PHARMACIA

Pharmacia 69(3): 745–753
DOI 10.3897/pharmacia.69.e86904

Research Article

Combination of selected Thai traditional pain relief medicinal plants with anti-inflammatory abilities in a protein denaturation assay

Orawan Theanphong[1], Pathom Somwong[2]

1 *Department of pharmacognosy, College of pharmacy, Rangsit University, Pathumthani, Thailand*
2 *Department of pharmaceutical chemistry, College of pharmacy, Rangsit University, Pathumthani, Thailand*

Corresponding author: Pathom Somwong (pathom.s@rsu.ac.th)

Received 24 May 2022 ♦ **Accepted** 26 June 2022 ♦ **Published** 8 August 2022

Citation: Theanphong O, Somwong P (2022) Combination of selected Thai traditional pain relief medicinal plants with anti-inflammatory abilities in a protein denaturation assay. Pharmacia 69(3): 745–753. https://doi.org/10.3897/pharmacia.69.e86904

Abstract

Crateva adansonii DC, *Maerua siamensis* (Kurz) Pax, and *Mallotus repandus* (Willd.) Müll. Arg. have long been used as ingredients in compound herbal medicines to relieve pain in Thailand. In this study, an albumin denaturation inhibition experiment was used to assess the anti-inflammatory properties of the ethanolic extracts of these plants and their mixture. Lupeol, the active molecule responsible for the anti-inflammatory activity, was chosen as a chemical marker for the extracts. All plant extracts demonstrated anti-inflammatory potential. Their IC_{50} values ranged from 1.19 to 7.31 mg/mL. This blend showed the strongest anti-inflammatory effect, with a 0.5-fold increase in activity when compared to diclofenac. Lupeol, an anti-inflammatory agent, is one of the chemical constituents of the selected medicinal plants. Its content ranged from 0.04 to 8.60% w/w, as determined by HPLC in this study. It means that the plants, alone and in combination, are a good source of herbs for further pharmacological study and product development.

Keywords

Crateva adansonii, *Maerua siamensis*, *Mallotus repandus*, anti-inflammatory activity, lupeol

Introduction

Crateva adansonii DC is a medicinal plant that is native to Africa and tropical Asia and belongs to the Capparaceae family. The leaves and bark of the plant have traditionally been widely used in India as herbal remedies for the treatment of inflammation and diabetes (Rathinavel et al. 2017, 2021). In Cameroon, folk healers have utilized the bark to treat uterine complaints and inflammatory illnesses (Zingue et al. 2016). In Thailand, it has been used by traditional practitioners to treat muscle discomfort, and the plant has been included on the National List of Essential Medicines, which was issued by the Ministry of Public Health, as a group of traditional medicines for muscle and bone disorders (Bureau of Drug Control, Food and Drug Administration, Thailand 2015). The dried bark of the plant, which is widely available in traditional pharmacies across the country, is regularly used as one of the ingredients in polyherbal preparations such as powders, pills, and decoctions, in which a variety of pain-relieving medicinal herbs are combined (Narajeenrone et al. 2020). Because of the plant's importance in traditional medicine, particularly in Africa and Asia, phytochemical studies of plant parts, particularly the bark and leaves, have been conducted in a systematic manner, revealing the presence of a variety of bioactive compounds such as polyphenols, flavonoids, steroids, and terpenoids, as well as the potential for antioxidant and anti-inflammatory activities (Rathinavel

et al. 2018; Atchou et al. 2020; Dathong and Maneechai 2020). Although *C. adansonii* has been shown to have pharmacological effects that contribute to its traditional use as a pain reliever and has more value in traditional therapy, another medicinal plant known as *Maerua siamensis* (Kurz) Pax (synonym: *Crateva mucronulata* Kuntze), which also belongs to the Capparaceae family, has rarely been studied. The plant is indigenous to and widely distributed in Indo-China countries, especially in Thailand. Its roots have long been used as an herbal remedy for tonics, pain alleviation, and diuretics by Thai folk healers (Chansuwanit and Chanprasert 1994). According to the most recent research on the phytoconstituents and biological activity of *M. siamensis*, the isolated alkaloids and terpenoids from the plant were active as potential larvicide agents. This research shows that the plant has the potential to evolve into beneficial plants for pharmacological purposes that are not limited to conventional applications (Nobsathian et al. 2018). Among the traditional pain-relieving herbs used in Thailand, *Mallotus repandus* (Willd.) Müll. Arg., a Euphorbiaceae family medicinal plant, is one of the most well-known for its use as a critical feature in herbal mixtures for tonics and the treatment of muscle soreness for knee osteoarthritis (Pongsaree et al. 2020). The stems of this plant are used as a crude drug and are also listed on the National List of Essential Medicines as an herbal medicine (Bureau of Drug Control, Food and Drug Administration, Thailand 2015). Due to the plant's anti-inflammatory, analgesic, and antioxidant properties, which have been shown in several studies, researchers are keen to ascertain the value of the plant in medications (Hasan et al. 2018; Mondal et al. 2020; Sriset et al. 2021). While pain-relieving medicinal plants such as *C. adansonii*, *M. siamensis*, and *M. repandus* have long been utilized in Thai traditional pharmacy as crude drugs, they are not used in solitude. These plants are frequently employed in polyherbal combinations in herbal therapy (Pongsaree et al. 2020). In consideration of this, we intend to investigate the pharmacological activity of the extracts obtained from a blend of these medicinal plants in comparison to the bioactivity of a single plant extract using an albumin denaturation inhibition assay to evaluate their anti-inflammatory potencies *in vitro*. Furthermore, we propose identifying a phytochemical compound from each plant species and a mixture that would be responsible for anti-inflammatory activity. Although these plants consist of a large number of active chemicals, one of the most notable is lupeol, a pentacyclic triterpene that has the potential to be a therapeutic anti-inflammatory agent (Saha et al. 2020; Liu et al. 2021) and was discovered in all of these plants (Nobsathian et al. 2018; Mondal et al. 2020; Rathinavel et al. 2021). As a basis, the compound was chosen as a chemical marker in this investigation. Our previous research on the anti-inflammatory effects of Thai herbal pain relievers led us to hypothesize that the active extracts of the plants that were subjected to this study might contain lupeol (Somwong and Theanphong 2021). Thus, chemical analysis was carried out in this work, utilizing the Soxhlet apparatus and HPLC-DAD to extract and measure the lupeol content of plant samples, respectively. Additionally, a relationship between the anti-inflammatory effects of plant extracts, both single and combined, and the amount of the selected anti-inflammatory agent was also established in this study.

Materials and methods

Chemicals and reagents

Nanjing Spring & Autumn Biological Engineering Co., Ltd. (Nanjing, China) provided the lupeol (99% purity). Honeywell Burdick & Jackson™ (North Carolina, US) provided all the analytical reagents for the HPLC analysis. S.N.P. Scientific Co., Ltd. (Bangkok, Thailand) furnished all disposable accessories for the HPLC apparatus. Manose Health and Beauty Research Center Co., Ltd. (Chiang Mai, Thailand) granted diclofenac diethylammonium, a positive control, and all of the essential chemical reagents used in the *in vitro* anti-inflammatory assay.

Plant material

In August 2021, *C. adansonii* bark (CA), *M. siamensis* roots (MS), and *M. repandus* stems (MR) were obtained from Thai traditional medicine and pharmacy stores. These plant materials were verified by one of the authors, Asst. Prof. Dr. Orawan Theanphong, by comparing these crude drugs to genuine specimens stored at the Department of Pharmacognosy, College of Pharmacy, Rangsit University, Thailand, using macroscopic and microscopic techniques documented in the Thai Herbal Pharmacopoeia (Department of Medical Sciences, Ministry of Public Health, Thailand 2018). The specimens that were verified to be factual were given the numbers RSU 0095, RSU 0096, and RSU 0097, respectively.

Preparation of plant extracts and solutions

The individual samples, including CA, MS, and MR, were pulverized to obtain the powdered samples. Each sample was precisely weighed (10 g) into a Soxhlet apparatus thimble. For 3 hours, 300 mL of 100% ethanol was used for extraction. Furthermore, the powdered samples of each plant were allocated and combined evenly in order to create a combination formula (MX). The blend was weighed (30 g) and extracted similarly to each crude powdered substance. The ethanolic extract was reduced to drought in a rotary evaporator. Each sample's concentrated extract was precisely weighed and reconstituted with small volumes of methanol, then diluted with the same solvent and progressively adjusted to yield a sample solution with a concentration of 10 mg/mL in a stock volumetric flask. This procedure was repeated for each sample in triplicate.

In vitro anti-inflammatory activity

In this study, a protein denaturation assay was conducted to investigate the *in vitro* anti-inflammatory potential of

the dried plant extracts of CA, MS, MR, and MX using the method of Chandra et al. (2012). Each test's reaction mixture contained 0.2 mL of fresh hen's egg albumin, 2.8 mL of phosphate buffered saline (pH 6.4), and 2 mL of different doses of the tested extracts diluted in 20% tween to yield final concentrations of 0.25, 0.5, 1, 2, and 4 mg/mL. Similarly, 2 mL of the examined extracts were substituted with double-distilled water and diclofenac diethylammonium to provide the study's control and positive control, respectively. In an incubator, the reaction mixtures were heated to 70 °C for 5 minutes. After cooling at room temperature, the bioactivity of the mixtures was evaluated at a wavelength of 660 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan). Using a formula revealed in a recognized study, the percentage inhibition of protein denaturation was computed from the absorbance of tested samples compared to the control (Chandra et al. 2012). The IC_{50} value was used to express the potency of the anti-inflammatory effect for all examined extracts and diclofenac diethylammonium as a reference standard. The sample concentration for 50% inhibition of albumin denaturation was estimated by graphing the percentage inhibition versus sample concentration. Also, one-way analysis of variance (ANOVA) and Tukey's multiple comparison post-hoc test (PSPP, GNU Project) were used to consider the statistical significance at a value of $P < 0.05$ of the IC_{50} values across the groups that were tested.

Preparation of standard solutions

A stock standard solution containing 500 µg/mL of lupeol was prepared by dissolving lupeol (25 mg) in methanol and then placing the solution in a volumetric flask of 50 mL. Standard solutions were prepared by diluting the stock standard solution with methanol. They were made at concentrations ranging from 10 to 400 µg/mL.

HPLC apparatus and conditions

For the chromatographic procedure, an HPLC apparatus (1260 Infinity Series, Agilent Technologies, US) with an equipped photodiode array detector (DAD) was employed. OpenLab ChemStation software (Agilent Technologies, US) was used to control the instruments. A 0.45 µm nylon membrane was used to filter the sample and working standard solutions prior to analysis. Isocratic elution of the mobile phase, which included methanol and acetonitrile in a ratio of 90:10, was used to separate the 20-µL samples on the Accucore™ XL C18 packed column (250 mm × 4.6 mm i.d., 4 µm, Thermo Scientific, US) in the HPLC system. The HPLC instrument's column chamber was kept at room temperature while the mobile phase flow rate was set at 1.0 mL/minute. DAD recorded a chromatogram of the analyzed materials and collected absorbance data for the lupeol compound at a wavelength of 210 nm. Each injection was given a time limit of 12 minutes for analysis on the HPLC system.

Method verification

The analytical method employed in this study was based on that proposed by Somwong and Theanphong (2021). Prior to performing the analysis, the analytical procedure was verified. The accuracy and precision of the applied analytical method were tested by adding a known amount of lupeol (80–120%w/w) to the three different plant extracts and the mixture. The recovery and the relative standard deviation (RSD) percentages were assessed. Additionally, the specificity of the analytical approach was established to ensure that it was suitable for evaluating the relevant plant extracts.

Quantification of lupeol in extracts

The concentration of lupeol was measured by using a linear regression equation that was obtained from the calibration curve of working standard solutions. Each sample was analyzed in triplicate and the lupeol concentration was expressed as grams per hundred grams of the crude extract. The contents were displayed together with their standard deviations (SD). The differences in lupeol concentrations across the sample groups were assessed using one-way analysis of variance (ANOVA) combined with Tukey's multiple comparison post-hoc test (PSPP, GNU Project), with a significance level of $P < 0.05$ found for all the tested groups.

Results and discussion

Evaluation of *in vitro* anti-inflammatory activity

The anti-inflammatory impact of plant extracts was tested *in vitro* against the denaturation of egg albumin in the current study. The percentage inhibition of albumin denaturation was computed using linear regression models derived from the inhibition efficiency against sample concentrations as shown in Fig. 1 (A–E). Samples CA, MS, MR, and their mixture formula (MX) displayed inhibitory efficacy on protein denaturation assays, evidenced by the IC_{50} values shown in Table 1. In that sense, MX was found to be more anti-inflammatory than CA, MS, and MR. When compared to diclofenac, a nonsteroidal anti-inflammatory drug that was used as a positive control, MX, CA, MS, and MR showed differences

Table 1. Concentrations of various extracts of individual plant samples and their combination formula resulted in a 50% inhibition of the protein denaturation assay.

Samples	IC_{50}, mg/mL
C. adansonii bark (CA)	5.10 ± 0.46
M. siamensis roots (MS)	5.44 ± 0.18
M. repandus stems (MR)	7.31 ± 0.07
Combined plants (MX)	1.19 ± 0.02
Diclofenac diethylammonium	0.59 ± 0.02

Mean ± SD, $n = 3$.

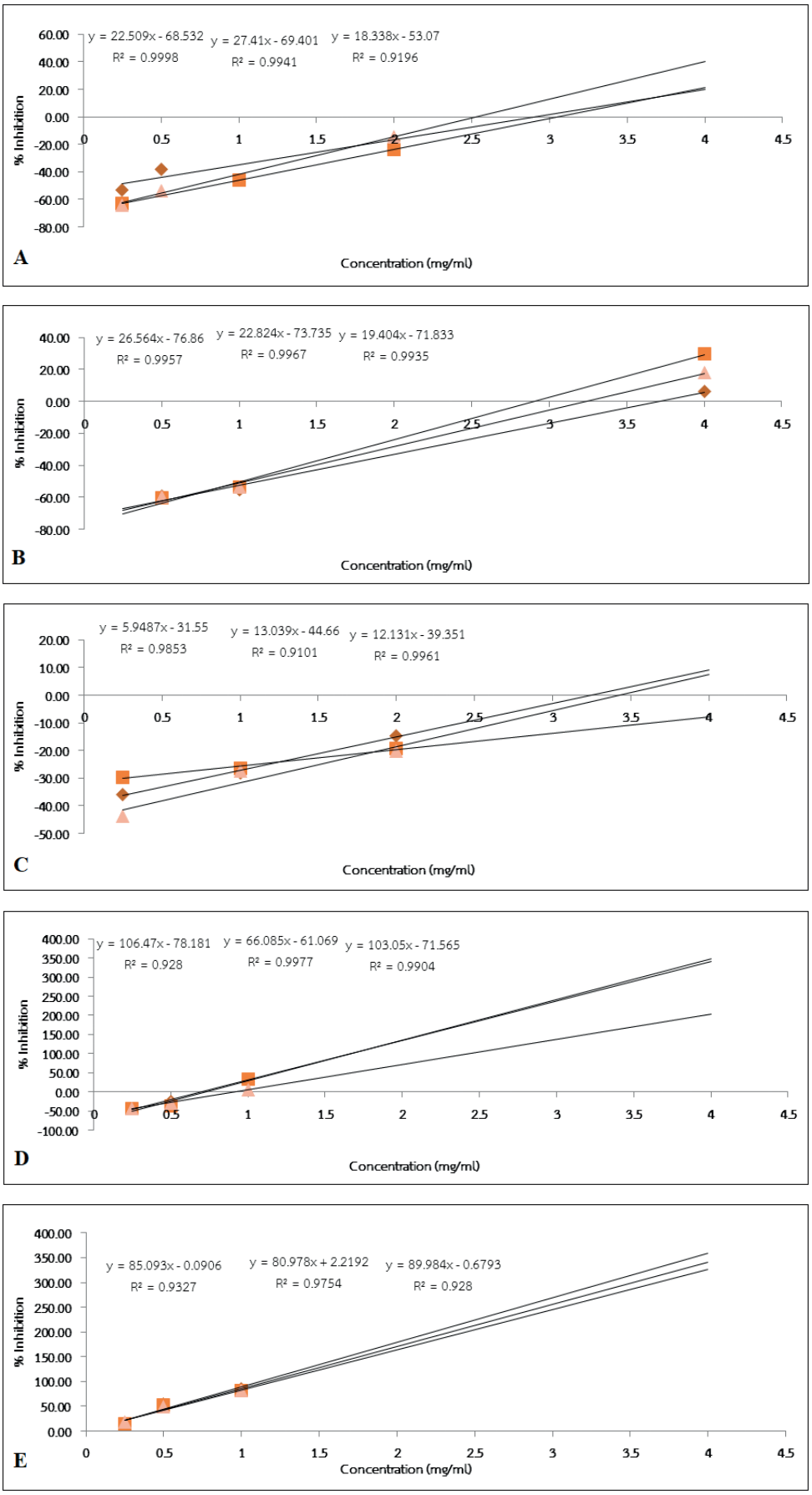

Figure 1. Plots of various concentrations of different extracts: *C. adansonii* bark (**A**), *M. siamensis* roots (**B**), *M. repandus* stems (**C**), and the mixture formula (**D**) and the positive control (**E**) against the percentage of inhibition of the protein denaturation assay. The triangular, diamond-shaped, and square markers on each plot's lines denoted the three experimental replicates.

in their anti-inflammatory activities, which were further investigated statistically. The anti-inflammatory activity of MX, a blend that incorporated CA, MS, and MR equally in a formula, was the strongest. Its inhibitory effect on albumin denaturation was also found to be greater and significantly different ($P < 0.05$) when compared to the inhibitory effects of each individual sample of CA, MS, and MR. Remarkably, the statistical difference in anti-inflammatory activity between the MX sample and the anti-inflammatory drug diclofenac was found to be insignificant ($P = 0.49$). Furthermore, the effects of CA and MS samples, which are members of the same medicinal plant family, Capparaceae, were revealed to be identical ($P = 0.79$). The statistical difference observed in this study among the examined groups revealed that all of the samples were potent differently on this anti-inflammatory examination, with the exception of the pairs of CA and MS, as well as MX and diclofenac. Thus, the studied extracts and the positive control can be arranged from strong to weak anti-inflammatory action in the following order: diclofenac>MX>CA, MS>MR. This finding demonstrated that the extract from the mix of three active plant parts, including *C. adansonii* bark, *M. siamensis* roots, and *M. repandus* stems, had the most potent anti-inflammatory effect. This analysis verifies the conventional use of these plants in Thai herbal medicine by providing scientific evidence why they had never been utilized as a single treatment. They should be utilized as ingredients in herbal pain-relief preparations by combining them with other medicinal herbs, as they had been traditionally. However, an extensive pharmacological and phytochemical examination of the potential anti-inflammatory components in the mixture should be designed and carried out to support the mixture formula as a candidate for an effective alternative combination of an anti-inflammatory pharmaceutical preparation.

This study confirmed the anti-inflammatory properties of *C. adansonii* extracts, which had an important impact on various anti-inflammatory mediators such as inhibition of 5-lipoxygenase (5-LOX), cyclooxygenase (COX), and myeloperoxidase (MPO), which are key enzymes in the inflammatory process (Rathinavel et al. 2017; Umeti et al. 2019; Thirumalaisamy et al. 2020; Rathinavel et al. 2021). The anti-inflammatory impacts of *C. adansonii* extracts have been scientifically proven, indicating that the plant should not be limited to folkloric use but should be encouraged to be developed for use in analgesic and anti-inflammatory herbal medicines also. Although there are numerous scientific reports supporting the pharmacological effects of *C. adansonii* extracts, bioactivity studies of another Capparaceae medicinal plant that is specifically distributed in the Indo-China region, notably *M. siamensis*, have received limited attention. The plant was found to contain phytochemicals with potential activity in a larvicidal bioassay. Its activities might well be beneficial in preventing the dengue epidemic (Nobsathian et al. 2018). Our study discovered that *M. siamensis* roots have anti-inflammatory properties. It demonstrated a significant ability to inhibit protein denaturation, similar to the activity of the most well-known plant, *C. adansonii* bark, indicating that the plant is noteworthy for use in anti-inflammatory herbal treatments in the same manner as *C. adansonii* bark. This is the first instance that the extract of *M. siamensis* roots has been proven to have anti-inflammatory properties. Furthermore, this investigation was expanded to include the exploration of another well-known medicinal plant, *M. repandus*, which is a member of the Euphorbiaceae family and has been used in various traditional herbal recipes for tonics and pain treatment in Thailand (Pongsaree et al. 2020). Due to the activity observed in this study, the extract of the stems of this plant could also be provided as one of the anti-inflammatory herbal treatments. Although this study indicated that the plant has the potential for anti-inflammatory effects, consistent with previous reports (Hasan et al. 2014; 2018), researchers have been urged to conduct additional pharmacological tests to evaluate its importance in herbal medicine, including antioxidant, hepatoprotective, and antibacterial properties (Mondal et al. 2020; Sriset et al. 2021; Zhang et al. 2021). In this comparative study, *M. repandus* was found to have a less potent anti-inflammatory effect than *C. adansonii* and *M. siamensis* in our evaluation of the three pain-relieving medicinal plants. But the results could help support how the plant has been used in the past, which has been in compound herbal recipes for pain relief and reducing inflammation rather than in a single treatment. Even though the traditional uses of *C. adansonii* and *M. repandus* have already been shown to have certain effects, this study is the first to show that a mixture of these two plants and *M. siamensis* has an anti-inflammatory effect that is much stronger than those of either plant alone.

Determination of lupeol content in different plant extracts

Ground samples of CA, MS, MR, and MX were extracted individually with absolute ethanol using the Soxhlet device. After evaporating the extracts from each trial, they were accurately weighed to obtain the crude extracts, providing an extraction yield of 3.68, 6.92, 6.68, and 9.47%w/w, respectively. The extracts were further divided to prepare the sample solutions for the HPLC analysis, which was performed according to the method developed by Somwong and Theanphong (2021). The HPLC method was validated using the lupeol compound and standard-spiked technique prior to the chemical analysis of the plant extracts. The chromatograms obtained from that HPLC system showed the peak of lupeol at 9.6 minutes of retention time, and the signal of that marker was clearly separated from the other interferences in the plant extracts, as shown in Fig. 2 (A–D). As a result, the method was shown to be selective and competent for determining lupeol in these plant extracts. Furthermore, the analytical method used in this study is accurate and reliable, as evidenced by the recovery and relative standard deviation percentages of the verification performed on all plant samples, which showed acceptable values in the ranges of

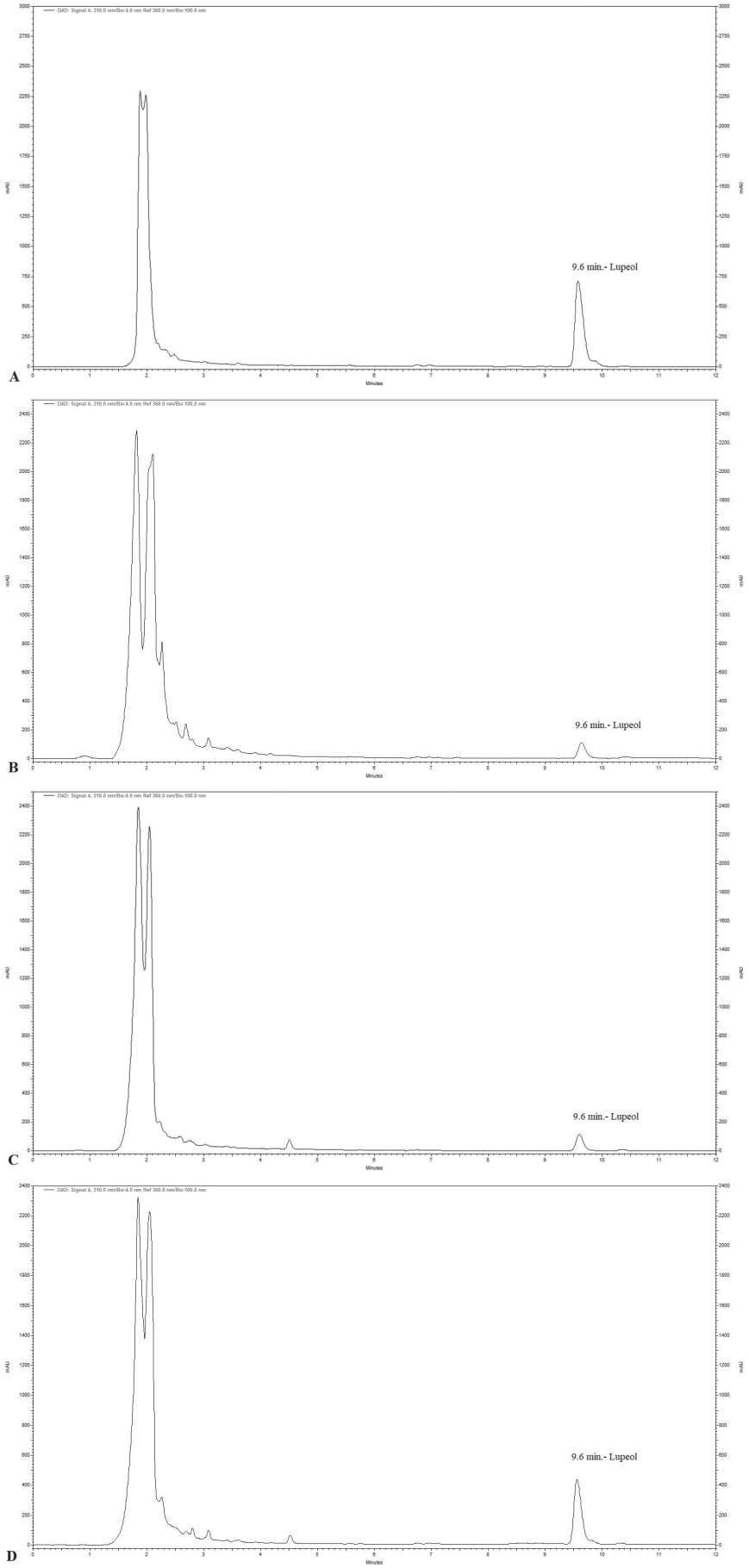

Figure 2. The HPLC chromatograms demonstrate the peak for lupeol compound at a retention time of 9.6 minutes from different extracts: *C. adansonii* bark (**A**), *M. siamensis* roots (**B**), *M. repandus* stems (**C**), and the mixture formula (**D**).

91.71–101.88 and 0.15–1.73%, respectively. The validated method was then used to determine the concentration of lupeol in all plant extracts, and the percentage of lupeol in each sample was calculated and shown in Table 2. Among the three plants, CA had the highest lupeol yield (8.38 ± 0.26%w/w), while MR had the lowest lupeol content (0.04 ± 0.01%w/w). Lupeol was found in all plant extracts, but the amount of lupeol in each extract varied greatly. The significant difference in lupeol content in extracts of three plant species, as well as their mixture, was investigated in our study. One-way ANOVA revealed that there were significant differences in lupeol content between CA, MS, MR, and MX extracts ($P < 0.05$, $F = 905.84$, $df = 3, 8$). The results of Tukey's post-hoc test revealed that the amount of lupeol in the tested samples varied significantly, with the exception of the content between the two pairs of CA and MX, as well as MS and MR, which revealed no noticeable difference in the content of lupeol compound at the significant values of 0.78 and 1.00, respectively. The lupeol yields in CA (8.38 ± 0.26%w/w) and MX (8.60 ± 0.50%w/w) were found to be uniform because the amount of lupeol in CA was found to be predominant, whereas the lupeol content in MS and MR extracts was found to be 100–200 times lower than that in the CA extract. Thus, the observed yield of lupeol in the combination most likely corresponded to the estimated yield of that constituent in CA. This discovery showed that the amount of lupeol in the CA extract affected the quantity of lupeol found in the mixture, and that the CA extract could have played a significant role in the formulation of these selected plants. In addition, our results indicate that lupeol is present in sufficient quantities in the examined plants and their mixtures to serve as a chemical marker for the HPLC analysis of their crude drug quality control. Based on the reviewed analytical method for exploring the active ingredients in CA, this study could help to show that the pentacyclic triterpene lupeol is a noteworthy chemical marker for the analysis because of its abundance in this plant extract (Tchimene et al. 2016; Thirumalaisamy et al. 2020; Rathinavel et al. 2021). In the extracts of MS and MR, a small amount of lupeol was found, which shows that the chemical analysis of the molecule in these plants is ordinary, as revealed in previous reports (Nobsathian et al. 2018; Mondal et al. 2020). However, this is the first study to quantify and confirm that their extracts contain the active compound lupeol, which makes them valuable for anti-inflammatory usage. Furthermore, this finding could facilitate the investigation of additional phytochemical components and their relevant medicinal effects in plant extracts.

Table 2. Content of lupeol compound of the examined plant extracts.

Samples	Content of lupeol, %w/w
CA	8.38 ± 0.26
MS	0.06 ± 0.01
MR	0.04 ± 0.01
MX	8.60 ± 0.50

Mean ± SD, $n = 3$.

Comparing the amount of lupeol expressed in CA, MS, and MR extracts with their relative anti-inflammatory ability revealed that the CA sample with the highest level of lupeol also exhibited the highest inhibition effect against protein denaturation, with an IC_{50} value of 5.10 ± 0.46 mg/mL. This result indicates that the potential of the plant extracts to counter inflammation was associated with the amount of the active compound lupeol existing in the plants. However, a comparison of CA and MS samples revealed that the quantified lupeol in their extracts differed substantially. The content of lupeol in MS extract (0.06 ± 0.01%w/w) was found to be about 100 times lower than in CA extract, but they both had similar anti-inflammatory activity. This shows that the activity of the MS extract was affected not only by the lupeol compound but also by the other active ingredients. The presence of indole glycosides, cappariloside A and B, a flavonoid chrysoeriol, and a phenylpropanoid cinnamic acid, which were isolated from *M. siamensis* in a previous report (Nobsathian et al. 2018), might well be attributed to the anti-inflammatory ability of MS extract in our observation. These phytoconstituents have already been demonstrated to be active molecules with anti-inflammatory properties (Li et al. 2018; Pontiki and Hadjipavlou-Litina 2019; Yoon and Park 2021). Likewise, the intriguing action was also found in the MR sample, whereas its extract had the smallest amount of lupeol (0.04 ± 0.01%w/w), which was comparable to MS and much lower than CA samples. Based on this result, it seems likely that the other active constituents of the MR extract might work synergistically with the lupeol compound to respond to the activity. Previous reports on phytochemical studies of *M. repandus* stem extracts revealed that the plant contains an isocoumarin bergenin as one of its active constituents (Rivière et al. 2010; Sriset et al. 2021). Bergenin was examined in various anti-inflammatory models and was proven to be an efficient anti-inflammatory compound (Oliveira et al. 2019). The compound has been thoroughly studied in terms of pharmacology and has emerged as a promising candidate for therapeutic uses (Mehta et al. 2022). Due to the anti-inflammatory agents that were identified as chemical constituents of *M. siamensis* and *M. repandus*, as well as the abundant lupeol compound found in *C. adansonii*, there could be a reason to support the anti-inflammatory activity of the mixture of these three plants, which was the most potent in our observation. The existence of these active phytoconstituents in the mixture might even increase its activity to a level comparable to that of diclofenac, a nonsteroidal anti-inflammatory drug used as a positive control in this study. Our finding suggests that a variety of chemical compounds might be responsible for the anti-inflammatory effect of the mixture formula. Therefore, an extensive study on the phytochemical analysis of other promising anti-inflammatory agents, as well as the use of multi-chemical markers for performing HPLC analysis in the mixture, should be considered in order to develop this combination into an effective pharmaceutical herbal compound for the treatment of inflammation.

Conclusion

This study demonstrates the efficacy of ethanolic extracts from herbal materials including *C. adansonii* bark, *M. siamensis* roots, and *M. repandus* stems, as well as their blending formula, in an *in vitro* anti-inflammatory assay. The study shows that the anti-inflammatory ability evident in all of these plants could support their traditional use as ingredients in herbal recipes for analgesic and anti-inflammatory purposes. This investigation was the first one to illustrate the anti-inflammatory action of a mixture formula of medicinal plants, and it also indicated that the extract from this blending is beneficial to developing as one of the most effective anti-inflammatory herbal remedies due to its highest potency toward an anti-inflammatory test, observed similarly to diclofenac in our study. This report outlined an effective tool for quantifying the lupeol compound, which could be used as a marker in the chemical analysis for the quality assessment of these plant crude drugs, and is the first to confirm the existence of the anti-inflammatory triterpene lupeol content in these plant samples and their mixture formula. It also exemplifies the relationship between the amount of lupeol found in each plant species and its anti-inflammatory abilities, which can be used as a basis for future research on anti-inflammatory properties, particularly for the mixture recipe.

Conflict of interest

The authors have no competing interests to declare.

Acknowledgements

The authors are grateful to the Rangsit University Research Institute for their financial support and the Rangsit University College of Pharmacy for supplying the essential equipment for this study.

References

Atchou K, Lawson-Evi P, Metowogo K, Bakoma B, Eklu-Gadegbeku K, AkliKokou K, Gbeassor M (2020) Antihyperglycaemic and antioxidant activities of *Crataeva adansonii* DC. ssp. *adansonii* leaves extract on ICR mice. Journal of Drug Delivery and Therapeutics 10(1-s): 30–38. https://doi.org/10.22270/jddt.v10i1-s.3855

Bureau of Drug Control, Food and Drug Administration, Thailand (2015) Herbs in national list of essential medicines. Ministry of Public Health, Nonthaburi, Thailand.

Chandra S, Chatterjee P, Dey P, Bhattacharya S (2012) Evaluation of *in vitro* anti-inflammatory activity of coffee against the denaturation of protein. Asian Pacific Journal of Tropical Biomedicine 2(1): S178–S180. https://doi.org/10.1016/S2221-1691(12)60154-3

Chansuwanit N, Chanprasert C (1994) Medicinal plants and local-used plants in Paknampran, Pranburi district, Prachup-khiri-khan province. Region 7 Medical Journal 13(2): 145–164.

Dathong S, Maneechai S (2020) Antioxidant activities of *Crateva adansonii* DC. subsp. trifoliata (Roxb) Jacobs. RMUTP Research Journal 14(1): 48–57. https://doi.org/10.14456/jrmutp.2020.5

Department of Medical Sciences, Ministry of Public Health, Thailand (2018) Thai Herbal Pharmacopoeia 2018. Keawjawjom Printing & Publishing Suan Sunandha Rajabhat University, Bangkok, Thailand.

Hasan M, Uddin N, Hasan R, Mahmudul Islam AFM, Hossain M, Rahman AB, Hossain S, Chowdhury IA, Rana S (2014) Analgesic and anti-inflammatory activities of leaf extract of *Mallotus repandus* (Willd.) Muell. Arg. BioMed Research International 2014: 539807. https://doi.org/10.1155/2014/539807

Hasan M, Uddin N, Sana T, Hossain M, Mondal M, Kanta IJ, Choudhuri MSK (2018) Analgesic and anti-inflammatory activities of methanolic extract of *Mallotus repandus* stem in animal models. Oriental Pharmacy and Experimental Medicine 18(2): 139–147. https://doi.org/10.1007/s13596-018-0312-3

Li Z, Zhao J, Zhou H, Li L, Ding Y, Li J, Zhou B, Jiang H, Zhong N, Hu W, Yang Z (2018) Capparilosside A shows antiviral and better anti-inflammatory effects against influenza virus via regulating host IFN signaling, *in vitro* and *vivo*. Life Sciences 200: 115–125. https://doi.org/10.1016/j.lfs.2018.03.033

Liu K, Zhang X, Xie L, Deng M, Chen H, Song J, Long J, Li X, Luo J (2021) Lupeol and its derivatives as anticancer and anti-inflammatory agents: Molecular mechanisms and therapeutic efficacy. Pharmacological Research 164: 105373. https://doi.org/10.1016/j.phrs.2020.105373

Mehta S, Kadian V, Dalal S, Dalal P, Kumar S, Garg M, Rao R (2022) A fresh look on bergenin: Vision of its novel drug delivery systems and pharmacological activities. Future Pharmacology 2(1): 64–91. https://doi.org/10.3390/futurepharmacol2010006

Mondal M, Hossain MM, Hasan MR, Tarun MTI, Islam MAF, Choudhuri MSK, Islam MT, Mubarak MS (2020) Hepatoprotective and antioxidant capacity of *Mallotus repandus* ethyl acetate stem extract against D-galactosamine-induced hepatotoxicity in rats. ACS Omega 5(12): 6523–6531. https://doi.org/10.1021/acsomega.9b04189

Narajeenrone K, Wattanarangsan J, Chaisri S, Pilakasiri K, Akarasereenont P, Booranasubkajorn S (2020) Optimizing the method for quantification of apigenin and quercetin in the Thai herbal Sattakavata formula (SVF) by ultra performance liquid chromatography coupled with mass spectrometer (UPLC-MS). Thai Journal of Pharmaceutical Sciences 44(2): 99–106.

Nobsathian S, Bullangpoti V, Kumrungsee N, Wongsa N, Ruttanakum D (2018) Larvicidal effect of compounds isolated from *Maerua siamensis* (Capparidaceae) against *Aedes aegypti* (Diptera: Culicidae) larvae. Chemical and Biological Technologies in Agriculture 5: 8. https://doi.org/10.1186/s40538-018-0120-5

Oliveira GA, Araujo AK, Pacheco G, Oliveira AP, Carvalho JL, Chaves LS, Sousa GC, Lopes ALF, Silva PC, Leódido ACM, Nogueira KM, Souza LKM, Araújo TL, Nicolau LAD, Medeiros JVR (2019) Anti-inflammatory properties of bergenin in mice. Journal of Applied Pharmaceutical Science 9(7): 69–77. https://doi.org/10.7324/JAPS.2019.90709

Pongsaree R, Vajaradul Y, Fakkham S, Kamoltham T (2020) Comparison in efficacy and safety of Koklan mixture formula 3 and diclofenac for pain relieving in primary knee osteoarthritis. Systematic Reviews in Pharmacy 11(11): 1457–1465. https://doi.org/10.31838/srp.2020.11.206

Pontiki E, Hadjipavlou-Litina D (2019) Multi-target cinnamic acids for oxidative stress and inflammation: Design, synthesis, biological evaluation and modeling studies. Molecules 24(1): 12. https://doi.org/10.3390/molecules24010012

Rathinavel T, Ammashi S, Jayaseelan B, Mani M (2017) Evaluation and assessment of anti-inflammatory mediators in the leaf extracts of *Crateva adansonii* DC. International Journal of Advanced Science and Engineering 3(4): 442–450.

Rathinavel T, Ammashi S, Muthusamy G (2018) Screening of anti-inflammatory phytocompounds from *Crateva adansonii* leaf extracts and its validation by *in silico* modeling. Journal of Genetic Engineering and Biotechnology 16(2): 711–719. https://doi.org/10.1016/j.jgeb.2018.03.004

Rathinavel T, Iqbal MN, Kumarasamy S (2021) Lupeol from *Crateva adansonii* DC exhibits promising enzymes inhibition: Play a crucial role in inflammation and diabetes. South African Journal of Botany 143: 449–456. https://doi.org/10.1016/j.sajb.2021.08.023

Rivière C, Nguyen Thi Hong V, Tran Hong Q, Chataigné G, Nguyen Hoai N, Dejaegher B, Tistaert C, Nguyen Thi Kim T, Vander Heyden Y, Chau Van M, Quetin-Leclercq J (2010) *Mallotus* species from Vietnamese mountainous areas: Phytochemistry and pharmacological activities. Phytochemistry Reviews 9(2): 217–253. https://doi.org/10.1007/s11101-009-9152-6

Saha S, Profumo E, Togna AR, Riganò R, Saso L, Buttari B (2020) Lupeol counteracts the proinflammatory signalling triggered in macrophages by 7-keto-cholesterol: New perspectives in the therapy of atherosclerosis. Oxidative Medicine and Cellular Longevity 2020: 1232816. https://doi.org/10.1155/2020/1232816

Somwong P, Theanphong O (2021) Quantitative analysis of triterpene lupeol and anti-inflammatory potential of the extracts of traditional pain-relieving medicinal plants *Derris scandens*, *Albizia procera*, and *Diospyros rhodocalyx*. Journal of Advanced Pharmaceutical Technology & Research 12(2): 147–151. https://doi.org/10.4103/japtr.JAPTR_13_21

Sriset Y, Chatuphonprasert W, Jarukamjorn K (2021) *In vitro* antioxidant potential of *Mallotus repandus* (Willd.) Muell. Arg stem extract and its active constituent bergenin. Songklanakarin Journal of Science & Technology 43(1): 24–30. https://doi.org/10.14456/sjst-psu.2021.4

Tchimene MK, Nwaehujor CO, Ezenwali M, Okoli CC, Iwu MM (2016) Free radical scavenging activity of lupeol isolated from the methanol leaf extract of *Crateva adansonii* Oliv. (Capparidaceae). International Journal of Pharmacognosy and Phytochemical Research 8(3): 419–426.

Thirumalaisamy R, Ameen F, Subramanian A, Selvankumar T, Alwakeel SS, Govarthanan M (2020) *In-vitro* and *in-silico* anti-inflammatory activity of lupeol isolated from *Crateva adansonii* and its hidden molecular mechanism. International Journal of Peptide Research and Therapeutics 26: 2179–2189. https://doi.org/10.1007/s10989-019-10006-5

Umeti CC, Onajobi FD, Obuotor EM, Anyasor GN, Esan EB (2019) Anti-inflammatory properties and gas chromatography-mass spectrometry analysis of ethyl acetate fraction of *Crateva adansonii* DC leaves. American Journal of Physiology, Biochemistry and Pharmacology 9(1): 1–12. https://doi.org/10.5455/ajpbp.20181226090820

Yoon HS, Park CM (2021) Chrysoeriol ameliorates COX-2 expression through NF-κB, AP-1 and MAPK regulation via the TLR4/MyD88 signaling pathway in LPS-stimulated murine macrophages. Experimental and Therapeutic Medicine 22: 718. https://doi.org/10.3892/etm.2021.10150

Zhang XC, Zhu L, Li XY, Liu LC, Lai PX (2021) Chemical composition, and evaluation of antibacterial, antibiofilm and synergistic effects with conventional antibiotics of essential oil from *Mallotus repandus*. Records of Natural Products 15(4): 324–329. https://doi.org/10.25135/rnp.217.20.10.1854

Zingue S, Cisilotto J, Tueche AB, Bishayee A, Mefegue FA, Sandjo LP, Nde CBM, Winter E, Michel T, Ndinteh DT, Awounfack CF, Silihe KK, Tanekou TTM, Creczynski-Pasa TB, Njamen D (2016) *Crateva adansonii* DC, an African ethnomedicinal plant, exerts cytotoxicity *in vitro* and prevents experimental mammary tumorigenesis *in vivo*. Journal of Ethnopharmacology 190: 183–199. https://doi.org/10.1016/j.jep.2016.06.004