



Research article

Extraction of active compounds from traditional herbal compress ingredients for hydrogel pain relief patch development

Waleepan Rakitikul^{a,*}, Jinda Sirita^a, Suthada Khattiya^b, Anyanee Katepan^b, Sasiwimon Puphoung^b, Pintip Kammas^c, Rachchanon Yamsri^b

^a Faculty of Science and Technology, Chiang Rai Rajabhat University, Chiang Rai 57100, Thailand

^b Faculty of Management, Chiang Rai Rajabhat University, Chiang Rai 57100, Thailand

^c Graduate School, Chiang Rai Rajabhat University, Chiang Rai 57100, Thailand

Article Info

Article history:

Received 11 November 2025

Revised 19 June 2026

Accepted 8 July 2026

Available online 30 September 2026

Keywords:

Gel patches,

Herbal compress extracted,

Hydrogel,

Pain relief patches,

Pectin,

Abstract

Importance of the work: The modification of traditional herbal compresses into easy-to-use patches provides a more convenient alternative while delivering equivalent benefits to other commercial products.

Objectives: To extract active compounds for herbal compresses for the development of pain relief patches.

Materials and Methods: Samples of the Thai herb 'plai' (*Zingiber cassumunar* Roxb.), turmeric (*Curcuma longa* L.) and *Centella asiatica* (L.) Urb., were extracted using steam distillation, water extraction or frying with coconut oil. Then, the active compounds were analyzed using gas chromatography-mass spectrometry (GC-MS) and Fourier-transform infrared spectroscopy (FTIR) to identify their components. Subsequently, these extracts were incorporated into pain relief patches formulated with a pectin and sodium alginate base.

Results: The extracts from the three plants were analyzed using GC-MS, identifying γ -terpinene at 11 min, (E)-1-(3,4-dimethoxyphen) butadiene at 29 min and levomenthol at 18 min as pain-relieving compounds. The FTIR analysis of the extracts and pain relief patches detected functional groups, consisting of C=C bending, C-O stretching, N-O stretching, C=O stretching, C-H stretching and O-H stretching. An irritation test using the 4 hr closed patch method at 0 hr, 4 hr, 24 hr, 48 hr and 72 hr resulted in no significant ($p > 0.05$) skin irritation.

Main finding: The extracts were incorporated effectively into a pectin-sodium alginate hydrogel matrix, forming structurally stable patches, as confirmed by scanning electron microscopy-energy-dispersive X-ray spectroscopy and FTIR analyses. Skin irritation testing demonstrated excellent biocompatibility, while volunteer-based evaluation showed significant pain reduction after application. Based on these results, the developed hydrogel patch represented a practical and user-friendly alternative to conventional herbal compresses, bridging traditional medicine with modern transdermal delivery systems.

* Corresponding author.

E-mail address: em_waleepan_r@ccru.ac.th (W. Rakitikul)

Introduction

The increasing prevalence of chronic pain due to changing modern lifestyles has led to rising healthcare costs (Gaskin and Richard, 2012). Traditional Thai herbal medicines, rooted in local wisdom, with the use of natural products, including herbs, are gaining popularity due to their cost-effectiveness and safety compared to synthetic chemicals (Ernst, 2003). The Tasud Herbal Enterprise Group has collaborated with Chiang Rai Rajabhat University, Thailand to develop herbal-based products aimed at improving community quality of life and economic sustainability, as part of initiatives to promote Smart Local CEOs and enhance competitiveness through innovative products, including herbal patches, by 2024 (Phichaphibul et al., 2022). In recent years, various modifications of traditional herbal compresses have been explored, such as prepackaged semi-finished compresses and modernized forms that are more user-friendly and aesthetically appealing (Phichaphibul et al., 2022). In addition, research has focused on developing herbal hydrogel patches for targeted applications, such as pain relief for knees (Promsungwong et al., 2020; Thongthamachat et al., 2021) and reducing inflammation and stress (Srikun, 2018; Waehayi et al., 2023). These studies have highlighted the potential of herbal extracts, including *Zingiber cassumunar* Roxb. known as ‘plai’ in Thai, turmeric (*Curcuma longa* L.) and *Centella asiatica* (L.) Urb., in delivering therapeutic benefits when incorporated into novel delivery systems such as hydrogel patches. Typically, extraction methods for key herbs in traditional compresses, such as plai, kaffir lime (*Citrus hystrix* DC) and *Ce. asiatica*, involve hydrodistillation, steam distillation or alcohol extraction, followed by gas chromatography-mass spectrometry (GC-MS) analysis to identify active compounds. For example, sabinene is the major compound in plai (Mektrirat et al., 2020), curcumin in turmeric (Ching et al., 2014) and β -pinene and D-limonene in kaffir lime extracted with water and ethanol (Le et al., 2020). *Ce. asiatica* contains high levels of compounds such as 2-dodecen-1-yl (-) succinic anhydride (15.29%) and benzenemethanol derivatives (Rahamouz Haghighi et al., 2022). Furthermore, herbal hydrogel patches have shown promise in clinical applications. For example, Waehayi et al. (2023) developed a patch containing *Ce. asiatica*, *Andrographis paniculata*, lavender essential oil and gelatin that substantially reduced stress in 35 student participants. Similarly, Srikun (2018) formulated transdermal patches using dill extracts, which effectively reduced inflammation without adverse reactions. Additionally, Vildanova et al. (2023) demonstrated the effectiveness of biodegradable hydrogels made from charge-complementary polyelectrolyte chains, using chitosan and pectin, for drug delivery. The present study aimed to extract active compounds from traditional herbal compress ingredients (plai, turmeric, *Ce. asiatica*) and subsequently to incorporate them into hydrogel pain relief patches to enhance usability, therapeutic efficiency and economic value.

Material and Methods

Preparation of herbal materials

The herbal ingredients (turmeric, plai, *Ce. asiatica* and kaffir lime) were prepared using air-drying or oven-drying and were then ground into fine powders prior to extraction. All plant materials were sourced from the Tasud Herbal Enterprise Group, Thailand and identified by a qualified botanist before use.

Extraction of active compounds

Three extraction methods were used according to the physicochemical properties of the target compounds in each plant material. The turmeric powder was extracted using distilled water at a powder-to-solvent ratio of 1:3 (weight per volume, w/v) to yield the aqueous extract. Dried rhizome powder of plai was subjected to steam distillation using a Clevenger-type apparatus for 4 hr and the essential oil layer was collected and dried over anhydrous sodium sulfate. *Ce. asiatica* was fried in coconut oil to extract the lipophilic active compounds. In addition, kaffir lime essential oil was obtained using steam distillation of the dried peel and incorporated into the hydrogel formulation at a concentration of 0.50% (volume per volume). Subsequently, the extracted solvents from the water-based and steam distillation processes were concentrated using rotary evaporation at 50°C under reduced pressure and all concentrated extracts were stored in amber glass bottles at 4°C for later analysis.

Gas chromatography-mass spectrometry and Fourier-transform infrared spectroscopy analyses

To identify the active compounds, the extracts were dissolved in dichloromethane and analyzed using GC-MS with a PerkinElmer Clarus 690 GC system (PerkinElmer Inc.; Waltham, MA, USA) coupled with an SQ8 mass-selective detector. The GC-MS analysis utilized a PerkinElmer Elite-5MS column (PerkinElmer Inc.; Waltham, MA, USA), with a length of 30 m, a 250 μ m internal diameter and 0.25 μ m film thickness, an injector temperature of 250°C and helium as the carrier gas at 137895 Pa. The oven temperature was programmed from an initial 60°C, increasing at 5°C/min to a final temperature of 250°C. Mass spectra were collected in the range 60–600 m/z using electron ionization at 70 eV.

Functional group analysis of the herbal extracts and the formulated hydrogel patches was performed using Fourier-transform infrared spectroscopy (FTIR) with a Bruker Vertex 80v spectrometer (Bruker Corporation; Ettlingen, Germany) under dry nitrogen conditions. Spectra were recorded over the range 500–4000 cm^{-1} to identify characteristic absorption bands corresponding to the key functional groups present in the samples.

Hydrogel formulation

Two hydrogel formulations were prepared for comparison. The first formulation (Gel 5) contained pectin, sodium alginate, saline solution (1 M sodium chloride, NaCl) and the herbal extracts. The second formulation (Gel 5-1) additionally incorporated chitosan alongside pectin, sodium alginate, saline solution and the same amounts of the herbal extracts.

In both formulations, the dry ingredients were combined and mixed thoroughly to form Mixture A. Separately, NaCl was dissolved in distilled water to prepare a 1 M saline solution. Mixture A was

added gradually to the saline solution with constant stirring for approximately 2 hr until a clear, homogeneous solution (Mixture B) was obtained. Then, the herbal extract oils — turmeric (10%, v/v), plai (5%, v/v), *Centella asiatica* (3%, v/v) and kaffir lime (0.50%, v/v) — were added to Mixture B and stirred until uniformly dispersed. Mixture B was poured into Petri dishes and dried either at 50°C for 8 hr or air-dried at ambient temperature to form hydrogel sheets. The oven-dried (50°C) formulation was used for all subsequent characterization and testing (FTIR, SEM-EDS, skin irritation and pain relief evaluation) reported in this study, while the air-drying option was included as a practical, low-cost alternative to allow community enterprise partners without access to a drying oven to reproduce the formulation. Both formulations were characterized using FTIR to confirm the successful incorporation of the herbal extracts. However, the addition of chitosan in Gel 5-1 resulted in a harder, less pliable gel compared with Gel 5, which was softer and adhered to the skin more effectively; Gel 5 was therefore selected for all subsequent characterization and testing, including SEM-EDS, skin irritation and pain relief evaluation.

Microstructural characterization (scanning electron microscopy-energy-dispersive X-ray spectroscopy)

The surface morphology and elemental composition of the sodium alginate-pectin composite films incorporated with NaCl were examined using a scanning electron microscope (SEM; JEOL JSM-IT500; JEOL Ltd; Japan) equipped with an energy-dispersive X-ray spectroscopy (EDS) system (Oxford Instruments; UK). The film specimens were cut into pieces of approximately 5 mm × 5 mm and mounted on aluminum stubs using double-sided conductive carbon tape. To minimize charging effects during observation, the samples were sputter-coated with a thin conductive layer of gold (approximately 10 nm) using an SC7620 sputter coater (Quorum Technologies Ltd; UK).

The SEM images were recorded under high-vacuum mode at an accelerating voltage of 15 kV and a working distance of approximately 10 mm, using a secondary electron detector. Images were captured at magnifications of 250× and 30,000× to observe both macroscopic and microstructural features of the films. Surface topography and morphological uniformity were examined to identify the distribution of polymer domains and crystalline NaCl particles.

The EDS analysis was conducted under the same instrumental conditions as the SEM imaging. Multiple regions of each sample were analyzed to ensure representative and reproducible results. The elemental spectra were collected and quantified using the Aztec software (Oxford Instruments; UK), applying ZAF correction (atomic number, absorption and fluorescence) for semi-quantitative evaluation. Elemental mapping was performed to visualize the spatial distribution of carbon (C), oxygen (O), sodium (Na) and chlorine (Cl) across the film surface. The co-localization of Na and Cl signals confirmed the presence of sodium chloride microcrystals embedded in or adhered to the film matrix. Results were reported in both weight percent (wt%) and atomic percent (at%) units.

Skin irritation test

Skin irritation of the Gel 5 hydrogel patch was assessed using the standardized 4 hr closed patch test. Sodium lauryl sulfate solution (SLS, 20% w/v) served as the positive control, while untreated skin was used as the negative control. The erythema index (EI), a quantitative measure of skin redness (Saknite et al., 2016) was recorded using a Mexameter probe at post-application time points of 0 hr, 4 hr, 24 hr, 48 hr and 72 hr. The relative percentage change in EI was calculated using Equation 1:

$$\text{Relative difference} = [(\text{Value after} - \text{Value before}) / \text{Value before}] \times 100 \quad (1)$$

Statistical differences in the EI among the treatment groups at each time point were evaluated using one-way analysis of variance, followed by Tukey's *post hoc* test to determine pairwise differences among groups. Statistical significance was set at $p < 0.05$. All analyses were conducted using the IBM (2019) software.

Pain relief evaluation

The pain relief efficacy and user satisfaction of the Gel 5 hydrogel patch were evaluated in a volunteer study. In total, 10 volunteers aged 24–60 yr were recruited, who had reported musculoskeletal pain in areas such as the legs, calves, neck or shoulders. Hydrogel patches (2.5 cm × 2.5 cm) were applied directly to the pain sites and left in place for 30–60 min. Pain intensity was assessed before and after patch application using a numerical rating scale (NRS), ranging from 0 (no pain) to 10 (worst imaginable pain). In addition, overall volunteer satisfaction with the hydrogel patches, including ease of use and perceived effectiveness, was evaluated through a structured questionnaire. Statistical analysis of pain scores was performed using a paired t test to compare NRS scores before and after patch application, with significance tested at $p < 0.05$. All analyses were conducted using the IBM (2019) software.

Ethics statements

This study was approved by the Ethics Committee of Chiang Mai Rajabhat University (Approval no. IRBCMURU 2024/323.10.07).

Results and discussion

Identification of active compounds

The GC-MS analysis of the herbal extracts revealed the presence of key volatile organic compounds known for their anti-inflammatory and analgesic properties. Chromatograms A–E (Fig. 1) identified the separation and identification of several major bioactive compounds, with chromatogram A showing kaffir lime essential oil (extracted from *C. hystrix*, using steam distillation): Major compounds identified using GC-MS were β-pinene (retention time approximately 10.8 min) and D-limonene (approximately 11.5 min), consistent with another reported profile of kaffir lime essential oil (Le et al., 2020). Chromatogram B showed kaffir lime aqueous extract (water-soluble fraction), where the dominant peak at 18.16 min was confirmed based on NIST library matching as levomenthol, with a secondary peak at

17.20 min corresponding to (+)-2-bornanone (camphor). The plai extract (chromatogram C) contained notable peaks corresponding to terpinen-4-ol, γ -terpinene and sabinene, which contribute to its analgesic effects (Hart et al., 2000). The *Ce. asiatica* oil extract (chromatogram D) showed major peaks of fatty acid derivatives and sesquiterpenes, particularly those known for reducing muscle tension and pain (Saha et al., 2013). The turmeric extract (chromatogram E) had the strong presence of curcuminoids, including curcumin and its derivatives, which are widely recognized for their anti-inflammatory activities (Williamson and Hoggart, 2005). Thus, the extraction methods used (steam distillation for plai, water extraction for turmeric, oil frying for *Ce. asiatica* oil extract) proved effective in isolating the key active components. The concentration process using rotary evaporation ensured that the active compounds were preserved for further integration into the hydrogel patch. These findings were consistent with the expected profiles from the literature (Burton et al., 2009; Higashi et al., 2010), confirming the efficacy of the extraction techniques. Notably, the GC-MS analysis in the present study was conducted primarily for compound identification rather than quantification. Therefore, precise extraction yield data and quantitative concentration comparisons across the three extraction methods (water extraction, steam distillation, oil frying) were not determined in the present work. This represents a limitation of the study and future investigations should include gravimetric yield measurements and comparative analysis of active compounds across different extraction techniques to optimize further the formulations and to improve reproducibility.

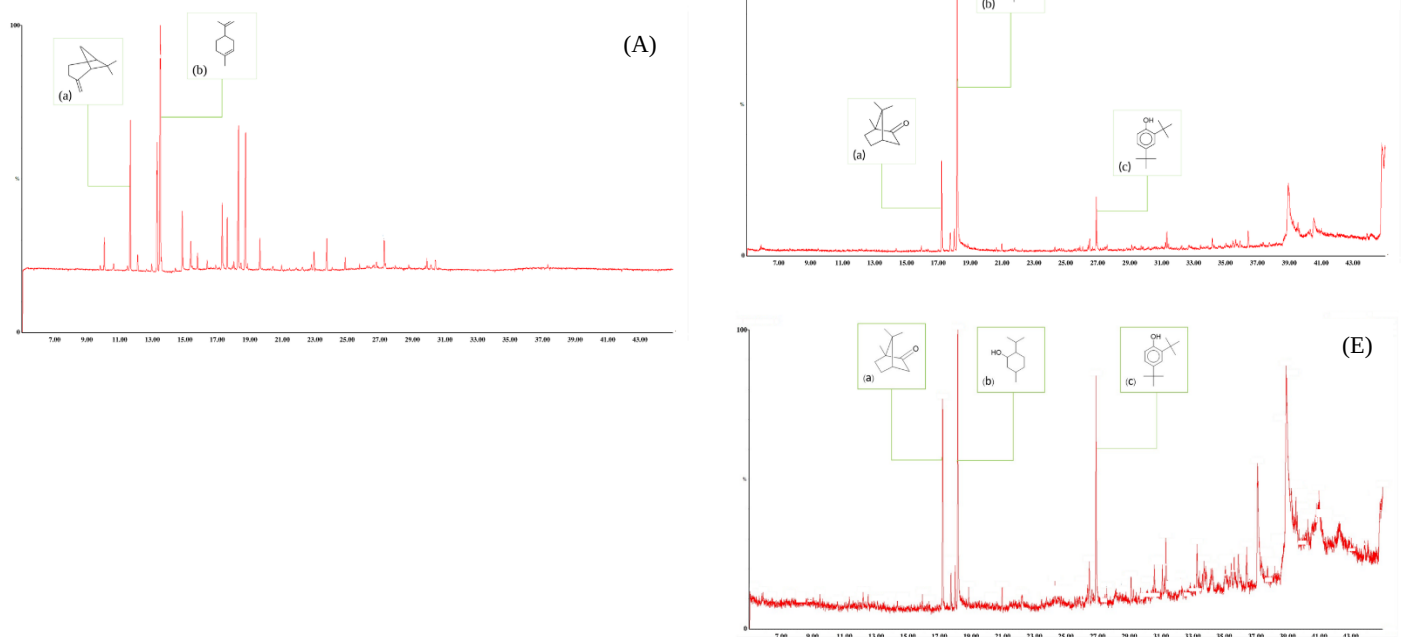


Fig. 1 Chromatograms of the individual herbal extracts used in this study: (A) kaffir lime oil; (B) water-soluble kaffir lime; (C) plai oil; (D) *Centella asiatica* oil; and (E) turmeric oil.

Hydrogel formulation and analysis

The Gel 5 hydrogel formulation effectively incorporated the herbal extracts, while maintaining the desired physical properties (Fig. 2) and chemical properties. The FTIR analysis revealed characteristic functional groups corresponding to polysaccharides (pectin and sodium alginate) and herbal compounds, which confirmed the successful integration. The well-organized polymeric network of the hydrogel matrix ensured sustained release of active ingredients, thereby enhancing its therapeutic potential. To further validate the structural integrity and uniform distribution of the incorporated bioactive compounds, surface and compositional analyses were performed based on SEM-EDS. Additionally, a skin toxicity assay was conducted to evaluate the hydrogel's biocompatibility and safety for dermal application.

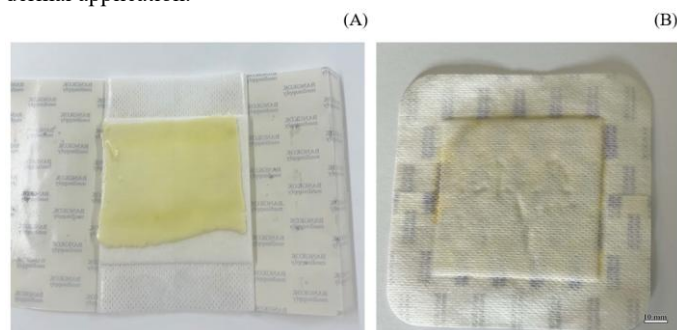


Fig. 2 Gel 5 patch composed of pectin and all extracted components: (A) general appearance; and (B) the same patch photographed alongside a calibrated ruler for scale reference. Scale bar (B) = 10 mm

Microstructural characterization and elemental mapping of composite films

At lower magnification (250 $\times$), as shown in Fig. 3A, the film surface appeared continuous and homogeneous with slight undulations, indicating good miscibility between the alginate and pectin polymers. However, there were small crystalline particles embedded within the polymer matrix, which were attributed to NaCl crystallites formed during solvent evaporation. These salt domains were distributed irregularly throughout the surface, suggesting partial migration of NaCl toward the upper film layer during the drying process.

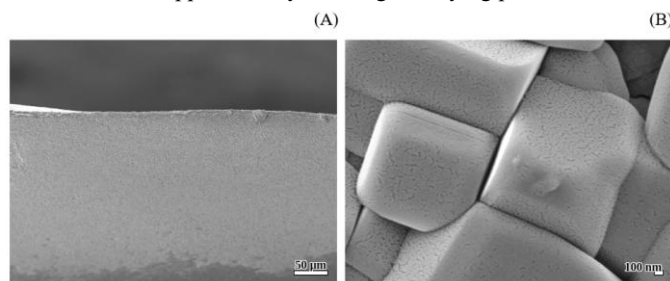


Fig. 3 Scanning electron micrographs of Gel 5 sodium alginate-pectin composite films containing NaCl showing: (A) cross-sectional morphology with micro-roughness related to NaCl crystallization and polymeric phase interaction at 250 $\times$ magnification (scale bar = 50 μ m); and (B) uniform

polymeric surface with visible salt particles at 30,000 $\times$ magnification (scale bar = 100 nm)

At higher magnification (30,000 $\times$), as shown in Fig 3B, the microstructure revealed a dense polymeric network with distinct granular features. The surface had micro-roughness and fine cavities, which could be related to the partial phase separation between alginate and pectin chains due to differences in their gelling and ionic crosslinking behaviors. Similar surface features have been reported in alginate-pectin and alginate-starch films incorporating inorganic salts or polyol plasticizers, where ionic interactions between the Na⁺ ions and carboxylate groups (-COO⁻) modified the local film morphology and mechanical flexibility (Galus and Lenart, 2013; Li et al., 2020).

The occurrence of NaCl microcrystals was confirmed by the EDS analysis (Fig. 4), showing high Na and Cl signals correlated in the same regions of the surface. These observations supported the conclusion that the residual NaCl particles were retained within or on the surface of the film matrix, contributing to the heterogeneity and potential alteration of moisture permeability and tensile strength.

The elemental mapping (Fig. 4) demonstrated clearly high and co-localized intensities of the Na and Cl signals, indicating the accumulation or crystallization of NaCl on the film surface after the drying process. This observation was consistent with another SEM-EDS study on polymeric films containing added salts, where Na/Cl co-localization corresponded to salt crystal domains on the matrix surface (Wilson, 2012).

The EDS analysis of the sodium alginate-pectin composite film incorporated with NaCl and the herbal extracts (turmeric, plai, *Centella asiatica* and kaffir lime essential oils) revealed the overall elemental composition, as shown in the EDS Map Sum spectrum (Fig. 5)

The relatively high carbon content (Table 1) reflected the polymeric backbone of the alginate-pectin matrix and the contribution of the carbon-rich essential oil constituents. However, notably, EDS has inherent limitations in quantifying light elements, such as C and O, due to their low X-ray generation efficiency and absorption effects.

Table 1 Quantitative results of elemental distribution and average weight percentages

Element	Signal Type	Line	Wt%	Wt% Sigma	Atomic %	Standard Name
C	EDS	K series	~16.5	0.39	~33.2	SiC
O	EDS	K series	~6.1	0.24	~8.9	SiO ₂
Na	EDS	K series	~18.0	0.30	~18.3	Albite
Cl	EDS	K series	~32.8	0.51	~21.7	NaCl
Au	EDS	M series	~26.6	0.45	~17.9	Au foil
Total			100		100	

Wt% = weight percentage; ~ = approximately; EDS = energy-dispersive X-ray spectroscopy Au = gold as added sputter coat.

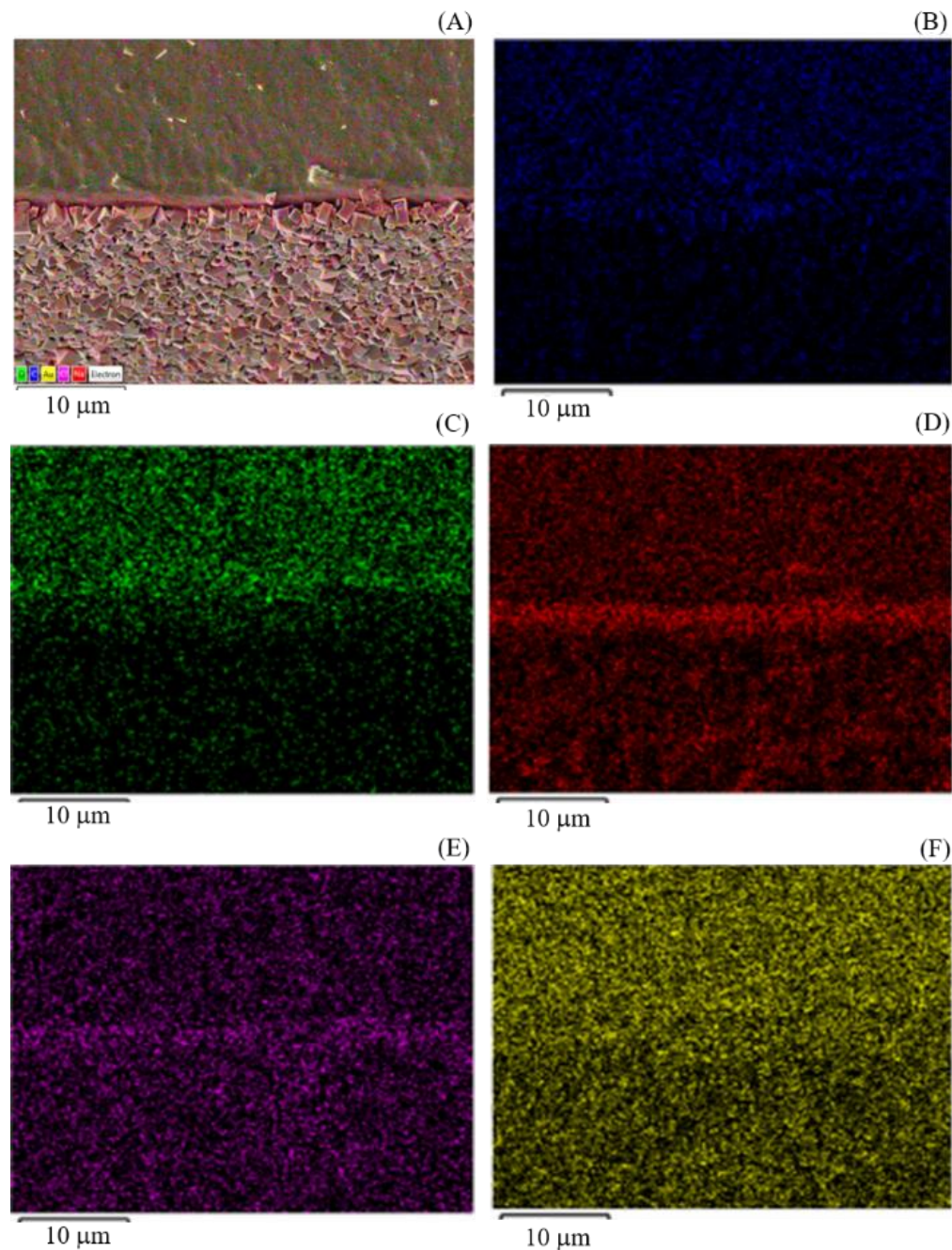


Fig. 4 Energy-dispersive X-ray spectroscopy (EDS) layered mapping of sodium alginate-pectin hydrogel film containing sodium chloride: (A) scanning electron microscopy-EDS layered image showing cross-sectional morphology and elemental overlay; (B) elemental distribution map of carbon (C K α 1,2); (C) elemental distribution map of oxygen (O K α 1); (D) elemental distribution map of sodium (Na K α 1,2); (E) elemental distribution map of chlorine (Cl K α 1); and (F) elemental distribution map of gold (Au M α 1), showing overall uniform dispersion of Na and Cl, corresponding to sodium chloride within the film matrix. Scale bar = 10 μ m

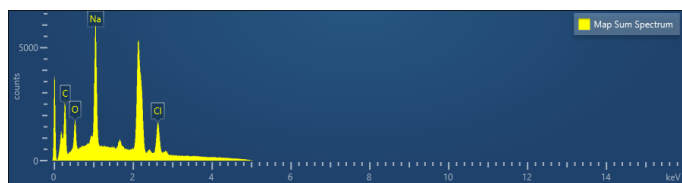


Fig. 5 Energy-dispersive X-ray spectroscopy (EDS) spectrum and elemental composition of sodium alginate-pectin hydrogel film containing NaCl, showing distinct peaks corresponding to carbon (C), oxygen (O), sodium (Na) and chlorine (Cl), indicating presence of NaCl and polysaccharide constituents within hydrogel matrix.

Functional structural investigation using Fourier-transform infrared spectroscopy

The FTIR spectra identified the functional groups present in the herbal extracts and the hydrogel formulations (Gel 5 and Gel 5-1). The broad peak observed around $3200\text{--}3600\text{ cm}^{-1}$ corresponded to the O-H stretching vibrations, which are characteristic of hydroxyl groups present in the herbal extracts and polysaccharide components, such as pectin and sodium alginate (Gulrez et al., 2011). The intensity and broadness of this peak for the Gel 5 and Gel 5-1 indicated the effective incorporation of phenolic compounds from the herbal extracts into the hydrogel matrix. Additionally, the absorption band around $1700\text{--}1750\text{ cm}^{-1}$ was attributed to C=O stretching, typically associated with carbonyl groups found in esters, aldehydes or carboxylic acids (Fernandez-Barbero et al., 2009). This peak (evident in the spectra of the herbal extracts such as turmeric and plai) remained prominent in the hydrogel formulations, confirming the retention of active compounds. The region around $1500\text{--}1600\text{ cm}^{-1}$ displayed C=C stretching vibrations, indicating the presence of aromatic compounds such as curcuminoids from turmeric and sesquiterpenes from plai, which contribute to their anti-inflammatory and analgesic properties (Hart et al., 2000; Jurenka, 2009). The peaks observed in the range $1000\text{--}1300\text{ cm}^{-1}$ corresponded to C-O stretching, indicative of the ether or ester bonds in the polysaccharides and confirmed the successful integration of the hydrogel base with the herbal extracts (Gulrez et al., 2011). Furthermore, the peaks within $1200\text{--}1400\text{ cm}^{-1}$ corresponded to C-N stretching vibrations, likely originating from nitrogen-containing functional groups in the herbal extracts. The spectra for Gel 5 and Gel 5-1 had overlapping features of the herbal extracts and hydrogel components, with slight shifts and peak broadening suggesting strong interactions between the matrix and the active compounds. These interactions were likely due to hydrogen bonding between the hydroxyl groups of the polysaccharides and the functional groups of the extracts and enhanced the structural integration. Notably, no new peaks or major changes were observed, confirming that the chemical structure of the active compounds remained intact, ensuring their bioactivity. This structural stability supported the effectiveness of the Gel 5 formulation as a pain relief patch. Comparing the results to other studies, the observed peaks aligned with the FTIR characteristics of curcumin, terpinen-4-ol and other bioactive compounds from the turmeric and plai extracts. In addition, the GC-MS analysis confirmed the presence of 2,4-di-tert-butylphenol (retention time of 26.89 min) in both the turmeric and kaffir lime aqueous extracts. In addition, there was a phenolic

compound in the FTIR spectra of the herbal extracts and hydrogel formulations whose characteristic O-H stretching vibration ($3200\text{--}3600\text{ cm}^{-1}$) was consistent with the broad absorption (Fig. 6).

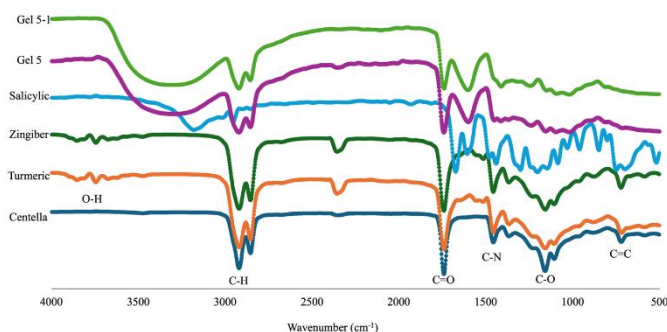


Fig. 6 Fourier-transform infrared spectra showing functional groups in extracts and gel patches

Furthermore, curcumin and its derivatives, identified as major constituents of the turmeric extract, are well-known polyphenols whose O-H and C=O stretching bands align with the FTIR peaks at $3200\text{--}3600\text{ cm}^{-1}$ and $1700\text{--}1750\text{ cm}^{-1}$, respectively (Ching et al., 2014). In addition, the polysaccharide matrix had similar features to other reported alginate-pectin systems used in sustained-release formulations (Ward and Georgiou, 2011). Overall, the FTIR analysis validated the successful incorporation of the herbal extracts into the hydrogel matrix and suggested that the formulation was structurally stable and suitable for controlled release applications, enhancing the therapeutic efficacy of the pain relief patch.

Although the release kinetics of the active compounds from the developed hydrogel were not evaluated in the present study, based on the SEM and FTIR results, there was a porous polymeric network and intermolecular interactions that may support sustained release behavior. Future studies should investigate the release profile using quantitative analysis under simulated conditions.

Skin toxicity and pain relief efficacy

The 4 hr closed patch test indicated that the Gel 5 hydrogel patch was non-irritating to human skin (Table 2), as the EI for the Gel 5 hydrogel patch was significantly lower than the positive control (20% SLS), which was consistent with the reported safety profiles of similar herbal formulations (Yip and Tam, 2008). In the pain relief evaluation, volunteers reported a significant reduction in pain levels based on the NRS. Before application, the pain scores were in the range 4–8, indicating moderate-to-severe pain. After 30–60 min of patch application, the scores reduced to 0–3, indicating mild-to-no pain. In addition, the participants reported high satisfaction levels with the hydrogel patches in terms of ease of use and effectiveness. Statistical analysis using a paired t test confirmed that the reduction in NRS pain scores before and after hydrogel patch application was significant, supporting the therapeutic efficacy of the formulation. However, the present study had notable limitations, including a small sample size ($n = 10$) and the absence of a placebo control group, which may limit the generalizability of the findings. These limitations should be addressed

Table 2 Erythema index values for volunteer skin redness over time after application of various test samples

Sample	Time after patch application (hr)				
	0	4	24	48	72
Gel 5 patch	214.50±25.30	219.96±27.62 ^a	217.5±31.62 ^a	213.96±25.76	216.67±26.65 ^a
20%w/v SLS	212.50±38.07	253.67±39.52 ^b	234.42±46.63 ^b	221.25±39.70	219.83±37.81 ^b
Negative control	211.21±28.83	211.13±32.72 ^a	207.33±30.35 ^a	212.42±35.33	210.46±40.35 ^a

20%w/v SLS = 20% weight per volume of sodium lauryl sulfate = positive control; Negative control = untreated skin.

Values (mean ± SD, $n = 10$) within the same column followed by different lowercase superscripts are significantly different ($p < 0.001$), based on Tukey's post hoc test

by future randomized controlled trials with larger sample sizes and appropriate placebo controls to further validate the clinical efficacy and safety of the herbal hydrogel pain relief patch.

The EI values in Table 2 provided an insight into the skin irritation potential of the tested gel patch compared to the positive control (20% w/v SLS) and the negative control. At time point 0, the EI values for all samples were comparable, indicating no initial irritation. Over time, distinct trends were observed between the samples. The gel patch had a relatively stable EI across all time points, with only slight fluctuations that remained within the margin of error (from 214.5 ± 25.30 at 0 hr to 216.67 ± 26.65 at 72 hr), indicating minimal irritation. In contrast, the positive control (20% w/v SLS) had a significant increase in EI from 212.5 ± 38.07 at 0 hr to a peak of 253.67 ± 39.52 at 4 hr, highlighting the strong irritation potential of SLS, commonly used as a benchmark for assessing skin irritants. The EI values gradually decreased over time but remained higher than those of the gel patch and control group, confirming prolonged irritation effects. The negative control group maintained consistently low EI values across all time points, from 211.21 ± 28.83 at 0 hr to 210.46 ± 40.35 at 72 hr, indicating no irritation and validating the baseline for comparison. The *post hoc* comparisons using Tukey's test revealed that 20% w/v SLS had a significantly higher EI than both the gel patch and the negative control ($p = 0.0001$). The gel patch was not significantly different from the negative control ($p > 0.05$). Overall, the gel patch showed consistently lower EI values compared to the SLS-containing positive control, with the EI values of the former remaining close to those of the negative control. Based on these results, the herbal-based gel patch was non-irritating and suitable for topical application. These findings aligned with other studies reporting the low skin irritancy of herbal-based patch formulations (Yip and Tam, 2008; Sarikaputi et al., 2019; Promsungwong et al., 2020) and provided further support regarding the potential of the hydrogel formulation in the present study as an effective topical pain relief alternative. This analysis underscored the potential of the gel patch as a effective alternative for pain relief applications.

Conclusions

The active compounds extracted from turmeric, plai and *Ce. asiatica*, identified using GC-MS and FTIR analyses, were compounds recognized as having anti-inflammatory and analgesic properties. The Gel 5 hydrogel pain relief patch developed from these extracts provided significant pain relief with minimal skin irritation. These findings supported the potential of integrating traditional herbal medicine with modern delivery systems for effective pain management. Statistical comparisons were performed separately at each post-application time point among the three treatment groups: at each time point where superscripts differ, the Gel 5 and negative control groups were both significantly different from 20% w/v SLS ($p = 0.0001$), while Gel 5 and the negative control did not differ significantly from each other.

However, quantitative data on extraction yields and compound concentration levels were not included in the present study, as the GC-MS analysis was focused on compound identification to support formulation development. This recognized limitation should be addressed in future studies that should quantify extraction yields, compare efficiencies across the different extraction techniques use and determine the precise concentrations of active compounds in each to optimize further the hydrogel formulation and strengthen the evidence base for its therapeutic application. Furthermore, the pain relief evaluation was limited by its small sample size ($n = 10$) and the absence of a placebo control group, which may affect the robustness of the clinical conclusions. Future randomized controlled trials are recommended with larger participant cohorts and appropriate placebo controls to provide stronger clinical validation of the analgesic efficacy of the developed Gel 5 hydrogel patch.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The Research and Development Institute, Chiang Rai Rajabhat University provided financial and research support. The Faculty of Science and Technology provided laboratory facilities and technical assistance throughout this study. The PBP-CMU Electron Linac Laboratory, Plasma and Beam Physics Research Center, Department of Physics and Materials Science, Faculty of Science, Chiang Mai University supported with the FTIR analysis.

References

- Burton, A.R., Birznies, I., Bolton, P.S., Henderson, L.A., Macefield, V.G. 2009. Effects of deep and superficial experimentally induced acute pain on muscle sympathetic nerve activity in human subjects. *J. Physiol.* 587: 183–193.
- Chaiprateep, A. 2018. Analgesic patch from Thai herb. Pathum Thani: College of Thai Traditional Medicine, Rajamangala University of Technology Thanyaburi, Thailand. [in Thai]
- Ching, W.Y., Yusadli, B.Y., Wan-Amarina, W.N.B. 2014. Extraction of essential oil from *Curcuma longa*. *J. Food Chem. Nutr.* 2: 1–10. Department of Medical Sciences, Ministry of Public Health. 2003. Compilation of Toxicology Research Works of the Institute of Thai Traditional Medicine. The Religious Affairs Press. Bangkok, Thailand. [in Thai]
- Ernst, E. 2003. Herbal medicines put into context. *Br. Med. J.* 327: 881–882. doi.org/10.1136/bmj.327.7420.881
- Fernández-Barbero, A., Suárez, I., Sierra-Martín, B., Fernández-Nieves, A., de Las Nieves, F., Marquez, M., Rubio-Retama, J., López-Cabarcos, E. 2009. Gels and microgels for nanotechnological applications. *Adv. Colloid Interface Sci.* 147–148: 88–108. doi.org/10.1016/j.cis.2008.12.004
- Galus, S., Lenart, A. 2013. Development and characterization of composite edible films based on sodium alginate and pectin. *Journal of Food Engineering.* 115(4): 459–465. doi.org/10.1016/j.jfoodeng.2012.03.006
- Gaskin, D.J., Richard, P. 2012. The economic costs of pain in the United States. *J. Pain* 13: 715–724. doi.org/10.1016/j.jpain.2012.03.009
- Gulrez, S.K.H., Al-Assaf, S., Phillips, G.O. 2011. Hydrogels: methods of preparation, characterisation and applications. In: Carpi, A. (Ed.). *Progress in Molecular and Environmental Bioengineering - From Analysis and Modeling to Technology Applications*. IntechOpen. pp. 117–151.
- Hart, P.H., Brand, C., Carson, C.F., Riley, T.V., Prager, R.H., Finlay-Jones, J.J. 2000. Terpinen-4-ol, the main component of the essential oil of *Melaleuca alternifolia* (tea tree oil), suppresses inflammatory mediator production by activated human monocytes. *Inflamm. Res.* 49: 619–626.
- Higashi, Y., Kiuchi, T., Furata, K. 2010. Efficacy and safety profile of a topical methyl salicylate and menthol patch in adult patients with mild to moderate muscle strain: A randomized, double-blind, parallel-group placebo-controlled, multicenter study. *Clin. Ther.* 32: 34–43. doi.org/10.1016/j.clinthera.2010.01.016
- IBM Corp. 2019. IBM SPSS Statistics for Windows, version 26.0. IBM Corp. Armonk, NY.
- Jungyampin, S. 2017. Development of gelatin mixed with shellac for use as transdermal gallic acid patch. Master's thesis, Rajamangala University of Technology Thanyaburi. Bangkok, Thailand. [in Thai]
- Jurenka, J.S. 2009. Anti-inflammatory properties of curcumin, a major constituent of *Curcuma longa*: a review of preclinical and clinical research. *Altern. Med. Rev.* 14: 141–153.
- Le, X.T., Ha, P.T.H., Phong, H.X., Hien, T.T., Ngan, T.T.K. 2020. Extraction of essential oils and volatile compounds of kaffir lime (*Citrus hystrix* DC) by hydrodistillation method. *IOP Conf. Ser. Mater. Sci. Eng.* 991: 012024. doi.org/10.1088/1757-899X/991/1/012024.
- Li, L.C., Wong, R.W., Rabie, A.B. 2009. Clinical effect of a topical herbal ointment on pain in temporo-mandibular disorders: a randomized placebo-controlled trial. *J. Altern. Complement Med.* 15: 1311–1317. doi.org/10.1089/acm.2009.0129
- Mektrirat, R., Yano, T., Okonogi, S., Katip, W., Pikulkaew, S. 2020. Phytochemical and safety evaluations of volatile terpenoids from *Zingiber cassumunar* Roxb. on mature carp peripheral blood mononuclear cells and embryonic zebrafish. *Molecules* 25: 613. doi.org/10.3390/molecules25030613
- Phichaphibul, P., Sirita, J., Duangtang, P., Thipyothin, A., Yotsunthon, P., Srisom, P., Tanma, S., Rakitikul, W., Phichaphibul, C., Supapolthorn, T., Phongnumkul, A. 2022. Innovation in herbal compress products to create community identity in Wiang Kaen District, Chiang Rai Province. Research report. Chiang Rai Rajabhat University. Chiang Rai, Thailand. [in Thai]
- Piyachaturawat, P., Glinsukon, T., Chanjarunee, A. 1985. Antifertility effect of *Citrus hystrix* DC. *J. Ethnopharmacol.* 13: 105–110.
- Promsungwong, P., Kodchomphu, S., Eungsamran, W., Sihamongkol, T. 2020. Effect of herbal gel patch from ginger and turmeric on knee pain relief among the elderly in Khlong Nueng Subdistrict, Pathum Thani Province. In: The 11th National and International Conference on Research Presentation. [in Thai]
- Rahamouz Haghighi, S., Yazdinezhad, A., Bagheri, K. 2022. Volatile constituents and toxicity of essential oils extracted from aerial parts of *Plantago lanceolata* and *Plantago* major growing in Iran. *Pharm. Biomed. Res.* 8: 205–224.
- Saha, S., Guria, T., Singha, T., Maity, T.K. 2013. Evaluation of analgesic and anti-inflammatory activity of chloroform and methanol extracts of *Centella asiatica* Linn. *ISRN Pharmacol.* 2013: 789613. doi.org/10.1155/2013/789613
- Saknite, I., Zavorins, A., Jakovels, D., Spigulis, J., Kisis, J. 2016. Comparison of single-spot technique and RGB imaging for erythema index estimation. *Physiol. Meas.* 37: 333–346.
- Sarikaputi, A., Napavichayan, S., Aramwit, P. 2019. Efficacy and safety of hydrogel patch from silk fibroin protein containing methyl salicylate and menthol for muscle pain relief in volunteers. *Huachiew Chalermprakiet Science and Technology Journal.* 5: 1–15. [in Thai]
- Srikun, P. 2018. Design and development of topical formulation from local plants in Thailand in the form of transdermal patch for anti-inflammatory effect. Burapha University, Thailand. [in Thai]
- Tanaka, T. 1981. Gels. *Sci. Am.* 244: 124–138. doi.org/10.1038/scientificamerican0181-124

- Thongthamachat, P., Fakkham, S., Yatrakorn, A., Phoonthananivatkul, B. 2021. Development of herbal hydrogel patch for knee pain relief among patients at Bang Mae Nang Health Promoting Hospital, Nonthaburi Province. *Journal of Public Health and Health Science*. 14: 1–15. [in Thai]
- Vildanova, R.R., Petrova, S.F., Kolesov, S.V., Khutoryanskiy, V.V. 2023. Biodegradable hydrogels based on chitosan and pectin for cisplatin delivery. *Gels* 9: 342.
- Waehayi, H., Somali, F., Towealo, K., Kumearoh, K. 2023. Effectiveness of lavender gel patch on stress relaxation among supporting staff at Yala Rajabhat University. *Yala Rajabhat University Journal*. 18: 52–61. [in Thai]
- Ward, M.A., Georgiou, T.K. 2011. Thermoresponsive polymers for biomedical applications. *Polymers* 3: 1215–1242.
- Williamson, A., Hoggart, B. 2005. Pain: A review of three commonly used pain rating scales. *J. Clin. Nurs*. 14: 798–804.
- Yip, Y.B., Tam, A.C.Y. 2008. An experimental study on the effectiveness of massage with aromatic ginger and orange essential oil for moderate-to-severe knee pain among the elderly in Hong Kong. *Complement Ther. Med*. 16: 131–138.