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Chemical Fingerprint Using FTIR and HPLC as Qualitative Analysis in the Study of Propagation of *Labisia pumila* Var. *Alata*

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Labisia pumila, Fourier Transform Infrared spectroscopy (FTIR), second derivative infrared spectroscopy, macroscopic fingerprint, High Performance Liquid Chromatography (HPLC).

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Abstract

Labisia pumila, known as kacip fatimah is a traditional herbs widely used for women. The herb was used as a post partum medicine to help contract the birth channel. From previous research, 120 clones of *Labisia pumila* var. *alata* was collected from three different locations and 30 clones of the herbs was found having high yielding of total phenolic content (TPC). In this study, one clone from each location was selected for further analyses, which are TA14 from Kuala Berang, TSA13 from Kemaman and DA20 from Gua Musang. Fourier Transform Infrared spectroscopy (FTIR) associated with second derivative infrared spectroscopy was applied to identify the chemical fingerprint of each clone of *Labisia pumila*. This analytical method is highly rapid and effective for analysis of medicinal herbs. Second derivative IR spectroscopy could enhance the spectral resolution by amplifying tiny differences in the IR spectrum. In this method, the whole chemical property of the sample can be revealed and shown in the IR spectrum. A total of ten absorption peaks were obviously present in the IR spectra which can be used to characterize the species. The IR spectra shows the presence of broader peak at frequencies of range 3266 – 3338 cm^{-1} which attributable to the alcohol group. This study also attempts to develop HPLC fingerprint of the selected clones. Observation on HPLC spectra shows the presence of one distinct peak at retention time of 12.30, 12.99 and 12.93 min, respectively in each clone. This compound will be characterized and will be used as reference compound in quality assessment in plant breeding.

1. Introduction

Labisia pumila or popularly known as kacip fatimah is a member of the small genus of slightly woody plants of the family Myrsinaceae [1]. This plant has been utilized by many generations of Malay women for the purpose of inducing and facilitation of labor. It is also used as a postpartum medication in the form of mixed preparation to help in the contraction of uterus. Further, this plant is reported to delay fertility and to help regain body strengths [3].

Previous research has reported antioxidant properties from this plant [7]. Plants antioxidants are believed to play a role in protection against a variety diseases and delaying ageing processes. There are several compounds which contribute to the antioxidant properties, these includes polyphenols, anthocyanins and flavonoids [6]. The aim of the present study is to develop the chemical fingerprint of *Labisia pumila* var. *alata* using FTIR and HPLC.

FTIR represents a simple and rapid methodology which is non-destructive for analytes [10]. The spectra result from transitions between quantized vibrational energy states. One of the greatest strengths of infrared spectrometry is that samples in all phases of matter can be examined. Otherwise, it has the ability to implement both qualitative and quantitative analysis of interrogated samples [5]. FTIR can analyze the chemical characteristics of the whole ingredients in complicated mixture systems [4].

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Besides, a non-destructive and rapid identification method focusing on macroscopic fingerprint and selective method using HPLC will developed. HPLC is a popular method for the analysis of herbal medicines because it is easy to learn and use and is not limited by the volatility or stability of the sample compound. In general, HPLC can be used to analyze almost all the compounds in the herbal medicines [11]. Peaks in the chromatogram can be identified directly by comparisons with literature data or standard compound.

2. Materials and Methods

2.1. Plant Materials

Leaf of L. pumila var. *alata* (TSA13, DA20 and TA14) was collected from herbal greenhouse located in FRIM.

2.2. FTIR analysis

Spectrum 100 FTIR system (Perkin Elmer), equipped with a DTGS detector. IR spectra were recorded from an accumulation of 16 scans in range of 4000 cm^{-1} – 450 cm^{-1}. Dried leaf were ground into powder and then blended with KBr powder, ground again and press into a tablet. Each sample was run for triplicates. IR spectra for all samples were collected.

2.3 HPLC Analysis

Each of resulting solution were filtered through membrane filter (pore size 0.45 μm) prior to analysis. The samples were analyzed by means of HPLC system (Waters Delta 600 with 600 controller) with Photodiode array detector (Waters 996). A Phenomenex-Luna (5 μm x 4.6 i.d. x 250 mm) was used and for elution of constituents, two solvents denoted as A and B was employed. A was 0.1% aqueous Formic acid and B was acetonitrile. Initial conditions were 85% A and 15% B with a linear gradient reaching 60% B at 30 minutes. The flow rate used was 1.0ml/min and the injection volume was 10 μl.

3. Results and Discussion

3.1. 1D FTIR spectra analysis

The FTIR spectra of three clones of *Labisia pumila* var. *alata* are shown in Fig. 1. Comparison of the FTIR spectrum between the three clones indicates the differences of the main constituent for the three clones seem to be similar. As shown in Fig. 1a, 1b and 1c, the strongest peak at 3340 cm^{-1}, 3339 cm^{-1} and 3262 cm^{-1} respectively assigned to the O-H stretching vibration [8]. Peak at 2920 cm^{-1}, 2922 cm^{-1} and 2923 cm^{-1} for the three clones assigned as symmetrical vibration of C-H group while the peaks at 2851 cm^{-1} for clones DA20 and TSA13 and 2852 cm^{-1} for clone TA14 indicates as asymmetrical vibration of C-H [8]. In the range of 900 – 1800 cm^{-1}, peaks at 1448 cm^{-1}, 1445 cm^{-1} and 1446 cm^{-1} are due to the asymmetrical bending of C-H in cyclic alkanes [9]. The FTIR spectra show the similarities of the three clones of *Labisia pumila* var.*alata* in the IR profile. However, the analyses continued on the second derivative spectra to see clearly the spectral resolution of the clones. Preliminary assignments of the three clones of *Labisia pumila* var. *alata* are shown in the Table 1.

3.2. Second derivative FTIR spectra

Generally, the second derivative infrared spectrum can obviously enhance the spectral resolution and amplify tiny differences of IR spectrum [2]. The differences in second derivative spectra of all clones were clearly seen in the range of 970 – 1425 cm^{-1} as highlighted in the Fig. 3. Though the 1D FTIR of all clones showed similarities between three clones, the second derivatives spectra further analysed the dissimilarities of these clones. In the range of 1425 – 1800 cm^{-1} (refer to: Figure 4), a sharp absorption peak at 1442 cm^{-1} showed a high intensity of clone TA14 was a characteristic feature to distinguish clearly the three clones of *Labisia pumila* var. *alata*. The dissimilarities may be due to the environmental factors such as climates, soil humidity, soil nutrients, light intensities and temperature [12].

3.3. HPLC analysis

The HPLC fingerprint of the three clones showed that they have a same major compound which was appeared at retention time of 12.30, 12.99 and 12.93 min. Both HPLC profile of TA14 and DA20 show the presence of two other major peaks at 20.169 and 19.715 min respectively. This compound will be characterized and will be used as reference compound in quality assessment in plant breeding.

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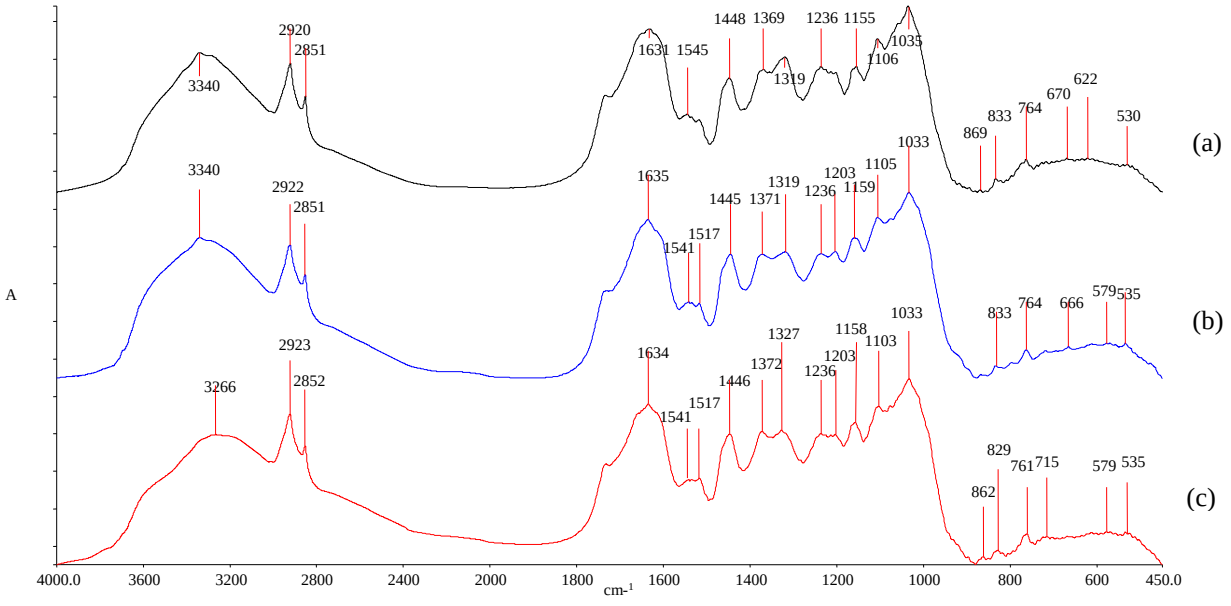

Figure 1: FT- IR spectra of the extracts of *L.pumila* : (a) clone TSA13, (b) clone DA20, (c) clone TA14

Table 1: The preliminary assignment of three clones of *Labisia pumila*

Frequencies [cm^{-1}] (DA20)	Frequencies [cm^{-1}] (TSA13)	Frequencies [cm^{-1}] (TA14)	Functional group
3340	3340	3266	*v(O-H, N-H)*
2922	2920	2923	v_{as}*(C-H)*
2851	2851	2852	v_s(C-H)
1635	1631	1634	*v*(C=O) main, *v*(C=C), δ_{as}(N-H)
1445	1448	1447	δ_{as}(C-H)
1371	1369	1369	δ_{as}(C-H)
1236	1236	1236	δ_s(C-CO), v(C-O),v_{llas}(=C-O-C), v_f(C-C)
1159	1155	1158	*v*(C-H)
1033	1035	1033	v_{ll}(=C-O-C), v_f(C-C)

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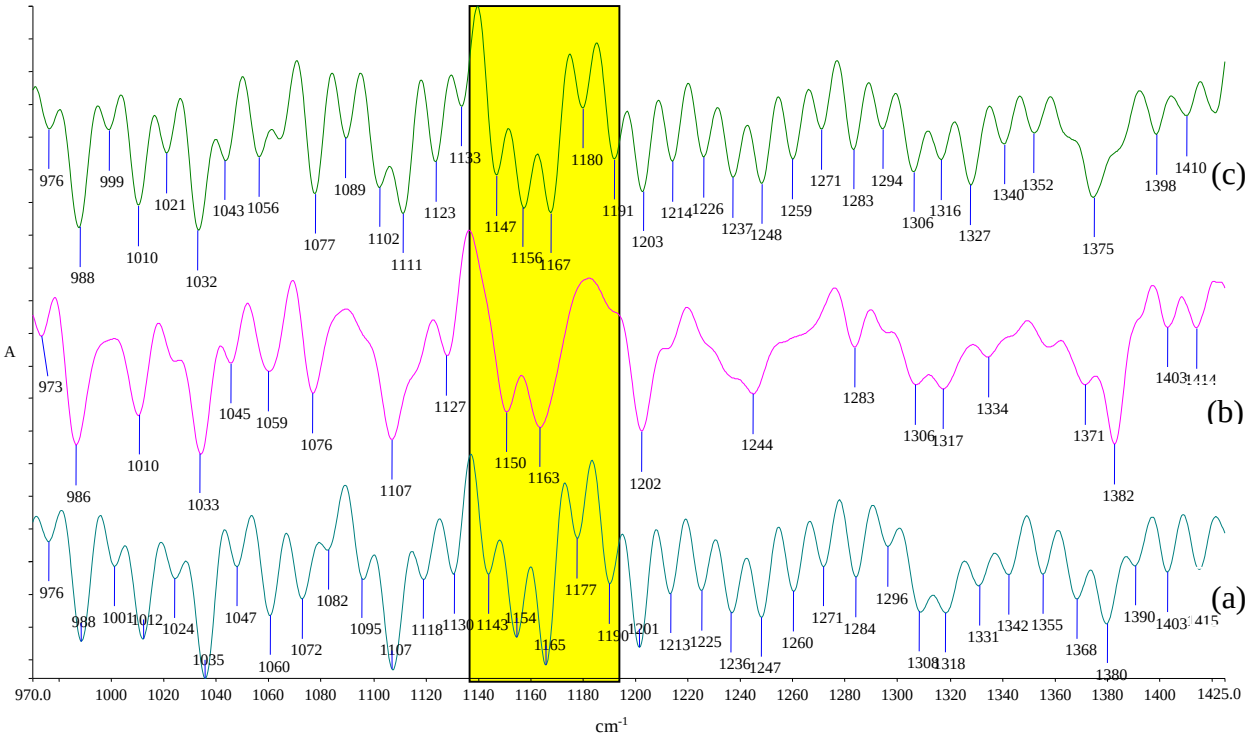

Figure 2: Second derivative spectra of the extracts of *L.pumila* in the range of 970cm^{-1} – 1425 cm^{-1} (a) clone TSA13, (b) clone DA20, (c) clone TA14

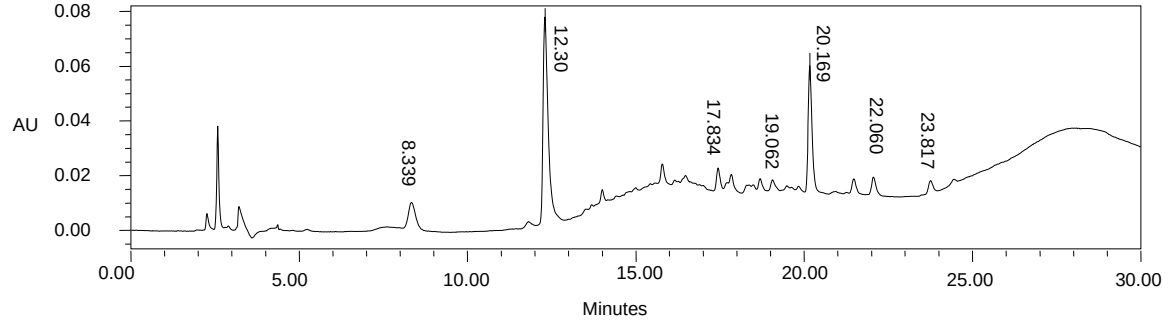

Figure 3: HPLC chromatogram of *L. pumila* for clones TA14

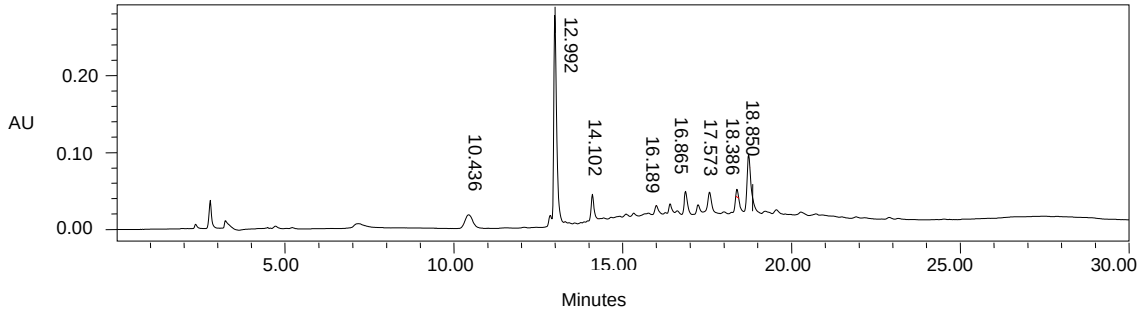

Figure 4: HPLC chromatogram of *L. pumila* for clones TSA13

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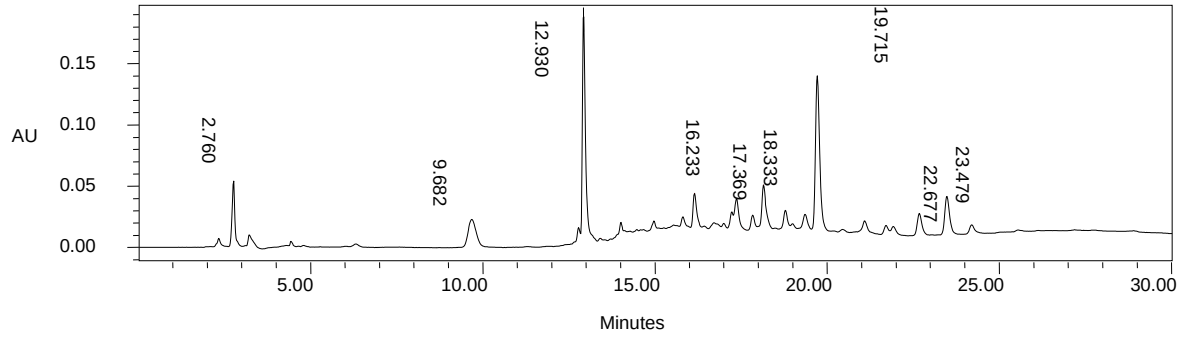

Figure 5: HPLC chromatogram of *L.pumila* for clones DA20

4. Conclusions

In this study, the chemical fingerprint profiles for three clones of L. pumila var. *alata* (TSA13, DA20 and TA14) have been successfully developed. The developed method will be used in the quality assessment study of plant breeding.

Acknowledgement

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