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DIFFERENTIATION OF THE WHITE AND PURPLE FLOWER FORMS OF *Orthosiphon aristatus* (Blume) Miq. BY 1D and 2D CORRELATION IR SPECTROSCOPY

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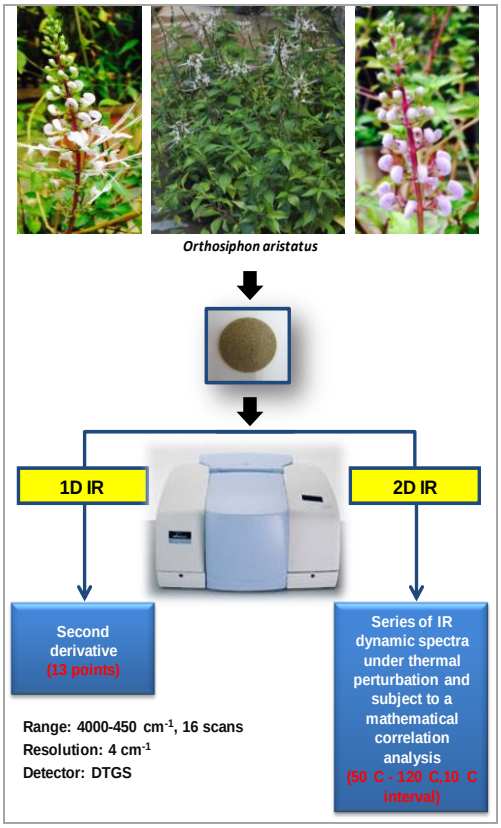
Graphical abstract



Abstract

Orthosiphon aristatus (Blume) Mig. is also known as Misai kucing or Kumis kucing. The plant is traditionally used to treat tonsilities, fever, menstrual disorders, gonorrhea, syphilis, gallstones, rheumatism, diabetes, hypertension, epilepsy, edema, hepatitis jaundice and for diuretic activity. The objective of this work was to differentiate the white and purple flower forms of *O. aristatus* (Blume) by multi-steps infrared (IR) macro-fingerprinting. The analyses included Fourier transform infrared spectroscopy (FT-IR), second derivative IR spectroscopy and two-dimensional correlation infrared (2D IR) spectroscopy with thermal perturbation. A distinctive absorption peak at 1384 cm^{-1} from the purple flower form clearly differentiated the two forms of *O. aristatus.* Further differentiation was made by the appearance of a strong auto-peak at 1580 cm^{-1} for the white flower form and at 1546 cm^{-1} for the purple flower form. Such results indicate that the multi-steps infrared macro-fingerprinting has the potential for the development of a fast and reliable analytical methodology for distinguishing the white and purple flower forms of *O. aristatus* (Blume) Miq. effectively.

Keywords: *Orthosiphon aristatus,* FT-IR, 2D IR correlation spectroscopy

Abstrak

Orthosiphon aristatus (Blume) Miq. juga dikenal sebagai Misai kucing atau Kumis kucing. Tumbuhan ini digunakan secara tradisional untuk merawat tonsilitis, demam, gangguan haid, gonorea, sifilis, batu karang, penyakit sendi, kencing manis, tekanan darah tinggi, epilepsi, edema, hepatitis, penyakit kuning serta aktiviti diuretik. Tujuan kajian ini adalah untuk membezakan di antara *O. aristatus* (Blume) jenis bunga putih dan bunga ungu dengan menggunakan cap-jari makro inframerah (IR) pelbagai langkah. Kaedah analisis termasuk spektroskopi inframerah (FT-IR), spektroskopi IR terbitan kedua dan spektroskopi korelasi inframerah dua dimensi (2D IR) dengan kaedah pemanasan. Puncak penyerapan tersendiri pada 1384 cm^{-1} daripada bunga ungu jelas membezakan kedua-dua warna *O. aristatus*. Pembezaan lanjut dapat dilihat dengan kemunculan puncak auto yang kuat pada 1580 cm^{-1} untuk bunga putih dan pada 1546 cm^{-1} untuk bunga ungu. Hasil itu menunjukkan bahawa cap-jari makro inframerah pelbagai langkah berpotensi untuk dibangunkan sebagai satu kaedah analisis yang cepat dan boleh dipercayai untuk membezakan *O. aristatus* (Blume) Miq. jenis bunga putih dan bunga ungu secara berkesan.

Kata kunci: *Orthosiphon aristatus,* FT-IR, spektroskopi korelasi 2D IR

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1.0 INTRODUCTION

Fourier transform infrared (FT-IR) spectroscopy is one of the most widely used methods to identify chemical constituents and elucidate their structures. With some incomparable advantages, such as fast, accurate, good reproducibility and needs less sample preparations, FT-IR has been used as a requisite method to identify medicines in Pharmacopoeia of many countries. The fingerprint characters and extensive applicability to various types of samples also has made FT-IR to play an important role in pharmaceutical analysis [1].

Likewise, two-dimensional infrared (2D IR) correlation spectroscopy is also used extensively in various research fields [2]. The method based on thermal perturbation was introduced by Noda [3, 4]. 2D correlation spectra will enhance the spectral resolution by spreading peaks over the second dimension, and simplify the complex spectra consisting of many overlapped peaks, as well as identify various inter- and intra-molecular interactions through selective correlation of IR peaks.

Orthosiphon aristatus is a herbaceous shrub, which grows up to 1.5 m in height. The stem is quadrangle, reddish in colour, erect and branced profusely. The petiole is reddish purple in colour and about 0.3 cm in length. Leaves are arranged in opposite pairs, simple, green and glabrous with a lanceolate leaf blade and serrate margin. The leaf apice is acuminate with an acute leaf base. The flowers are campanulate in shape, white to bluish in colour with long far exerted filaments like 'cat's whiskers' as suggested by the vernacular name [5]. It is one of the popular herbs used by the traditional Malay herbal practitioners for treating ailment related to bladder and kidneys [6, 7]. It is also used traditionally in treating gout, diabetes and rheumatism. It is reported that the intake of *Orthosiphon* tea could increase the alkalinity in kidney or bladder stones [8]. Clinical studies showed that the diuretic effect of Misai kucing had no influence on sodium excretion [9]. Besides the diuretic property, this herb also contains ample potassium to replace that is lost from the body during the diuretic process. It is also known that this species has two types of flower colours, white and purple. Differences in its flower colour may lead to the presence of different chemical constituents and may contribute to different potential of biological activities. The main groups of chemical constituents present in *O. aristatus* are flavonoids, organic acids and terpenoids.

Therefore, this study aims to differentiate the white and purple flower forms of *O. aristatus* by multi-steps IR macro-fingerprinting. The developed fingerprints will be useful as a quality control method for the selection and authentication of the raw material supply as well as their derived products.

2.0 EXPERIMENTAL

2.1 Plant Materials

Dried leaves of the white and purple flower forms of *O. aristatus* were bought from the supplier.

2.2 Apparatus

IR spectra were recorded on a Spectrum 100 Fourier transform-infrared (FT-IR) spectrometer (Perkin Elmer, CA, USA), equipped with a mid-infrared deuterated triglycine sulphate (DTGS) detector. The spectra were obtained in the frequency range of 4000–450 cm^{-1} with a resolution of 4 cm^{-1} and with a total accumulation of 16 scans. Portable programmable temperature controller (4000 series™ High Stability Temperature Controller, Specac, Ltd.) was used in the range of 50–120°C.

2.3 FT-IR Spectroscopy and 2D IR Spectroscopy

The dried leaves were ground and sieved with 150 μm mesh. Exactly 2 mg of each sample was mixed with 100 mg of potassium bromide (KBr) powder and the mixture was further ground and pressed into a 13 mm diameter disc. 1D FT-IR spectra were recorded from a total of 16 scans in the 4000–450 cm^{-1} range with resolution of 4 cm^{-1}. The second derivative IR spectra were obtained by using Savitzky-Golay filter through 13-point smoothing. Savitzky-Golay smoothing aimed for minimum distortion by least squares fitting a cubic polynomial. For the measurement of 2D IR spectra, each sample disc was placed in the sample pool connected with a temperature controller. The dynamic 2D IR spectra were collected at different temperatures from 50 to 120°C at interval of 10°C. 2D IR correlation spectra were acquired by treatment of the series of temperature-dependant dynamic spectra with 2D IR correlation analysis employing Softdoc software developed by Tsinghua University (Beijing, China). Each sample was analyzed in triplicates.

3.0 RESULTS AND DISCUSSION

3.1 1D FT-IR Spectral Analysis

FTIR spectroscopy is a rapid and simple methodology that is non-destructive for the analytes [10]. The IR spectra of the leaves of the white and purple flower forms of *O. aristatus* are shown in Figure 1a. Generally, the two spectra showed similar IR absorption peaks stating that their chemical properties were not distinctively different. Nevertheless, some variations in term of shape and intensities could be observed among the samples in the expanded region of 2000–900 cm^{-1} (Figure 1b). A distinctive strong and sharp

absorption peak at 1384 cm^{-1} in the purple flower form and the presence of peaks at 1528 cm^{-1} and 1444 cm^{-1} in the white flower form clearly differentiated the two forms of *O. aristatus*.

As shown in Figure 1a, the strongest peak can be seen at 1627–1641 cm^{-1} assignable to the conjugated C=O stretching vibration in carbonyl compounds which may be characterized by the presence of high content of terpenoids and flavonoids. The presence of narrow and sharp peaks at ~2924 cm^{-1} and 2852 cm^{-1} were assigned to C–H and C–H (methoxy compounds) stretching vibrations respectively [11]. A total of eighteen absorption peaks were obviously present in the IR spectra which can be used to characterize the white and purple flower forms of *O. aristatus* (Table 1).

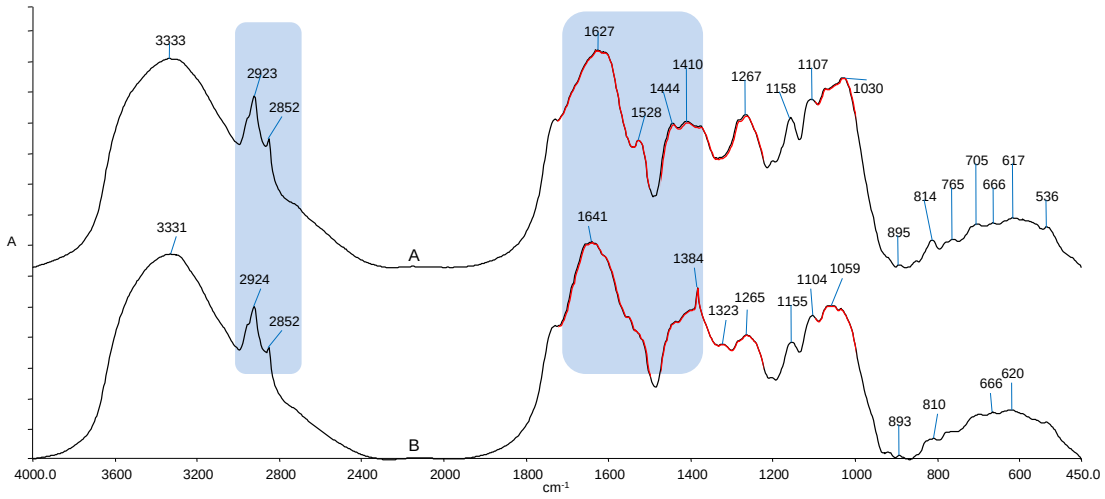

Figure 1a IR spectra of the leaves of *O. aristatus*, (A) white flower form and (B) purple flower form

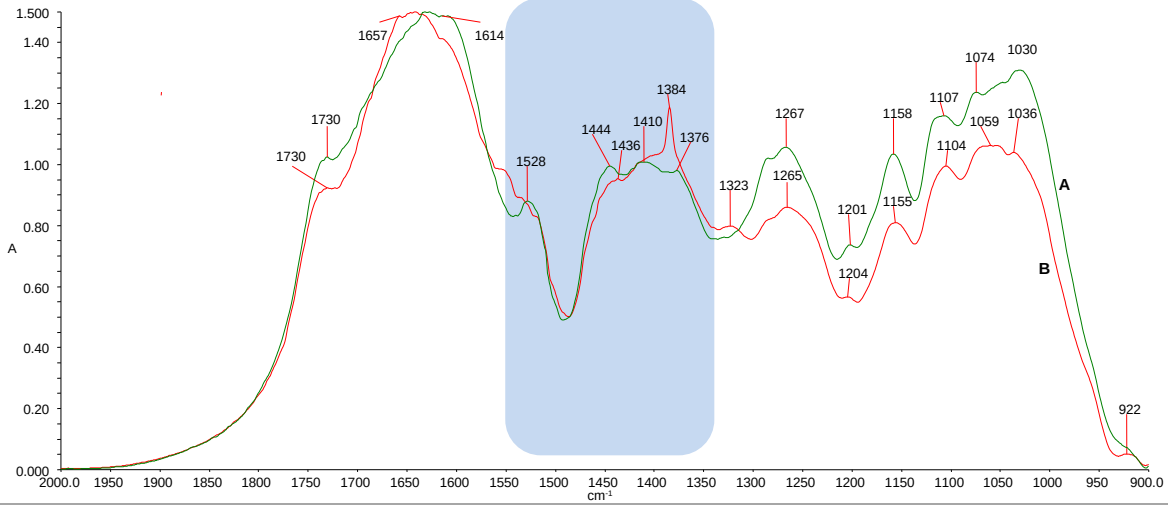

Figure 1b Expanded IR spectra in the region of 2000–900 cm^{-1} for the leaves of *O. aristatus*, (A) white flower form and (B) purple flower form

Table 1 Preliminary assignment of IR spectra of *O. aristatus*

Wavenumber (cm^{-1})	Base group and vibration mode	Main attribution	References
3331–3333	ν(O–H)	Hydroxyl	19
2923–2924	ν_{as}(C–H)	Methylene	19
2852	ν_s(C–H)	Methylene	19
1627–1641	ν(C=C)	Aromatic benzene ring	20
1528	ν_{rf}(ar), ν_{rf}(ha)	Flavonoids, anthraquinones	18
1384–1444	δ(C–H)	Methyl, flavonoids	16
1323	δ(O–H)	Hydroxyl	16
1265–1267	ν(C–O)	Phenolic hydroxyl	16
1155–1158	ν(C–O), δ(C–OH)	Lipid, tertiary alcohol groups	16
1104–1107	δ(C–H)	Methyl or phenyl	16
1030–1059	ν_s(C–O)	=C–O–C of aromatics	16
893–895	γ(C–H)	End methylene	16
536–814	γ(C–H)	Benzene ring	19

Note: ν, stretching or vibration; ν_{as}, asymmetrical; ν_s, symmetrical; ν_{rf}, ring frame; δ, in plane deformation; ar, aromatic; ha, heteroaromatic

3.2 Second Derivative IR Spectral Analysis

Second derivative IR spectra could enhance the spectral resolution by amplifying tiny differences in the IR spectrum as well as resolving some overlapped absorption peaks [1, 12, 13]. The spectral data in the region 1550 – 1180 cm^{-1} showed many dissimilarities between the two flower forms of *O. aristatus* (Figure 2), whilst the same region in Figure 1a showed only a few obvious absorption peaks.

There was no evidence of characteristic peaks at 1541 cm^{-1}, 1376 cm^{-1}, 1365 cm^{-1}, 1330 cm^{-1}, 1308 cm^{-1} and 1239 cm^{-1} for the leaves of purple flower form as compared to the leaves of the white flower form while a sharp absorption peak appeared at 1384 cm^{-1} in the leaves of the purple flower form. Dissimilarity was observed in the intensity of absorption peaks between the two flower forms. The peaks at 1529 cm^{-1}, 1499 cm^{-1}, 1454 cm^{-1}, 1287 cm^{-1} and 1203 cm^{-1} appear with higher intensities in the white flower form as compared to that of the purple flower form. Thus the two flower forms of *O. aristatus* can be distinguished from each other by studying their peak positions at 1541 cm^{-1}, 1384 cm^{-1}, 1376 cm^{-1}, 1365 cm^{-1}, 1330 cm^{-1}, 1308 cm^{-1} and 1239 cm^{-1}.

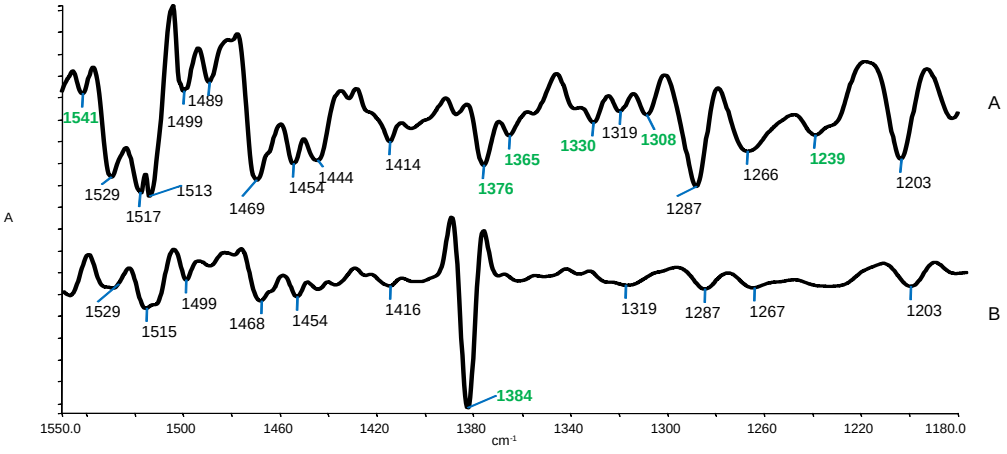

Figure 2 Second derivative IR spectra in the range of 1180–1550 cm^{-1} of *O. aristatus*, (A) white flower form and (B) purple flower form

3.3 Two-Dimensional Correlation IR Spectral Analysis

The 2D correlation IR analysis is the study of spectrum through the vibration with external perturbation and is able to enhance the resolution of the spectra which cannot be acquired from the 1D FT-IR and second derivative IR spectra. Therefore 2D correlation IR spectroscopy based on thermal perturbation reveals molecular vibrating behavior of relative group of molecule during the temperature perturbation [14, 15]. In synchronous spectrum, the auto-peaks on the diagonal line show the self-correlativity and susceptibility of some normal vibration of functional groups with the increasing temperature. The cross-peak located at the off-diagonal position reveals the relativity of intensity variations of a pair of group vibrations corresponding to their frequencies [1, 15]. Cross-peaks appear when the dynamic variations of the IR spectrum at two different wavenumbers are correlated or anti-correlated to each other. A positive cross-peak represents either simultaneous increase or decrease, of different group of molecules under an external perturbation [16, 17].

In order to obtain enhanced spectral resolution, we carried out the synchronous 2D IR spectroscopy under thermal perturbation from 50°C to 120°C. The synchronous 2D IR correlation spectra in the region 1800–1000 cm^{-1} are shown in Figure. 3. Comparison based on the positions of auto-peaks as the third step of identification, revealed the presence of the strongest auto-peak at 1580 cm^{-1} for the white flower form and at 1546 cm^{-1} for the purple flower form of *O. aristatus*. There were four obvious auto-peaks for the white flower form while five obvious auto-peaks were observed in the purple flower form (Table 2). The 2D correlation IR synchronous spectra showed relatively stronger cross-peak at (1654 cm^{-1}, 1579 cm^{-1}) for the white flower form. Conversely, a weaker cross-peak was observed at (1392 cm^{-1}, 1217 cm^{-1}) in the purple flower form but not in the white flower form.

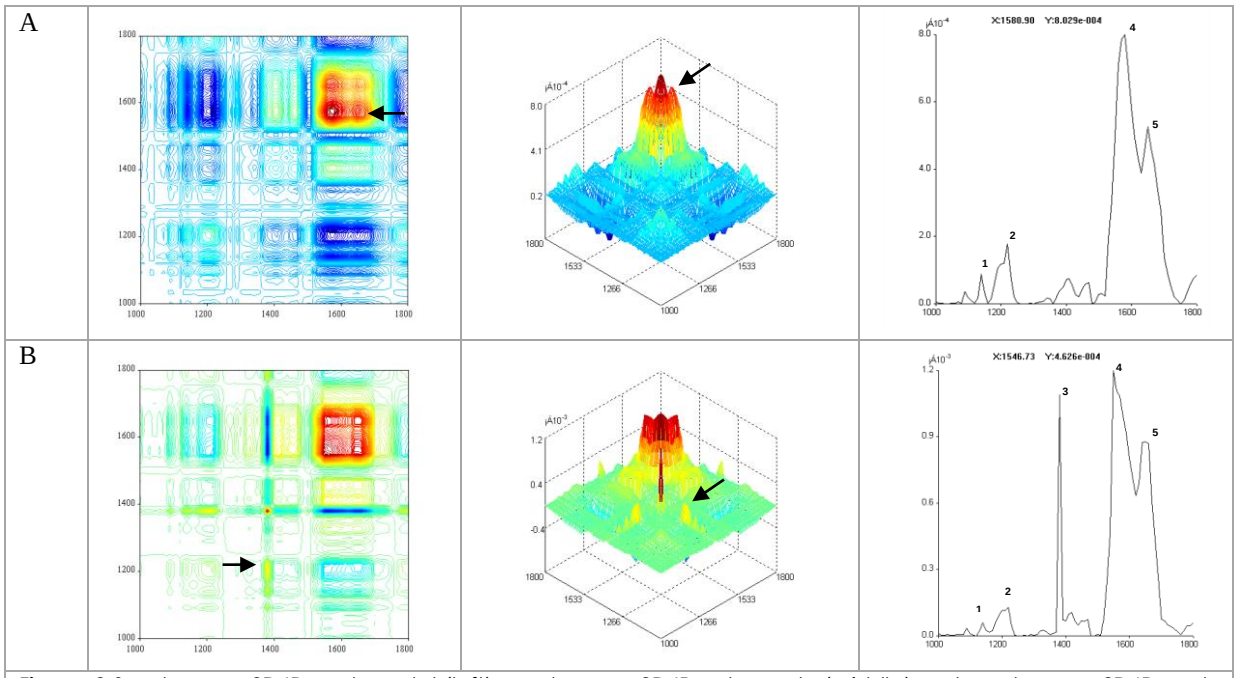

Figure. 3 Synchronous 2D IR contour plot (left), synchronous 2D IR auto-peaks (middle) and synchronous 2D IR mesh plot (right) in the range of 1800–1000 cm^{-1} for *O. aristatus*, (A) white flower form and (B) purple flower form. Cross-peaks are indicated by the arrows

Table 2 Characteristic auto-peaks in the range of 1800–1000 cm^{-1} for the white and purple flower forms of *Orthosiphon aristatus*

Peak number	Wavenumber (cm^{-1})	
	White flower form	Purple flower form
1	1175	1169
2	1280	1283
3	-	1384
4	1580	1546
5	1682	1685

4.0 CONCLUSION

This study showed that the 1D IR spectral features of the white and purple flower forms of *O. aristatus* (Blume) exhibited slight variations but more apparent features were observed in their second derivative IR spectra. Generally, the white and purple flower forms can be distinguished from each other through the typical peaks in the second derivative IR spectra. The 2D correlation IR spectral analysis provided additional information on their similarities and dissimilarities under thermal perturbation. The results supported the use of a combined approach of FT-IR with second derivative IR that is non-destructive and 2D correlation IR spectroscopy as an alternative tool for the quality control of herbal materials.

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