BCT17
Botanical Conference of Thailand

12 - 14 June 2025

Faculty of Science, NU

# Proceeding

งานประชุมวิชาการพฤกษศาสตร์แห่งประเทศไทย ครั้งที่ 17

# THE 17TH BOTANICAL CONFERENCE OF THAILAND

*Green for Sustainability and Community*

**Organized by**

**Sponsors**


BCT17
Botanical Conference of Thailand

# สารบัญ

# คณะผู้จัดงานประชุมวิชาการพฤกษศาสตร์แห่งประเทศไทยครั้งที่ 17

**สมาคมพฤกษศาสตร์**

**ในพระบรมราชินูปถัมภ์**

**มหาวิทยาลัยนเรศวร**

https://www.nu.ac.th

**จุฬาลงกรณ์มหาวิทยาลัย**

https://www.chula.ac.th

**มหาวิทยาลัยเกษตรศาสตร์**

https://www.ku.ac.th/th

ภาควิชาชีววิทยา คณะวิทยาศาสตร์
มหาวิทยาลัยนเรศวร

Center of Excellence in Agricultural Biotechnology
Naresuan University

Center of Excellence for Biodiversity
Naresuan University

Center of Excellence for Innovation and Technology for Detection and Advanced Materials

# ผู้สนับสนุนงานประชุมวิชาการพฤกษศาสตร์แห่งประเทศไทยครั้งที่ 17





GenePlus
AUTHORIZED DISTRIBUTOR

# THE ICON OF LIFE SCIENCE INNOVATION

MGI


DNBSEQ-E25


DNBSEQ-G400


DNBSEQ-G99

HAMILTON


Hamilton Microlab VANTAGE Liquid Handling System
Hamilton Microlab STARV


Hamilton Microlab Prep

iontorrent
by Thermo Fisher Scientific


Ion GeneStudio™ S5 Systems


Ion Torrent™ Genexus™ System

qualitype


appliedbiosystems
by Thermo Fisher Scientific
for HUMAN IDENTIFICATION


RapidHIT™ ID System


SeqStudio™ Genetic Analyzer


QuantStudio™ 5
Real-Time PCR System


SeqStudio Flex Genetic Analyzer

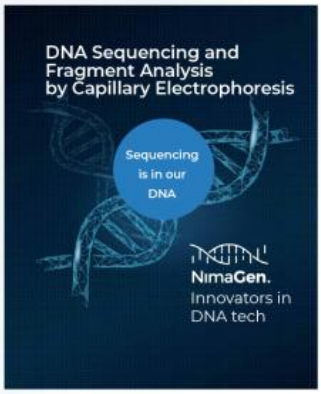
azure biosystems


AZURE CIELO™
REAL-TIME PCR

RainSure
SCIENTIFIC


Cartridge Scanner


Sample Prep Station




IDT
INTEGRATED DNA TECHNOLOGIES


Yourgene Health

Yourgene's LightBench with Ranger Technology provides and automated high throughput gel electrophoresis platform for DNA size selection.

FOR PREGNANT WOMEN
the IONA test
non-invasive prenatal screen: safe, fast, accurate

TRUPCR®
ALL PCR Panel Kit
for detection of
DIAGNOSTIC AND PROGNOSTIC
markers of acute lymphoblastic leukemia (ALL)



PlantScreen™ Conveyor and Robotic System
High Throughput Plant Phenotyping

**GENE PLUS CO., LTD.**
TEL. 02 274 1291 - 5, 02 692 9330
www.gene-plus.com info@gene-plus.com
Line: @geneplus FB: geneplusdotcom

GENEPLUS is a professional distributor company in biotechnology. We are exclusive distributor for Applied Biosystems™ which is a global leader in providing innovative instrument systems to accelerate academic and clinical research, drug discovery and development, pathogen detection, environmental analysis, and forensic DNA analysis.

Proceeding No. OPH10

# สูตรตำรับและลักษณะเฉพาะของยาหม่องไพล (*Zingiber montanum* (J.Koenig) Link ex A.Dietr.) ที่เสริมด้วยสารออกฤทธิ์ทางชีวภาพจากสารสกัดดอกกัญชง (*Cannabis sativa* L.)

# Formulation and Characterization of *Zingiber montanum* (J.Koenig) Link ex A.Dietr. Balm Enriched with Bioactive Compounds from *Cannabis sativa* L. Flower Extract

Panha Pen[1], จิดาภา สมบุญไชย[1], ชนิตา แก้วกอง[1], วริษรา จอมแก้ว[1], วิภาษณีย์ ธรรมเภรี[1], จำนงค์ ธัญญสิทธิ์[1], ภัทรวดี สุ่มทอง นาคมี[1,*]
Panha Pen[1], Jidapha Somboonchai[1], Chanita Kaewkong[1], Waritsara chomkaeo[1], Wipasanee Thamparee[1], Jamnong Tanyasit[1], Pattarawadee Sumthong Nakmee[1,*]

[1] ภาควิชาทรัพยากรและสิ่งแวดล้อม คณะวิทยาศาสตร์ ศรีราชา มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตศรีราชา, 199 หมู่ 6 ตำบลทุ่งสุขลา อำเภอศรีราชา จังหวัดชลบุรี 20230 ประเทศไทย
[1] Department of Resources and Environment, Faculty of Science at Sriracha, Kasetsart University Sriracha Campus, 199 Moo 6, Thung Sukhla, Sriracha District, Chonburi 20230, Thailand
Correspondence: pattarawadee.sum@ku.th

**บทคัดย่อ:** การศึกษานี้มุ่งเน้นในการสกัดดอกกัญชง (*Cannabis sativa* L. สายพันธุ์ Charlotte Angle) เพื่อให้ได้สารแคนนาบิไดออล (CBD) นำมาเป็นส่วนผสมในยาหม่องไพล และตรวจสอบสาร (E)-1-(3,4-dimethoxyphenyl) butadiene (DMPBD) ซึ่งเป็นสารออกฤทธิ์ต้านการอักเสบในสารสกัดไพล (*Zingiber montanum* (J.Koenig) Link ex A.Dietr.) ที่ใช้ทำยาหม่อง จากนั้นวิเคราะห์ลักษณะทายกายภาพของยาหม่อง ได้แก่ สี ความสามารถในการกระจายตัว และ ฟองอากาศในเนื้อยาหม่องไพล สูตรตำหรับต่างๆ ผลการวิเคราะห์สียาหม่องทั้งสามสูตรตำหรับ ได้แก่ สีเหลือง (A7) สีเขียว (A9) และสีขาวนวล (A12) ด้วยเครื่อง colour analyzer พบว่าไม่มีการเปลี่ยนแปลงอย่างมีนัยสำคัญตลอดระยะเวลา 8 เดือนในการเก็บรักษาที่อุณหภูมิห้องในที่มืด ส่วนการทดสอบความสามารถในการกระจายตัวของเนื้อยาหม่องโดยใช้แผ่นสไลด์ แสดงให้เห็นว่าสูตรตำรับยาหม่อง A9 และ A12 มีการกระจายตัวและมีเนื้อสัมผัสที่เรียบเนียนกว่าสูตรตำรับยาหม่อง A7 นอกจากนั้นการวิเคราะห์ด้วยกล้องจุลทรรศน์เชิงซ้อนที่กำลังขยายเลนส์วัตถุ 10X ยืนยันว่าไม่มีฟองอากาศในเนื้อยาหม่องทั้ง 3 สูตรตำหรับ ดังนั้นการเติมสารสกัดดอกกัญชงในยาหม่องไพลทำให้สีของยาหม่องคงตัวและสามารถเก็บรักษาได้เป็นระยะเวลา 8 เดือน เนื้อสัมผัสเรียบเนียนและไม่มีฟองอากาศ อีกทั้งยังเสริมสรรพคุณของสารออกฤทธิ์ทางชีวภาพ ได้แก่ CBD เพื่อการพัฒนาเป็นผลิตภัณฑ์ยาหม่องสมุนไพรที่ได้มาตรฐานต่อไป

คำสำคัญ: แคนนาบิไดออล, ซีบีดี, สียาหม่อง, dimethoxyphenyl butadiene, DMPBD

**Abstract:** This study focused on the extraction of *Cannabis sativa* L. (variety Charlotte Angle) flowers to obtain cannabidiol (CBD) and combine with Plai (*Zingiber montanum* (J.Koenig) Link ex A.Dietr.) extract to obtain (E)-1-(3,4-dimethoxy phenyl) butadiene (DMPBD) which were essential ingredients in Plai balm. Color, spread ability and texture of Plai balm formulations were analyzed. The result indicated no significant change of any colors over eight months storage which were yellow (A7), green (A9) and off-white (A12). Spread ability tests using glass slide showed that Plai balm formulations A9 and A12 exhibited a smoother texture compared to A7. The compound microscopic analysis using 10X objective lens confirmed no bubbles in all 3 balm formulations. Thus, the incorporation of *C. sativa* flower extract into Plai balm contributed to color stability and extended shelf life up to eight months, while maintaining a smooth texture without air bubbles. Additionally, it enhanced the bioactive properties, particularly CBD, supporting the development of a standardized herbal balm product.

Keywords: cannabidiol, CBD, balm color, dimethoxyphenyl butadiene, DMPBD

## 1. Introduction

*Cannabis sativa* L. which belongs to the family Cannabaceae is a shrub which compose of several groups of secondary metabolites, concluding at least 104 cannabinoids, 120 terpenoids (61 monoterpenes, 52 sesquiterpenoids, and 5 triterpenoids), 26 flavonoids, and 11 steroids among 545 identified compounds [1]. However, CBD has been richly illustrated anxiety, cancer and cancer chemotherapy side effects, inflammatory bowel disease, osteoarthritis, seizures, inflammatory, neurologic disease [2].

*Zingiber montanum* (J.Koenig) Link ex A.Dietr., also known as Plai in Thai, are extensively served in food, herbal medicines, and Thai balm products. Genus *Zingiber* consists of about 85 species which belong to the Zingiberaceae family. *Z. montanum* has been widely used as anti-inflammatory, antifungal, antioxidant, antibacterial agent [3], antidiabetic, hepatoprotective, neuroprotective, anticancer [4], antiviral, immunomodulatory, antihistaminic, anticholinesterase, and smooth muscle relaxant activities. While (E)-1-(3,4-dimethoxyphenyl) butadiene (DMPBD) was the bioactive compound for anti-inflammatory activity [5-6]. Similarly, many phenylbutanoids, flavonoids and terpenes have been also found in *Zingiber* sp. rhizomes [7].

The research focus on extracting *Cannabis sativa* L. (variety Charlot Angle) to obtain CBD and incorporate it into *Zingiber montanum* (Plai) balm with DMPBD as a bioactive compound to enhance therapeutic properties. And evaluate the balm's color stability, spread ability, and texture, supporting its potential as a standard balm product.

## 2. Material and Methods

### 2.1. Plants Sample Preparation and Extraction

2.1.1 *Cannabis sativa*

Flowers of *C. sativa* variety Charlotte Angle were collected from Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus in January 2024 and stored at -20°C under dark conditions. The plant materials were ground and made a maceration extraction with hexane for 3 days with 15 minutes of sonication per day. The liquid extract was filtered under the vacuum. After that hexane was evaporated using rotary evaporator at 50°C to obtain the crude extract and preserved in -20 ℃ for the next experiment.

2.1.2 *Zingiber Montanum*

Rhizomes of *Z. montanum* (BK No. 086014) were harvested in February 2024 from Nan Province, Thailand. The outer bark was removed, and the cleaned rhizomes were washed, shredded into small pieces, and then oven-dried at 60℃ for 16 hours. The dried plant material was divided into two portions. The first portion was fried using the traditional method at 110–120℃ for 15 minutes to extract *Z. montanum* oil, which was then collected and stored at room temperature for balm preparation. The second portion of dried rhizomes was macerated in hexane for three days, with daily sonication for 15 minutes. The extract was then filtered under vacuum, and the solvent was evaporated using a rotary evaporator to obtain the hexane crude extract.

### 2.2. Balm Preparation

(E)-1-(3,4-dimethoxyphenyl) butadiene (DMPBD) in *Z. montanum* hexane extract was investigated by dissolving in chloroform-d at the final volume of 700 µL (50 µL crude extract and 650 µL solvent) for 400 $MH_z$ $^{1}H$ NMR and $^{13}C$ NMR spectroscopy (Bruker, Germany) according to the method [8]. Evaluation of Cannabinoids from *C. sativa* variety Charlotte Angle flower extract using HPLC (Agilent infinity II 1260, USA) to derive the concentration of 11 cannabinoids especially total THC and total CBD. Formulation of *Z. montanum* balms (20%w/w *Z. montanum* extract) with and without *C. sativa* flower extract (0.02%w/w) are shown in Table 1.

**Table 1** The formulation of *Zingiber montanum* balm

| formulation | Plai extract | | *C.sativa* flower extract | Base of balm | Cooling/Warming Agents |
|---|---|---|---|---|---|
| | Traditional | Hexane | | | |
| A7 | ✓ | - | - | ✓ | ✓ |
| A9 | ✓ | - | ✓ | ✓ | ✓ |
| A12 | - | ✓ | ✓ | ✓ | ✓ |

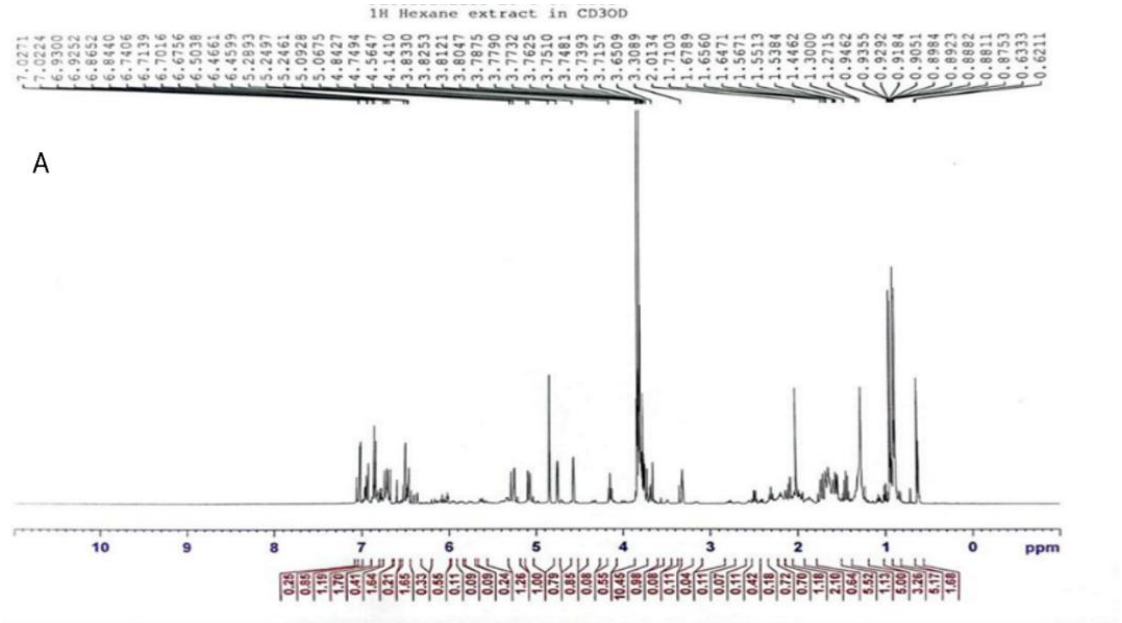
### 2.3 Quality and Stability Test of *Z. montanum* Balm

The color and volatile compounds of the balm were evaluated at 0, 4, and 8 months after storage, while the spread ability test was conducted after produce. Balm color was analyzed using a spectrophotometer (HunterLab ColorFlex EZ, USA) at wavelengths ranging from 380 to 530 nm. A HunterLab colorimeter with the CIELAB color space was used, measuring L*, a*, and b* values. The L* value (lightness) ranges from 0 (pure black) to 100 (pure white or completely transparent for transmission). The a* value represents the red-green axis, where positive values indicate red, negative values indicate green, and 0 is neutral. The spread ability of *Z. montanum* balm was assessed at room temperature using glass slides, following the method [9]. Additionally, a compound light microscope was used to examine the balm's texture.

## 3. Results and Discussion

### 3.1. DMPBD in Z. *montanum* rhizome extract

The hexane extract of Z. *montanum* rhizome has determined the bioactive compound, DMPBD. $^{1}$H NMR spectrum (400 MHz, chloroform-d1) of DMPBD; **δ**(ppm) = 6.99-6.90 (m, $^{2}$H, ArH), 6.82 (d, J = 8.1 Hz, $^{1}$H, ArH), 6.73-6.62 (m, $^{1}$H, ArCH), 6.56–6.42 (m, $^{2}$H, ArCH=CH), 5.33 (m, $^{1}$H, CH=CHH), 5.08 (m, $^{1}$H, CH=CHH), 4.74 (s, $^{3}$H, $OCH_3$), 4.56 (s, $^{3}$H, $OCH_3$) (Figure 1A, B). While the $^{13}$C NMR spectrum (400 MHz, chloroform-d1) of DMPBD; **δ**(ppm)= 149.98, 149.03, 137.40, 132.49, 130.52, 127.72, 119.06, 115.36, 111.48, 109.11, 55.38, 55.21. The results indicated that the hexane extract of *Z. montanum* rhizome contained DMPBD which exhibited for antioxidant, anti-inflammatory effect, and reduce muscle pain and ankle sprain [11-12]. The quantitative analysis of DMPBD might be the marker for quality control of *Z. montanum* balm for industrial scale production.



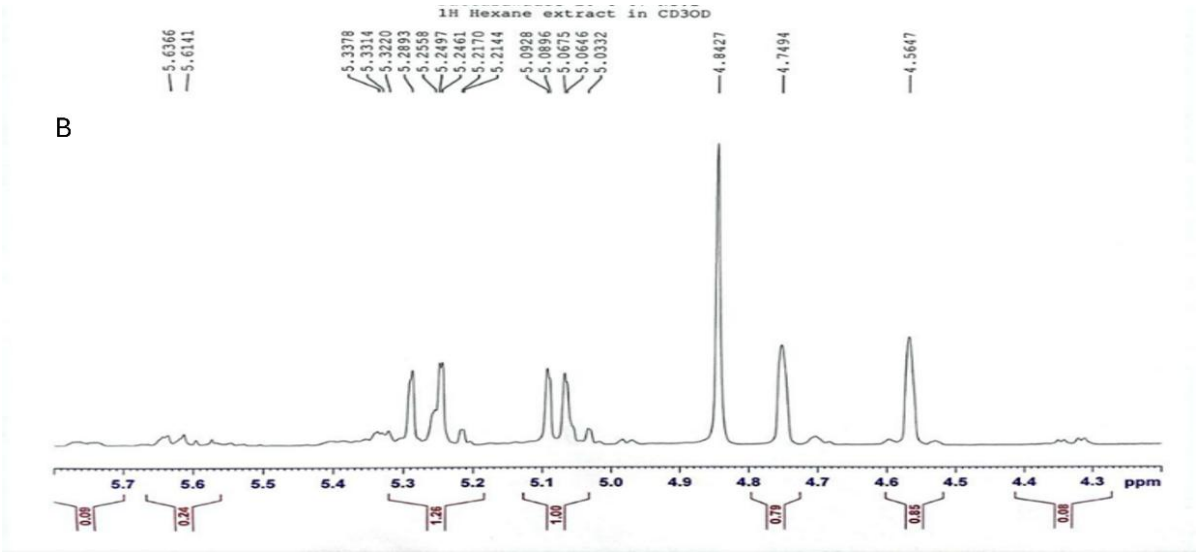



**Figure 1** $^{1}$H-NMR spectrum of DMPBD (A) and the marker spectrum of DMPBD at δ = 4.3-5.7 ppm (B)

### 3.2. Cannabinoids from *Cannabis sativa*

The hexane extract of *C. sativa* flower presented the concentration of 11 cannabinoids by HPLC (Table 2). The results found both cannabinoid and cannabinoid acid in the extract. The analytical results indicated that *C. sativa* 'Charlotte Angle' flower contained high amount of cannabidiol (42.12 % dry weight of total CBD) than tetrahydrocannabinol (1.99% dry weight of total THC) which is undoubtedly beneficial for the development of pharmaceutical products. Transdermal CBD has gained attention for its potential health benefits due to its ability to deliver cannabinoids directly into the bloodstream without the need for inhalation or ingestion. CBD has been shown to reduce pain and inflammation by interacting with the body's endocannabinoid system (ECS) and CB2 receptors, which are involved in pain perception. Although transdermal research is limited, CBD is known to reduce anxiety by influencing serotonin receptors (5-HT1A) in the brain. Some users report calming effects when using transdermal CBD patches, which may provide consistent, long-lasting delivery without psychoactive effects. Thus, transdermal CBD is a promising option for pain, inflammation, anxiety, anti-bacteria, skin conditions, and sleep disorders [13–15]

The effects of tetrahydrocannabinol (THC) on psychological and addictive behaviors have been extensively studied, primarily focusing on inhalation and oral consumption methods. Consequently, it remains unclear whether transdermal application of THC would elicit similar psychological effects or potential for addiction as observed with inhalation or oral consumption. There is currently insufficient scientific evidence to determine the effects of transdermal THC applications on psychology and addictive behavior. Further research is necessary to elucidate these effects and to assess the safety and efficacy of transdermal THC delivery systems [16–18]

**Table 2** The concentration of cannabinoids from *Cannabis sativa* 'Charlotte Angle' flower extract.

| compounds | CBDV | CBDA | CBGA | CBG | CBD | THCV | CBN | D9-THC | D8-THC | CBC | THCA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Potency (mg/g) | 0.28 | 335.86 | 5.65 | 4.44 | 126.67 | ND | 0.46 | 10.45 | ND* | 6.86 | 10.8 |

*ND = Not detected

### 3.3 Color Analyzer

The color of *Z. montanum* balm formulations (Figure 3) were analyzed at 0, 4, and 8 months after storage at room temperature. The b* value represents the blue-yellow axis, with positive values indicating yellow, negative values indicating blue, and 0 being neutral. The results showed that the color of all *Z. montanum* balm formulations remained stable over the 8-month period (Figure 4). The color parameters of carrot and beetroot lip balms were investigated and found no spots or color changes when stored at room temperature [9,19]. However, some studies, such as ingredient degradation in balm formulations can contribute to color changes over time [20].

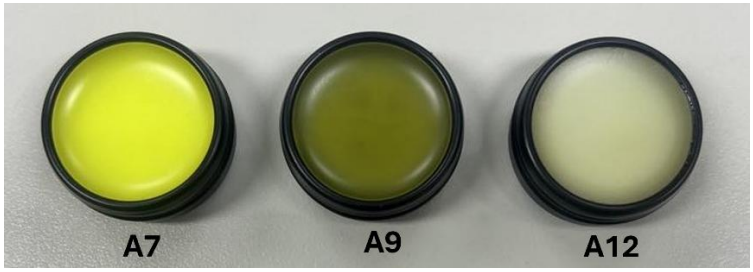



**Figure 3** *Z. montanum* balm (traditional extract) without cannabis extract (A7), with cannabis extract (A9) and *Z. montanum* balm (hexane extract) with cannabis extract (A12).

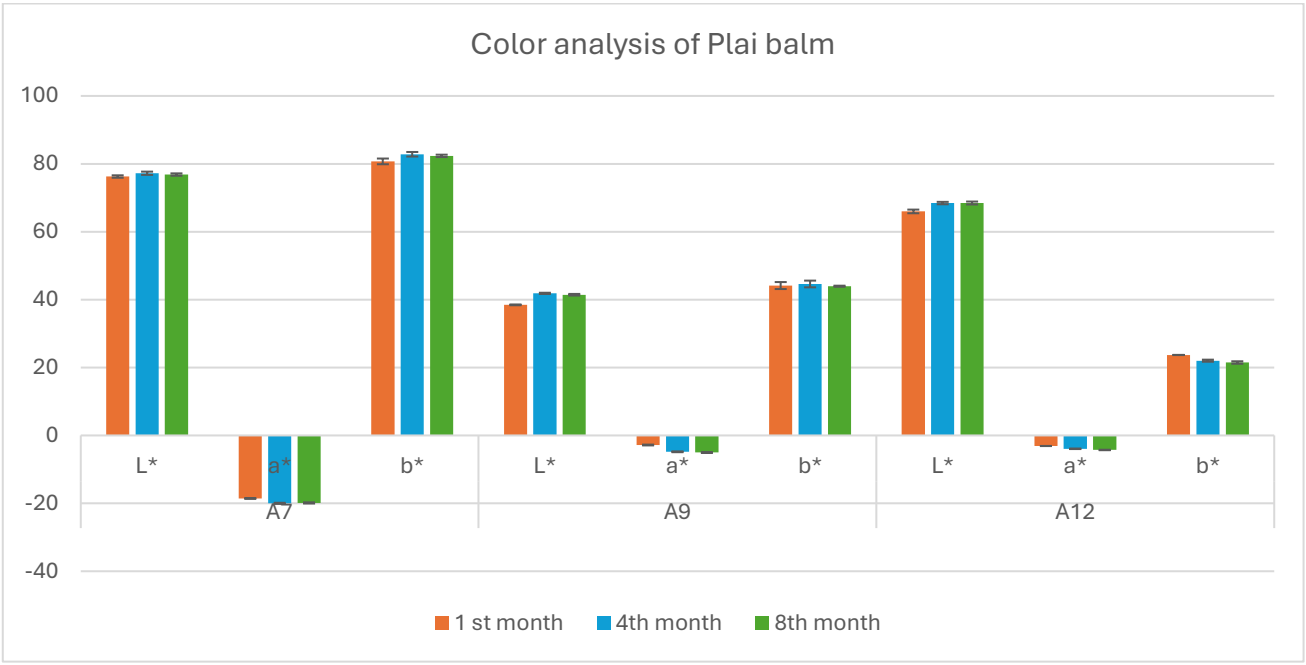



**Figure 4** The Color analysis of *Z. montanum* balm A7, A9 and A12 formulations at 1st, 4th, and 8th month after storage. L* is light color (+ lighter, - darker), a* red to green (+ red, - green) and b* is yellow to blue (+ yellow, - blue).

### 3.4 Spread ability of *Z. montanum* balm

The spread ability test was conducted by applying *Z. montanum* balms, stored at room temperature, onto glass slides. The spread ability was evaluated visually (Table 3) and under a microscope (Figure 5). The *Z. montanum* balm formulations A9 and A12 exhibited a smoother texture compared to A7. Azmin et al. [9] and Visht et al. [21] reported that lip balm initially had a good texture for spreading under normal temperature conditions but deteriorated by the second week. Additionally, the lip balm stored at chiller temperature showed poorer spread ability compared to storage at room temperature during both the first and second weeks. However, microscopic examination revealed no bubbles in the *Z. montanum* balm formulations A7, A9, and A12. Notably, the A9 formulation exhibited more color variation than A7 and A12,

likely due to its composition. The A9 balm contained both traditionally extracted *Z. montanum* rhizome, which has a yellow hue, and *C. sativa* L. flower extract, which appears green.

**Table 3** Spread ability of various formulations of *Z. montanum* balm at 25 °C

| Treatments | 0 days | 5 days | 10 days |
|---|---|---|---|
| A7 | I | B | B |
| A9 | G | G | I |
| A12 | G | G | I |

Remark: G = Good (smooth, consistent, flawless application, no fragmentation, and no Thai balm distortion), I = Intermeddle (consistent, few leaves that are fragmented, proper application, and minimal Thai balm deformation),
B = Bad (not uniform, leaves a lot of pieces, is applied improperly, and causes Thai balm to distort).

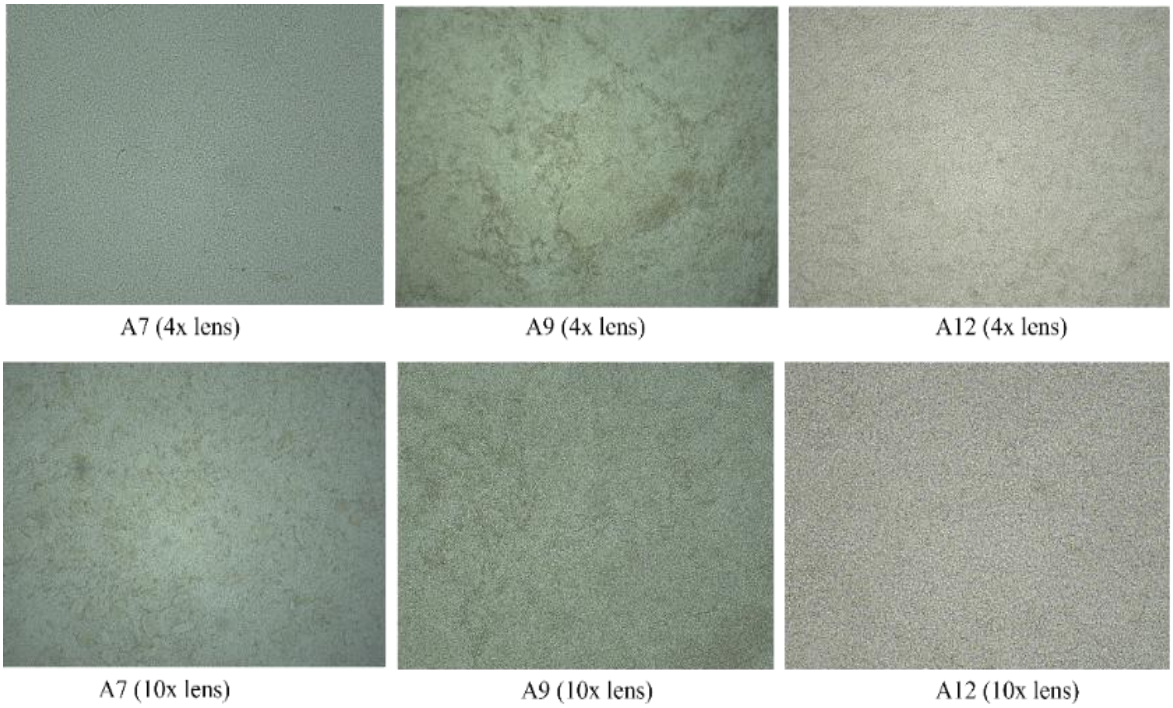



**Figure 5** Balm texture was performed using compound light microscope with 4x and 10x objective lens.

## 4. Conclusions

The balm formulations were prepared using *Zingiber montanum* rhizome extract and combined with *C. sativa* L. flower extract to enhance their pharmaceutical properties. *Z. montanum* extract contained DMPBD, a bioactive compound known for its antioxidant, anti-inflammatory, and muscle pain-relieving effects, as well as its ability to aid in ankle sprain recovery. Adding *C. sativa* L. flower extract further enriched the balm with bioactive compounds, including cannabidiol (CBD), for enhanced anti-inflammatory benefits.

Stability analysis based on color analysis at 0, 4, and 8 months showed that formula A7 had the highest yellow intensity, formula A9 had the highest green intensity, and formula A12 had the highest brightness (whiteness) compared to A7 and A9. Notably, no significant color changes were observed in any formulation after 8 months of storage. A spread ability test, conducted visually and under 4× and 10× objective lenses magnification of a compound light microscope, revealed that formulas A9 and A12 had a smoother texture than A7. These findings suggest that the *Z. montanum* balm,

with the addition of *C. sativa* L. flower extract, not only enhances bioactive properties but also maintains good texture and green color stability for at least 8 months. Thus, the further study is the development of a standardized medicinal balm product.

**Acknowledgements:** This research grant was supported by Kasetsart University Sriracha Campus, Chonburi, Thailand.

## References

1. Jin, D, Dai, K, Xie, Z, Chen, J (2020) Secondary Metabolites Profiled in Cannabis Inflorescences, Leaves, Stem Barks, and Roots for Medicinal Purposes. Sci Rep. 10(1):3309.
2. Wakshlag, JJ, Cital, S, Eaton, SJ, Prussin, R, Hudalla, C (2020) Cannabinoid, Terpene, and Heavy Metal Analysis of 29 Over-the-Counter Commercial Veterinary Hemp Supplements. Vet Med Res. 11:45–55.
3. Alolga, RN, Wang, F, Zhang, X, Li, J, Tran, LSP, Yin, X (2022) Bioactive Compounds from the Zingiberaceae Family with Known Antioxidant Activities for Possible Therapeutic Uses. Antioxidants, 11:1281.
4. Thepthong, P, Rattakarn, K, Ritchaiyaphum, N, Intachai, S, Chanasit, W (2023) Effect of Extraction Solvents on Antioxidant and Antibacterial Activity of *Zingiber montanum* Rhizomes. ASEAN J Sci Tech. 26(3):1–9.
5. Jeenapongsa, R, Yoorathaworn, K, Pongprayoon, U, Sriwatanakul, K (2003) Anti-inflammatory activity of (E)-4-(3,4-dimethoxyphenyl)butadiene from *Zingiber cassumuar* Roxb. J Ethnopharmacol. 87, 143-148.
6. Devkota, HP, Paudel, KR, Hassan, MM, Dirar, AI, Das, N, Adhikari-Devkota, A, Echeverría, J, Logesh, R, Jha, NK, Singh, SK, Hansbro, PM, Chan, Y, Chellappan, DK, Dua, K (2021) Bioactive compounds from *Zingiber montanum* and their pharmacological activities with focus on zerumbone. Appl Sci. 11(21):1–24.
7. Hassan, MM, Adhikari-Devkota, A, Imai, T, Devkota, HP (2019) Zerumbone and kaempferol derivatives from the rhizomes of *Zingiber montanum* (J. Koenig) link ex a. dietr. from Bangladesh. Separations, 6(2):31.
8. Seaho B, Lekwongphaiboon C, Inthakusol W, Prateeptongkum S, Harnying W, Berkessel A, Duangdee N (2024) NMR-based stability evaluation of (E)-1-(3',4'-dimethoxy phenyl) butadiene (DMPBD) from *Zingiber cassumunar* Roxb. rhizome. Phytochem Anal. 35(3):579-585.
9. Azmin, M, Huda, SN, Sulaiman, NS, Binti Yosri, NA, Mat Nor, MS, Abdullah, PS (2021) Stability analysis of carrot-based natural moisturising lip balm. Chem Eng Trans. 83:49–54.
10. Chongmelaxme, B, Sruamsiri, R, Dilokthornsakul, P, Dhippayom, T, Kongkaew, C, Saokaew, S, Chuthaputti, A, Chaiyakunapruk, N (2017) Clinical effects of *Zingiber cassumunar*: A systematic review. CTHMES 35:70–77.
11. Chienthavorn, O, Poonsukcharoen, T, Pathrakorn, T (2011) Pressurized liquid and superheated water extraction of active constituents from *Zingiber cassumunar* Roxb. rhizome. Sep Sci Technol 46(4):616–624.
12. Leelarungrayub, J, Manorsoi, J, Manorsoi, A (2017) Anti-inflammatory activity of niosomes entrapped with Plai oil (*Zingiber cassumunar* Roxb.) by therapeutic ultrasound in a rat model. Int J Nanomedicine. 12:2469–2476.
13. Ferreira, BP, Costa, G, Mascarenhas-Melo, F, Pires, PC, Heidarizadeh, F, Giram, PS, Mazzola, PG, Cabral, C, Veiga, F, Paiva-Santos, AC (2023) Skin applications of cannabidiol: sources, effects, delivery systems, marketed formulations and safety. Phytochem Rev. 22:781–828.
14. Hamelink C, Hampson A, Wink DA, Eiden LE, Eskay RL (2005) Comparison of cannabidiol, antioxidants, and diuretics in reversing binge ethanol-induced neurotoxicity. J Pharmacol Exp Ther. 314(2):780-8.
15. Varadi, G, Zhu, Z, Crowley, HD, Moulin, M, Dey, R, Lewis, ED, Evans, M (2023) Examining the Systemic Bioavailability of Cannabidiol and Tetrahydrocannabinol from a Novel Transdermal Delivery System in Healthy Adults: A Single-Arm, Open-Label, Exploratory Study. Adv Ther 40(1):282–293.
16. D'Souza DC, Perry E, MacDougall L, Ammerman Y, Cooper T, Wu YT, Braley G, Gueorguieva R, Krystal JH. (2004) The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. Neuropsychopharmacol. 29(8):1558-72.
17. D'Souza DC, Ranganathan M, Braley G, Gueorguieva R, Zimolo Z, Cooper T, Perry E, Krystal J. (2008) Blunted psychotomimetic and amnestic effects of delta-9-tetrahydrocannabinol in frequent users of cannabis. Neuropsychopharmacol. 33(10):2505-16.
18. Mahmoudinoodezh, H, Telukutla, SR, Bhangu, SK, Bachari, A, Cavalieri, F, Mantri, N. (2022) The Transdermal Delivery of Therapeutic Cannabinoids. Pharmaceutics, 14(2).
19. Azmin, SNHM, Jaine, NIM., Nor, MSM. (2020) Physicochemical and sensory evaluations of moisturizing lip balm using natural pigment from Beta vulgaris. Cogent Eng. 7:1, 1788297.
20. Fernandes, A.R., Dario, M.F., Pinto, C.A.S.O., Kaneko, T.M., Baby, A.R. Velasco, M.V.R. (2013). Stability evaluation of organic Lip Balm. Braz. J. Pharm. Sci. 49 (2), 293-299.
21. Visht, S, Salih, SS, Mohammed, DA, Abduljabbar, AA, Hama, SJ, Khudhair, IA (2024) Formulation and Evaluation of Lip Balm Using Different Herbal Pigments. Pharmacogn. Res, 16(2), 367–375.

Proceeding No. OYS01

# การศึกษาปริมาณผลึกแคลเซียมออกซาเลตในผักพื้นเมือง จังหวัดอุบลราชธานี

# The Calcium Oxalate Crystals in Local Vegetable from Ubon Ratchathani Province, Thailand

รชยา ไทยาภิรมย์ [1], ประศาสตร์ เกื้อมณี [2,*]
Rachaya Thaiyapirom [1], Prasart Kuemanee [2,*]

[1] โรงเรียนสาธิตมหาวิทยาลัยศรีนครินทรวิโรฒ ปทุมวัน, กรุงเทพฯ ๑๐๓๓๐
[1] Satitpatumwan Demonstration School, Bangkok 10330
[2] มหาวิทยาลัยเกษตรศาสตร์ ภาควิชาพฤกษศาสตร์, กรุงเทพฯ ๑๐๙๐๐
[2] *Department of Botany Faculty of Science Kasetsart University, Bangkok 10900
Correspondence: fscipsk@ku.ac.th

**บทคัดย่อ:** ศึกษาชนิดและความหนาแน่นของผลึกแคลเซียมออกซาเลตในผักพื้นบ้านจังหวัดอุบลราชธานี จำนวน 25 ชนิด โดยศึกษาในใบลำดับที่ 1-5 จากปลายยอดใช้เทคนิคการทำ Tissue clearing จากนั้นนำไปศึกษาด้วยกล้องจุลทรรศน์แบบใช้แสง ผลการศึกษาพบการปรากฏของผลึกในใบพืช จำนวน 19 ชนิด รูปร่างของผลึกที่พบมี 5 แบบ ได้แก่ รูปดาว รูปปริซึม รูปแท่ง รูปเข็ม และรูปทราย พบการปรากฏของผลึกมากบริเวณชั้นมีโซฟิลล์ของใบ และตามแนวเส้นใบ ยิ่งไปกว่านั้นผลึกในใบแก่มีขนาดใหญ่และเยอะกว่ากว่าในใบอ่อน ออกซาเลตที่อยู่ในรูปผลึกจะถูกดูดซึมโดยร่างกายได้น้อยกว่าออกซาเลตที่ละลายน้ำ ดังนั้นการบริโภคผักที่มีผลึกอาจลดความเสี่ยงต่อการเกิดนิ่วมากกว่าผักที่ไม่มีผลึก

คำสำคัญ: ผักพื้นบ้าน ผลึกแคลเซียมออกซาเลต

**Abstract:** This study examined the types and densities of calcium oxalate crystals in 25 local vegetables from Ubon Ratchathani province, Northeastern Thailand were investigated. The young and mature leaves of 1st-5th from shoot tip were used in this study. The samples were subjected to a tissue cleaning technique. The specimens were observed under a compound light microscope. The crystals were found in 19 of 25 species. Five types of crystals were classified including druse, prismatic, styloid, raphide, and crystal sand. The crystals were predominantly found in the mesophylls and along the veins. Moreover, crystal sizes were larger and a lot more in mature leaves compared to younger ones. Oxalate in crystal form is less absorbed by the body than soluble oxalate. Hence, the consumption of vegetables containing calcium oxalate crystals may pose a lower risk of urolithiasis than the vegetable without calcium oxalate crystals.

Keywords: Local vegetables, Calcium oxalate crystals

## 1. Introduction

Kidney stones in the urinary tract are a public health problem found throughout the world. The worldwide prevalence of kidney stones is increasing globally. The factors that are affecting prevalence and incidence include age, gender, race, ethnicity, occupation, climate, geography, systemic diseases, diabetes, vascular disease, chronic kidney disease, and dietary risk factors relevant to kidney stones [1]. According to the Ministry of Public Health report in 2021, the incidence of stones in the urinary tract is very common in the northern and northeastern regions [2]. Northeast Thailand reported Kidney stones one-year-prevalence rates of 16.9% [3]. A study in the past in Ubon Ratchathani province found 3.8% of urinary tract stones [4] and at present the incidence of urinary tract stones in urinary patients from Sunpasitthiprasong Hospital, Ubon Ratchathani Province, found patients receiving treatment at the Urological Surgery Department in 2021, 396 cases per year, and in 2022, there were 479 cases. It can be seen that urinary tract stones have an increasing trend and are a public health problem in the country. Therefore, it is necessary to focus on preventing the occurrence of stones in the urinary tract.

Calcium oxalate crystal is the cause of urinary tract stones which are most commonly found in Thailand [5]. In normal individuals, approximately half of urinary oxalate is derived from diet and half from endogenous synthesis [6]. Consumption of foods containing water-soluble dietary oxalate has an effect on the formation of stones in the urinary tract. Oxalate combines with calcium ions to form calcium oxalate stones in the urinary tract [7], which reported that the regular consumption of foods high in oxalate is at risk of developing stones in the urinary tract [8].

Oxalates present in plants come in two forms: soluble oxalate and insoluble oxalate (crystalline CaOx), which together form the total oxalates [9]. These oxalates are mostly stored in plant tissues as calcium oxalate crystals [10]. In general, oxalates are mainly accumulated in leaves and least accumulated in the stems [11]. The insoluble oxalate or calcium oxalate crystals found in plants come in five forms: prismatic crystals, raphide crystals, druse crystals, styloid crystals, and crystal sands. The form and appearance of calcium oxalate is characteristic of each type of plant or specific group. Therefore, it can be used to classify plant taxonomy [12]. Consumption of foods with high amounts of insoluble oxalate is not likely to be harmful to health, but it may cause irritation when eaten [13,14].

In the past, calcium oxalate crystals in local vegetables have been studied, such as research on local vegetables 20 species in Nong Khai Province by making the leaf blades clear with a solution of sodium hydroxide (NaOH) [15], calcium oxalate crystals were found in the leaves of 12 types of plants, which had 5 shapes: druse, prismatic, raphide, styloid and sand crystal. The density of crystals tends to decrease in older leaves [16].

Another research studied 20 types of local vegetables in Nakhon Ratchasima province, by making the leaf blades clear with sodium hydroxide (NaOH) solution [15], calcium oxalate crystals were found in the leaves of 15 types of plants and in the stems of 13 plants. There are four types of crystals found in both leaves and stems: druse crystal, prismatic, styloid, and raphide. The density of most crystals tends to decrease in older leaves. [17].

The other research studied some medicinal plants in Ubon Ratchathani Province by cutting across the leaf blades and staining them with safranin dye. It was found that the distribution of calcium oxalate crystals found in the leaves differed in each type of plant. Crystals spread in the epidermis tissue and the mesophyll layer: consisting of druse crystals, prismatic, and raphide. [18]

A similar research on local vegetables, in the central district of Chiang Mai province, was studying and comparing the size and quantity of crystals. By cutting crosswise (free-hand section), it was found that plants could be grouped according to the calcium oxalate crystals found in 4 groups: druse crystals, prismatic, raphide, and sand crystals. Plants can be divided according to the amount of crystals found into 4 groups: a lot of crystals, medium crystallinity, few crystals, and the group that did not find crystals [19].

From past studies of crystals, the appearance is still missing the size and density of calcium oxalate crystals at the same time and does not yet cover all types of vegetables that are consumed. Therefore, the objective of this research was to study the types, size, and density of calcium oxalate crystals in some local vegetables consumed in Ubon Ratchathani Province. This research used the technique of Tissue Clearing [20], which the results from studying the crystals can help in classifying plants and identifying species. The size and density of crystals in each type of local vegetable can be used as a primary guideline for choosing to consume local vegetables with high density and large insoluble calcium oxalate crystals as an indicator for less absorption to decrease the risk of developing urinary tract stones.

## 2. Material and Methods

### 2.1. Selected plant samples

Twenty five samples of local vegetables collected from the market in Central District, Ubon Ratchathani Province.

**Table1** Showing samples of plants used for research

| Scientific name | Common name | Vernacular name | Family name |
|---|---|---|---|
| *Adenanthera pavonina* L. | Growing Sandalwood | ผักอีล่ำ | Fabaceae |
| *Allium tuberosum* Rottler ex Spreng. | Chinese chives, Garlic chives | กุยช่าย | Amaryllidaceae |
| *Amaranthus gangeticus* L. | Elephant-head Amaranth | ผักโขมใหญ่ | Amaranthaceae |
| *Amaranthus lividus* Lin. | Pigweed | ผักโขมเล็ก | Amaranthaceae |
| *Anethum graveolens* L. | Dill | ผักชีลาว | Apiaceae |
| *Brassica juncea* (L.) Czern and Coss | Indian/Chinese mustard | ผักหิ่น | Brassicaceae |
| *Brassica oleracea* L. Group Acephala | Kale, Collard greens | ผักคะน้า | Brassicaceae |
| *Careya arborea* Roxb. | Wild guava | กระโดน | Lecythidaceae |
| *Cratoxylum formosum* (Jacq.) Benth. & Hook.f. ex Dyer subsp. formosum | Mempat tree | ผักติ้ว | Hypericaceae |
| *Emilia sonchifolia* (L.) DC. ex Wight | Lilac tasselflower | *ผักลิ้นปี่* | Asteraceae |
| *Eryngium foetidum* L. | *Culantro* | ผักชีฝรั่ง | Apiaceae |
| *Ipomoea aquatica* Forssk. | Swamp morning glory, Water spinach | ผักบุ้ง | Convolvulaceae |
| *Ipomoea batatas* (L.) Lam. | Sweet potato | มันเทศ | Convolvulaceae |
| *Limnocharis flava* (L.) Buchenau | Yellow bur-head, Yellow sawah lettuce, Yellow velvetleaf | ผักพาย | Alismataceae |
| *Limnophila aromatica* (Lam.) Merr. | Rice Paddy Herb | ผักแขยง | Plantaginaceae |

| | | | |
|---|---|---|---|
| *Ludwigia adscendens* (L.) H.Hara | Creeping water primrose | ผักพังพวย | Onagraceae |
| *Manihot esculenta* Crantz | Cassava | *มันสำปะหลัง* | Euphorbiaceae |
| *Marsilea crenata* C.Presl | Water-clover | ผักแว่น | Marsileaceae |
| *Melientha suavis* Pierre | Phak wan | ผักหวาน | Opiliaceae |
| *Monochoria vaginalis* (Burm.f.) C.Presl ex Kunth var. angustifolia G.X Wang | Oval-leaf Monochoria | ผักอีฮิ้น | Pontederiaceae |
| *Moringa oleifera* Lam. | Drumstick tree/ Horseradish tree | มะรุม | Moringaceae |
| *Oroxylum indicum* (L.) Benth. ex Kurz | Broken Bones Tree, Midnight horror | ลิ้นฟ้า | Bignoniaceae |
| *Piper sarmentosum* Roxb. | Betel Leaf | ช้าพลู | Piperaceae |
| *Syzygium gratum* (Wight) S.N. Mitra | Eugenia (Phak Mek) | ผักเสม็ด | Myrtaceae |
| *Vigna unguiculata* (L.) Walp. subsp. sesquipedalis (L.) Verdc. | Asparagus pea, Yard-long bean | ถั่วฝักยาว | Fabaceae |

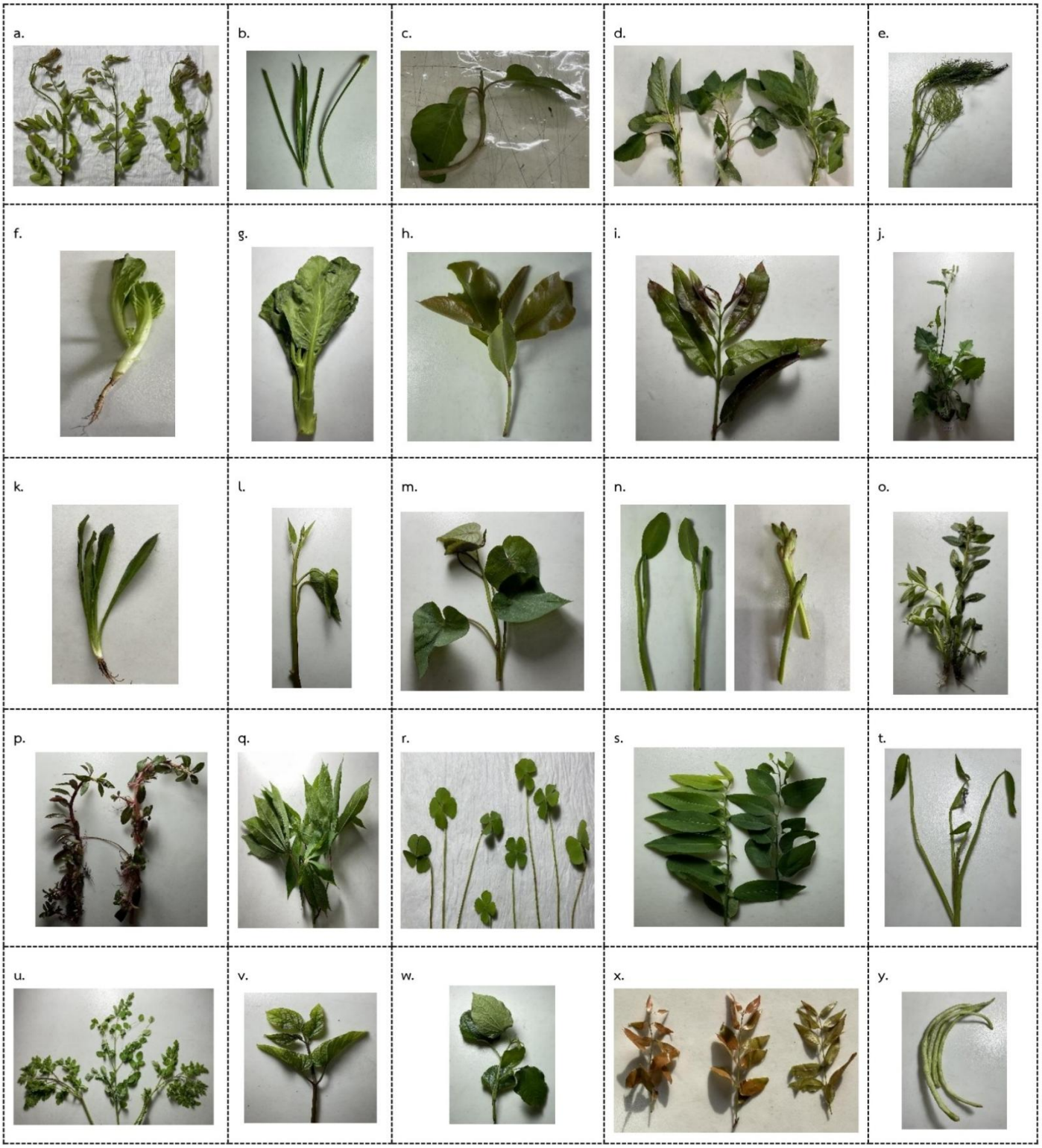



**Table 2** Local vegetable a. *Adenanthera pavonina* L., b. Allium tuberosum Rottler ex Spreng., c. *Amaranthus gangeticus* L., d. *Amaranthus lividus* Lin., e. *Anethum graveolens* L., f. *Brassica juncea* (L.) Czern and Coss, g. *Brassica oleracea* L. Group Acephala, h. *Careya arborea* Roxb., i. *Cratoxylum formosum* (Jacq.) Benth. & Hook.f. ex Dyer subsp. formosum, j. *Emilia sonchifolia* (L.) DC. ex Wight, k. *Eryngium foetidum* L., l. *Ipomoea aquatica* Forssk., m. *Ipomoea batatas* (L.) Lam., n. *Limnocharis flava* (L.) Buchenau, o. *Limnophila aromatica* (Lam.) Merr., p. *Ludwigia adscendens* (L.) H.Hara, q. *Manihot esculenta* Crantz, r. *Marsilea crenata* C.Presl, s. *Melientha suavis* Pierre, t. *Monochoria vaginalis* (Burm.f.) C.Presl ex Kunth var. angustifolia G.X Wang, u. *Moringa oleifera* Lam., v. *Oroxylum indicum* (L.) Benth. ex Kurz, w. *Piper sarmentosum* Roxb., x. *Syzygium gratum* (Wight) S.N. Mitra, และ y. *Vigna unguiculata* (L.) Walp. subsp. sesquipedalis (L.) Verdc.

### 2.2. Crystal appearance and Crystal density

All samples were prepared as permanent slides for study under a light microscope according to the following steps.

1. Take the leaves of plants number 1 - 5 from the tip of the shoot and cut the leaves to size 5 x 5 square millimetres. The samples were divided into 2 groups: leaves of plants number 1 - 2 (young leaves) and 3 - 5 (old leaves).
2. Put the sample into a beaker containing 3 ml of 10% v/v KOH and bring to a boil for 3 minutes.
3. Rinse with tap water 3 times, soaking for about 5 minutes each time.
4. Soak in NaClO 6% w/w for 30 minutes.
5. Rinse with tap water 3 times, soaking for about 5 minutes each time.
6. Extract water from the tissue with ethanol. Using concentrations of 30%, 50%, 70%, 95% and 100% respectively, each step takes approximately 10 minutes.
7. Soak in a mixture of absolute ethanol and xylene (ratio 1:1).
8. Soak in pure xylene for 3 hours.
9. Seal the slide (mount) with permount.
10. Analyze crystal types, measure size, measure density and record images under a compound light microscope (Olympus-CH30, Japan).

### 2.3. Checking and recording data

1.1 Studying the characteristics and size of calcium oxalate crystals. The size of calcium oxalate crystals was measured at 40X magnification in young and old leaves. 20 random crystals were measured in each plant's leaf and plant's type to find the mean crystal's size. 1 ocular scale equals 2.4 micrometers.

1.2 Count the number of crystals under the microscope. 5 random counts in each plant's leaf and plant's type to find the mean crystal's density. Random counts crystals were performed per frame of the ocular micrometer at 100× magnification, and densities were grouped as follows.

1.2.1 Crystal size (micrometer)
- No crystals found (0/-)
- Small crystal (1 – 10)
- Median (10 – 20)
- Large crystals (20 – up)

1.2.2 Number/amount of crystals (per $mm^2$)
- No crystals found (0/-)
- Few crystals (1 – 40)
- Median (40 – 80)
- Have a lot of crystals (80 – up or uncountable)

## 3. Results and Discussion

### 3.1. Research results

From a study of 25 local vegetables in Ubon Ratchathani Province; calcium oxalate crystals were found scattered in the leaf tissues of 19 studied plants, classified (1) Druse crystals located within mesophyll cells, which are spherical

aggregates of numerous sharp-tipped crystals, form a star-like shape and are typically large enough to occupy almost the entire cell. Found druse crystals in 12 plants: Pigweed, Elephant-head Amaranth with crystal also gather up inside vascular bundle, Phak wan, Rice Paddy Herb, Culantro, Broken bones tree, Lilac tasselflower, Wild guava (epidermis), Drumstick tree, Eugenia (Phak mek), Cassava, and Water-clover. (2) Prismatic crystals have a double sided pyramid (bipyramidal) and a long rectangular prism (styloid) inside the cell. Found bipyramidal prismatic crystals in 2 plants: Dill and Chives, and found styloid in 1 plant: Yardlong beans. (3) Raphide crystals are long, needle-like rods with sharp ends on both sides. Often gathered together in bundles or groups with a different number of crystals with one raphide for one cell. Found raphide crystals in 2 plants: Creeping water primrose and Oval-leaf monochoria. (4) Crystal sands are similar to sand grains, and found crystal sand in 2 plants: Betel leaf and Indian mustard. (5) No crystals found in 6 plants: Kale, Mempat tree, Sweet potato, Growing sandalwood, Morning glory, and Yellow bur-head.

**Table 3** Types and distribution of calcium oxalate crystals found in leaves of the selected medicinal plants

| Scientific names | Location | Crystal types[1] | Size (micron) | | Density ($mm^2$) | |
|---|---|---|---|---|---|---|
| | | | young | mature | young | mature |
| *Adenanthera pavonina* L. | Mesophyll | -[2] | -[2] | -[2] | -[2] | -[2] |
| *Allium tuberosum* Rottler ex Spreng. | Mesophyll | Prismatic (Bipyramidal) and druse | 0.17 ± 0.41 | 7.27 ± 1.8 | 24.00 ± 12.14 | 78.80 ± 21.92 |
| *Amaranthus gangeticus* L. | Mesophyll and vascular bundle | Druse | 19.92 ± 3.77 | 31.15 ± 10.87 | 123.40 ± 14.24 | 111.25 ± 41.13 |
| *Amaranthus lividus* L. | Mesophyll | Druse | 13.20± 1.248 | 39.84 ± 13.0 | 23.25 ± 27.43 | 75.73 ± 34.40 |
| *Anethum graveolens* L. | Mesophyll | Bipyramidal | 2.30 ± 0.22 | 6.70 ± 1.68 | 170 | 120.05 ± 71.42 |
| *Brassica juncea* (L.) Czern. | Mesophyll | Crystal sand | 1.2 | 2.06 ± 0.98 | 33 | *1 |
| *Brassica oleracea* L. Group Acephala | Mesophyll | -[2] | -[2] | -[2] | -[2] | -[2] |
| *Careya arborea* Roxb. | Epidermis | Druse | 2.47 ± 0.26 | 5.44 ± 1.03 | 60.50 ± 38.89 | 61.00 ± 15.53 |
| *Cratoxylum formosum* (Jacq.) Benth. & Hook.f. ex Dyer subsp. formosum | Mesophyll | -[2] | -[2] | -[2] | -[2] | -[2] |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Emilia sonchifolia* (L.) DC. ex Wight | (Palisade) Mesophyll | Druse | 2.4 | 14.76 ± 2.33 | Too small | 11.56 ± 14.25 |
| *Eryngium foetidum* L. | Mesophyll | Druse | 9.48 ± 2.59 | 17.16 ± 2.90 | 17.13 ± 15.41 | 7.60 ± 9.71 |
| *Ipomoea aquatica* Forssk. | Mesophyll | [2] | [2] | [2] | [2] | [2] |
| *Ipomoea batatas* (L.) Lam. | Mesophyll | [2] | [2] | [2] | [2] | [2] |
| *Limnocharis flava* (L.) Buchenau | Mesophyll | [2] | [2] | [2] | [2] | [2] |
| *Limnophila aromatica* (Lam.) Merr. | Mesophyll and along vascular bundle | Druse | [2] | 13.20 ± 3.07 | [2] | 16.00 ± 4.53 |
| *Ludwigia adscendens* (L.) H.Hara | Mesophyll | Raphides and druse | 72.00 ± 3.12 | 119.88 ± 24.19 | 1.75 ± 0.96 | 12.80 ± 9.66 |
| *Manihot esculenta* Crantz | Mesophyll | Druse | 4.39 ± 0.62 | 7.03 ± 1.99 | 83.86 ± 35.47 | 75.00 ± 37.03 |
| *Marsilea crenata* C.Presl | Mesophyll | Druse | 9.91 ± 1.66 | | 15.77 ± 7.82 | |
| *Meliantha suavis* Pierre | Mesophyll | Druse | 19.56 ± 3.24 | 24.55 ± 3.10 | 96.50 ± 1.29 | 110.00 ± 1.83 |
| *Monochoria vaginalis* (Burm.f.) C.Presl ex Kunth var. angustifolia G.X Wang | Mesophyll | Raphides | 32.09 ± 8.69 | 32.28 ± 9.65 | 48.20 ± 27.06 | 7.80 ± 8.52 |
| *Moringa oleifera* Lam. | Along vascular bundle | Druse | 9.24 ± 1.94 | 11.88 ± 1.66 | 9.5 ± 3.87 | 87.10 ± 34.39 |
| *Oroxylum indicum* (L.) Benth. ex Kurz | Vascular Bundle | Small Druse | 4.66 ± 0.77 | 4.56 ± 0.62 | 25.08 ± 33.93 | 44.20 ± 32.51 |
| *Piper sarmentosum* Roxb. | Mesophyll | Crystal sand | 1.25 ± 0.31 | | | [*1] |
| *Syzygium gratum* | Along vascular bundle | Druse | 6.6 ± 2.14 | 1.79 ± 1.66 | 13.67 ± 18.24 | 181.40 ± 124.99 |

| | | | | |
|---|---|---|---|---|
| *Vigna unguiculata* (L.) Walp. subsp. sesquipedalis (L.) Verdc. | Endocarp | Prismatic (Styloid) | 4.44 ± 0.89 x 17.89 ± 3.70 | 438.24 ± 461.33 |

*1 uncountable crystal sand which can't accurately count under a light microscope.

-2 no crystal found.

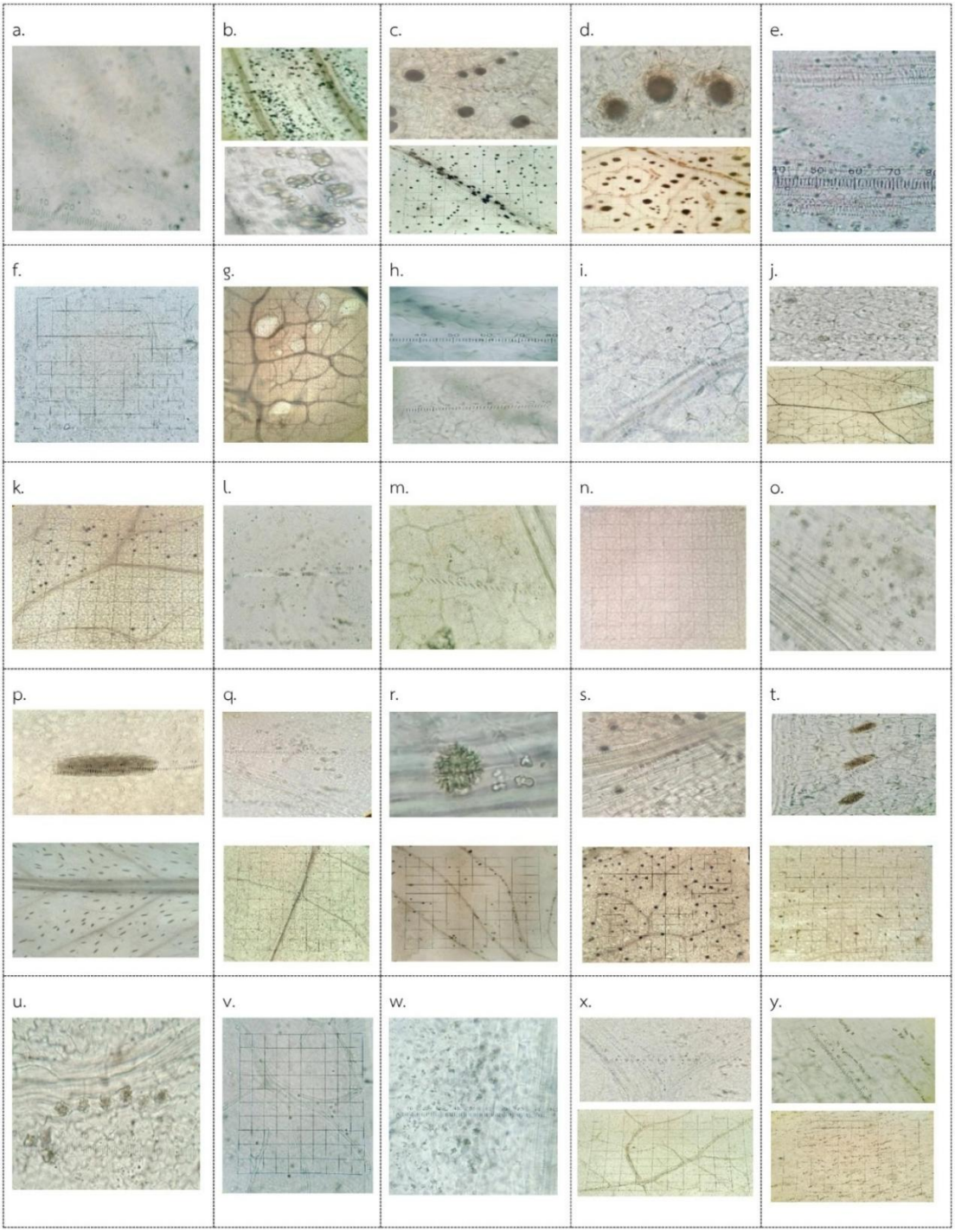



**Table 4** Calcium oxalate crystal in local vegetable a. *Adenanthera pavonina* L., b. Allium tuberosum Rottler ex Spreng., c. *Amaranthus gangeticus* L., d. *Amaranthus lividus* Lin., e. *Anethum graveolens* L., f. *Brassica juncea* (L.) Czern and Coss, g. *Brassica oleracea* L. Group Acephala, h. *Careya arborea* Roxb., i. *Cratoxylum formosum* (Jacq.) Benth. & Hook.f. ex Dyer subsp. formosum, j. *Emilia sonchifolia* (L.) DC. ex Wight, k. *Eryngium foetidum* L., l. *Ipomoea aquatica* Forssk., m. *Ipomoea batatas* (L.) Lam., n. *Limnocharis flava* (L.) Buchenau, o. *Limnophila aromatica* (Lam.) Merr., p. *Ludwigia adscendens* (L.) H.Hara, q. *Manihot esculenta* Crantz, r. *Marsilea crenata* C.Presl, s. *Melientha suavis* Pierre, t. *Monochoria vaginalis* (Burm.f.) C.Presl ex Kunth var. angustifolia G.X Wang, u. *Moringa oleifera* Lam., v. *Oroxylum indicum* (L.) Benth. ex Kurz, w. *Piper sarmentosum* Roxb., x. *Syzygium gratum* (Wight) S.N. Mitra, และ y. *Vigna unguiculata* (L.) Walp. subsp. sesquipedalis (L.) Verdc.

### 3.2. Discussion

Crystals play an important role in plants; crystal formation removes oxalic acid, which is a waste product from the metabolic process of cells, and is a regulator of calcium ions in cells [21]. In addition, crystals also help reduce insects and herbivores [22], especially plants with raphides will contain mucilage and proteinase together, causing irritation to animals [23,24].

Oxalate is contained in plants, presented in 2 forms: soluble oxalate and insoluble oxalate. They are mainly accumulated in plant tissues in the form of calcium oxalate crystals [10]. The shape of the crystals that are classified as calcium oxalate crystals has many forms, including raphide, druse, prismatic, crystal sand, and styloid [25,26]. The crystal shape and distribution of calcium oxalate crystals in plants is genetic and can be used to identify certain plant groups [27,28].

For human beings, oxalate is an anti-nutrient and an excessive consumption of oxalate-rich foods inhibits mineral absorption or creates calcium oxalate, which increases the risk of some diseases, such as kidney stones, in the digestive systems [11,29,30]. Oxalate is the main substance that causes kidney stones in the urinary tract, especially calcium oxalate stones which found up to a percentage 75 of stones in the urinary tract [31]. If there is a large amount of calcium oxalate crystals in plants, there will be a lot of oxalate as well [32]. Total oxalate and soluble oxalate are more in young leaves than in old leaves, and in younger plants, there is a higher amount of oxalate than in older plants. [33,34].

When soluble oxalates are present in the plant, oxalic acid can combine with calcium to form insoluble calcium oxalate crystals. This process reduces the amount of soluble oxalate available in the plant tissue. Therefore, a high level of insoluble calcium oxalate may indicate that a significant portion of the soluble oxalate has been converted into an insoluble form [35]. Several pathways for oxalate production were proposed, including glycolate/glyoxylate oxidation, cleavage of ascorbate, and hydrolysis (breakdown) of oxaloacetate: intermediate in photosynthesis and cellular respiration pathway [36]. From the study of oxalate metabolic pathway genes in spinach [37]: The high oxalate concentrations in spinach were because of the high transcription levels of the genes that were involved in oxalate biosynthesis, under normal growth conditions. Oxalate degradation gene had high levels of expression in the mature leaf because of higher levels of oxalate accumulate more in older leaves [38]. Oxalate could be toxic to plants and could induce programmed cell death in plant organs [39,40]. Consequently, the plant organs had to maintain a high expression level for the genes from the oxalate degradation pathway, in order to maintain the steady state, so as to reduce the harm from the excess oxalate in the mature leaf. The overexpression of the oxalate degradation genes could remarkably reduce the oxalate content in plants [41-43]. Oxalate degradation genes appeared to be important factors in the regulation of oxalate content in spinach. Furthermore, a large amount of oxalate (about 60%) was found to be stored in an insoluble form, such as calcium oxalate. The amount of insoluble oxalates in mature leaves is higher than immature leaves. [37] Therefore, the oxalate degradation steps, especially oxalate degradation genes, The calcium oxalate not only played a very important role in changing the concentration of calcium but also in regulating the oxalate levels [44].

Oxalate was already created in the young leaves, but the gene that converted the soluble into insoluble was switched on in the mature leaves, which can infer that soluble oxalate has been degraded or changed into insoluble more in the mature leaves. Failure to detect crystals does not indicate whether the plant has a small amount or less oxalate than the type in which crystals were detected. This is because oxalate in that plant may be in the form of soluble calcium oxalate more than form of calcium oxalate crystals [16]. The amount of oxalate that has a greater effect on the amount of calcium absorption and the formation of stones in the urinary tract is the soluble oxalate, which oxalate combines with calcium ions to form insoluble oxalate calcium crystals [16]. Although in this study cannot tell how much soluble oxalate is present in each type of plant, because the oxalate content analyzed in this study was the amount of insoluble calcium oxalate crystals. But from the study of all oxalate content it was found that some species without crystals found contained higher total oxalate content than the species in which crystals were observed. [16]

Leaves with no crystals found may indicate that oxalate may be accumulated in soluble oxalate form, the form that is available for body absorption and developing into calcium oxalate kidney stone. Kale, Mempat tree, Sweet potato, Growing sandalwood, Morning glory, and Yellow bur-head are vegetables that do not find crystals. Vegetables that are found with large size or large amounts of crystals in the mature leaves, most of the crystals are presented all over mesophyll layer and along the leaf vein, such as Phak wan, Creeping water primrose, but especially in Pigweed and Elephant-head amaranth that addition calcium oxalate crystals are accumulated in bundle sheath cells along the leaf vein. Vegetables with median size and high density are Chives: as the prismatics are stuck together forming a large group complex crystals decreasing the risk of absorption, and Yardlong beans. With high density can suggest that a lot of soluble oxalate has been changed into insoluble form. As insoluble oxalate substances are less absorbed, and the larger the crystal size, the less absorption will occur [21].

Vegetables with median size but lower density have crystals mainly occurring along the leaf vein and few of the crystals are found in mesophyll layer, which include Oval-leaf monochoria, Water-clover, Lilac tasselflower, Culantro, and Drumstick tree with medium density. Betel leaf and Indian mustard contain crystal sand which are very small in size but high density inside the cells. Broken bones tree, Dill, Rice paddy herb, Wild guava, Eugenia (Phak mek) and Cassava are plants that contain small and lower density crystals, which the crystals are mainly aggregated along the leaf vein.

Pigweed, Elephant-head amaranth, Phak wan, Drumstick tree, and Culantro have similar druse crystals with star shape. In Pigweed and Elephant-head amaranth, crystals were found distributed throughout the mesophyll layers, and crystal accumulation was also found in cells along the leaf veins, which have a greater accumulation of crystals. Crystals in Phak Wan and Culantro are smaller and evenly distributed throughout the leaves and in pak wan also has crystal distribution along the leaf veins. Drumstick tree has druse mostly accumulated in the cells along the leaf veins.

Water-clover and Wild guava have a small druse: star-shaped crystal, but especially in Wild guava, druse crystals are very small that can only see at 400x magnification as also having small amounts. In Broken bones tree and Eugenia (Phak mek) have small circular druse crystals that are found scattered along the leaf veins, similar with Broken bones tree's younger leaves which can indicate that crystal is starting to form. Cassava also has a circular druse crystal but distributes randomly in the mesophyll layer and also along the leaf vein. Lilac tasselflowers mostly do not find crystal, but can rarely find druse in some leaves.

Raphide is found in the leaves of Creeping water primrose and Oval-leaf monochoria, but in Creeping water primrose is found a larger and denser crystal than in Oval-leaf monochoria leaves. In some Creeping water primrose's leaves found druse along with raphide. Prismatic crystal is found in Chives, Dill, and Yardlong beans. Both Chives and Dill have a bipyramidal crystal, but in Chives; crystals are larger and densely packed together than Dill. Some chive's leaves have druse instead of prismatic crystal. Yardlong beans have styloid crystals which are highly organized in the endocarp. Betel leaf and Indian mustard have crystal sand which consist of many very small crystals clumped inside cells. From this study it was found that the mature leaves have a larger crystal than young leaves.

When comparing the results of this study with the other report [16] who studied the presence of calcium oxalate crystals and oxalate content in some vegetables from Nong Khai province. It was found that the results of the study were similar among the same plants studied. Calcium oxalate crystals were found similarly in plants with the same genus, like Pigweed and Elephant-head Amaranth (Genus *Amaranthus*) with druse in the mesophyll layer, in genus *Brassica*: Chinese cabbage reported that no crystals were found and in this study, also no crystals were found in kale, in genus *Limnophila*: Rice paddy herb found druse, in genus *Marsilea*: in the study it was reported that no crystals were found but Water-clover in this study found druse, in genus *Monochoria*: Oval-leaf monochoria found raphide in the mesophyll cells of the leaves, and in genus *Syzygium*: *Syzygium gratum* found prismatic, but Eugenia (Phak mek) in this study found small circular druse along the leaf veins.

This study of 25 local vegetables of Ubon Ratchathani Province aim was to survey the presence and total amount of calcium oxalate crystals in some vegetables consumed in Ubon Ratchathani Province. The results of the study, in addition

to gaining additional information from those who have previously studied some local vegetables, each plant has different size and amount of calcium oxalate crystal which can further make a classification combines with future study to make an actual recommendation for consumption of vegetables which may reduce the risk of developing kidney stone from dietary oxalate factor.

## 4. Conclusions

From studying the presence: size and density of calcium oxalate crystals in the leaves of local vegetables commonly consumed in Ubon Ratchathani Province, found that 19 plants found crystals and 6 plants do not found crystal, which can be classified into group according to their crystal size and amount of crystal in the mature leave. The vegetables with large crystals and high density are Pigweed, Elephant-head amaranth, Phak wan, Creeping water primrose, Chives, and Yardlong beans. Median size with lower density, including Oval-leaf monochoria, Water-clover, Lilac tasselflower, Culantro, and Drumstick tree. Vegetables with smaller crystal size include Betel leaf, Indian mustard, Broken bones tree, Dill, Rice paddy herb, Wild guava, Eugenia (Phak Mek), and Cassava. Lastly, vegetables with no crystal include Kale, Mempat tree, Sweet potato, Growing sandalwood, Morning glory, and Yellow bur-head. From this study, it can be concluded that there are larger crystals in older leaves than in young leaves, as in some plants, crystals have just begun to form and have not yet been formed in young leaves. Each plant has their own characteristic of calcium oxalate crystal. Each plant has a different size and amount of calcium oxalate crystal which can further combine with future study for making an actual recommendation for consumption of vegetables which may reduce the risk of developing kidney stones from dietary oxalate factors.

## 5. Suggestions

However, vegetables are considered nutritious foods, containing vitamins, minerals and high in dietary fiber. And it is the main food of Thai people in every region, including in Ubon Ratchathani province, that is influenced by the food consumption culture of Thai people from Vietnamese descent. This makes it a province where vegetables, especially fresh vegetables, are an ingredient or side dish in food. In addition, the consumption of vegetables is commonly eaten as cooked vegetables. Boiling or blanching will partially reduce the amount of oxalate [34]. In addition to avoiding the consumption of vegetables that contain soluble oxalate You should drink at least 2-3 liters of water per day and a high calcium dietary.

This research lacks data on the amount of water-soluble oxalate, which is more easily absorbed by the body than calcium oxalate crystal, but there are difficulties in the analysis, which addition of the data about the soluble oxalate content inside the plant might be necessary for accurate clarification.

This study is only a pre-study to find information on crystal size for the benefit of further research to solve the problem of stones in the urinary tract. The data indicates that the shoots (younger leaves) contain small crystals that are more easily absorbed by the body than the large crystals in older leaves. This would be a primary resource for further research that studies about the calcium oxalate kidney stone prevention that studies the absorption of crystals to be used as a way to prevent disease occurrence.

**Acknowledgements:** I would like to express my deepest gratitude to the Department of Botany at Kasetsart University, for providing facilities and guiding me through my botany journey. I am also sincerely thankful to Ms. Tassanee Klinhom, Senior Professional Nurse and Head of the Urology Surgery Ward at Sunpasittiprasong Hospital, Ubon Ratchathani, for her incredible support in gathering vegetable samples from the Ubon Ratchathani market. Her dedication to patient care, especially for those suffering from recurrent kidney stones, has been a driving force in this research. Her assistance has

provided me with a meaningful opportunity to explore the role of calcium oxalate crystals in local vegetables, aiming to address a significant health concern in Thailand.

## References

1. Stamatelou, K., & Goldfarb, D. S. (2023). Epidemiology of Kidney Stones. *Healthcare (Basel, Switzerland)*, *11*(3), 424.
2. Dueanphen Yoheuang, Napaphat Mongkhonakeomorn, Pakaporn Sangthong, Watcharin Sophap, Nuchanat Inchai, & Bandit Nuannate. (2021). Annual morbidity report, 2021 (1st ed.). Digital Health Group, Strategy and Planning Division, Office of the Permanent Secretary, Ministry of Public Health, Ministry of Public Health.
3. Romero, V., Akpinar, H., & Assimos, D. G. (2010). Kidney stones: a global picture of prevalence, incidence, and associated risk factors. *Reviews in urology*, *12*(2-3), e86–e96.
4. Halstead, S. B., & Valyasevi, A. (1967). Studies of bladder stone disease in Thailand: III. Epidemiologic studies in Ubol Province. *The American Journal of Clinical Nutrition, 20*(12), 1329–1339.
5. Sathit Siriboonrit. (2017). Pathophysiology of kidney stones. In Manin Asawachintachit, Ekarin Chotikawanich, Monthira Tanthanuch and Apirak Santikamkul (editors), stones in the urinary tract (1st printing). (P. 1-48). Association of Urological Surgeons of Thailand. Under the Royal Patronage.
6. Mitchell, T., Kumar, P., Reddy, T., Wood, K. D., Knight, J., Assimos, D. G., & Holmes, R. P. (2019). Dietary oxalate and kidney stone formation. *American journal of physiology. Renal physiology*, *316*(3), F409–F413.
7. Holmes RP. Goodman HO, Assimos D. (2004). Contribution of dietary oxalate to urinary oxalate excretion. *Kidney Int*. 2001;59:270-276.
8. Boonthong, T., & Muktabhant, B. (2018). Factors associated with the occurrence of urinary stone disease of adults living in Takrai – Nalare Village, Nasi Nuan Subdistrict, Muang District, Mukdahan Province. *Journal of Sakon Nakhon Hospital, 21*(1), 56–64.
9. Cai, X., Ge, C., Xu, C., Wang, X., Wang, S., & Wang, Q. (2018). Expression Analysis of Oxalate Metabolic Pathway Genes Reveals Oxalate Regulation Patterns in Spinach. *Molecules*, *23*(6), 1286.
10. Massey, L. K. (2007). Food oxalate: factors affecting measurement, biological variation, and bioavailability. *Journal of the American Dietetic Association* 107: 1191-1194.
11. Noonan, S. C., & Savage, G. P. (1999). Oxalate content of foods and its effect on humans. *Asia Pacific Journal of Clinical Nutrition, 8*(1), 64–74.
12. Wiroj Kaesornbua, Daralaporn Tanoochon, & Anisthan Srinuan. (2023). Patterns and Distribution of Calcium Oxalate Crystals in Leaves of Some Medicinal Plants in Ubon Ratchathani Province [รูปแบบและการกระจายของผลึกแคลเซียมออกซาเลตในใบพืชสมุนไพรบางชนิดในจังหวัดอุบลราชธานี]. *Ubon Ratchathani University Journal of Science and Technology*, 25(2), 38–48. (In Thai)
13. Savage, G.P., Vanhanen, L., Mason, S.M. and Ross, A.B. (2000). Effect of cooking on the soluble and insoluble oxalate content of some New Zealand foods. *Journal of Food Composition and Analysis.* 13:201–206.
14. Wanyo, P., Huaisan, K., Boothaisong, S., Nitisuk, P., Wongpreedee, P., & Chamsai, T. (2018). Effect of hot-air drying and vacuum drying on oxalate contents of *Limnophila aromatica* and *Limnophila geoffrayi*. *Food and Applied Bioscience Journal, 6*(2), 65–75.
15. Lersten, M. R. and Curtis, J. D. (2001). Idioblast and other unusual internal foliar secretory structures in Scrophulariaceae. *Plant Systematics and Evolution*, 227: 63-73.
16. Narumon Phiuphuen. (2014). Calcium oxalate crystals and oxalate content in some vegetables in Nong Khai Province. *Khon Kaen University Science Journal*, 42 (4), 820-829
17. Yasumin Kengkaeng, Phetcharat Jongthonglang, and Pusadee Promprasit. (2022). Detection of calcium oxalate crystals in local vegetables. Ban Mai Subdistrict, Khonburi District, Nakhon Ratchasima Province. *Journal of Science and Technology Research Nakhon Ratchasima Rajabhat University*, 7 (2), 75-82
18. Wirot Kesornbua, Daranporn Tanuchan, and Anittha Srinuan. (2023). Pattern and distribution of calcium oxalate crystals in the leaves of some medicinal plants in Ubon Ratchathani Province. *Journal of Science and Technology Ubon Ratchathani University*, 25 (2), 38-48
19. Warunee Na Lampang. (2015). Comparative study of calcium oxalate crystals in local vegetables from Mueang District, Chiang Mai Province [Master's thesis, Chiang Mai University]. Chiang Mai: Graduate School, Chiang Mai University.
20. Prasart Kuemanee. (2015). Detection of Crystals in Some Plants [การตรวจหาผลึกในพืชบางชนิด]. In *Proceedings of the 9th Botanical Conference of Thailand* (pp. 293–300). Department of Botany, Faculty of Science, Chulalongkorn University.
21. Franceschi, V.R. & Horner, H.T. (1980). Calcium oxalate crystals in Plants. *Botanical Review* 46: 361-427.
22. Finley, D.S. (1999). Patterns of calcium oxalate crystals in young tropical leaves: A possible role as an anti- herbivory defense. *Review of Biology Tropical* 47: 27-31.
23. Kaush, A.P. & Horner, H.T. (1983). The development of mucilaginous raphides crystals in young leaves of *Typha angustifolia* L. (Typhaceae). *American Journal of Botany* 70: 691-705.
24. Bradbury, J.H. & Nixon, R.W. (1998). The acridity of raphides from edible aroids. *Journal of Science and Food Agriculture* 76: 608-616.
25. Esau, K. (1977). Anatomy of Seed Plants. 2nd edition. Hoboken: John Wiley & Sons, Inc.
26. Franceschi, V.R. and Nakata, P.A. (2005). Calcium oxalate in plants: formation and function. *Annual Review of Plant Biology* 56: 41-71.
27. Nakata, P.A. (2002). Calcium oxalate crystal morphology. *Trends in Plant Science* 7: 324.
28. Nakata, P.A. (2003). Advances in our understanding of calcium oxalate crystal formation and function in plants. *Plant Science* 164: 901-909.

29. Holmes, R. P., Goodman, H. O., & Assimos, D. G. (2001). Contribution of dietary oxalate to urinary oxalate excretion. *Kidney International, 59*(1), 270–276.
30. Holmes, R. P., & Kennedy, M. (2000). Estimation of the oxalate content of foods and daily oxalate intake. *Kidney International, 57*(4), 1662–1667.
31. Hesse, A. and Siener, R. (1997). Current aspects of epidemiology and nutrition in urinary stone disease. *World Journal of Urology* 15: 165-171.
32. Nakata, P.A. and McConn, M.M. (2007). Calcium oxalate content affects the nutritional availability of calcium from Medicago truncatula leaves. *Plant Science* 172: 958-961.
33. Kitchen JW, Burns EE (2006). The effect of maturity on the oxalate content of spinach (*Spinacia oleraceae* L.). *Journal of Food Science* 30(4):589–593.
34. Oscarsson, K. V. and Savage, G. P. (2007). Composition and availability of soluble and insoluble oxalates in raw and cooked taro (*Colocasia esculenta* var. Schott) leaves. *Food Chemistry* 101: 559-562.
35. Tuazon-Nartea, J., & Savage, G. P. (2013). Investigation of Oxalate Levels in Sorrel Plant Parts and Sorrel-Based Products. *Food and Nutrition Sciences*, *2013*(8), 838–843.
36. Cai, X. F., Xu, C. X., Wang, X. L., Ge, C. H., & Wang, Q. H. (2015). The oxalic acid in plants: Biosynthesis, degradation, and its accumulation regulation (Review in Chinese). *Plant Physiology Journal, 51*(3), 267–272.
37. Cai, X., Ge, C., Xu, C., Wang, X., Wang, S., & Wang, Q. (2018). Expression Analysis of Oxalate Metabolic Pathway Genes Reveals Oxalate Regulation Patterns in Spinach. *Molecules*, *23*(6), 1286.
38. Kaminishi, A., & Kita, N. (2006). Seasonal change of nitrate and oxalate concentration in relation to the growth rate of spinach cultivars. *HortScience, 41*(6), 1589–1595.
39. Kim, K. S., Min, J. Y., & Dickman, M. B. (2008). Oxalic acid is an elicitor of plant programmed cell death during *Sclerotinia sclerotiorum* disease development. *Molecular Plant-Microbe Interactions, 21*(5), 605–612.
40. Errakhi, R., Meimoun, P., Lehner, A., Vidal, G., Briand, J., Corbineau, F., Rona, J. P., & Bouteau, F. (2008). Anion channel activity is necessary to induce ethylene synthesis and programmed cell death in response to oxalic acid. *Journal of Experimental Botany, 59*(11), 3121–3129.
41. Foster, J., Kim, H. U., Nakata, P. A., & Browse, J. (2012). A previously unknown oxalyl-CoA synthetase is important for oxalate catabolism in *Arabidopsis*. *The Plant Cell, 24*(3), 1217–1229.
42. Chakraborty, N., Ghosh, R., Ghosh, S., Narula, K., Tayal, R., Datta, A., & Chakraborty, S. (2013). Reduction of oxalate levels in tomato fruit and consequent metabolic remodeling following overexpression of a fungal oxalate decarboxylase. *Plant Physiology, 162*(1), 364–378.
43. Walz, A., Zingen-Sell, I., Loeffler, M., & Sauer, M. (2008). Expression of an oxalate oxidase gene in tomato and severity of disease caused by *Botrytis cinerea* and *Sclerotinia sclerotiorum*. *Plant Pathology, 57*(3), 453–458.
44. Franceschi, V. R., & Nakata, P. A. (2005). Calcium oxalate in plants: Formation and function. *Annual Review of Plant Biology, 56*, 41–71.

Proceeding No. PPH12

# การศึกษาฤทธิ์ต้านจุลชีพของสารสกัดจากพืชสมุนไพรจากตำรับยาพื้นบ้านไทย

# Investigating medicinal plant extracts from Thai traditional recipes for their antimicrobial effects

กัญธรส ปัทมปราณี [1], วิชาณี แบนคีรี [1], วรพนธ์ ชัยกีรติศักดิ์ [2], ธนิษฐา ฉัตรสุวรรณ [3], ธิติ สุทธิยุทธ์ [1,*]
Kantaros Padmapani [1], Wichanee Bankeeree [1], Vorrapon Chaikeeratisak [2] , Tanittha Chatsuwan [3], Thiti Suttiyut [1,*]

[1] ภาควิชาพฤกษศาสตร์ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[1] Department of Botany, Faculty of Science, Chulalongkorn University
[2] ภาควิชาชีวเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[2] Department of Biochemistry, Faculty of Science, Chulalongkorn University
[3] ภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
[3] Department of Microbiology, Faculty of Medicine, Chulalongkorn University
Correspondence: thiti.sut@chula.ac.th

**บทคัดย่อ:** ภาวะดื้อยาปฏิชีวนะเป็นปัญหาสำคัญในระดับโลก ซึ่งส่งผลกระทบต่อประสิทธิภาพในการรักษาโรคติดเชื้อ แนวทางหนึ่งที่มีศักยภาพในการแก้ไขปัญหาดังกล่าวคือ การศึกษาสารออกฤทธิ์ทางชีวภาพจากพืช ซึ่งมีความหลากหลายของโครงสร้างโมเลกุลและอาจนำไปพัฒนายาต้านจุลชีพจากธรรมชาติ งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาศักยภาพของพืชสมุนไพรไทย 10 ชนิดที่ใช้ในภูมิปัญญาพื้นบ้านในการรักษาอาการไข้และการติดเชื้อ โดยเตรียมสารสกัดหยาบด้วยเอทานอล 95% จากพืชอบแห้งด้วยวิธีการแช่ (maceration) จากนั้นนำมาทดสอบฤทธิ์ต้านจุลชีพต่อ *Escherichia coli* ATCC 25922 (แบคทีเรียแกรมลบ) และ *Staphylococcus aureus* ATCC 25923 (แบคทีเรียแกรมบวก) ซึ่งเป็นตัวแทนของแบคทีเรียทั้งสองชนิดด้วยวิธี disk diffusion ผลการทดลองพบว่าสารสกัดจากกระดอม (*Trichosanthes costata* Blume) และขี้กาแดง (*Trichosanthes bracteate* (Lam.) Voight) แสดงฤทธิ์ในการยับยั้งการเจริญของเชื้อ *E. coli* และ *S. aureus* ได้ดีกว่าสารสกัดจากพืชชนิดอื่น ผลการศึกษานี้แสดงให้เห็นถึงศักยภาพของพืชสมุนไพรไทยในการพัฒนาเป็นแหล่งของสารต้านจุลชีพจากธรรมชาติในอนาคต

คำสำคัญ: พืชสมุนไพรไทย, การดื้อยา, การทดสอบฤทธิ์ยับยั้งเชื้อจุลชีพ

**Abstract:** Antibiotic resistance is becoming a major global health issue, posing challenges to the treatment of infectious diseases. One promising strategy to address this problem is the study of bioactive compounds derived from plants, which are rich sources of structurally diverse molecules with potential for antimicrobial drug development. This study aimed to evaluate the antibacterial potential of 10 Thai medicinal plants traditionally used in folk medicine for treating fever and infections. Ethanolic crude extracts were prepared from dried plant materials using the maceration method and tested for antimicrobial activity against *Escherichia coli* ATCC 25922 (Gram-negative) and *Staphylococcus aureus* ATCC 25923 (Gram-positive) which is representative of both bacterial species using the disk diffusion method. The results showed that crude extracts from Ribbed Orange Gourd (*Trichosanthes costata* Blume) and Bitter Snake Gourd (*Trichosanthes bracteate* (Lam.) Voight) exhibited inhibitory effects on the growth of both *E. coli* and *S. aureus* compared to other plant extracts. This study indicates that most of the selected Thai medicinal plant extracts possess sufficient antibacterial potential and should be further investigated for their potential development as natural antimicrobial agents in the future.

Keywords: Thai medicinal plant, antibiotic resistance, antimicrobial activity

## 1. Introduction

The increasing global demand for antibiotics has become a critical concern due to their essential role in treating bacterial infections. This growing need is driven by factors such as population growth, improved access to healthcare, and extensive use in agriculture [1]. Although antibiotics have greatly contributed to reducing mortality, their misuse and overuse have resulted in antibiotic resistance, allowing bacteria to adapt and evade treatment. The spread of resistance genes among bacterial populations poses a serious threat to human health [2]. This resistance complicates infection management, raises healthcare costs, and threatens public health worldwide [3]. Despite the pressing demand for new antibiotics, their development is hindered by high research costs, long timelines, and limited funding opportunities [4]. To overcome this challenge, a global effort is required, including responsible antibiotic use, expanded research initiatives, and the exploration of alternative treatment options.

Plant-derived antibiotics have emerged as a promising alternative in the fight against antibiotic resistance. Plants naturally produce a range of bioactive compounds that protect them from pathogens, many of which have demonstrated antimicrobial properties [5]. Unlike conventional antibiotics, plant-derived substances often act through different mechanisms, making them effective even against resistant strains. Studies have shown that these natural compounds hold great potential for treating resistant infections while posing fewer risks to the environment [6]. Additionally, plants may offer a sustainable and resource-efficient source for drug development.

Thailand introduced antibiotics in the late 1940s to early 1950s, following the global success of penicillin and other antibiotics. With support from international organizations such as the World Health Organization (WHO), antibiotics were rapidly integrated into the national healthcare system to combat infectious diseases like tuberculosis, cholera, and pneumonia [7]. While these efforts significantly improved public health, the widespread and prolonged use of antibiotics soon raised concerns about resistance, a problem that continues to pose serious health risks today [8]. At the same time, Thailand's unique geographic location and diverse ecosystems have made it a center of biodiversity, home to a wide variety of medicinal plants [9]. This rich natural heritage offers a valuable opportunity to discover plant-based treatments, particularly for antibiotic-resistant infections [10]. Many of these plants have been used in traditional Thai medicine for generations, yet their pharmacological properties remain largely underexplored [9].

Hence, systematic research into these traditional remedies may uncover effective antimicrobial agents while preserving cultural knowledge and supporting sustainable drug discovery. In this study, traditional Thai medicinal knowledge was reviewed from three key sources: Thai Traditional Medicine Formulary, the medical recipes of Luang Pu Sook, and inscriptions at Wat Ratchaorosaram Ratchaworawihan. Plants traditionally used for fever and infectious diseases were identified, with emphasis on those frequently cited but lacking scientific reports on antimicrobial activity. Selected plants were then screened against *S. aureus* and *E. coli* to explore their potential as natural antimicrobial agents. This approach highlights the value of traditional knowledge in guiding the search for novel treatments to combat against antibiotic-resistant bacteria.

## 2. Material and Methods

### 2.1. Selection of Thai medicinal plants

Research was conducted on medicinal formulations from the *Thai Traditional Medicine Formulary* (Chailom, 2021), *Luang Pu Suk's Herbal Medicine Textbook*, and the *National Thai Traditional Medicine Pharmacopoeia, 2021 Edition*, focusing on remedies for fever and infections. ten types of plants were selected for testing, as show in Table 1 which includes folk medicine formulas, King Narai's medical formulas, Krad medical recipes, the formulas of Luang Intharaya, the formulas of Muen Chamnarn Phaitaya (Ploy Phaitayanon), and various ancient Thai traditional medicine formulas, as well as the National Formula

**Table 1.** The medicinal plants used in the study of the inhibitory effects on *E. coli* and *S. aureus*

| Common name | Scientific name | Abbreviation | Family | Part used |
|---|---|---|---|---|
| Hogvine | *Camonea umbellate* (L.) A.R.Simões & Staples | CU | Convolvulaceae | root |
| Strychnine Tree | *Strychnos lucida* R.Br. | SL | Loganiaceae | stem |
| Harrisonia | *Harrisonia perforata* (Blanco) Merr. | HP | Simaroubaceae | root |
| Ribbed Orange Gourd | *Trichosanthes costata* Blume | TC | Cucurbitaceae | fruit |
| Bitter Snake Gourd | *Trithianes bracteata* (Lam.) Voigt | TB | Cucurbitaceae | root |
| Milky Vine | *Alyxia reinwardtii* Blume | AR | Apocynaceae | bark |
| Chalut | *Tinospora baenzigeri* Forman. | TB | Menispermaceae | leaf |
| Curtain Creeper | *Tarlmounia elliptica* (DC.) "H.Rob., S.C.Keeley, Skvarla & R.Chan" | TE | Asteraceae | root |
| White vine | *Urceola minutiflora* (Pierre) D. J. Middleton | UM | Apocynaceae | root |
| Tropical finger-grass | *Digitaria ciliaris* (Retz.) Koel. | DC | Poaceae | whole plant |

### 2.2. Frequency of citation (FC) calculation

The collected data were processed to determine the frequency of citation (FC). The FC indicates the local importance of a specific plant species. It is calculated by dividing the number of each plant name precented in the drug recipe (P) by the total number of recipe (N)

$$FC = \frac{P}{N}$$

### 2.3. Preparation of medicinal plants

The medicinal plants were prescribed by a licensed traditional medicine practitioner from Thaprachan Herbs Co., Ltd., which complies with GMP standards. The dried medicinal plants were finely ground using a hammer mill with a 6-millimeter sieve and then further ground using a grinder (modified from the method of [11])

### 2.4. Extraction of crude extracts from medicinal plants

A total of ten types of finely ground medicinal plants were weighed at 100 grams each and subjected to extraction using the maceration method with 95% ethanol (at a ratio of 1:5 by weight to volume). The extraction process was conducted for approximately 72 hours at room temperature. During this time, the mixture was maintained in a sealed container and stirred periodically.

Following the extraction, the plant residues were filtered out using No.1 filter paper, retaining only the clear liquid. This liquid was then subjected to solvent evaporation using a rotary vacuum evaporator set at 45 degrees Celsius, resulting in a thick, viscous crude extract. The extract was weighed, and the percentage yield was calculated in relation to the weight of the medicinal plants used in the extraction (% yield) [11].

For testing, 640 milligrams of the crude extract was weighed and dissolved in 100% dimethyl sulfoxide (DMSO) until fully dissolve0d. The solution was then diluted with water to achieve a final concentration of 3% DMSO, and the concentration of the crude extract was subsequently calculated.

$$\%\ \text{yield} = \left(\frac{\text{actual yield (gram)}}{\text{theoretical yield (gram)}}\right) \times 100$$

### 2.5. Bacteria culture preparation

*E. coli* and *S. aureus* bacteria were cultured on Mueller Hinton agar (MHA) and incubated at 37°C for 18 hours. Single colonies were then isolated and inoculated into Mueller Hinton broth (MHB), followed by incubation at 37°C with shaking at 180 rpm for 18 hours. The optical density (OD) was measured using a spectrophotometer at a wavelength of 600 nanometers, and the $OD_{600}$ was adjusted to 0.3 using MHB as the diluent.

### 2.6. Antibacterial activity assay

The antibacterial activity was evaluated using the disk diffusion method. A 100-microliter aliquot of bacterial suspension, adjusted to the appropriate concentration, was pipetted onto Mueller Hinton agar (MHA) plates, and the bacterial cells were evenly spread across the surface using a triangular glass spreader. Sterile 6-millimeter diameter filter paper discs were immersed in crude plant extracts at various concentrations and placed on the agar surface. Kanamycin, at a concentration of 2.4 µg/mL, served as the positive control, while 3% DMSO was used as the negative control. The plates were incubated at 37°C for 18 hours, after which the diameters of the inhibition zones were measured and compared with the control groups (adapted from [12]).

## 3. Results and Discussion

### 3.1. Medicinal plant selection guided by ethnobotany

A total of 138 medicinal plants were compiled from the *Thai Traditional Medicine Formulary*, *Luang Pu Suk's Herbal Medicine Textbook*, and the *National Thai Traditional Medicine Pharmacopoeia 2021 Edition*. Redundant entries of medicinal plants utilized across various formulations were removed. Medicinal plants were selected based on both traditional use and research gap. Species commonly used for fever and infectious diseases were identified from classical Thai medical texts, with priority given to those frequently cited but lacking scientific evidence of antimicrobial activity. Plants mentioned across multiple sources (Luang Pu Sook's recipes, the National Pharmacopoeia, and Thai Traditional Medicine Formulary) were considered especially promising due to their consistent traditional use. This overlap suggests cultural consensus and strengthens their potential as candidates for antimicrobial screening. In addition, the frequency of citation (FC) was calculated to further guided the selection (Table 2).

**Table 2** The percentage yield and FC of crude extracts relative to the dry weight (% yield) of selected medicinal plants.

| Scientific name | Abbreviation | Part used | Percentage yield (%) | FC |
|---|---|---|---|---|
| *Camonea umbellata* | CU | root | 3.05 | 0.03 |
| *Strychnos lucida* | SL | stem | 4.60 | 0.09 |
| *Harrisonia perforata* | HP | root | 7.80 | 0.28 |
| *Trichosanthes costata* | TC | fruit | 9.02 | 0.22 |
| *Trichosanthes bracteata* | TB | root | 2.81 | 0.03 |
| *Alyxia reinwardtii* | AR | bark | 14.89 | 0.03 |
| *Tinospora baenzigeri* | TB | leaf | 5.40 | 0.06 |
| *Tarlmounia elliptica* | TE | root | 7.52 | 0.03 |
| *Urceola minutiflora* | UM | root | 6.59 | 0.03 |
| *Digitaria ciliaris* | DC | whole plant | 3.84 | 0.06 |

The investigation revealed that out of 138 plant species, 14 species were consistently utilized across all three sources. Among these, only three remain untested: *Capparis micracantha*, *Trichosanthes costata*, and *Harrisonia perforate*, as presented in Figure 1. In addition to these 3 species, 7 plant species without antimicrobial activity reported were selected based on their FC values (Table 2).

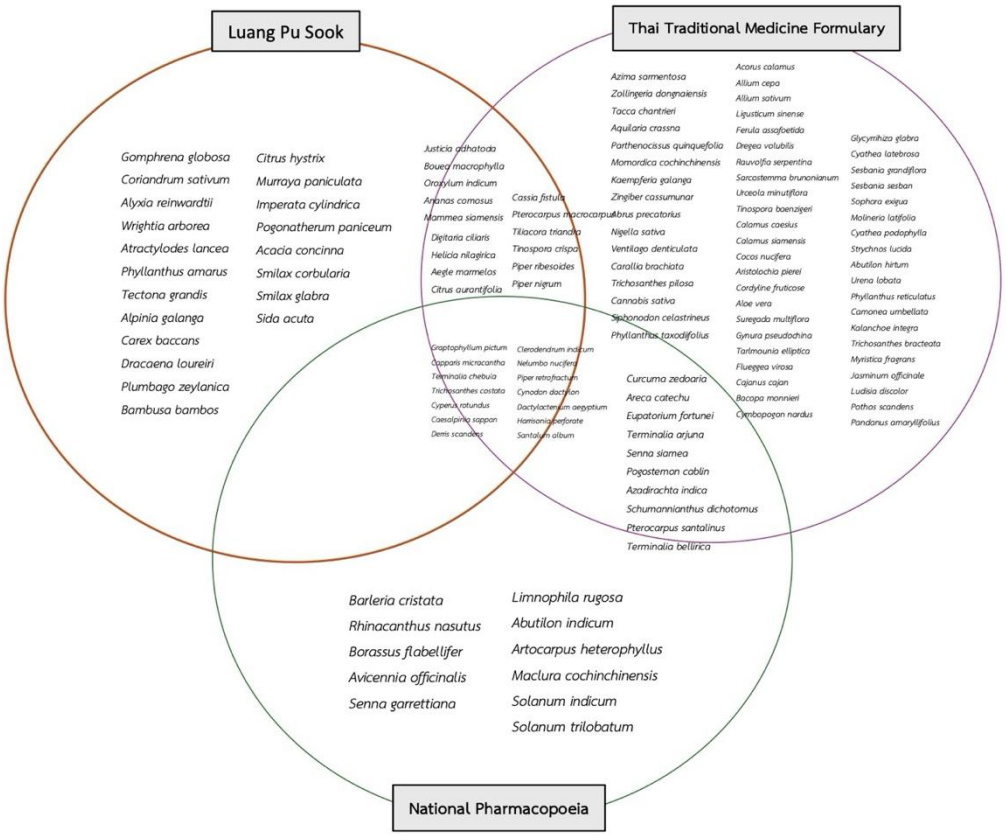



**Figure 1**. An analysis of 138 medicinal plant species with overlapping usage across three traditional pharmacopoeias

Moreover, we further analyzed the taxonomic distribution of the complied plant species based on their Family. The three most frequently employed plant families in these formulations were identified: the Fabaceae, which was the most predominant, followed by the Poaceae, Apocynaceae, and Euphorbiaceae, as illustrated in Figure 1. According to the report by Phumthum et al. [9], the Fabaceae family was identified as the most frequently utilized in the treatment of various health conditions, including digestive system disorders, infections and infestations, nutritional deficiencies, musculoskeletal disorders, and genitourinary system disorders. The prevalent use of plants from this family may be attributed to their high diversity and widespread distribution throughout Thailand [13].

The random selection of plants for pharmacological research is often inefficient in terms of both time and cost. A more targeted and strategic approach would be to investigate traditional herbal formulations that have been historically used to treat illnesses such as fever [12]. These remedies, rooted in long-standing cultural practices, can provide valuable insight into potentially effective treatments. By analyzing the distribution of plant families and their overlapping usage across different traditional drug recipes from various regions of Thailand, researchers may increase the chances of identifying plants with relevant pharmacological activity while reducing resource use. This ethnobotanical approach highlights the value of integrating traditional medicinal knowledge with modern scientific research to address current healthcare challenges.

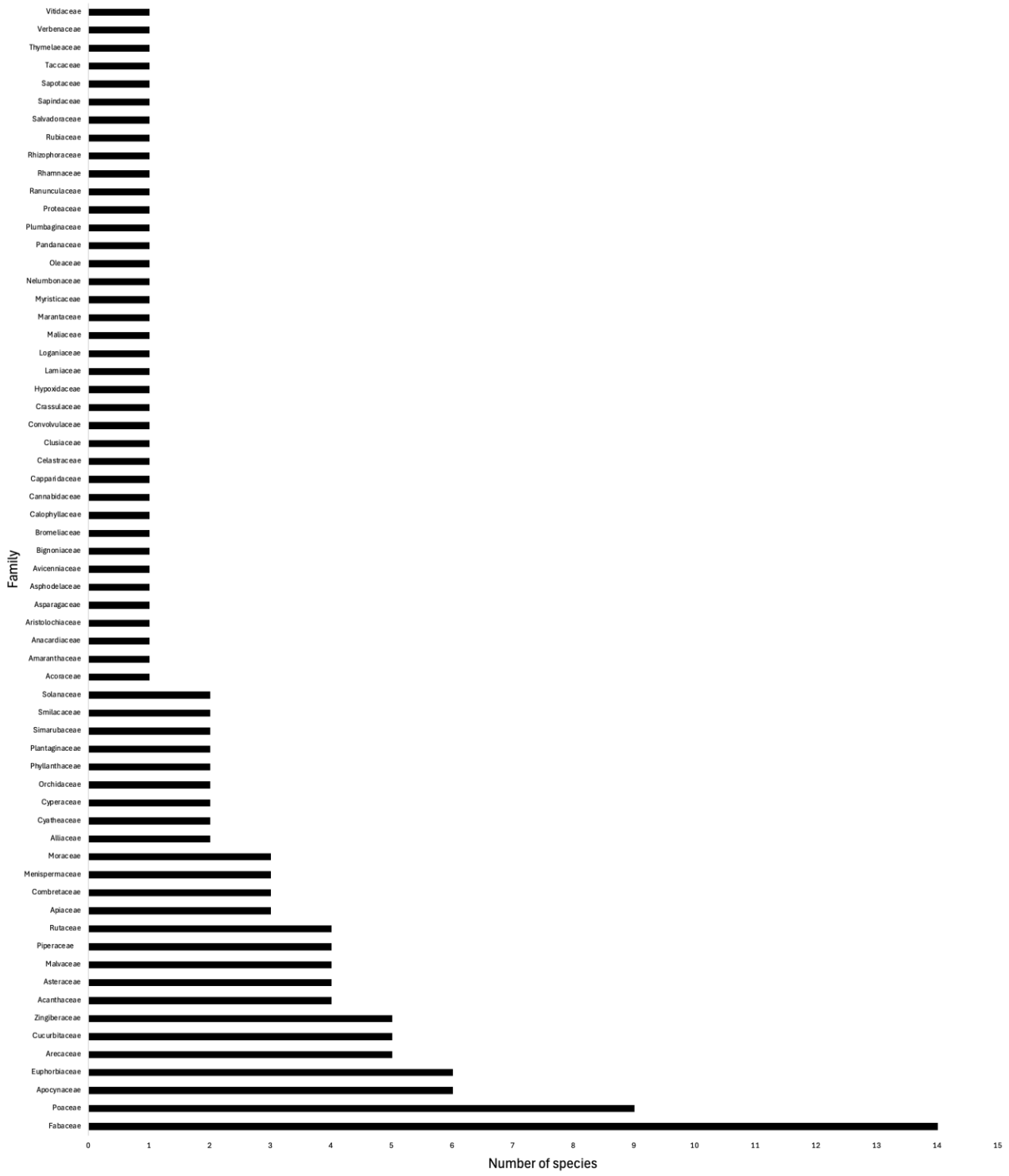



**Figure 2.** Bar chart showing the number of plant usage classified by family

### 3.2. The inhibitory effect on bacterial growth

Disk diffusion assays were conducted to evaluate the antibacterial activity of the ten crude extracts against *E. coli* and *S. aureus*. Extracts from the fruit of *T. costata* and the root of *T. bracteata* showed the most pronounced inhibitory effects (Figure 3, Table 3). Although the extracts produce inhibition zones, their activity was weak compared to the kanamycin control. Nevertheless, both Gram-positive and Gram-negative bacteria were susceptible to a limited extent.

While the observed effects were weak, the identification of activity in traditionally used species remains significant. This study is the first to report antimicrobial activity in *T. costata* and *T. bracteata*. These findings support the potential of the Cucurbitaceae family, which includes genera such as *Cucurbita* [14] and *Trichosanthes* [15], known for producing antimicrobial compounds like cucurbitacins and flavonoids. While the exact bioactive molecules and mechanisms remain unknown, these results highlight the need for further studies on this plant group, including compound isolation and mode-of-action analysis to elucidate the nature and mechanism of these inhibitory effects.

Traditional medicinal formulations often contain multiple ingredients with complementary pharmacological properties, such as antipyretic or antibacterial effects. In contrast, this study examined individual plant species individually, which may partly explain the weak antibacterial activity observed in *T. costata* and *T. bracteata.* This difference from traditional polyherbal practices may have overlooked potential synergistic effects that enhance therapeutic efficacy.

Although fever is often infection-related, non-infectious causes such as autoimmune diseases, cancers, inflammatory disorders, and drug reactions are also common [16]. Since plant selection in this study was based on traditional use for fever, some species may exert primarily antipyretic rather than antimicrobial effects. Additionally, the weak activity observed may be due to the low concentration of bioactive compounds in the crude extracts. Further purification and bioactivity-guided fractionation may enhance the detection of pharmacologically potent constituents.

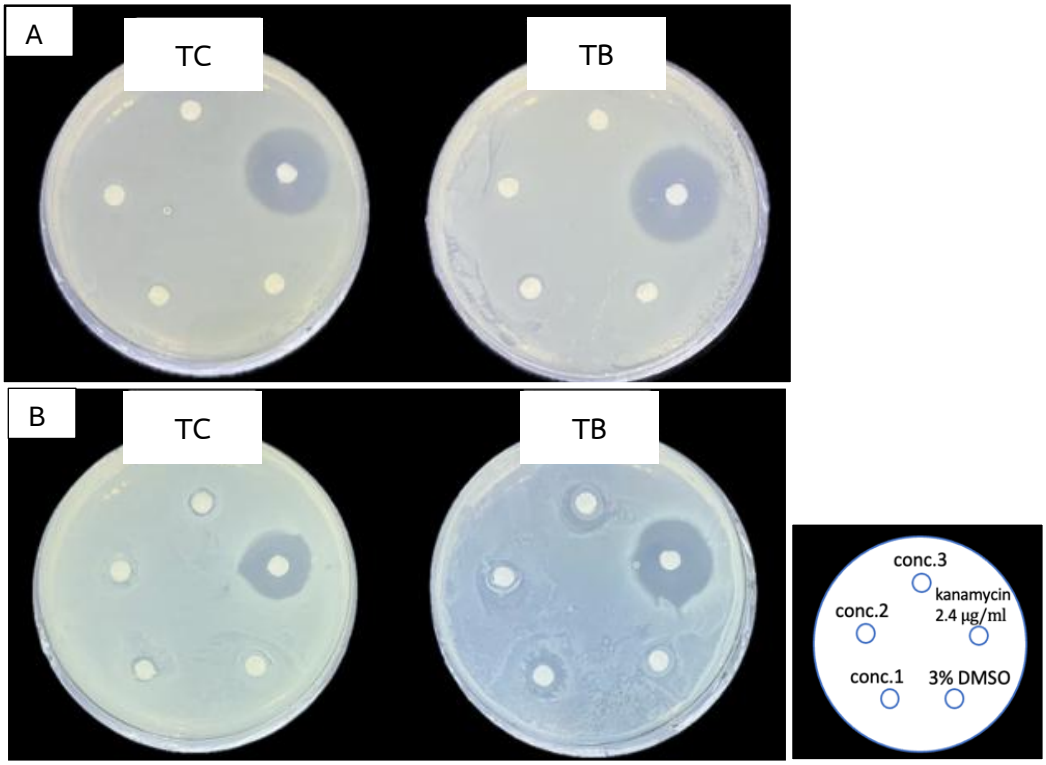



**Figure 3**. Antibacterial activity test of crude extracts from *T. costata* and *T. bracteata* against *E. coli* (A) and *S. aureus* (B) using the disk diffusion method.

**Table 3** Results of the antibacterial activity test of crude extracts from medicinal plants using the disk diffusion method

| ฉ | Abbreviation | *Escherichia coli* | | | | | *Staphylococcus aureus* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Concentration of crude extract | | | | | Concentration of crude extract | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| *Camonea umbellata* | CU | - | - | - | - | - | - | - | - | - | - |
| *Strychnos lucida* | SL | - | - | - | - | - | - | - | - | - | - |
| *Harrisonia perforata* | HP | - | - | - | - | - | - | - | - | - | - |
| *Trichosanthes costata* | TC | + | - | - | - | - | + | + | + | - | - |
| *Trichosanthes bracteata* | TB | + | - | - | - | - | + | - | - | - | - |
| *Alyxia reinwardtii* | AR | - | - | - | - | - | - | - | - | - | - |
| *Tinospora baenzigeri* | TB | - | - | - | - | - | - | - | - | - | - |
| *Tarlmounia elliptica* | TE | - | - | - | - | - | - | - | - | - | - |
| *Urceola minutiflora* | UM | - | - | - | - | - | - | - | - | - | - |
| *Digitaria ciliaris* | DC | - | - | - | - | - | - | - | - | - | - |
| kanamycin (2.4 µg/ml) | | +++ | | | | | +++ | | | | |
| 3% DMSO | | - | | | | | - | | | | |

Concentration 1 corresponds to the highest concentration, followed by concentration 2, concentration 3, and concentration 4, with concentration 5 representing the lowest concentration.

## 4. Conclusions

This study applied an ethnobotanical approach to identify Thai medicinal plants traditionally used for treating fever and infections. Based on frequency of use and absence of prior studies, ten species were selected for preliminary screening of antibacterial activity. Among these, crude ethanol extracts from the fruit of *Trichosanthes costata* and the root of *Trichosanthes bracteata* demonstrated the most notable inhibitory effects against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. To our knowledge, this is the first report of antimicrobial activity in these two species. These findings support the potential of traditional medicinal plants as sources of antimicrobial agents. Further studies focusing on the isolation and characterization of active compounds are warranted to better understand their therapeutic potential and underlying mechanisms of action.

**Acknowledgements:** This work would not have been possible without the support and encouragement of many people, to whom I am deeply grateful. I extend my sincere thanks to my dissertation committee. The value of their guidance cannot be overstated. Prof. Dr. Manit Kidyoo and Asst. Prof. Dr. Anchalee Chaidee offered thoughtful insights and steady wisdom that helped shape this research. I am especially indebted to my supervisor, Dr. Thiti Suttiyut, whose expertise, encouragement, and careful mentorship guided me through each stage of this project. This research was

generously supported by the Department of Botany, Faculty of Science, Chulalongkorn University. Finally, I am grateful to my family and friends for their constant emotional support throughout this journey.

## References

1. Ventola C.L. 2015. The antibiotic resistance crisis: part 1: causes and threats. **Pharmacy and therapeutics** 40(4): 277.
2. World Health Organization. 2014. World health statistics 2014 **World Health Organization**.
3. Garcia M., Lipskiy N., Tyson J., Watkins R., Esser E.S., Kinley T. 2020. Centers for Disease Control and Prevention 2019 novel coronavirus disease (COVID-19) information management: addressing national health-care and public health needs for standardized data definitions and codified vocabulary for data exchange. **Journal of the American Medical Informatics Association : JAMIA** 27(9):1476–1487.
4. Brown E., Wright G. 2016. Antibacterial drug discovery in the resistance era. **Nature** 529: 336–343.
5. Balandrin M.F., Klocke J.A., Wurtele E.S., Bollinger W.H. 1985. Natural plant chemicals: sources of industrial and medicinal materials. **Science** 228(4704): 1154–1160.
6. Wink M. 2015. Modes of action of herbal medicines and plant secondary metabolites. **Medicines** 2(3): 251–286.
7. Sumpradit N., Wongkongkathep S., Malathum K. et al. 2021. Thailand's national strategic plan on antimicrobial resistance: Progress and challenges. **Bulletin of the World Health Organization** 99: 661–673.
8. Saeloh D., Visutthi M. 2021. Efficacy of Thai plant extracts for antibacterial and anti-biofilm activities against pathogenic bacteria. **Antibiotics** (Basel, Switzerland). 2021; 10 (12): 1–9.
9. Phumthum M. & Balslev H. 2020. Anti-infectious plants of the Thai Karen: A meta-analysis. **Antibiotics**, 9(6): 298.
10. Sukkasem K., Panthong S., Itharat A. 2016. Antimicrobial Activities of Thai Traditional Remedy "Kheaw-Hom" and Its Plant Ingredients for Skin Infection Treatment in Chickenpox. **Journal of the Medical Association of Thailand** 99(4): 116–123.
11. Todtong P. 2023. Effects of Medicinal Plant Extracts as Pre-milking Teat Dip to Reduce Teat-end Bacterial Load in Lactating Dairy Cow. **Journal of Mahanakorn Veterinary Medicine** 17(2): 271–28.
12. Quave C.L. 2016. Antibiotics from nature: traditional medicine as a source of new solutions for combating antimicrobial resistance. **AMR Control** 98: 102.
13. Matuschek E., Brown D.F., Kahlmeter G. 2014. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. **Clinical microbiology and infection** 20(4): O255–O266.
14. Salehi B., Capanoglu E., Adrar N., Catalkaya G., Shaheen S., Jaffer M., ... & Capasso R. 2019. Cucurbits plants: A key emphasis to its pharmacological potential. **Molecules** 24(10): 1854.
15. Ali M.A., Sayeed M.A., Islam M.S., Yeasmin M.S., Khan G.R.M.A.M., & Muhamad I.I. 2011. Physicochemical and antimicrobial properties of Trichosanthes anguina and Swietenia mahagoni seeds. **Bulletin of the Chemical Society of Ethiopia** 25(3).
16. Ogoina D. 2011. Fever, fever patterns and diseases called 'fever'–a review. **Journal of infection and public health** 4(3): 108–124.


BCT17
Botanical Conference of Thailand