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Investigating antioxidant and antibacterial activity of Thai local herbal extracts for development reducing muscle pain product

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Abstract The results revealed that Turmeric and Ginger extracts had the high antioxidant efficacy by DPPH assay at IC_{50} = 0.57±0.01 and 0.61±0.01 mg/mL, respectively., The ABTS assay showed that Turmeric, Ginger and Plai extract had IC_{50} = 0.22±0.00, 0.23±0.00 and 0.30±0.01 mg/mL, respectively. In addition, turmeric and ginger extracts was determined FRAP value at 105.85±1.99 and 137.94±3.11 mg TE/g sample, respectively. The TPC and TFC of turmeric extract exhibited the highest TPC at 63.07 mg GAE/g sample and TFC at 555.94 mg QE/g sample, respectively. Antibacterial assay was evaluated by agar well diffusion technique against *Staphylococcus aureus.* It was found that the turmeric extract had the highest activity, with a zone of inhibition at 7.33±0.29 mm. These results indicated that five herbal extracts exhibited both antioxidant and antibacterial effects could be used as ingredients for development in reducing pain product.

Keywords: Plant extract, Antioxidant activity, Antibacterial activity

Introduction

Thai herbal medicine comprises more than 200 medicinal plants that are recorded in Thai Pharmacopoeia. Most of these plants are local in the country and are implemented in traditional herbal formulas. *Curcuma longa* L. (Tumeric), *Zingiber officinale* Roscoe (Ginger), *Z. cassumunar* Roxb. (Plai), *Crimum asiaticum* L. (Crinum Lily), and *Morinda citrifolia* L. (Indian Mulberry) are the frequently used herbs. These plants have been proven to possess anti-inflammatory and antioxidant properties and pain-relieving characteristics. They are also utilized in combination herbal formulas such as Triphala and Sahasthara to cure fever, joint pain, and muscle inflammation (Kakatum, 2012;

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Thongphichai *et al*., 2025). These herbs have high proportion of bioactive compounds including phenolic acids, flavonoids, terpenoids, alkaloids, and essential oils in high concentration. They are healing because they alter the levels of inflammation and oxidative stress in the body.

Tumeric rhizomes contain curcuminoids with curcumin, demethoxycurcumin and bisdemethoxycucumin. Curcumin has a strong antioxidant and anti-inflammatory effect. It reduces the response of cells to inflammation and reduces the generation of pro-inflammatory substances (Burapan *et al*., 2020; de Oliveira Filho *et al*., 2021). Ginger rhizomes contain gingerols, shogaols, and paradols, which have anti-inflammatory and immunomodulatory effects. They inhibit the enzymes responsible from inflammation and pain and also reduce oxidative stress. The stress markers respond to ginger which allows the herb to suppress muscle pain and tiredness according to Mao *et al*. (2019) and Ayustaningwarno *et al*. (2024). The rhizomes of plai contain a special kind of phenylbutenoids which include cassumunarins A, B, and C which target vital proteins in inflammation and cell death pathways. The compounds in this herb have been shown the anti-inflammatory and pain-relieving properties and the essential oil of plai has been found to be as effective as diclofenac in reducing topical edema and pain (Han *et al*., 2021; Gundom *et al*., 2025). The anti-inflammatory effects of crinum lily leaves extract are attributed to alkaloids and flavonoids which are present in the leaves. According to scientific research, parthenolide, a commonly known anti-inflammatory drug, is less effective in suppressing inflammation than thermally processed *C. asiaticum* extracts (Kongkwamcharoen *et al*., 2021). The leaves of Idian mulberry have been found to have antioxidant, antibacterial, and anti-inflammatory properties which are useful in protecting tissues against oxidative damage and promote tissue healing (Zhou and Huang, 2024; Hou *et al*., 2025;).

These herbs aren't just anti-inflammatory but also contain powerful antioxidants. These antioxidants reduce oxidative stress that leads to muscle fatigue and tissue damage and slow healing. Strenuous physical activity or injury produces reactive oxygen species (ROS) which damage cells and aggravate inflammation. The antioxidants that occur in herbs can neutralize ROS, maintain cell health and promote recovery of tissues. The extracts of *Boesenbergia rotunda*, *Centella asiatica* and Triphala shown in laboratory experiments to be very high in antioxidant activity as indicated by DPPH, ABTS and FRAP assays (Sihanata *et al*., 2023). Such herbs contain flavonoids, and among them are quercetin, kaempferol, and rutin which eliminate free radicals and inhibit fat damage (Panche *et al*., 2016), and phenolic acids like gallic acid and caffeic acid which are powerful reducers (Shahidi and Ambigaipalan, 2015). Thai herbs also

provide essential oils that are antioxidant, antimicrobial and stabilise topical formulations (Burt, 2004).

Despite the medicinal advantages of the Thai herbal medicine, a lack of experimental studies has been conducted on the antioxidant and anti-inflammatory properties of the local herbs in the treatment of muscle pain. This study examined the antioxidant and antibacterial properties of Thai herbal extracts as their possible anti-inflammatory agents.

Materials and methods

Plant extraction

The herbal plants such as plai (*Zingiber montanum*), tumeric (*Curcuma longa*), ginger (*Z. officinale*), Indian mulberry leaves (*Morinda citrifolia*), and crinum lily leaves (*Crinum asiaticum*) was dried at 45°C in hot air oven until reaching stable moisture content and then ground into powder using a mechanical grinder. The modified extraction protocol, based on Thititanaapipong *et al*. (2025), involved macerating each plant powder with 95% ethanol at a 1:5 (w/v) ratio. The mixtures were incubated at room temperature (28 ± 2°C) for 72 h. Following incubation, the resulting extract was filtered through Whatman No. 1 filter paper. The solvent was then removed by rotary evaporation (Büchi Rotavapor R–205, Germany) at 40°C.The obtained extracts were analyzed for total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity.

Total phenolic contents

The total phenolic contents (TPC) of the extracts were determined using a modified Folin-Ciocalteu colorimetric method based on the procedure described by Shao *et al*. (2014). In brief, 0.1 mL of the appropriately diluted extract was mixed with 1.5 mL of Folin-Ciocalteu reagent, which was diluted 1:10 in distilled water. After 15 min, 1.5 mL of 7.5% sodium carbonate (Na_2CO_3) solution was added. The reaction mixture was kept in the dark at room temperature for 90 min. Absorbance was then measured at 725 nm using a spectrophotometer (Genesys 30, Thermo Scientific, USA). The results were expressed as milligrams of gallic acid equivalents per gram of dry sample (mg GAE/g DW).

Total flavonoid contents

Total flavonoid contents (TFC) were determined by a modified aluminum chloride colorimetric assay as described by Haile and Kang (2019). After that 0.1 mL of diluted extract was combined with 0.2 mL 5% Sodium Nitrite ($NaNO_2$) solution and allowed to stand for 5 min. The next step was the addition of 0.3 mL of 10% Aluminum Chloride ($AlCl_3$) solution and the mixture was left at room temperature for 6 min. Then, 2.5 mL of 1 M sodium hydroxide (NaOH) solution was added, and the reaction mixture was incubated in the dark for 10 min. Absorbance was measured at 510 nm using a spectrophotometer. The results were reported in milligrams of quercetin equivalents per gram of dry sample (mg QE/g DW).

Antioxidant activity

Three tests (DPPH, ABTS, and FRAP) were used to test the antioxidant properties of the sample. Through the DPPH test, the sample was combined with 0.1 mM DPPH solution in methanol, allowed to stand in the dark at room temperature for 30 min, then the absorbance was measured at 517 nm (Chen et al., 2021). In the ABTS test, the $ABTS^{\bullet+}$ radical cation was created using potassium persulfate, the mix was let stand for 12-16 h, diluted to an absorbance of 0.70 ± 0.02 at 734 nm, reacted with the sample for 6 min, then absorbance at 734 nm was measured (Re *et al*., 1999). The FRAP test was performed by mixing the sample with freshly prepared FRAP reagent (consisting of acetate buffer, TPTZ, and $FeCl_3$), incubating at 37 °C for 30 min, and measuring absorbance at 593 nm (Santos *et al*., 2017). DPPH and ABTS radical scavenging assays were reported as IC_{50} values and FRAP assay was reported as Trolox equivalent antioxidant capacity (TEAC).

Determination of curcuminoids, 6-gingerol, genipin, and lycorine using high performance liquid chromatography (HPLC)

The optimized parameters which were used as a final method for the estimation of curcuminoids, 6-gingerol, genipin, and lycorine, as shown in Table 1.

Table 1. Chromatographic condition for curcuminoids, 6-gingerol, genipin and lycorine analysis

Condition	Curcuminoids	6-gingerol	Genipin	Lycorine
Solvent A	DI water	DI water	DI water	1% acetic acid in DI
Solvent B	Acetonitrile	Acetonitrile	Acetonitrile	Acetonitrile
Ratio	50:50	25:75	75:25	80:20
Stationary phase	Zorbax SB-C18 (5 µm particle size, 4.6 x 150 mm internal diameter)			
Wavelength	425 nm	280 nm	240 nm	280 nm
Run time	10 min	10 min	10 min	10 min
Flow rate	1.0 mL/min	1.0 mL/min	1.0 mL/min	1.0 mL/min
Injection volume	10 µL	10 µL	10 µL	10 µL
Temperature	30°C	30°C	30°C	40°C
Mode of operation	Gradient elution	Gradient elution	Gradient elution	Gradient elution
References	Jayaprakasha *et al*. (2002)	Ali *et al*. (2008)	Chen *et al*. (2008)	Emir *et al*. (2017)

Antimicrobial activity

Antibacterial activities of herbal plant extract were evaluated a against of *Staphylococcus aureus.* The bacterium was initially cultured on tryptic soy broth (TSB) and incubated with shaking (100 rpm) at 37°C for 24 h.

Agar well diffusion assay

Agar well diffusion experiment was performed on tryptic soy agar (TSA) plates using a modified protocol of Wattanuruk and Detraksa (2023) to test the antibacterial effect of herbal extracts. The isolate suspensions prepared to 10^6 CFU/mL were evenly distributed on the surface of the agar. Sterile wells (~6 mm) were created and 50 µL (0.05 g/mL) of each extract at specific concentration was dispensed into separate wells, together with positive control (amoxycillin) and solvent control. Plates were incubated at 37 °C for 24 h and inhibition zones were measured in millimeters (mm) with a calliper. The intensity of the inhibition was determined as weak (<5 mm), moderate (5-10 mm), strong (10-20 mm) and very strong (>20 mm) (Dewi and Mardhiyani, 2021).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The herbal extract was determined to have MIC and MBC through the broth microdilution technique, after minor modifications to the method described by Jung *et al*. (2022). Two-fold serial dilutions of each extract were prepared in TSB, and each well received bacterial suspensions adjusted to the population of bacteria was 10^6 CFU/mL. After 18 h of incubation at 37 °C, the MIC was

determined to be the minimum concentration of extract that completely inhibited visible bacterial growth. To determine the MBC, aliquots (10 uL) from the wells at the MIC and higher concentrations were transferred onto TSA plates without extract, followed by incubation for 24 h, and the minimum concentration that gave ≥ 99.9% reduction in bacterial colonies was taken as the MBC. Amoxycillin was used as positive controls and the wells containing only solvent were used as negative controls to test the assay.

Statistical analysis

All experiments were conducted in triplicate. The results were given as mean±standard deviation (SD). A completely randomized design (CRD) was used in the statistical analysis. The data were analyzed with IBM SPSS Statistics® and the differences between mean values of treatments were tested using Duncan's New Multiple Range Test (DMRT). Values were considered significant at $P < 0.05$.

Results

The study used five local Thai herbal powders, i.e., Plai (*Z. cassumunar*), Turmeric (*C. longa*), Ginger (*Z. officinale*), Indian Mulberry leaves (*M. citrifolia*), and Crinum Lily leaves (*C. asiaticum*). The researchers visually examined these powders as shown in Figure 1. Each of the powders exhibited distinctive colors and textures. Plai had a bright yellow color, Tumeric had orange-red coloring, Ginger and Crinum Lily leaves were light brown in color, and Indian Mulberry leaves powders had greenish-blue color.

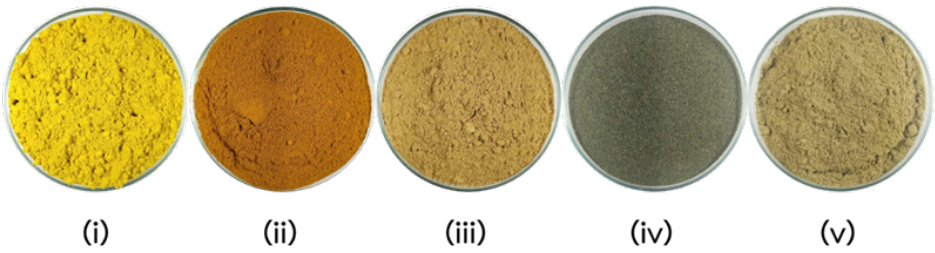

Figure 1. Herbal plant powder (i) Plai, (ii) Tumeric, (iii) Ginger, (iv) Indian Mulberry leaves, and (v) Crinum Lily leaves

TPC, TFC, and antioxidant activities of herbal plants extract

Five Thai herbal extracts were determined, and this includedas Plai, Turmaric, Ginger, Indian Mulberry leaves, and Crinum Lily leaves. Turmeric showed the one with the greater TPC content, then followed by Ginger, Plai, Indian Mulberry leaves, and Crinum Lily leaves. Turmeric was found to have the maximum TFC content while Indian Mulberry leaves were at the second position followed by Ginger, Plai and Crinum Lily leaves. Turmeric tested better with the DPPH test, closely followed by Ginger, with values of 0.57±0.01 and 0.61±0.01 mg/mL. Plai is moderately active (1.14±0.03 mg/mL) whereas Indian Mulberry leaves and Crinum Lily leaves are very lowly effective (2.59±0.15 and 3.58±0.36 mg/mL), respectively. Turmeric, Ginger, and Plai were highly active in the ABTS test (0.22±0.00, 0.23±0.00, and 0.30±0.00 mg/mL, respectively), and Plai had an active level of 0.31±0.01 mg/mL. The leaves of the Indian Mulberry and Crinum Lily obtained reduced ABTS radical scavenging efficacy at 2.94±0.11 mg/mL and 3.13±0.12 mg/mL, respectively. The Ginger samples have the largest antioxidant content at 137.94±3.11 mg TE/g sample, followed by Turmeric at 105.85±1.99 mg TE/g sample and Plai at 71.38±0.83 mg TE/g sample according to the FRAP assay results. The leaves of Indian Mulberry and Crinum Lily exhibit reduced FRAP values of 17.23±0.13 and 13.65±0.76 mg TE/g sample.

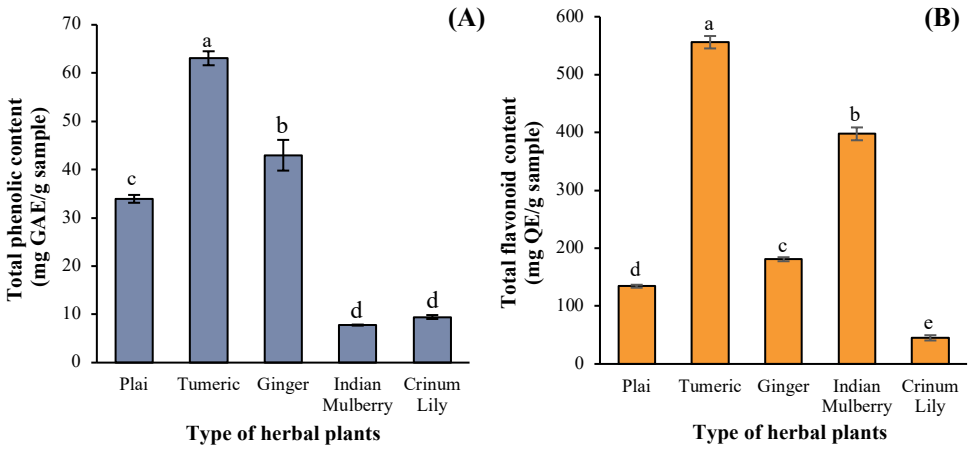

Figure 2. Effect of different herbal plant extract on the TPC and TFC

Table 2. Antioxidant capacities of herbal plants extract

Crude extracts	DPPH assay IC_{50} (mg/mL)	ABTS assay IC_{50} (mg/mL)	FRAP assay (mg TE/g sample)
Trolox	0.17±0.00	0.11±0.00	-
Plai	1.14±0.03 b	0.30±0.01 a	71.38±0.83 c
Tumeric	0.57±0.01 a	0.22±0.00 a	105.85±1.99 b
Ginger	0.61±0.01 a	0.23±0.00 a	137.94±3.11 a
Indian Mulberry leaves	2.59±0.15 c	2.94±0.11 b	17.23±0.13 d
Crinum Lily leaves	3.58±0.08 d	3.13±0.12 c	13.65±0.76 e

Determination of active ingredients of each herbal plant by HPLC

The quantitative analysis of active ingredients in five Thai herbal extracts revealed unique phytochemical profiles based on their medicinal value as demonstrated in Table 3. The extract of Turmeric contains curcuminoids at the highest levels with bismethoxycurcumin at 210.65±0.36 mg/g and methoxycurcumin at 154.35±0.24 mg/g and curcumin at 201.97±0.38 mg/g. Plai extract was also found to have curcuminoids though at reduced amounts with bismethoxycurcumin (1.70±0.01 mg/g) and methoxycurcumin (10.89±0.03 mg/g) and curcumin (77.86±0.53 mg/g). Ginger extract contains a high level of 6-gingerol (315.14±4.22 mg/g) which is a pain relieving and anti-inflammatory substance. The leaves of Indian Mulberry were found to have genipin at 5.06±0.31 mg/g and Crinum Lily were found to have lycorine at 53.25±4.97 mg/g, which is an alkaloid.

Table 3. Active ingredient of herbal plants extract

	Active ingredients (mg/g sample)				
	Plai	Tumeric	Ginger	Indian Mulberry	Crinum Lily
Bismethoxycurcumin	1.70±0.01	210.65±0.36	-	-	-
Methoxycurcumin	10.89±0.03	154.35±0.24	-	-	-
Curcumin	77.86±0.53	201.97±0.38	-	-	-
6-Gingerol	-	-	315.14±4.22	-	-
Genipin	-	-	-	5.06±0.31	-
Lycorine	-	-	-	-	53.25±4.97

Determination of herbal plant extract on against S. aureus using agar well technique

The sensitivity test in *S. aureus* was conducted using the agar well diffusion method. The most effective antibiotic property was produced by amoxicillin, which is the reference substance, with a concentration of 0.05 g/mL and a large inhibition zone of 34.00±0.50 mm. The greatest zone of inhibition produced by the herbal extracts was that of Turmeric (7.33±0.29 mm), followed by Ginger, (5.33±0.58 mm), and Plai (1.83±0.29 mm). Indian Mulberry leaves and Crinum Lily leaves did not possess such zones of inhibition in the test conditions (Table 4).

Table 4. Efficiency evaluation of herbal plants extracts in inhibiting growth of *S. aureus* using agar well diffusion technique

Crude extracts	Inhibition zone (mm)
Amoxycillin	34.00±0.50
5% DMSO	ND
Plai extract	1.83±0.29 c
Tumeric extract	7.33±0.29 a
Ginger extract	5.33±0.58 b
Indian Mulberry leaves extract	ND
Crinum Lily leaves extract	ND

ND = Not detected

Determination of MIC and MBC of herbal plant extract on against S. aureus

We looked at the MIC and MBC of five different Thai herbal extracts. Turmeric demonstrated the highest antibacterial effect against *S. aureus* since both MIC and MBC values were about 0.16 g/mL. Plai, Ginger, and Indian Mulberry leaves had the same MIC of 1.28 g/mL, but the MBC was higher, at 2.56 g/mL. There was the least antibacterial effect of Crinum Lily leaves, having MIC and MBC values of 2.56 and 5.12 g/mL, respectively. The research findings show that Turmeric and Ginger extract demonstrated strong antibacterial effect because they stop and kill *S. aureus* (Figure 3).

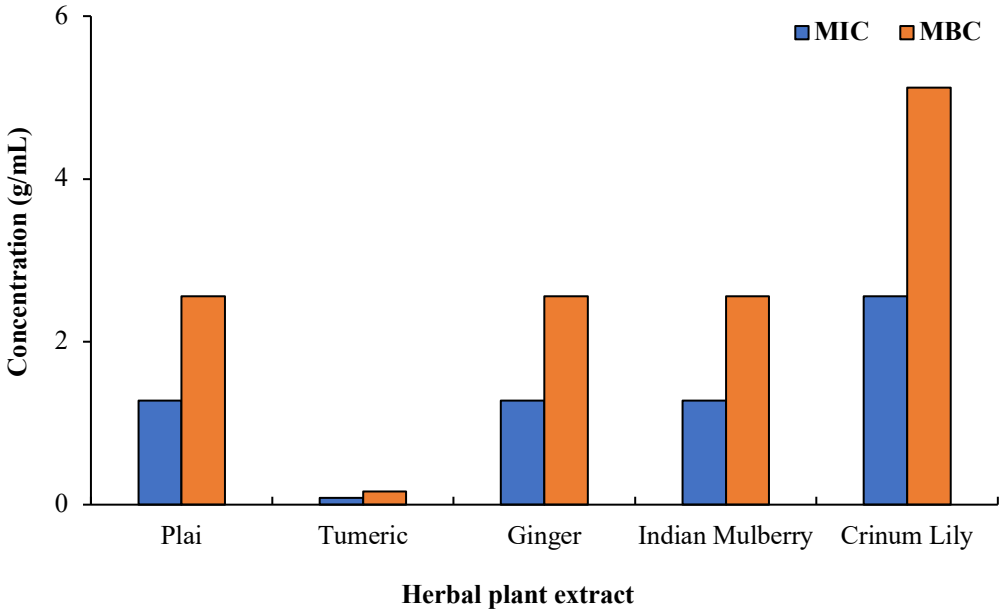

Figure 3. Determination of MIC and MBC of herbal plant extract on against *S. aureus*

Discussion

The results of the study showed that the Thai extracts of herbs, especially Turmeric (*C. longa*) and Ginger (*Z. officinale*), had strong antioxidant and antibacterial effects, which may be useful in muscle pain-relieving products. Our results are consistent with the previous studies that indicate that curcuminoids and gingerols are powerful bioactive ingredients that have anti-inflammatory effects and can scavenge free radicals (Burapan *et al*., 2020; Mao *et al*., 2019). The DPPH, ABTS, and FRAP tests results showed that Ginger had the highest FRAP value while Turmeric had the lowest IC50 values in DPPH and ABTS tests. The results are similar to those of Sihanat *et al*. (2023), found that Turmeric and Ginger extracts had high antioxidant activity because of their high phenolic and flavonoid content. Turmeric had the most significant TPC and TFC content among all the herbs studied. According to the report of Shahidi and Ambigaipalan (2015), who discovered that phenolic acids and flavonoids contribute to the antioxidant protection of the body. The extracts of Indian mulberry (*M. citrifolia*) and Crinum Lily (*C. asiaticum*) were found to have weak antioxidant and antibacterial activities. The findings of this study contradict the previous studies that reported that the leaves of *M. citrifolia* possess considerable

biological activity (Zhou and Huang, 2024). The variation in the content of phytochemicals and their effectiveness may be attributed to the extraction method, the part of the plant used, or the environmental conditions.

The antibacterial tests performed against *S. aureus* established that Turmeric has the largest inhibitory zone and the next two are Ginger and Plai (*Z. montanum*). Also the report of Wattanuruk and Detraksa (2023) observed that Turmeric extract had a high degree of inhibition of the pathogenic bacteria in aquatic organisms. The effect of Turmeric in killing bacteria was stronger, which was further supported by the MIC and MBC values, as was shown by Jung *et al*. (2022) who demonstrated that *C. longa* has a low MIC value against methicillin-resistant *S. aureus*.

It was also interesting, as has been shown by Han *et al*. (2021), that Plai exhibited a moderate antioxidant and antibacterial activity, yet the content of curcuminoids in it was much lower than that of Turmeric, which suggests that other compounds, such as cassumunarins, may be responsible for its bioactivity. Crinum Lily had the least antibacterial property even though it had lycorine content. The reason why it had weak antibacterial activity is because of low extract concentration or poor solubility of the active compounds.

In conclusion, the results of this study demonstrate that Turmeric and Ginger can be used as two powerful natural ingredients to make new products that will be used to relieve muscle pain because of their antioxidant and antibacterial activities. The future studies should examine the combined action of extracts, better extraction methods and test on animals their effectiveness and safety. Furthermore, the uniformity of active ingredients and the stability of the formulation should be checked to make it more effective in the treatment and commercially viable.

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Conflicts of interest

The authors declare no conflict of interest.

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