samples were obtained from a fruit and vegetable market in Okada, Benin city, Edo State, in January 2024 and then taken to the Herbarium unit, Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin (UNIBEN) where they were identified by a seasoned plant taxonomist; Prof Henry Akinnibosun Adewale (FLS, MRSB; London) as Ginger rhizomes (*Zingiber officinale*) and Nutmeg seeds (*Myristica fragrans*) with voucher specimen numbers of UBH-Z384 and UBH-M537 respectively.

Figure 1: Ginger rhizomes

Figure 2: Nutmeg seeds

Drugs and Chemicals

Distilled water, Methanol, Ethyl acetate, Ginger (*Zingiber officinale*) Rhizomes, Nutmeg (*Myristica fragrans*) seeds, Aspirin powder (Acetylsalicylic acid, Loba Chemie Laboratory Reagents and Fine Chemicals, India), Acetic Acid (Sigma-Aldrich Laborchemikalien GmbH D30926 USA), Formaldehyde (BDH chemicals), Pentazocin

(Tazowin, WellSpring Pharmaceutical Private Limited, Bijapur India).

Animals

Swiss Albino Mice (*Mus musculus)* of both sexes with a weight range of 20-30 g were used in this study. These animals were procured from the animal house unit of the Department of Pharmacology and Toxicology, Faculty of Pharmacy. They were housed in plastic cages and maintained in the same facility within the Department of Pharmacology and Toxicology, UNIBEN, Benin City. All animals were allowed a Two (2) weeks acclimatization period before commencement of the study during which they were maintained under a natural 12-hour light and dark cycle at room temperature of approximately 27±5ºC. They were provided with a standard diet of Rodent pellet feed (Chikun Grower Pellet Feed by Oasis Farms & Agro limited, Anambra, Nigeria) and granted access to clean tap water *ad libitum*. The beddings/cages of the mice were cleaned daily and the care and treatment of the animals was strictly in adherence to the guidelines outlined in the "Guide for the care and use of laboratory animals (National Research Council, 2010) and the Public Health Service policy on Humane Care and use of Laboratory animals (Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2015)". Ethical approval was sourced from the Faculty of Pharmacy Ethics committee, UNIBEN with ethical approval number: EC/FP/024/11.

Extraction of the Plant Material

The Ginger rhizomes (*Zingiber officinale*) was chopped to tiny bits and air-dried for 4 days in the Laboratory unit, Department of Pharmacognosy, Faculty of Pharmacy, Benin City away from direct sunlight. When properly dried, the sample was then pulverized to powder and the weight was taken. About 500 g of the powdered ginger sample was suspended in 2500 ml of distilled water in a jar and allowed to macerate for 48 hours with periodic shaking and stirring. After 48 hours, it was then filtered to separate the residue from the filtrate using a filter paper and a funnel. The filtrate was then concentrated to paste form using a water bath, transferred to a sample bottle and preserved in a refrigerator. The Nutmeg seeds (*Myristica fragrans*) were pulverized to powder and the weight was taken. About 500 g of the powdered sample was suspended in 1200 ml of distilled water in a jar and allowed to macerate for 24 hours with periodic shaking and stirring. After 24 hours, it was then filtered to separate the residue from the filtrate using a filter paper and a funnel. The filtrate was then concentrated to paste form using a water bath, transferred to a sample bottle and preserved in a refrigerator.

PHYTOCHEMICAL SCREENING

The following qualitative examination of phytochemical content was carried out according to known methods[13]: Flavonoids, alkaloids, resin, tannins, glycosides and saponin.

PHARMACOLOGICAL SCREENING

Acetic Acid-induced Writhing Test

Mice were divided into 7 groups of 5 mice each. Group 1 received a single dose of distilled water (0.20 ml) orally with the aid of an oro-gastric tube and syringe, the 2nd group served as the standard test group receiving acetylsalicylic acid suspended in 5% Acacia preparation (100 mg/kg) orally using an oro-gastric tube, the next three (3) groups received the combined aqueous extracts of *Myristica fragrans* seeds and *Zingiber officinale* rhizome (150, 300 and 400 mg/kg for each group) orally and simultaneously respectively. The 6th and 7th groups were administered *Zingiber officinale* extract (ZOR) alone at oral doses of 150 and 300 mg/kg, respectively.

Thirty (30) minutes after, intraperitoneal injection of Acetic acid (10 ml/kg, 1%v/v in Normal saline) was administered to all mice and they were observed for writhes which consists of constriction of the abdominal muscles alongside stretching of the hind limbs. The writhes were counted at five (5) minutes interval for 30 minutes. Analgesic effect was expressed as significant reduction in the number of writhes in comparison with the control group[14].

Formalin- induced pain test

According to the Formalin-induced pain test model described by Shibata *et al.,* [15], *35* adult mice were selected at random, weighed and allocated to seven (7) groups of five (5) mice each. The first group of mice served as the control group with the mice in that group receiving distilled water (0.2 ml each) orally with the aid of an oro-gastric tube and syringe, The second group served as the standard group with the mice in that group receiving pentazocine (10 mg/kg) intraperitoneally and the third, fourth and fifth groups received the combined aqueous extracts of *Myristica fragrans* seeds and *Zingiber officinale* rhizome (150, 300 and 400 mg/kg respectively) orally and simultaneously. The 6th and 7th groups received *Zingiber officinale* extract (ZOR) alone at 150 and 300 mg/kg, respectively.

Thirty (30) minutes after, subcutaneous administration of 0.02 ml of 1% formalin to the right hind paw was done. The time (in seconds) spent by each mouse licking or biting the injected paw was taken as an indicator for pain response. Responses

were measured for five (5) minutes after injecting formalin to represent the "Neurogenic pain phase" and 15-30 minutes after injecting formalin to represent the "inflammatory pain phase". Analgesic effect was expressed as a reduction in the time spent licking or biting the injected paw.

Tail Immersion Test Model

According to the procedure proposed by D'Amour *et* al.[16], and modified by Jansen[17], 25 adult Swiss albino mice were randomly selected, weighed and allotted to five (5) groups of five (5) mice each. In this model, the combined extracts were used. The tail of the mice in all the groups were immersed in a water bath set at 55 ± 1ºC in order to measure their baseline response to thermal stimulus expressed as a flick of the tail, a withdrawal time of 120 seconds was set and any mouse with no response at this time were screened out of the experiment and replaced with another. The first group of mice served as the control group receiving distilled water (0.20 ml) orally with the aid of an oro-gastric tube, pentazocine (10 mg/kg) was administered intraperitoneally to the mice in the second group which served as the standard test group, the third, fourth and fifth groups received the combined aqueous extracts of *Myristica fragrans* seeds and *Zingiber officinale* rhizome (150, 300 and 400 mg/kg respectively) orally and simultaneously with the aid of an oro-gastric tube. At 30, 90, 120, 150, 180 minutes respectively after administering each drug, the response to thermal stimuli were measured by recording the time (in seconds) the tail spent immersed in the water bath set at 55 ± 1ºC before a flick is expressed.

Statistical analysis

The data obtained from the experiment were all expressed as Mean ± S.D (Standard Deviation) and percentage inhibition was calculated using the values obtained. For each of the test models in the experiment, "n" was used to represent the number of animals. Statistical analysis was conducted using One-way Analysis of Variance (ANOVA) followed by the Tukey's Post Hoc Test for data comparison. Statistical significance was defined as P values less than 0.05 ($P<0.05$).

RESULTS

PHYTOCHEMICAL SCREENING

The qualitative examination of phytochemical contents of Ginger rhizomes and Nutmeg seeds yielded the following results as presented in table 1.

Table 1: Phytochemical evaluation of *Zingiber officinale* (Ginger) and *Myristica fragans* (Nutmeg) extracts

TESTS	GINGER			NUTMEG		
	Distilled water	Ethyl acetate	Methanol	Distilled water	Ethyl acetate	Methanol
ALKALOIDS	+	-	-	+	+	+
TANNINS	+	+	+	+	+	-
TERPENOIDS	+	+	+	+	+	+
PHENOLIC COMPOUNDS	+	-	+	-	+	+
FLAVONOIDS	+	+	-	+	+	-
SAPONINS	+	+	+	+	-	+
GLYCOSIDES	+	+	+	+	+	+
REDUCING SUGARS	-	+	_	-	+	+
CARBOHYDRATES	+	+	+	+	+	+
PROTEINS	+	+	+	+	-	+

(+) = Positive. **(-)** = Negative

Acetic acid induced writhing test in mice

Acetic acid-induced writhing test results are showed in Table 2. The *Zingiber Officinale* Extract (ZOR) produced a significant inhibition for writhing reflex in mice ($p < 0.05$) in comparison to the control group. In this experiment, the inhibitory effect was dose-dependent in that the higher dose (300 mg/kg) had greater inhibition compared to the lower dose (150 mg/kg). The effect of the extract compares well with the control drug, aspirin at a dose of 100 mg/kg which also presented a significant decrease ($p<0.05$) of writhing. On combined use of ginger and nutmeg, a better inhibitory effect on acetic acid induced mouse writhing was not observed in comparison to when ginger was used alone. Rather the use of ginger alone at 300 mg/kg out- performed the combined use of

nutmeg and ginger and aspirin the reference drug. Results are also shown in table 2. The control group (distilled water) showed the highest number of writhes, followed by the combined extracts in a dose dependent manner showing reduction in the number of writhes. Interesting it was observed that the use of ginger alone had the least number of writhes especially at 300 mg/kg.

The combined aqueous extracts at all doses though showed some level of pain inhibition, however ginger alone had the highest pain inhibition (79.59%) at 300 mg/kg.

TAIL IMMERSION TEST MODEL

The effect of combined aqueous extracts of *Zingiber officinale* rhizome and *Myristica fragrans* seeds on the animal reaction time based on the tail immersion test carried out is presented in table 4. At all tested times, the 300 mg/kg dose of both extracts showed the highest immersion time before animal's response indicating the highest level of pain inhibition, however these inhibitory effects were only significant ($p<0.05$) in comparison to the control at the 30th, 90th and 180th min. The inhibitory effect of the extract was not dose-dependent as the 300 mg/kg dose showed the highest level of inhibition compared to the 400 mg/kg. From the results obtained from the acetic acid induced mouse writhing and formalin induced pain tests, with ginger administration alone outperforming the combined extracts of ginger and nutmeg, the combined extract alone was investigated in this model.

Table 2: Effect of aqueous extracts of Ginger (*Zingiber officinale*) rhizome and in combination with Nutmeg (*Myristica fragrans*) seeds on acetic acid- induced mouse writhing.

Drugs (mg/kg)	Number of writhes	Percentage inhibition
Distilled Water (0.2ml)	76.20 ± 16.75	-
ZOR(150)	16.50±3.78[a]	66.32%
ZOR(300)	10.00±2.80[a]	79.59%
MFS & ZOR (150)	65.80 ± 12.15[b]	13.65 %
MFS & ZOR (300)	37.75 ± 19.75[b]	50.46 %
MFS & ZOR (400)	39.75 ± 20.91[b]	47.83 %
Aspirin (100)	25.60 ± 16.44[b]	66.40 %

The values obtained are expressed as Mean writhes ± S.D (n = 5, per group). [a] $P<0.001$ and [b] $P<0.05$ significantly different from Distilled water (Control) in the One-Way Analysis of Variance (ANOVA) followed by comparison of data using the Tukey's Post hoc test.

MFS = *Myristica fragrans* seed extract; ZOR = *Zingiber officinale* rhizome extract

FORMALIN-INDUCED PAIN TEST

The findings of the formalin induced pain test are shown in Table 3. The data demonstrates considerable ($p < 0.05$) inhibitory actions of the *Zingiber officinale* extract (ZOR) in the second phase of the test: Phase 2 (inflammatory phase) compared to the control group. At the inflammatory stage, with both doses of the extract, there was inhibition, though at varying degrees and statistically significant ($p<0.05$) compared to the control with the 300 mg/kg dose demonstrating a greater effect, comparable to the effect of pentazocine (10 mg/kg), the standard drug. When ginger was combined with nutmeg, the combined aqueous extracts also showed significant ($p<0.05$) reduction in formalin-induced neurogenic pain in mice compared with the control group (distilled water). This effect of the combined extracts was not dose-dependent as the 300 mg/kg dose of the combined aqueous extracts gave the highest inhibitory effect with a percentage inhibition of 53.07% while the 400 mg/kg dose gave a percentage inhibition of 50.50 %. Pentazocine, the standard reference drug showed significant inhibition at 10mg/kg dose with a percentage inhibition of 54.26 %. However on comparison of the effect of ginger alone and when combined with nutmeg, a similar trend observed in the acetic acid mouse writhing was seen, as the ginger extract alone significantly ($p<0.001$) outperformed the combined extracts of ginger and nutmeg in both the central and inflammatory phases of the formalin model.

Table 3: Effect of aqueous rhizome and seed extracts of *Zingiber officinale* and in combination with *Myristica fragrans* respectively on formalin induced pain test

Drugs/extracts (mg/kg)	Time spent licking the paw (sec)	Time spent licking the paw (sec)	Percentage inhibition (%)	Percentage inhibition (%)
	PHASE 1 (0-5 min)	PHASE 2 (15-30 min)	PHASE 1 (0-5 min)	PHASE 2 (15-30 min)
Distilled water (0.2 ml)	101.0 ± 20.28	212.8 ± 61.15	-	-
ZOR(150)	45.18 ± 2.16[b]	21.60 ± 0.57[a]	55.26	89.80
ZOR(300)	44.50 ±17.67[b]	29.00 ± 7.07[a]	55.94	86.30
MFS & ZOR (150)	88.00 ± 33.53	107.00 ± 27.99[b]	12.87	49.72
MFS & ZOR (300)	47.40 ± 13.13[a]	43.60 ± 37.74 [a]	53.07	79.51
MFS & ZOR (400)	50.00 ± 22.32[b]	53.40 ± 25.71[b]	50.50	74.91
Pentazocine (10)	46.20 ± 16.18[a]	14.47 ± 20.61[a]	54.26	93.20

The values obtained are expressed as Mean ± S.D (n = 5, per group). [a] $P<0.001$ and [b] $P<0.05$ significantly different from Distilled water (Control in the One-Way Analysis of Variance (ANOVA) followed by comparison of data using the Tukey's Post hoc test. MFS = *Myristica fragrans* seed extract; ZOR = *Zingiber officinale* rhizome extract

Table 4: Effect of aqueous extracts of *Zingiber officinale* rhizome and *Myristica fragrans* seeds, on tail immersion test in mice

Drug/extracts (mg/kg)	RESULTS REACTION TIME (SECONDS)					
	0 min	30 min	90 min	120 min	150 min	180 min
DISTILLED WATER	2.71 ± 0.89	2.20 ± 0.89	2.52 ± 0.48	2.56 ± 0.37	2.71 ± 1.29	1.66 ± 0.40
MFS & ZOR 150	2.30 ± 0.90	1.52 ± 0.53	2.11 ± 0.34	1.87 ± 0.38	1.84 ± 0.78	1.18 ± 0.25
MFS & ZOR 300	2.13 ± 0.24	2.54 ± 0.68[b]	2.84 ± 0.83[b]	2.75 ± 0.73	2.54 ± 0.75	2.66 ± 0.90[b]
MFS & ZOR 400	2.01 ± 0.26	2.01 ± 0.43	1.60 ± 0.46	1.78 ± 0.56	1.96 ± 1.22	1.41 ± 0.28
PENTAZOCINE 10	2.70 ± 0.51	2.89 ± 0.76	3.13 ± 0.43	3.03 ± 0.50	2.30 ± 0.49	2.91 ± 0.89[b]

The values obtained are expressed as Mean ± S.D (n = 5, per group). There was no significant difference between the values of the test extracts except at 300 mg/kg at the times stated at $P<0.05$ in comparison with Distilled water (Control) in the One-Way Analysis of Variance (ANOVA) followed by comparison of data using the Tukey's Post hoc test. MFS = *Myristica fragrans* seed extract; ZOR = *Zingiber officinale* rhizome extract

DISCUSSION

The phytochemical screening of the *Zingiber officinale* rhizomes and *Myristica fragrans* seeds extract revealed the presence of a diverse array of secondary metabolites, including alkaloids, tannins, terpenoids, phenolic compounds, flavonoids, saponins, glycosides, carbohydrates, and proteins. This rich phytochemical profile underscores the potential pharmacological significance of ginger and nutmeg, aligning with their traditional use in various medicinal systems. Alkaloids flavonoids and saponins, known for their diverse biological activities, including analgesic, anti-inflammatory, and antimicrobial properties, were detected in the two extracts. The presence of these phytochemicals in the ginger rhizome and nutmeg seeds extracts supports their traditional use in various medicinal applications. The observed phytochemicals may contribute to the reported pharmacological activities of ginger and nutmeg, such as its anti-inflammatory and analgesic properties[18].

The acetic acid-induced mouse writhing test is a widely used method to evaluate peripheral analgesic activity in experimental models [19]. This test involves the administration of an irritant, such as acetic acid, which induces abdominal writhing in mice due to pain mediated by nociceptors and the release of inflammatory mediators like prostaglandins [20]. The fact that the ginger extract proved to have dose-dependent effect by suppressing the writhing phenomenon in this study and surpassing the combined used of nutmeg and ginger, indicates the analgesic potential of this extract. The 300 mg/kg dose showed a significantly higher inhibitory effect (79.59%) compared to the 150 mg/kg dose (66.32%), with results suggestive that the analgesic propert of the extract may be dependent on the amount of original phytochemicals present.

Therefore, the data from the acetic acid-induced writhing test evidently indicated that the aqueous extract of ginger rhizome has analgesic activity against visceral inflammatory pain. It also showed that ginger is better used alone than in combination

with nutmeg in pain management. The dose-dependent inhibition and comparable efficacy to aspirin suggest the presence of bioactive phytochemicals with potential anti-nociceptive and anti-inflammatory properties. The analgesic effect of the combined aqueous extracts of ginger rhizomes and nutmeg seeds increased with increase in dose up to 300 mg/kg, after which there appeared to be no further enhancement and rather a slight decline in efficacy at 400 mg/kg. The study showed that the combination of ginger and nutmeg extracts possesses notable analgesic property but did not confer additional benefits as the use of ginger alone gave the most prominent inhibitory effect on pain. The results obtained were comparable to conventional drugs like aspirin used in this model. These results support their use as natural alternatives for managing pain, especially for individuals seeking plant-based remedies with potentially fewer side effects than synthetic drugs. The mechanism behind the analgesic property of ginger may include modulating inflammatory pathways, blocking the release of inflammatory mediators. It is worth mentioning that the formalin test measures mainly acute pain and how this varies when the extract is used on conditions that cause chronic inflammatory pain is still unknown. Overall, the formalin pain model test outcome showed that the aqueous extract of ginger rhizomes alone exhibited good analgesic activity focused on alleviating both central and inflammatory pain, and it may act through peripheral mechanisms that include anti-inflammatory or antioxidant pathways. Nonetheless, more research should be done to show a clearer association between ZOR extract and acute and chronic neuropathic pain.. The antinociceptive activity of the combined extracts was a non-dose-dependent response; At a dose of 300 mg/kg, the combined extracts exhibited the most pronounced effects both at the neurogenic pain phase where there was a 53.07% reduction in licking/biting behavior compared to controls and at the inflammatory pain phase where there was a 79.51% reduction in licking/biting behavior compared to controls. Pentazocine, an opioid analgesic commonly used as a standard drug for comparison in such studies was administered at a dose of 10 mg/kg and it showed slightly higher inhibitory rates (54.26% at the neurogenic pain phase and 93.20% at the inflammatory pain phase) than the combined aqueous extracts of *Zingiber officinale* rhizome and *Myristica fragrans* seeds. These findings suggest that while pentazocine remains more effective overall, the combined extracts demonstrate comparable efficacy, particularly in reducing inflammatory pain during the late phase. The observed antinociceptive effects can be attributed to several bioactive compounds present in ginger and nutmeg. For instance, ginger contains compounds like gingerols and shogaols that exhibit anti-inflammatory and analgesic properties by inhibiting cyclooxygenase enzymes (COX-1 and COX-2) thereby reducing prostaglandin synthesis involved in inflammation [21], while Nutmeg seeds contains myristicin and eugenol—compounds known for their anti-inflammatory and sedative effects. Nutmeg has also been shown to modulate central nervous system activity by interacting with neurotransmitter systems such as GABAergic pathways [22]. The combination of the aqueous extracts of ginger and nutmeg likely enhanced efficacy due to complementary mechanisms—ginger targeting peripheral inflammation while nutmeg modulates central pain pathways. These findings highlight the potential use of aqueous extracts of *Zingiber officinale* rhizome (ginger) and *Myristica fragrans* seeds (nutmeg) as a natural alternative or adjunctive therapy for managing both acute neurogenic pain and chronic inflammatory pain conditions; however it is advised to use just the ginger rhizomes alone as combination with nutmeg did not offer any better activity.

Finally, the tail immersion test is a widely used method to evaluate the antinociceptive effects of drugs or compounds by measuring the latency of a mouse's response to thermal stimuli [23]. The results revealed that while the combination exhibited antinociceptive activity, its efficacy varied depending on the dose and time point. At 300 mg/kg dose, the pattern of inhibition was distinct from that seen with the lower dose; the highest inhibition (60.24%) occurred at the final measured time point (180 minutes), suggesting that this dose provided stronger and more sustained pain relief over time. This result indicates that increasing the dose enhanced both the magnitude and duration of antinociceptive effects. While the combined extracts have measurable pain-relieving property, they were less potent than pentazocine but ginger alone showed a higher potency than pentazocine under these experimental conditions. These findings suggest potential therapeutic applications for aqueous extracts of *Zingiber officinale* and *Myristica fragrans* as natural analgesics.

CONCLUSION

The data obtained from this research study showed the efficacy of *Zingiber officinale* rhizomes and *Myristica fragrans* seeds aqueous extracts alone and in combination on nociceptive and centrally mediated pain but it is advised to use ginger extract alone as it showed more prominent activity in pain management. The analgesic effect of the combined aqueous extracts of *Zingiber officinale* rhizome and

Myristica fragrans seeds are both centrally and peripherally mediated.

DECLARATION

The work described has not been published and is not under consideration for publication elsewhere

CONFLICT OF INTEREST: Authors declare no conflict of interest.

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