การประยุกต์ใช้อินฟราเรดสเปกโทรสโกปีร่วมกับวิธีทางเคโมเมตริก สำหรับพิสูจน์ชนิดที่ถูกต้องของเครื่องยาไทย–จันทน์ชะมด
Application of Infrared Spectroscopy–chemometric Methods for the Authentication of a Thai Crude Drug Chan-chamot

นิพนธ์ต้นฉบับ **Original Article**

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บทคัดย่อ

วัตถุประสงค์: จันทน์ชะมดเป็นเครื่องยาที่ใช้ในแพทย์แผนไทย ชื่อท้องถิ่นของพืชที่เป็นแหล่งที่มาของเครื่องยาชนิดนี้มีความซับซ้อน จึงอาจนำไปสู่การใช้สมุนไพรผิดชนิด การศึกษานี้มีวัตถุประสงค์เพื่อระบุและพิสูจน์ชนิดที่ถูกต้องของเครื่องยาจันทน์ชะมดที่มีจำหน่ายในท้องตลาดโดยใช้อินฟราเรดสเปกโทรสโกปี (IR) **วิธีการศึกษา:** เปรียบเทียบอินฟราเรดสเปกตรัมในช่วง 1,801 – 501 ซม$^{-1}$ ของสารสกัดแอซีโทนของตัวอย่างจันทน์ชะมดกับตัวอย่างแท้ด้วยวิธีการเคโมเมตริก ได้แก่ การวิเคราะห์ความคล้ายคลึง (SA) การวิเคราะห์แบบกลุ่มเชิงลำดับชั้น (HCA) และการวิเคราะห์องค์ประกอบหลัก (PCA) นอกจากนี้ได้พัฒนาแบบจำลอง PCA สำหรับ soft independent modeling of class analogy (SIMCA) เพื่อใช้ทำนายความถูกต้องของตัวอย่าง **ผลการศึกษา:** อินฟราเรดสเปกตรัมและสเปกตรัมแบบอนุพันธ์ที่สองของตัวอย่างจันทน์ชะมดและ *Mansonia gagei* มีความคล้ายคลึงกันสูง (R > 0.9) นอกจากนี้ HCA และ PCA ยังจัดให้อยู่ในกลุ่มเดียวกัน SIMCA ที่ใช้แบบจำลอง PCA ที่สร้างจากข้อมูลสเปกตรัมในช่วงของหมู่ฟังก์ชันหรือช่วงของลายนิ้วมือ สามารถแยกความแตกต่างระหว่างจันทน์ชะมดออกจากเครื่องยาชนิดอื่นที่มีโอกาสใช้ทดแทนด้วยความแม่นยำ 100.00% **สรุป:** การใช้อินฟราเรดสเปกโทรสโกปีร่วมกับวิธีทางเคโมเมตริกช่วยระบุและพิสูจน์ชนิดที่ถูกต้องว่าเครื่องยาจันทน์ชะมดมีที่มาจาก *M. gagei*

คำสำคัญ: จันทน์ชะมด; *Mansonia gagei*; อินฟราเรดสเปกโทรสโกปี; เคโมเมตริก; การพิสูจน์ชนิดที่ถูกต้อง

Abstract

Objective: Chan-chamot is a crude drug utilized in Thai traditional medicine. Its plant source has a complex vernacular naming system that can lead to misuse of this crude drug due to incorrect identification. This study aimed to identify and authenticate commercially available Chan-chamot crude drugs using infrared (IR) spectroscopy. **Methods:** The IR spectra in the ranges of 1,801 – 501 cm^{-1} obtained from the acetone extracts were compared with those of authentic samples using chemometric methods, i.e., similarity analysis (SA), hierarchical cluster analysis (HCA) and principal component analysis (PCA). PCA models for authentication were developed and subsequently used for soft independent modeling of class analogy (SIMCA). **Results:** The IR and second derivative spectra of Chan-chamot and *Mansonia gagei* demonstrated a high similarity (R > 0.9). Both HCA and PCA also grouped them into the same cluster. SIMCA based on PCA models constructed from either IR wavenumber in functional group or fingerprint regions successfully distinguished Chan-chamot from its potential substitutions with 100.00% accuracy. **Conclusion:** The combination of IR and chemometric methods effectively clarified the botanical identification and authentication of Chan-chamot as *M. gagei*.

Keywords: Chan-chamot; *Mansonia gagei*; infrared spectroscopy; chemometrics; authentication

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Introduction

"Chan-chamot" is a crucial botanical material or crude drug widely used in Thai traditional medicine for treating fever and supporting heart health.[1] However, the naming of this crude drug becomes complex, particularly when relying on vernacular names of its plant origin. Chan-chamot refers to at least two plant species: *Mansonia gagei* J.R.Drumm. (family Malvaceae) and *Aglaia silvestris* (M.Roem) Merr. (family Meliaceae). The former plant species (*M. gagei*) also has the vernacular names as "Chan-kao" and "Chan-hom". Interestingly, Chan-kao is also the vernacular names of other plants, including *Diospyros decandra* Lour. (family Ebenaceae), *Tarenna hoaensis* Pit. (family Rubiaceae), and *Santalum album* L. (family Santalaceae). Meanwhile, Chan-hom could also refer to *T. hoaensis*.[2] The confusion was more

complex when both Chan-kao and Chan-hom are used as names of other crude drugs, and their characteristics are very similar to Chan-chamot. Table 1 is the summary of this complex naming system.

Table 1 Probably botanical identification of Chan-chamot and its probably substituted crude drugs.

Crude drug	Probably botanical names
Chan-chamot	*Mansonia gagei* J.R.Drumm.
	Aglaia silvestris (M.Roem) Merr.
Chan-kao	*Mansonia gagei* J.R.Drumm.
	Tarenna hoaensis Pit
	Diospyros decandra Lour.
	Santalum album L.
Chan-hom	*Mansonia gagei* J.R.Drumm.
	Tarenna hoaensis Pit

Different plant species and crude drugs exhibit varying therapeutic efficacy and toxicity, emphasizing the need for caution in herbal product formulation to avoid the inadvertent use of incorrect crude drugs. Many studies have investigated the authentication of medicinal plants, with chemical fingerprint analysis emerging as a widely used method.[3-10] Chemical fingerprint can be established through various chromatography and spectroscopy techniques. In a previous survey of the crude drug known as Chan-chamot, it was identified as *M. gagei* using thin-layer chromatography (TLC).[11] In this study, infrared (IR) spectroscopy was suggested as another identification method. IR spectrum was considered as a macro-fingerprint representing to all chemical compositions in the extract, making it suitable for identification purpose. The advantages of IR spectroscopy include rapidity, non-destructiveness, simplicity, high precision, and reduced chemical usage. Coupled with chemometric methods, IR spectrum analysis enhances the identification of various traditional medicines.[12,13] Chemometrics is the scientific discipline that extracts and establishes relationships from chemical measurements using mathematical methods.[14] Pattern recognition is chemometric which aids in discerning similarities and differences among objective materials. For preliminary analysis, similarity analysis (SA) and hierarchical cluster analysis (HCA) are commonly employed.[15] Principal component analysis (PCA), a more comprehensive unsupervised technique, reduces the number of important variables to new components called principal components (PCs), thereby enhancing the clarity of similarity and difference.[16] To classify an unknown sample into a group, Soft independent modeling of class analogy (SIMCA) is employed. This method utilizes PCA to model the shape and position of the object formed by the samples in space for class definition.[15,17]

Chan-chamot is a brownish wood, which makes it difficult to distinguish from other crude drugs. Additionally, the complexity of its vernacular names could potentially lead to the substitutions and adulterations. Therefore, establishing a reliable authentication method that utilized more objective criteria is essential to accurately identify samples in practical applications. This study aimed to elucidate its botanical identification. The approach involved utilizing infrared IR fingerprint analysis coupled with chemometric techniques, including SA, HCA, and PCA. Additionally, a reliability prediction model using SIMCA was proposed for further authentication of this crude drug.

Methods

Plant materials

Sixteen samples of Chan-chamot (M1–M16, *M. gagei*), ten samples of Chan-kao (K1–K10) and ten samples of Chan-hom (H1–H10) were purchased from different Thai traditional drugstores throughout Thailand during 2012 to 2013 (Table 2). The voucher specimens were deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Silpakorn University, Thailand. Additionally, the authentic samples of *M. gagei* and *S. album*, were collected from Prachuap- Prachuap-Khiri-Khan Silvicultural Research

Table 2 Collection places of the samples.

Sample code	Collection place	Sample code	Collection place	Sample code	Collection place
M1	Bangkok	K1	Bangkok	H1	Bangkok
M2	Nakhon Pathom	K2	Nakhon Pathom	H2	Nakhon Pathom
M3	Nonthaburi	K3	Nonthaburi	H3	Nonthaburi
M4	Bangkok	K4	Bangkok	H4	Bangkok
M5	Nonthaburi	K5	Nonthaburi	H5	Nonthaburi
M6	Nonthaburi	K6	Nonthaburi	H6	Nonthaburi
M7	Phitsanulok	K7	Phitsanulok	H7	Nonthaburi
M8	Songkhla	K8	Nonthaburi	H8	Nonthaburi
M9	Uttaradit	K9	Songkhla	H9	Songkhla
M10	Bangkok	K10	Bangkok	H10	Uttaradit
M11	Nonthaburi				
M12	Chiang Mai				
M13	Uttaradit				
M14	Roi Et				
M15	Saraburi				
M16	Phichit				

Station, Royal Forest Department, Thailand. The authentic samples of *A. silvestris, T. hoaensis* and *D. decandra* were collected from Chantaburi Medicinal Plant Garden, Medicinal Plant Research Institute, Department of Medical Sciences,

Thailand. All samples were ground to powder, passed through a sieve mesh 0.180 mm, and stored at 4°C before use. All samples were prepared and measured for IR spectrum immediately after collection.

Sample preparation

Five grams of each sample was extracted with 100 ml of acetone by sonication for 30 min. All extracts were filtered and dried under reduced pressure using a rotary evaporator (BÜCHI, Switzerland).

Infrared spectroscopy

The sample extracts were applied as thin film on KBr disc (32x3 mm drilled) and analyzed by infrared spectroscopy (Nicolet 4700 FT-IR, USA) in an absorbance mode in the range of 4000–400 cm^{-1}, with 16 scans and the resolution of 4 cm^{-1}.

Chemometric methods

The IR spectra were exported in ASCII file format using the Omnic 7.2a software (Thermo Electron Corporation, USA). Subsequently, these spectra were imported into Unscrambler® X version 10.1 (Camo Process AS, Norway) for chemometric analyses, which included HCA, PCA and SIMCA. All IR spectral data within the range of 1801–501 cm^{-1} underwent pretreated with unit vector normalization, and transformed into second derivative spectra using the Savitzky-Golay method with a second-order polynomial and 15 smoothing points. SA was calculated by Pearson's correlation (R) using Microsoft Office Excel 2019 (Microsoft, WA, USA). For HCA, the Square Euclidean method was employed to measure distances between samples, and the single-linkage method was applied for sample clustering. All PCA models underwent full cross-validation. SIMCA, based on the appropriate PCA models, assessed the classification performance. Sensitivity and specificity were used to evaluate the correct identification of samples as Chan-chamot (M. gagei) and its substitutions, respectively, while accuracy indicated the overall correct identification of all samples.[18]

Results and Discussions

Identification of Chan-chamot

The extracts of Chan-chamot were prepared using different solvents (hexane, dichloromethane, ethyl acetate, acetone, and water), and their IR spectra were measured. Data in the range of 1801–501 cm^{-1} were compared to authentic samples, including *M. gagei*, *A. silvestris, D. decandra*, *T. hoaensis* and *S. album*. Pearson's correlation was used to evaluate the similarities among all spectral data, and the results are presented in Table 3. Among the Chan-chamot samples, the correlation coefficient values (R) of the IR spectra of the hexane extract varied widely from 0.04 to 1.00. This variation indicated a diverse non-polar constitution among the Chan-chamot samples, and then the hexane extract was unsuitable to use for identification. After applying the second derivative method to enhance fine details and make the differences more noticeable, acetone was the only solvent with a correlation exceeding 0.9 among the Chan-chamot samples (Table 4). As a result, the acetone extract was suitable for identification, and this study focused extensively only on the extract prepared with this solvent. Figures 1 display the IR and second derivative spectra of acetone extracts within the 1801–501 cm^{-1} range for all Chan-chamot and the authentic samples. The similarity result in Table 3 indicated that the IR spectra of Chan-chamot samples closely resembled *M. gagei* (R = 0.98 ± 0.01). However, the result remained uncertain because the correlation coefficients with most other authentic samples exceeded 0.7, indicating also a high positive correlation.[19] After the application of the second derivative (Table 4), the correlation coefficients between Chan-chamot samples and *T. hoaensis*, *A. silvestris* and *D. decandra* decreased to 0.5–0.7, whereas the correlation with *S. album* was only 0.23 ± 0.07. Notably, *M. gagei* was the only authentic sample that exhibited high similarity (R = 0.93 ± 0.05) to Chan-chamot. This result suggested that Chan-chamot was the botanical material originated from *M. gagei*.

Table 3 The similarity (R) between IR spectra of Chan-chamot sample extracts prepared using different solvent and authentic samples.

Sample	Solvent					
	Hexane	Dichloro-methane	Ethyl acetate	Acetone	Methanol	Water
Chan-chamot	0.59 ± 0.33 (0.04 – 1.00)	0.96 ± 0.03 (0.87 – 1.00)	0.92 ± 0.06 (0.78 – 1.00)	0.98 ± 0.02 (0.91 – 1.00)	0.91 ± 0.10 (0.68 – 1.00)	0.97 ± 0.02 (0.90 – 1.00)
M. gagei	0.59 ± 0.39 (0.02 – 0.97)	0.97 ± 0.02 (0.94 – 0.99)	0.94 ± 0.03 (0.86 – 0.98)	0.98 ± 0.01 (0.95 – 1.00)	0.93 ± 0.02 (0.88 – 0.96)	0.93 ± 0.03 (0.86 – 0.99)
A. silvestris	0.61 ± 0.30 (0.17 – 0.94)	0.75 ± 0.05 (0.61 – 0.83)	0.78 ± 0.05 (0.62 – 0.84)	0.82 ± 0.04 (0.71 – 0.87)	0.89 ± 0.03 (0.81 – 0.94)	N.D.
D. decandra	0.55 ± 0.21 (0.23 – 0.88)	0.88 ± 0.02 (0.86 – 0.91)	0.86 ± 0.05 (0.71 – 0.91)	0.84 ± 0.03 (0.81 – 0.90)	0.72 ± 0.13 (0.45 – 0.87)	0.95 ± 0.02 (0.89 – 0.98)
T. hoaensis	0.63 ± 0.30 (0.17 – 0.98)	0.86 ± 0.03 (0.81 – 0.92)	0.57 ± 0.15 (0.39 – 0.84)	0.84 ± 0.02 (0.81 – 0.89)	0.65 ± 0.09 (0.56 – 0.87)	0.94 ± 0.03 (0.87 – 0.97)
S. album	0.59 ± 0.20 (0.27 – 0.85)	0.63 ± 0.05 (0.56 – 0.74)	0.61 ± 0.08 (0.51 – 0.74)	0.61 ± 0.05 (0.52 – 0.68)	0.59 ± 0.13 (0.30 – 0.74)	0.96 ± 0.02 (0.91 – 0.97)

Note: Data are expressed as mean ± SD, with minimum to maximum values in parentheses.

Table 4 The similarity (R) between second derivative spectra of Chan-chamot sample extracts prepared using different solvent and authentic samples.

Sample	Solvent					
	Hexane	Dichloro-methane	Ethyl acetate	Acetone	Methanol	Water
Chan-chamot	0.50 ± 0.31 (0.04–0.99)	0.75 ± 0.14 (0.45–0.98)	0.80 ± 0.12 (0.50–0.99)	0.91 ± 0.06 (0.74–0.99)	0.82 ± 0.13 (0.49–0.98)	0.54 ± 0.28 (-0.02–0.91)
M. gagei	0.39 ± 0.32 (0.03–0.92)	0.79 ± 0.11 (0.62–0.94)	0.82 ± 0.06 (0.72–0.91)	0.93 ± 0.05 (0.80–0.99)	0.80 ± 0.06 (0.69–0.92)	0.19 ± 0.30 (-0.03–0.99)
A. silvestris	0.23 ± 0.24 (-0.02–0.66)	0.41 ± 0.09 (0.27–0.58)	0.53 ± 0.04 (0.46–0.61)	0.55 ± 0.08 (0.42–0.62)	0.46 ± 0.07 (0.28–0.55)	N.D.
D. decandra	0.22 ± 0.24 (-0.05–0.64)	0.52 ± 0.06 (0.43–0.63)	0.47 ± 0.08 (0.27–0.57)	0.72 ± 0.08 (0.51–0.77)	0.56 ± 0.07 (0.39–0.67)	0.51 ± 0.23 (0.01–0.73)
T. hoaensis	0.39 ± 0.28 (0.09–0.88)	0.59 ± 0.09 (0.47–0.87)	0.54 ± 0.21 (0.28–0.88)	0.72 ± 0.03 (0.67–0.77)	0.43 ± 0.05 (0.36–0.51)	0.44 ± 0.17 (0.02–0.63)
S. album	0.09 ± 0.13 (-0.05–0.32)	0.09 ± 0.10 (-0.02–0.35)	0.20 ± 0.90 (0.06–0.33)	0.23 ± 0.07 (0.14–0.40)	0.13 ± 0.06 (0.02–0.25)	0.10 ± 0.07 (-0.03–0.21)

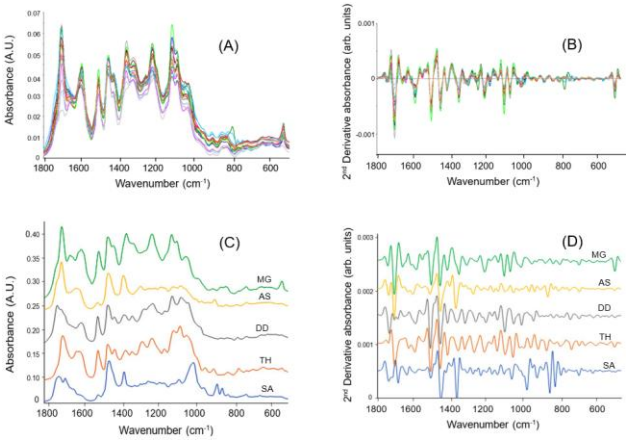

Figure 1 IR spectra and second derivative spectra in the range of 1801–501 cm^{-1} of (A, B) Chan-chamot, and (C, D) authentic samples (MG = *M. gagei*, AS = *A. silvestris*, DD = *D. decandra*, TH = *T. hoaensis* and SA = *S. album*).

To confirm the result, the exploratory analysis employing HCA was conducted. Figure 2 illustrates the dendrograms generated using the Square Euclidean distance and the single-linkage method to cluster the groups. Based on either the IR or secondary derivative spectra, all Chan-chamot samples clustered together in the same group as *M. gagei*, distinctly separate from the other four authentic samples.

The results of SA and HCA were also supported by the PCA method. PCA abstracted the complex spectral data into a reduced number of PCs. The first three PCs were used to provide a 3D-clustering visualization of score plots (Figure 3). These components explained 88% and 77% of the total variances in the IR and second derivative spectra, respectively. Consistent with HCA, PCA clustered all Chan-chamot samples with *M. gagei*. The combination of IR with these chemometric methods, i.e. SA, HCA and PCA, conclusively identified Chan-chamot as *M. gagei*. This result confirmed our previous study conducted using the TLC method.[11] A limitation of this identification approach was that closely related plant species may have similar chemical compositions, leading to comparable fingerprints and potential misidentification. However, the chance of confusing Chan-chamot as other species of *Mansonia* is minimal because *M. gagei* is the only *Mansonia* species present in Thailand.[2]

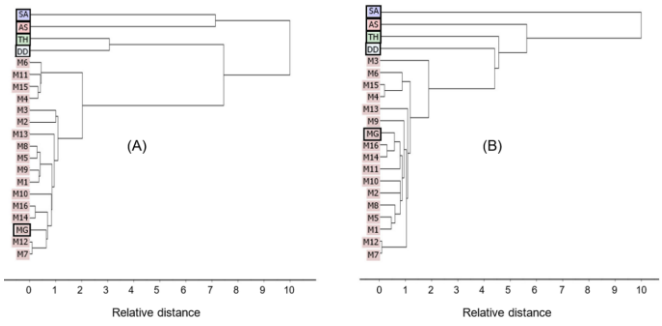

Figure 2 HCA dendrograms of Chan-chamot and authentic samples using (A) IR spectra and (B) second derivative spectra (M1–M16 = Chan-chamot samples, MG = *M. gagei*, AS = *A. silvestris*, DD = *D. decandra*, TH = *T. hoaensis* and SA = *S. album*).

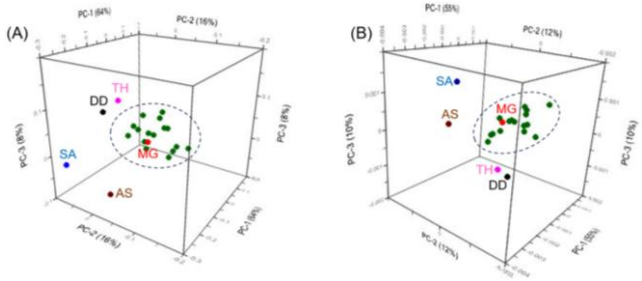

Figure 3 PCA score plots of Chan-chamot and authentic samples using (A) IR spectra and (B) second derivative spectra (green spots = Chan-chamot samples, MG = *M. gagei*, AS = *A. silvestris*, DD = *D. decandra*, TH = *T. hoaensis* and SA = *S. album*).

Development of the models for authentication

Following the successful application of the PCA method for the botanical identification of Chan-chamot, SIMCA classification was carried out to distinguish between the genuine (*M. gagei*) and possible substituted crude drugs. SIMCA relies on the well-constructed PCA models determined through full cross-validation. It is a supervising classification method suitable for dealing with limited sample numbers.[20]

The entire Chan-chamot sample was randomly divided into a training set (n = 10) and a testing set (n = 6). Authentic sample of *M. gagei* was also included in training set. The authentication fingerprint was featured by fundamental attributions of sameness and differences. To challenge the models, other potentially substituted crude drugs were included in the testing set. These included Chan-kao, previously identified as *T. hoaensis* (n = 10)[21], and Chan-hom, previously identified as *S. album* (n = 3), *S. spicatum* (n = 6) and *S. lanceolatum* (n = 1).[22] Additionally, authentic samples of *A. silvestris*, *D. decandra*, *T. hoaensis* and *S. album* were also supposed as substituted samples and included in the testing set.

The PCA models were developed from the training set using IR and second derivative spectra in the range of 1801–501 cm^{-1}. No outliers were detected based on the Hotelling T^2 statistic under the critical limit with p-value of 5%. Efficiency of the models was evaluated using the testing set, and the results were expressed in the terms of sensitivity, specificity, and accuracy. Sensitivity was the correct identification of Chan-chamot sample as *M. gagei*, whereas the ability to reject false samples was expressed as specificity. The overall correct identification was presented as accuracy. All these evaluation parameters of SIMCA, whether using IR or second derivative spectra achieved 100.00%. However, the models included a large number of variables (675 variables), some of which might be unnecessary and impact prediction ability in practical application. Then specific wavenumbers in the functional group region (1801–1545 cm^{-1}), and the fingerprint region (1543–930 cm^{-1} and 928–501 cm^{-1}) were trialed to reduce the complexity of the models. All models still gave 100.00% of all evaluated parameters.

Classification ability of each model was expressed as a graphical plot of Si vs. Hi (Figure 4). Si (sample distance to model) is a measure of how far a sample is from a modeled class. Hi (sample leverage) illustrates how a sample differs from other members of its class. All models demonstrated that all tested Chan-chamot samples were within the limits of both Si and Hi and belonged to the model, which corresponded to correct identification at a 5% significance level. In contrast, all false samples were located at the other quadrants. This suggested that a reliable model could be established using either functional group or fingerprint regions of IR data. In the functional group region, Chan-chamot (*M. gagei*) displayed a strong IR band at 1708 cm^{-1}, corresponding to the conjugated ketone functional group of mansonones, its major chemical constituents.[23] The fingerprint region is recognized as each chemical's unique characteristic. However, the range of 928–501 cm^{-1} exhibited limited and weak IR bands which might not be suitable for authentication.

Moreover, the reliability of the PCA model depends on sample size and variation in their quality. In future applications of routine work, an increasing number of samples will challenge the model. At the same time, more sample could be added to the model to increase sample size and improve its reliability.

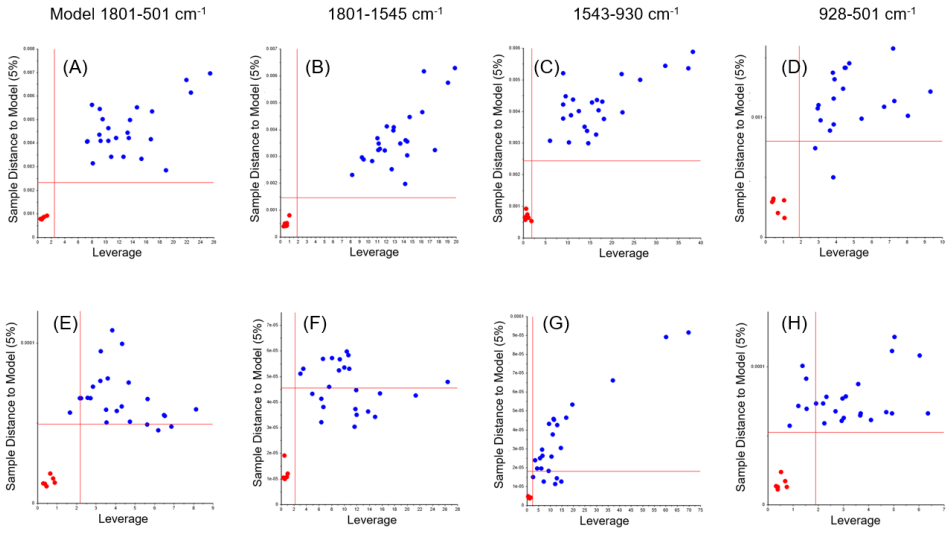

Figure 4 Si vs. Hi plots for Chan-chamot authentication using (A–D) IR spectra and (E–H) second derivative spectra (red spots = genuine *M. gagei* samples, blue spots = false samples).

Conclusion

The use of IR in combination with chemometric methods (SA, HCA and PCA) proved to be an effective method to identify and authenticate the crude drug Chan-chanmot (*M. gagei*). Both the genuine and possible substituted crude drugs were accurately classified using SIMCA. This method could be further applied for the quality assessment of other crude drugs used in traditional medicine.

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