Vasorelaxation Study and Tri-Step Infrared Spectroscopy Analysis of Malaysian Local Herbs

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Key Words
Malaysian local herbs, tri-step FTIR, vasorelaxation

Abstract
Objectives: The aim of this paper is to investigate the activities of Malaysian local herbs (Clinacanthus nutans Lindau, Strobilanthes crispus, Murdannia bracteata, Elephantopus scaber Linn., Pereskia bleo, Pereskia grandifolia Haw., Vernonia amygdalina, and Swietenia macrophylla King) for anti-hypertensive and vasorelaxant activity. An infrared (IR) macro-fingerprinting technique consisting of conventional fourier transform IR (FTIR), second-derivative IR (SD-IR), and two-dimensional correlation IR (2D-correlation IR) analyses were used to determine the main constituents and the fingerprints of the Malaysian local herbs.

Methods: The herbs were collected, ground into powder form, and then macerated by using three different solvents: distilled water, 50% ethanol, and 95% ethanol, respectively. The potentials of the extracts produced from these herbs for use as vasorelaxants were determined. Additionally, the fingerprints of these herbs were analyzed by using FTIR spectra, SD-IR spectra, and 2D-correlation IR spectra in order to identify their main constituents and to provide useful information for future pharmacodynamics studies.

Results: Swietenia macrophylla King has the highest potential in terms of vasorelaxant activity, followed by Vernonia amygdalina, Pereskia bleo, Strobilanthes crispus, Elephantopus scaber Linn., Pereskia grandifolia Haw., Clinacanthus nutans Lindau, and Murdannia bracteata. The tri-step IR macro-fingerprint of the herbs revealed that most of them contained proteins. Pereskia bleo and Pereskia grandifolia Haw. were found to contain calcium oxalate while Swietenia macrophylla King was found to contain large amounts of flavonoids.

Conclusion: The flavonoid content of the herbs affects their vasorelaxant activity, and the tri-step IR macro-fingerprint method can be used as an analytical tool to determine the activity of a herbal medicine in terms of its vasorelaxant effect.

1. Introduction
A large percentage of modern drugs are derived from plant sources. This includes well-known drugs such as aspirin, whose precursor, salicin, was originally derived from the bark and leaves of the willow (Salix spp.). Digitalis glycosides, which have been the dominant therapeutic agents in cardiovascular medicine for more than 200 years, originated from purple foxglove (Digitalis purpurea) [1]. The prevalent use of botanical drugs is further illustrated by the fact that a large number of anti-biotic, anti-diabetic, anti-hypertension, and anti-tumor drugs are derived from natural products. The widespread development of modern drugs from natural products and the increasing use of traditional botanical medicines are now an integral part of national health care systems in both developed and developing nations. The current trend seen in Europe and the United States of America is that herbal remedies are increasingly sought after by the population. However, the notion that people prefer herbal medicines due to
their costs being lower than those of most allopathic medicines or to a ‘nature-loving’ primal tendency is not entirely accurate. The trend may also be, to some extent, due to the side effects of synthetic allopathic drugs.

Herbal medicine has always been an asset to the people of Malaysia due to our country’s rich herbal resources. Therefore, bringing the consumption and use of such remedies into a valid framework for the rational scientific use of such medicines is imperative. Because Malaysia is a tropical country with plentiful rainforests, many herbs grow throughout the year. Most of the local herbs in Malaysia, such as Clinacanthus nutans Lindau, Strobilanthes crispus, Murrannia bracteata, Elephantopus scaber Linn., Pereskie bleo, Pereskie grandifolia Haw., Vernonia amygdalina, and Swietenia macrophylla King, are safe to consume and are non-toxic [2-5]. In addition, the leaves of these herbs possess many nutrients [1, 6]. Traditional medicine practitioners have been using Vernonia amygdalina as an anti-helminthic medicine, an anti-malarial medicine, a laxative, a digestive tonic, an orexigenic, a febrifuge, and a topical treatment of wounds [7]. Preclinical studies have shown that Strobilanthes crispus possesses anti-oxidant, free radical-scavenging, anti-cancer, anti-diabetic, anti-microbial, wound healing, and anti-ulcerogenic activities [8]. Based on previous research, various pharmacological activities of Swietenia macrophylla King have been established, which include anti-microbial, anti-inflammatory, anti-oxidant effects, anti-cancer, anti-tumor, and anti-diabetic activities [6]. The seeds of Swietenia macrophylla King are edible but have a bitter taste. Locals believe that the bitterer the taste is, the better the efficiency of the herb is. Murrannia bracteata is a local plant that possesses anti-oxidant hepatoprotective effects and is widely used in Malaysia as a traditional remedy for various diseases of the kidney and liver, including inflammation and cancer [9]. The whole plant of Elephantopus scaber Linn. has been tested for acute toxicity and has been found to have analgesic, anti-pyretic, anti-inflammatory, cardiovascular, diuretic, and laxative activities [10].

The knowledge of using herbal remedies to treat hypertension has been passed down for generations. However, these concepts have yet to be proven scientifically, and no evidence exists to prove that the local herbs possess anti-hypertension activity. Thus, a preliminary test to screen these local herbs and to determine their fingerprints.

2. Materials and Methods

The raw herbs of Clinacanthus nutans Lindau, Strobilanthes crispus, Murrannia bracteata, Elephantopus scaber Linn., Pereskie bleo, Pereskie grandifolia Haw., Vernonia amygdalina, and Swietenia macrophylla King were collected from Paya Terubong, Penang, Malaysia, and were identified by Dr. Rahmad Zakaria from the School of Biological Sciences, Universiti Sains Malaysia (USM). The voucher specimens of the herbs (Nos. 11700, 11701, 11702, 11703, 11704, 11705, 11706, and 11707, respectively) were deposited in the Herbarium of the School of Biological Sciences, USM. The phenylephrine and the acetylcholine used in this study were purchased from Acros Organic (Belgium) while the potassium bromide (KBr) was purchased from Merck (Germany). All drugs were dissolved in distilled water, and the stock solutions were stored at 4 – 8°C.

Adult male sprague-dawley (SD) rats weighing 250 g to 300 g were obtained from the Animal House Facility at Universiti Sains Malaysia. The rats were housed at room temperature with a 12-hour light and dark cycle and allowed free access to food and water. The rats were allowed to acclimatize in the animal transit room for a minimum period of one week before being used for the experiment. The investigation conforms to the guidelines of the Animal Ethics Committee of Universiti Sains Malaysia (Penang, Malaysia).

All herbs were washed to remove all trace particles and contaminants. The plant materials were then chopped into smaller pieces and dried in an oven. The dried plants were ground into a fine powder by using a milling machine and then underwent a maceration extraction process. The raw herbs were soaked in either water, 50% ethanol or 95% ethanol for 48 hours at 50°C. Each extract was then concentrated with a rotary evaporator under reduced pressure and subsequently freeze-dried. The samples were then stored in a desiccator at room temperature for further use.

An empty Petri dish filled with Krebs-Henseleit (Kreb's) solution was prepared. This solution consisted of (in mM) 118.0 NaCl, 4.7 KCl, 25.0 NaHCO3, 1.8 CaCl2, 1.2 NaH2PO4, 1.2 MgSO4, and 11.0 glucose. The aorta isolated from the rat was transferred to the Petri dish for the cleaning process. The nearby tissue and fat were removed from the aorta. The isolated aorta was then cut into 2 to 3 mm long strips and suspended horizontally in a tissue chamber containing 10 mL of the Kreb's solution. Special care was taken to avoid damage to the endothelium. Carbogen (95% O2 and 5% CO2) at 37°C was constantly bubbled through the Kreb's solution throughout the experiment. The aortic rings were allowed to equilibrate at an optimal tension of 1 g for 30 minutes. Throughout this period, the Kreb’s solution was replaced every 10 minutes, and the tension was readjusted to 1 g if needed. For confirmation of the condition of the aortic rings, they were pre-contracted with phenylephrine (1 µM), followed by relaxation with acetylcholine (1 µM). Then, 100 µL of plant extracts was added accumulatively into the tissue chambers at concentrations from 0.125 mg/mL to 128 mg/mL (equivalent to 0.00125 – 1.28 mg/mL in the tissue chambers). The changes in the contractile force were measured with a force-electricity transducer (GRASS Force-Displacement Transducer FT03 C Isometric Force Measurements). The signals, generated by the Labchart-5 software, were amplified, and the data were tabulated by using Microsoft Excel.
A spectrum 400 FTIR spectrometer (Perkin-Elmer) equipped with a Deuterated Tri-Glycine Sulfate (DTGS) detector was used to characterize the herbs. A tablet of KBr was used as the blank. The samples were pulverized and passed through a 200-mesh sieve. Each sample (about 2 – 3 mg) was mixed evenly with 100 mg of crystalline KBr. The mixture was ground and pressed into a tablet at a pressure of not more than 10 psi. After that, the spectra were calculated from a total of 16 co-added scans in the range of 400 – 4,000 cm⁻¹ with a resolution of 4 cm⁻¹. The interferences of water and carbon dioxide were eliminated online when scanning. The raw FTIR data were then processed by using the spectrum software of the Perkin-Elmer FTIR spectrometer (ver. 6.3.5). The FTIR spectrum was generally accepted when a transmission of 60% – 70% was achieved. Otherwise, the test was repeated with either the sample or with KBr added [11, 12].

All the SD-IR spectra were obtained after Savitzky-Golay polynomial fitting (13-point smoothing) of the original IR spectra taken at room temperature. To obtain the 2D-correlation IR spectrum, we placed the sample tablet into the sample pool with a programmable heated jacket controller (Model GS20730, Specac). In order to avoid the loss of or change in some of the unstable compositions, we collected the dynamic spectra at different temperatures ranging from room temperature to 150°C at intervals of 10 ± 2°C (with a rate of increase of 2°C/min). The full temperature scan took a total time of 50 minutes for each sample. Then, the 2D-correlation IR correlation spectra were obtained by treating the series of dynamic spectra with the 2D-correlation IR correlation analysis software developed by Tsinghua University, Beijing, China [12, 13].

The data are presented as means ± standard errors of the mean (SEMs), and n is the number of aortic rings isolated from the SD rats (n = 8). Relaxation was expressed as the percent relaxation of the phenylephrine-induced contraction.

3. Results

### 3.1. Vasorelaxant activity of different herbs with various extracts

The results in Table 1 indicate the screening data obtained by using pre-contracting aortic rings from SD rats treated with extracts of different local herbs in Malaysia. Each herb was extracted by using three different types of solvents: water, 50% ethanol, and 95% ethanol. The results showed that most of the local herb extracts exhibited significant vasorelaxant properties. The concentration of the extract is one of the factors causing relaxation of the rat aorta. However, the half maximal efficiency concentration (EC₅₀) for each solvent was different.

According to Table 1, the extracts that exhibited the best vasorelaxant activity were those from the batch of 95% ethanol extracts. Most of the ethanol extracts only required the lowest dosage to reduce the pre-contracting aortic rings, especially the ethanol extract of *Swietenia macrophylla* King (EC₅₀ was 5.60 ± 1.16 mg/mL), which was followed by *Vernonia amygdalina* and *Elephantopus scaber* Linn. with EC₅₀ values at 6.78 ± 0.66 and 11.47 ± 0.43 mg/mL, respectively. The EC₅₀ values of the extracts of *Pereskia bleo*, *Strobilanthes crispus*, *Pereskia grandifolia* Haw., *Murdannia bracteata*, and *Clinacanthus nutas* Lindau at doses of not more than 50 mg/mL were 20.11 ± 7.27, 21.41 ± 14.32, 28.87 ± 0.22, 31.98 ± 11.42, and 48.13 ± 7.56 mg/mL, respectively.

The 50% ethanol batch of extracts also exhibited vasorelaxant properties. Only 2.16 ± 0.49 mg/mL of the *Swietenia macrophylla* King 50% ethanol extract was required to reach 50% relaxation of the aortic ring, which was followed by the extracts of *Vernonia amygdalina*, *Pereskia bleo*, *Murdannia bracteata*, *Clinacanthus nutans* Lindau, and *Elephantopus scaber* Linn., for which the values of the EC₅₀ were 11.41 ± 1.50, 13.68 ± 4.57, 20.61 ± 10.27, 23.20 ± 4.37 and 37.05 ± 5.21 mg/mL, respectively. However, more than 50 mg/mL of the *Strobilanthes crispus* and *Pereskia grandifolia* Haw. 50% ethanol extracts were required to achieve 50% relaxation of the aortic rings, for which the EC₅₀ values were 56.48 ± 6.36 mg/mL and 62.11 ± 2.26 mg/mL, respectively.

### Table 1 Vasorelaxation screening of a pre-contracting aorta ring treated with herbal extracts

| Herbs                      | Water (mg/mL) | 50% Ethanol (mg/mL) | 95% Ethanol (mg/mL) |
|---------------------------|---------------|---------------------|---------------------|
| *Clinacanthus nutans* Lindau | 128.10 ± 14.82 | 23.20 ± 4.37        | 48.13 ± 7.56        |
| *Strobilanthes crispus*    | 39.46 ± 12.77 | 56.48 ± 6.36        | 21.41 ± 14.32       |
| *Murdannia bracteata*      | 166.91 ± 14.07 | 20.61 ± 10.27       | 31.98 ± 11.42       |
| *Elephantopus scaber* Linn.| 79.55 ± 19.49 | 37.05 ± 5.21        | 11.47 ± 0.43        |
| *Pereskia bleo*            | 14.31 ± 4.20  | 13.68 ± 4.57        | 20.11 ± 7.27        |
| *Pereskia grandifolia* Haw.| 77.69 ± 3.58  | 62.11 ± 2.26        | 28.87 ± 0.22        |
| *Vernonia amygdalina*      | 25.18 ± 8.58  | 11.41 ± 1.50        | 6.78 ± 0.66         |
| *Swietenia macrophylla* King | 3.46 ± 0.70   | 2.16 ± 0.49         | 5.60 ± 1.16         |

EC₅₀, half maximal efficiency concentration.
In general, a higher concentration of the water extracts of the herbs was required to achieve the same amount of relaxation induced by the 50% ethanol and the 95% ethanol extracts of the herbs. For the water extracts of Pereskia grandifolia Haw., Elephantopus scaber Linn., Clinacanthus nutans Lindau, and Murdannia bracteata, a higher dosage was required (more than 50 mg/mL) to achieve 50% relaxation of the aortic rings. However, the Swietenia macrophylla King water extract still produced a great vasorelaxant activity, with an EC50 value of 3.46 ± 0.70 mg/mL, followed by the water extracts of Pereskia bleo, Vernonia amygdalina, and Strobilanthes crispus.

The overall results in Table 1 show that Swietenia macrophylla King is the best choice for hypertension studies due to its having excellent vasorelaxant properties. All three types of Swietenia macrophylla King extracts showed significant vasorelaxant activity in the pre-contracting aortic ring assays, especially the 50% ethanol extract (EC50 = 2.16 ± 0.49 mg/mL). In short, most of the local herbal extracts showed significant vasorelaxant properties, especially Swietenia macrophylla King, Vernonia amygdalina, and Pereskia bleo. Therefore, further investigation of the vasorelaxation mechanism pathways of the herbs with the best potential will be carried out.

3.2. The tri-step IR macro-fingerprint analysis of Malaysian herbs

In this study, we used the tri-step FTIR identification method (conventional FTIR, SD-IR, and 2D-correlation IR methods) to determine the fingerprints of the herbs. The tri-step FTIR identification method by means of spectral resolution enhancement is used to progressively amplify the differences between the spectra in order to achieve a distinct identification of the sample. Conventional FTIR is used for a basic discrimination between the herbs and constitutes as a “primary identification”. SD-IR is used to amplify the differences obtained by using conventional FTIR spectroscopy with higher resolving power and constitutes a “secondary identification”. The 2D-correlation IR method can obtain information on the dynamics of the sample under external perturbation and will develop the classic spectrum in the second dimension. It has a higher resolution capability and constitutes a “tertiary identification” [14].

By using the tri-step FTIR identification method, we were able to obtain high-quality IR spectra based on the use of the SD-IR method to increase the apparent resolution of the spectrum. The enhanced expression of sample information obtained by using the 2D-correlation IR method
was used to identify distinctions between similar samples. This method was also able to verify the components within the sample herbs.

The conventional FTIR spectra of the herbs are shown in Fig. 1, and their characteristic absorption peaks are assigned in Table 2. Their SD-IR spectra and 2D-correlation IR spectra are shown in Fig. 2 and Fig. 3, respectively.

### 4. Discussion

#### 4.1. Assignments and comparison by using conventional FTIR spectra

Fig. 1 shows the FTIR spectra of *Clinacanthus nutans* Lindau, *Strobilanthes crispus*, *Murdannia bracteata*, *Elephantopus scaber* Linn., *Pereskia bleo*, *Pereskia grandifolia* Haw., *Vernonia amygdalina*, and *Swietenia macrophylla* King.

### Table 2 Peak assignments on the conventional FTIR spectra of Malaysian local herbs

| Peak (cm⁻¹) | Primary assignment | Possible compounds |
|------------|--------------------|--------------------|
| **Clinacanthus nutans** Lindau |                    |                    |
| 3366       | O-H, \( \nu \)     | Various            |
| 3409       | O-H, \( \nu \)     | Various            |
| 3413       | C-H, \( s_{\nu} \) | Alkene             |
| 3415       | C-H, \( s_{\nu} \) | Alkene             |
| 3422       | C-H, \( s_{\nu} \) | Alkene             |
| 3357       | C-H, \( s_{\nu} \) | Alkene             |
| 3300       | C-H, \( s_{\nu} \) | Alkene             |
| 2959       | C-H, \( s_{\nu} \) | Alkene             |
| 2923       | C-H, \( s_{\nu} \) | Alkene             |
| 2921       | C-H, \( s_{\nu} \) | Alkene             |
| 2920       | C-H, \( s_{\nu} \) | Alkene             |
| 2924       | C-H, \( s_{\nu} \) | Alkene             |
| 2919       | C-H, \( s_{\nu} \) | Alkene             |
| 2920       | C-H, \( s_{\nu} \) | Alkene             |
| 2925       | C-H, \( s_{\nu} \) | Alkene             |
| 2851       | C-H, \( s_{\nu} \) | Alkene             |
| 2852       | C-H, \( s_{\nu} \) | Alkene             |
| 2851       | C-H, \( s_{\nu} \) | Alkene             |
| 2850       | C-H, \( s_{\nu} \) | Alkene             |
| 2851       | C-H, \( s_{\nu} \) | Alkene             |
| 2854       | C-H, \( s_{\nu} \) | Alkene             |
| 1735       | C-O, \( \nu \)     | Ester              |
| 1734       | C-O, \( \nu \)     | Ester              |
| 1738       | C-O, \( \nu \)     | Ester              |
| 1649       | N-H, \( \delta \)  | Amide I            |
| 1647       | N-H, \( \delta \)  | Amide I            |
| 1627       | N-H, \( \delta \)  | Amide I            |
| 1638       | N-H, \( \delta \)  | Amide I            |
| 1654       | N-H, \( \delta \)  | Amide I            |
| 1549       | N-H, \( \delta \)  | Amide II           |
| 1547       | N-H, \( \delta \)  | Amide II           |
| 1432       | N-H, \( \delta \)  | Amide II           |
| 1412       | N-H, \( \delta \)  | Amide II           |
| 1416       | N-H, \( \delta \)  | Amide II           |
| 1399       | (O) C-H, \( \nu \) | Saccharides        |
| 1401       | (O) C-H, \( \nu \) | Saccharides        |
| 1376       | (O) C-H, \( \delta \) | Ester             |
| 1384       | (O) C-H, \( \delta \) | Ester             |
| 1382       | (O) C-H, \( \delta \) | Ester             |
| 1384       | (O) C-H, \( \delta \) | Ester             |
| 1315       | (O) C-H, \( \delta \) | Ester             |
| 1318       | C-O, \( \nu \)     | Calcium oxalate   |
| 1242       | C-O, \( \nu \)     | Calcium oxalate   |
| 1241       | C-O, \( \nu \)     | Calcium oxalate   |
| 1242       | C-O, \( \nu \)     | Calcium oxalate   |
| 1256       | C-O, \( \nu \)     | Calcium oxalate   |
| 1245       | C-O, \( \nu \)     | Calcium oxalate   |
| 1256       | C-O, \( \nu \)     | Calcium oxalate   |
| 1240       | C-O, \( \nu \)     | Calcium oxalate   |
| 1155       | C-O, \( \nu \)     | Calcium oxalate   |
| 1164       | C-O, \( \nu \)     | Calcium oxalate   |
| 1105       | C-O, \( \nu \)     | Calcium oxalate   |
| 1107       | C-O, \( \nu \)     | Calcium oxalate   |
| 1101       | C-O, \( \nu \)     | Calcium oxalate   |
| 1053       | C-O, \( \nu \)     | Calcium oxalate   |
| 1075       | C-O, \( \nu \)     | Calcium oxalate   |
| 1031       | C-O, \( \nu \)     | Calcium oxalate   |
| 1067       | C-O, \( \nu \)     | Calcium oxalate   |
| 1071       | C-O, \( \nu \)     | Calcium oxalate   |

FTIR, fourier transform infrared; \( \nu \), stretching; \( s_{\nu} \), symmetrical stretching; \( s_{\nu} \), asymmetrical stretching; \( \delta \), bending.
Figure 2 Second-derivative spectrum for each herb in the range of 1,800 – 800 cm⁻¹: (A) Clinacanthus nutans Lindau, (B) Strobilanthes crispus, (C) Murdannia bracteata, (D) Elephantopus scaber Linn., (E) Pereskia bleo, (F) Pereskia grandifolia Haw., (G) Vernonia amygdalina, and (H) Swietenia macrophylla King.
Figure 3 The 2D-correlation IR fingerprint of each herb in the range of 1,200 – 1,800 cm⁻¹: (A) Clinacanthus nutans Lindau, (B) Strobilanthes crispus, (C) Murdannia bracteata, (D) Elephantopus scaber Linn., (E) Pereskia bleo, (F) Pereskia grandifolia Haw., (G) Vernonia amygdalina, and (H) Swietenia macrophylla King.

2D-correlation IR, two-dimensional correlation infrared.
ephantopus scaber Linn., Pereskia bleo, Pereskia grandifolia Haw., Vernononia amygdalina, and Swietenia macrophylla King. The peaks that appeared at 3,400 – 3,300 cm\(^{-1}\) are O-H stretching vibration absorption peaks. The peaks nearby at 2,921 and 2,851 cm\(^{-1}\), are methylene C-H asymmetric and symmetric stretching vibration absorption peaks, respectively. The peak at 1,735 cm\(^{-1}\) is the carbonyl group C=O stretching vibration absorption peak. The peaks nearby at 1,640 cm\(^{-1}\) may contain OH bending vibration absorption and conjugated carbonyl stretching vibration absorption peaks. The absorption peaks within the range of 1,430 – 1,400 cm\(^{-1}\) region may contain a variety of ingredients, such as C=H bending vibration absorption and (O)C-H stretching vibration peaks, while the peaks in the range of 1,300 – 950 cm\(^{-1}\) are the absorption peaks for all types of C-O stretching (see Table 2).

In Fig. 1, all the spectra of the herbs, except that for Swietenia macrophylla King, are very similar. An obvious difference between them lies in the intensities of the absorption peaks in the range of 1,200 – 950 cm\(^{-1}\), corresponding to the peak intensity nearby at 1,640 cm\(^{-1}\). The absorption peaks of Strobilanthus crispus, Murdannia bracteata, and Elephantopus scaber Linn. (Figs. 1B, 1C, 1D) in the range of 1,200 – 950 cm\(^{-1}\) have higher intensities than the absorption peaks at 1,640 cm\(^{-1}\), in contrast with the peak intensities of other herbs. This indicates that Strobilanthus crispus, Murdannia bracteata, and Elephantopus scaber Linn. have a higher content of saccharides or sugar than the other herbs. In the spectra of Clinacanthus nutans Lindau and Pereskia bleo (Figs. 1A, 1E), both the 1,649 cm\(^{-1}\) and the 1,549 cm\(^{-1}\) absorption peaks appear simultaneously in their spectra. The shapes of these two absorption peaks correspond to those of the amide I and amide II proteins, indicating that these two herbs contain a certain amount of protein. In addition, the spectra of Pereskia bleo and Pereskia grandifolia Haw. (Figs. 1E, 1F) record the appearance of some absorption peaks at 1,318 cm\(^{-1}\), 780 cm\(^{-1}\), and 514(515) cm\(^{-1}\), which are due to the symmetrical stretching vibration and bending vibration absorption of the C-O bond of oxalate ions. This indicates that Pereskia bleo and Pereskia grandifolia Haw. contain a certain amount of calcium oxalate.

The spectrum of Swietenia macrophylla King is completely different from the spectra of the other herbs. This is because the material from the Swietenia macrophylla King plant that was used in this experiment was the seeds. The seeds of Swietenia macrophylla King have a high content of oil and show obvious characteristic peaks in its IR spectrum. The molecule of oil is a long-chain fatty-acid ester. The main functional groups contained within the extract are a methylene group and an ester carbonyl. The spectrum of Swietenia macrophylla King (Fig. 1H), due to the fact that the plant oil contains more unsaturated fatty acids, shows more C=C double bonds. Therefore, a significant alkene C-H stretching absorption peak (3,009 cm\(^{-1}\)) is observed in its spectrum. The peak at 2,925 cm\(^{-1}\) is the absorption peak due to the methylene C-H asymmetric stretching vibration, and the peak at 2,854 cm\(^{-1}\) is the absorption peak due to the methylene C-H symmetric stretching vibration. In addition, the peak at 1,746 cm\(^{-1}\) is the absorption peak for the ester carbonyl C=O stretching vibration while the peak appearing at 722 cm\(^{-1}\) (C-H bending vibration) indicates that Swietenia macrophylla King contains long-chain carbons (\(\text{C} \geq 4\)).

4.2. Comparison and discussion of the SD-IR spectra

The SD-IR spectra can resolve the problem of overlapping peaks, enhance the spectral characteristics, and improve the apparent resolution in the IR spectrum. Fig. 2 shows the SD-IR spectra of the eight Malaysian local herbs within the wave-number range of 1,800 – 800 cm\(^{-1}\), which contains the main absorption bands of the chemical constituents of the herbs. As shown in the figure, some relatively weak absorption peaks in the conventional IR spectra can be seen more clearly in the SD-IR spectra [14].

In Fig. 2, the intensities of the peak around 1,740 cm\(^{-1}\) (carbonyl C=O stretching vibration) among these eight herbs are not the same. The strongest peak is from Swietenia macrophylla King (Fig. 2H), indicating that it has the highest content of ester compounds. The appearance of the absorption peaks at 1,165 cm\(^{-1}\), 1,078 cm\(^{-1}\), 989 cm\(^{-1}\), 952 cm\(^{-1}\), 890 cm\(^{-1}\), and 826 cm\(^{-1}\) in the spectrum of Murdannia bracteata (Fig. 2C) can be ascribed to its containing much stachyose. In the SD-IR spectra of Pereskia bleo and Pereskia grandifolia Haw. (Figs. 2E, 2F), the absorption peaks at 1,317 cm\(^{-1}\) are very strong, once again showing that they have a high content of calcium oxalate.

In the SD-IR spectrum of Clinacanthus nutans Lindau (Fig. 2A), the absorption peaks at 1,739 cm\(^{-1}\), 1,715 cm\(^{-1}\), 1,635 cm\(^{-1}\) (C=O stretching vibration), 1,577 cm\(^{-1}\), 1,540 cm\(^{-1}\), 1,516 cm\(^{-1}\), 1,496 cm\(^{-1}\) (aromatic ring skeleton vibration), 1,472 cm\(^{-1}\), 1,375 cm\(^{-1}\) (C-H bending vibration), and 1,282 cm\(^{-1}\) (C-O stretching vibration) are stronger, which indicates that it contains more flavonoids. Strobilanthus crispus, Vernononia amygdalina, and Swietenia macrophylla King (Figs. 2B, 2G, 2H) also have absorption peaks due to C=O stretching (around 1,740 cm\(^{-1}\) and 1,660 cm\(^{-1}\)), aromatic ring skeleton vibration (around 1,545 cm\(^{-1}\)), and C-H bending vibration (around 1,470 cm\(^{-1}\)) in their spectra; therefore, they also contain flavonoids. The observation that the locations of their absorption peaks are a bit biased indicates that they contain different types of flavonoids. This array of different flavonoids will affect their vasorelaxant effect.

4.3. Comparison and discussion of the 2D-correlation IR spectra

Compared to conventional spectroscopic methods, 2D-correlation spectroscopy introduces an external perturbation. This will expand the conventional IR spectrum to a second dimension to obtain more information on the sample, thus increasing the spectral resolution. In the 2D-correlation IR experiments, the sample was subjected to a perturbation that changed the intramolecular and the intermolecular interactions within the sample. The perturbation will also affect the vibration frequency and the coupling effect of each group of molecules. Through an analysis of the changes in the spectra, we were able to obtain relevant information on the intramolecular interactions and intermolecular interactions of the functional groups [14]. By taking advantage of the differences in the responses for the different chemical groups under an ex-
ternal thermal perturbation, we obtained the 2D-correlation IR spectra of the eight local Malaysian herbs from the temperature-dependent IR spectra. We proceeded with an analysis of the synchronous spectra of these herbs due to the clear characteristic intensity change within the 1,200 to 1,800 cm⁻¹ region (Fig. 3).

In the synchronous spectrum obtained using the 2D-correlation IR method, the diagonal peak that results from the autocorrelation of perturbation-induced dynamic fluctuations of the IR signals is called the “auto peak”. It indicates the susceptibility of the corresponding absorbance bands to a given external perturbation. The cross peaks located at the off-diagonal positions reveal the relative intensity variations of a pair of group vibrations corresponding to their frequencies. A positive cross peak (red/green area) represents a consistent population change, either simultaneous increasing or decreasing, of different groups under an external perturbation. The more coordinated the intensity changes are, the stronger the cross peak is. In contrast, a negative cross peak (blue area) represents coordinated changes of the band intensities in the opposite directions [12].

The 2D-correlation IR spectra of the herbs in the range of 1,200 – 1,800 cm⁻¹ are shown in Fig. 3. The 2D-correlation IR spectra of Clinacanthus nutans Lindau, Strobilanthes crispus, Murdannia bracteata, Elephantopus scaber Linn., Persikia bleo, Pereskiia grandifolia Haw., and Vernonia amygdalina (Figs. 3A, 3B, 3C, 3D, 3E, 3F, 3G) are quite similar, with their main auto peaks being located in the range of 1,700 – 1,600 cm⁻¹, and 1,600 – 1,500 cm⁻¹, and with their having positive corresponding cross peaks. This indicates that these auto peaks should correspond to variations in the amide I protein and the amide II protein under heat perturbation. The specific locations, shapes and intensities of these auto peaks vary for different herbs. The information given above indicates that these seven herbs contain proteins. Their peaks are clearly shown in the 2D-IR spectra, proving that the 2D-correlation IR spectra have a higher resolution than the conventional FTIR and SD-IR spectra. In the 2D-correlation IR spectra of Murdannia bracteata (Fig. 3C), an auto-peak is also seen near 1,500 cm⁻¹ (aromatic ring stretching vibration), and its corresponding cross peaks are negative. This indicates that in the heating process, the stabilities of the proteins and the aromatic substances are different. The auto peaks around 1,617 cm⁻¹ and 1,326 cm⁻¹ correspond to the absorption peaks of calcium oxalate, which only appear in the 2D-correlation IR spectra of Persikia bleo and Persikia grandifolia Haw. (Figs. 3E, 3F), showing that these two herbs contain calcium oxalate. Both the auto peaks of calcium oxalate and the auto peaks within the range of 1,700 – 1,500 cm⁻¹ form positive cross peaks. This can be interpreted as calcium oxalate and proteins having the same thermal stability. While Persikia grandifolia Haw. (Fig. 3F) still has an auto peak at 1,382 cm⁻¹ (C-H bending), it forms negative cross peaks with the aforementioned auto peaks.

The 2D-correlation IR spectra of Swietenia macrophylla King (Fig. 3H) is significantly different from those of the other herbs, with its main auto peaks being located at 1,760 cm⁻¹, 1,741 cm⁻¹, 1,709 cm⁻¹, 1,630 cm⁻¹, 1,563 cm⁻¹, 1,500 cm⁻¹, and 1,467 cm⁻¹. These auto peaks can be divided into two groups by grouping the two auto peaks at 1,760 cm⁻¹ and 1,500 cm⁻¹ in one group, and the other remaining auto peaks in the other group. The corresponding cross peaks for every two auto peaks within the same group are positive (corresponding cross peaks of 1,760 cm⁻¹ and 1,500 cm⁻¹ are positive, as well as the corresponding cross peaks of 1,741 cm⁻¹, 1,709 cm⁻¹, 1,630 cm⁻¹, 1,563 cm⁻¹, and 1,467 cm⁻¹) while the cross peaks between two different groups of auto peaks are negative (auto peaks at 1,760 cm⁻¹ and 1,500 cm⁻¹ form negative cross peaks with those at 1,741 cm⁻¹, 1,709 cm⁻¹, 1,630 cm⁻¹, 1,563 cm⁻¹, and 1,467 cm⁻¹). The auto peaks near 1,760 cm⁻¹, 1,741 cm⁻¹, and 1,709 cm⁻¹ correspond to the C=O stretching vibration absorption of esters while the auto peaks near 1,630 cm⁻¹, 1,563 cm⁻¹, and 1,467 cm⁻¹ correspond to the flavonoid compounds. Above, Swietenia macrophylla King was shown to contain large amounts of flavonoids, which also explained why Swietenia macrophylla King had the highest vasorelaxant effect (see Table 1). In addition, the auto peak near 1,500 cm⁻¹ corresponds to the aromatic substance skeleton stretching vibration absorption. The absorption peaks near 1,500 cm⁻¹ form negative corresponding cross peaks with those at 1,709 cm⁻¹, 1,630 cm⁻¹, 1,563 cm⁻¹, and 1,467 cm⁻¹, indicating that the carbonyl group of esters and flavonoids are more sensitive to heat than the aromatic ring skeletons.

5. Conclusion

The overall results for various extracts (water extract, 50% ethanol extract, and 95% ethanol extract) of Malaysian local herbs in a vasorelaxation experiment showed that Swietenia macrophylla King had the highest vasorelaxant effect, followed by Vernonia amygdalina, Persikia bleo, Strobilanthes crispus, Elephantopus scaber Linn., Persikia grandifolia Haw., Clinacanthus nutans Lindau, and Murdannia bracteata. Based on a systematical analysis of the Malaysian local herbs by using the FTIR, SD-IR, and 2D-correlation IR methods, we discovered that most of the herbs contained proteins, that Persikia bleo and Persikia grandifolia Haw. contained calcium oxalate, and that Swietenia macrophylla King contained large amounts of flavonoids. According to the analysis of the FTIR spectra, we believe that the contents of flavonoids will affect the vasorelaxant activities of the herbs. This indicates that the tri-step IR macro-fingerprint method can be used as an analytical tool to determine the activity of a herbal medicine in relation to its vasorelaxant effect. The mechanism pathways utilized by herbs, such as Swietenia macrophylla King and Vernonia amygdalina with medicinal potential, to induce vasorelaxation effects will be investigated in future studies.

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Conflict of interest

The authors declare that there are no conflict of interest.

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References

1. Upton R, Graff A, Jolliffe G, Länger R, Williamson E. American herbal pharmacopoeia: botanical pharmacognosy-microscopic characterization of botanical medicines. Florida: CRC Press; 2010. 800 p.
2. Tan ML, Sulaiman SF, Najimuddin N, Samian MR, Muhammad TS. Methanolic extract of Pereskia bleo (Kunth) DC. (Cactaceae) induces apoptosis in breast carcinoma, T47-D cell line. J Ethnopharmacol. 2005;96(1-2):287-94.
3. Quasie O, Zhang YM, Zhang HJ, Luo J, Kong LY. Four new steroid saponins with highly oxidized side chains from the leaves of Vernonia amygdalina. Phytochem Lett. 2016;15:16-20.
4. Chen JJ, Huang SS, Liao CH, Wei DC, Sung PJ, Wang TC, et al. A new phragmalin-type limonoid and anti-inflammatory constituents from the fruits of Swietenia macrophylla. Food Chem. 2010;120(2):379-84.
5. Goh KL. Malaysian herbs. Port Klang: Percetakan Advanco Sendirian Berhad; 2000. 260 p.
6. Moghadamtousi SZ, Goh BH, Chan CK, Shabab T, Kadir HA. Biological activities and phytochemicals of Swietenia macrophylla King. Molecules. 2013;18(9):10465-83.
7. Ijeh II, Eijke C. Current perspectives on the medicinal potentials of Vernonia amygdalina Del. J Med Plants Res. 2011;5(7):1051-61.
8. Nurraihana H, Norfarizan-Hanoon N. Phytochemistry, pharmacology and toxicology properties of Strobilanthes crispus. Int Food Res J. 2013;20(5):2045-56.
9. Yam MF, Ang LF, Lim CP, Ameer OZ, Salman IM, Ahmad M, et al. Antioxidant and hepatoprotective effects of Murdannia bracteata methanol extract. J Acupunct Meridian Stud. 2010;3(3):197-202.
10. Poli A, Nicolau M, Simoes CM, Nicolau RM, Zanin M. Preliminary pharmacologic evaluation of crude whole plant extracts of Elephantopus scaber. part I: in vivo studies. J Ethnopharmacol. 1992;37(1):71-6.
11. Choong YK, Sun SQ, Zhou Q, Lan J, Lee HL, Chen XD. Verification of Ganoderma (lingzhi) commercial products by fourier transform infrared spectroscopy and two-dimensional IR correlation spectroscopy. J Mol Struct. 2014;1069:60-72.
12. Li JR, Sun SQ, Wang XX, Xu CH, Chen JB, Zhou Q, et al. Differentiation of five species of Danggui raw materials by FTIR combined with 2D-COS IR. J Mol Struct. 2014;1069:229-35.
13. Li D, Jin Z, Zhou Q, Chen J, Lei Y, Sun S. Discrimination of five species of Fritillaria and its extracts by FT-IR and 2D-IR. J Mol Struct. 2010;974(1-3):68-72.
14. Sun SQ, Zhou Q, Chen JB. Analysis of traditional Chinese medicine by infrared spectroscopy. Beijing: Chemical Industrial Press; 2010. 358 p.