Moxa Wool in different purities and different growing years measured by terahertz spectroscopy

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Abstract
Moxa wool is a traditional Chinese herbal medicine, which can warm channels to dispel coldness. At present, there is no unified index to evaluate purity and growing years of moxa wool in the market. Terpineol is one of the effective substances in the volatile oil of moxa wool. Here, we characterize the purity and growing years of moxa wool by studying terpineol. Gas chromatography-mass spectrometry (GC-MS) and high performances liquid chromatography (HPLC) are the methods for monitoring terpineol at present, all of which have defects of complicated procedures. We established linear fitting to distinguish the different purities of moxa wool through the intensities (areas) of terpineol the characteristic peaks, and the consequence presented the coefficient of determination (R²) were higher than 0.90. Furthermore, based on the characteristic peaks position of standard terpineol, the correlation model with the and purity and growing year of moxa wool was set up, thereby differentiate the quality of moxa wool. We have built the partial least squares (PLS) model of the growing years of moxa wool with high accuracy, and the determination coefficient is greater than 0.98. In addition, we compare the quantitative accuracy of Raman spectroscopy with terahertz technology. Finally, a new method of terahertz spectroscopy to evaluate quality of moxa wool were found. It provides a new idea for the identification of inferior moxa wool in the market and a new method for identifying the quality of moxa wool in traditional Chinese medicine.

1. Introduction
Wormwood has the effects of relieving dampness, blood stasis and swelling, which have been widely applied to traditional Chinese medicine [1,2]. Among them, moxa wool is an important wormwood product, which obtained by repeatedly processing wormwood to remove powder residue and its purity (wormwood-to-moxa wool ratios) and growing year determine its quality [3]. Generally, easily volatile substances in moxa wool decrease with the increase of purity and growing years of moxa wool. The difficult volatile
substances in moxa wool are the opposite. Generally, difficult volatile matters have expectorant, antiasthmatic and bacteriostatic curative effects. The price of moxa wool increases with the increase of curative effect. The prices of one year's growth period, two year's growth period, three year's growth period of moxa wool and so on increased by 20 yuan in turn. At present, there is no unified evaluation index in market to evaluate the purity and growing years of moxa wool. Terpineol is one of the effective substances in the volatile oil of moxa wool, which presents microbicide, anticancer and other biological actions [4]. The determination of terpineol in moxa wool is of great significance to distinguish moxa wool quality. Thus, we studied the purities and growing years of moxa wool through the change of terpineol.

Most current methods for detecting terpinol are usually chemical, such as GC-MS and HPLC [5,6]. The results of GC-MS analysis are accurate and reliable, but the pretreatment and analysis steps are complicated [7]. HPLC is on the basis of disconnected substances, which depends on the difference of adsorption characteristics and protein molecular size, and usually requires a long detection time and high sample preparation cost [8].

Besides, the aforementioned terpineol celluloid tested approaches, an increasing number of approaches of spectrum detection are applied to biological detection, consisting of infrared spectroscopy and Raman spectroscopy [9]. Infrared spectrum, on account of different substances have different absorption intensities and wavelengths, their reflected absorption peaks and intensities in infrared spectrum are different from each other [10,11]. Li et al. quantitatively analyzed volatile lignans by infrared spectroscopy [12]. Moni et al. use FTIR spectral analyzer to certificate the existence of alkaloids and tannins [13]. These consequences displayed that infrared spectroscopy technology supplied an valid and practicable method for the test of botanical physical message. Raman spectrum can reflect the difference of chemical composition of samples at molecular level [14]. Different detection method pairs are shown in Table 1. Yoon et al. studied Raman spectroscopy which is a new method to detect terpineol as an effective component in new green tea essential oil nanoemulsion [15]. These researches present that it is feasible to analyze moxa wool by terpineol characteristic absorption peak spectrum.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Pretreatment time</th>
<th>Required sample size</th>
<th>Prediction accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas chromatography-mass</td>
<td>20h</td>
<td>10ml</td>
<td>&lt;90% [16]</td>
</tr>
<tr>
<td>spectrometry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raman spectroscopy</td>
<td>3h</td>
<td>10ml</td>
<td>&lt;85% [17]</td>
</tr>
<tr>
<td>Terahertz spectroscopy</td>
<td>1h</td>
<td>2ml</td>
<td>&gt;95%</td>
</tr>
</tbody>
</table>

Happen along with development wake of modern ultrafast optics, THz spectroscopy is becoming gradually full-grown. Terahertz wave is electromagnetic wave, which own a frequency scope between 0.10-10.0 THz and the properties of infrared wave and microwave [18]. A great deal of biological samples has obvious THz characteristic absorption peak, and THz technology has added preponderance in the vibration and rotation level of macromolecules [19]. THz spectroscopy is in the extreme impressive to polar crowds. Inverse to Raman spectroscopy, which chiefly represents oscillation inside the molecule, the spectrum message in THz domain is ample at frail inside the molecule mutual and skeleton oscillation of macromolecules, which is straightway relevant to molecular configuration [20, 21]. For this reason, THz technology is more and more used to characterization and dissect of configuration.
The near-infrared spectrum bands overlap seriously, and the number of peaks in near-infrared spectrum is generally relatively large, and the same band is often overlapped by multiple groups, and the -CH$_3$ and -OH groups of terpineol overlap, which leads to the near-infrared spectrum of terpineol with severe overlapping peaks and wide peaks. Raman scattering area has great influence on the analysis, and the overlapping of different vibration peaks and Raman scattering intensity are easily influenced by optical parameters and other factors. The terahertz band lies between the millimeter wave band and the far-infrared band. From the energy point of view, the frequencies corresponding to the weak interaction between molecules, the skeleton vibration of macromolecules and the low-frequency vibration absorption of crystal lattice are just within the terahertz band. Therefore, we propose a new method to use terahertz technology to identify the quality of moxa wool by terpineol.

Recently, THz technology has been applied to study Chinese herbal medicines, including Yin et al. who used terahertz spectroscopy to study the biological molecular characteristics of baicalein, quercetin and other common flavonoids in 0.2-2.5THz band [22]. Yan et al. simulated the terahertz spectroscopy of glycyrrhizic acid by quantum chemical calculation method, and its absorption characteristic peaks matched with molecular vibration mode [23]. These studies show that the vibration modes of macromolecular compounds of these Chinese herbal medicines are matched with terahertz peaks. Kou et al. used THz spectroscopy to analysis of ginsenosides in Panax quinquefolium to differentiate herbal medicines [24]. The research shows that THz spectroscopy can be applied to test Chinese herbal medicines. At present, there is no literature report on terahertz spectroscopy used in terpineol detecting research.

Terpineol is an isomer, terahertz wave is confoundedly impressible to molecular structure, and the weak difference of isomers in terahertz wave absorption spectrum can be obviously different. We have obvious advantages in detecting isomer terpineol by terahertz spectroscopy. In addition, for further enhance prediction accuracy of the model of moxa wool, we improved sample preparation method. COC (cycloolefin copolymer) has extremely low transmission loss compared with polyethylene (PE) spectral information in terahertz band [25], and has the advantages of high transparency and low water absorption [26]. To solve these technical bottlenecks of terahertz measurement, we mixed cycloolefin copolymer (COC) instead of polyethylene (PE) with moxa sample and tabletted it. Further, in the process of detecting volatile liquid terpineol, we tried various methods, dropping terpineol on filter paper, silicon chip and weighing paper, and finally reducing the volatility of terpineol in the detection process by making a sample cell, and successfully detecting the terahertz spectrum of terpineol. It provides the possibility for the next step of modeling and improving the modeling accuracy.

During the research of this paper, we put forward a fast-nondestructive testing method of moxa wool based on terahertz spectrum. Firstly, the vibration absorption spectrum of terpineol molecule with high content in moxa wool was simulated by DFT, and the characteristic peaks position of terpineol in terahertz band were identified. Then, fourier transform infrared spectroscopy (FTIR) is used to collect THz spectra of moxa wool in different growing years and different purities. Find the characteristic peaks position of terpineol corresponding to moxa wool. Therefore, we established the purity model of moxa wool with peak heights and peak areas respectively. Then, we also use the PLS model, establishing the relevant model of the purities and growing years of the moxa wool and compare the prediction accuracy of the two models. Moreover, we have done Raman spectroscopy experiments to detect moxa wool in different growing years and different purities, and try to compare the quantitative accuracy of the two technologies.
The model based on the characteristic peaks of terpineol, which can accurately distinguish the growth years and purity of moxa wool. We can solve the problem of shoddy moxa wool in the current market, moreover identify the best quality of moxa wool for traditional Chinese medicine.

2. Materials and Methods

2.1 Chemical Reagent

The molecular formula of terpineol is C\textsubscript{10}H\textsubscript{18}O, and its molecular weight is 154.24, which is insoluble in water and purchased from Aladdin. The reagent purity is more than 98%, colorless viscous liquid, CAS number is 8000-41-7. All the tested moxa wool is extracted from the moxa sticks (Nanyang Hanyi Moxa Co., Ltd, Henan, China) with 18mm in diameter, 200mm in length and 21-22g in mass. The wormwood-to-moxa ratio of the moxa sticks with different storage years (five, eight and ten years) which are numbered as "2017", "2014" and "2012" respectively was 15:1, and the storage years of the moxa sticks in different wormwood-to-moxa ratios (10:1, 15:1 and 30:1) were 8 years.

2.2 Density functional theory

Density universal function theory (DFT) is a common method used to analyze molecular vibration and rotation [27]. The theoretical origin of terpineol peak position was explained by DFT combined with terpineol molecular structure, and the experimental data of terpineol was further explained. Because of its high precision and low computational complexity, the DFT plays a significant role in the analysis of molecular structure [28]. Using Gauss -09 package (revised version D.01, American Gauss Company) together with B3LYP hybridization function and 6-31G basis set, we calculated the absorption peak position of terpineol at THz frequency [29].

2.3 Sample preparation and spectral collection

Firstly, the moxa wool samples were ground, and the steel balls with diameter of 2mm and moxa wool were put into a grinding tube, and the moxa wool was ground for 190s by a grinder. (The parameter of the grinder machine is composed of frequency and grinding time, which are set to 70Hz and 190s.) Secondly, the ground moxa wool mixed with COC powder is pressed into tablets (20mg moxa wool mixed with 40mg COC), the quality of each tablet is controlled at 60mg, and the experimental quality loss is controlled within 1%. When tableting, press moxa wool powder in a die with a stress of 4 tons for two minutes to take shape a thin slice with a thickness of 1 mm.

For the same batch of different growing years moxa wool samples, three varieties with different growing years of 2012, 2014 and 2017 were selected, and each variety suppressed 15 samples, with a total of 45 samples from one batch. For different purities of moxa wool, which were 10:1, 15:1 and 30:1, each variety will also prepare 15 samples. We totally prepared three batches of moxa wool samples, including the different growing years and purities of moxa wool. Two of these three batches are used to build the moxa wool model, and another batch of moxa wool with different growing years and different purities is bought from the market to verify the accuracy of the model. Terahertz signal is easily assimilate by water vapor, so it is necessary to dry the internal part of the experimental apparatus before performing an experiment. Guarantee that the whole terahertz signal survey section is in an environment with moisture content less than 3% [30]. Fourier transforms infrared spectrometer (FTIR) was applied to take a measurement to the absorption spectrum. Water cooled mercury lamp is as light source. FTIR has a signal-to-noise ratio of surpassing 10000:1 and a frequency domain of 0.9-20.0THz. The resolution of the spectrometer is 4 cm\textsuperscript{-1} and every spectroscopy is the average of 64 sample sweeps applied to a sweep velocity of 5kHz in the view of 64 backdrop sweeps. In the process of collecting spectral data, the apparatus is full of nitrogen to eliminate the influence of moisture on terahertz spectral collection. FTIR has excellent scanning...
accuracy, and the jitter rate is under 3% in the measurement process. Before measuring the spectrum, it is necessary to scan the background and eliminate the background, and record the scanning results by using the spectrum software OPUS. In the process of gathering moxa wool spectra, 3 divergent places were gathered for each tablet, 5 times for each point, and then the average value was taken.

In addition, the advantages and disadvantages of terahertz spectra are compared by Raman spectroscopy, which is consistent with the sample preparation method of terahertz spectroscopy. For moxa wool samples from different growing years (2012, 2014 and 2017), three batches of samples were prepared. For different purities of moxa wool samples (10:1, 15:1, 30:1), there are 45 samples in one batch (15 samples in each proportion) and 3 batches in total. Using laser confocal micro Raman spectroscopy, the optical effectiveness is rise above 30.0%, the spectrum resolving power is exceed 0.40 wave number, the acinic resolving power is super than 1 micron. We employ 532.0nm optical maser as Raman optical maser and 2.50% strainer. The time of acq and RTD are 5.0s and 1.0s. As to every last sample slice, we gather 3 different situations and every last slice was gathered 5 times and thereafter equalized.

2.4 Partial least squares algorithm

PLS, a dimension reduction technique, is used to maximize the covariance between the prediction (independent) matrix x and the prediction (correlation) matrix y on each component of the dimension reduction space. PLS improves the original least squares algorithm, and can not use all variable data in calculation. The principal component extraction algorithm is applied to drop dimension of the original data, so as to extract metadata to the maximum extent and extract the main information from the residual error.

3. Results

3.1 Molecular simulation of terpineol

The spectral information of terpineol molecules in terahertz band is calculated and simulated by Gaussian-09 software and density functional theory [31-33]. In addition, the origin of characteristic absorption peaks of the terpineol were simulated go through assisting of optical window [34].
Fig. 1. (a) THz absorption spectroscopy of terpineol on the basis of DFT; (b) THz absorption spectroscopy of terpineol on the basis of experiment; Typical vibration modes at (c) 4.17THz; black, grey and red atoms represent carbon, hydrogen, and oxygen atoms, respectively. The blue arrows indicate the vibration direction of atoms, and the length of the arrow indicates the vibration amplitude of atoms.

The absorption spectrum of terpineol received by theoretical calculation is shown in Fig. 1a, and the absorption spectrum of terpineol received by molecular simulation is shown in Fig. 1b. In the experimental results of terpineol, terpineol is mixed according to the isomer ratio of 1:1:1, including α-Terpineol, β-Terpineol and γ-Terpineol. As shown in Fig. 1a, through molecule simulating, we can find that there are four characteristic peaks of terpineol, which are situated at 4.13, 5.01, 5.60 and 6.26THz. The results of the experiment from Fig. 1b, we can find five characteristic peaks, which situated at 4.22, 4.57, 4.76, 5.09 and 6.21THz. By the viewable window of Gaussian, we can find that this oscillation at 4.13THz is causal for folding vibration of molecular as shown in Fig. 1c. We compare the simulational consequences of terpineol with the tested consequences to further analyze the standard terpineol, we discovered that the absorption peaks 4.13THz and 5.01THz calculated theoretically by the standard terpineol are equivalent to the absorption peaks 4.22THz and 5.09THz detected experimentally, respectively. The absorption peaks at 4.13THz and 5.01THz of simulated spectral are weakly red shift contrast to the measured spectral. The reason of red-shifted hydrogen bond is mainly due to elongation and contraction effect [35]. The combination of covalent bonds and
electronegative atoms is the main combination mode of hydrogen atoms. We observe that
the absorption peak position of 5.60THz calculated by theory is not reflected in the
spectrum detected by experiment, which may be connected to temperature through
analysis [36]. In molecular systems, hydrogen bonds are easily impinged by temperature,
which further leads to molecular vibration frequency become different, thus leading to the
frequency shift of peak position [37]. In addition, the absorption peaks at 4.57THz and
4.76THz detected in the experiment did not present in simulated consequences. The
standard terpineol detected in the experiment is a multimolecular system, which includes
not only the atomic vibration of single molecule, but also the association between
molecules [38]. The interaction between molecules in a multi-molecular system will lead
to the absorption of characteristic peaks of terahertz waves [39]. Therefore, the theoretical
model verifies that terpineol can be detected in terahertz spectrum range, which lays a
foundation for the follow-up research of moxa wool.

3.2 Study on different purities of moxa wool

Afterwards, deleting baseline and delineating by thickness of samples, terahertz
spectroscopy of moxa wool in different purity were displayed at Fig. 2. From Fig. 2a, we
know that moxa wool has four characteristic absorption peaks in the 4-6.5THz band,
which are 4.22, 4.57, 5.09 and 6.28THz respectively.

We compared the absorption peaks of moxa wool with terpineol. The absorption
peaks of moxa wool at 4.22, 4.57, 5.09 and 6.28THz correspond to that of terpineol at
4.22, 4.57, 5.09 and 6.21THz. Among them, compared with the standard terpineol, the
corresponding absorption peak at 6.28THz on moxa wool is offset by 0.07THz. Through
analysis, when the absorption peaks of some hydrogen-containing groups overlap with
those of some groups, it is possible to deuterate the hydrogen of the functional groups, so
that the absorption peaks shift to low wavenumber [40].

Different states of the same molecule, their interactions between molecules are
different, which leads to different detected spectra [41]. Generally, the wave number of the
spectral band measured in gaseous state is the highest, and the rotating fine structure of the
vibrating spectral band can be observed. At this time, the moxa wool is in solid state, and
the wave number will be lower than that of terpineol measured in liquid state. Therefore,
the frequency shift of moxa wool peaks may be caused by many factors.
Fig. 2. (a) THz absorption spectral on the basis of the different purities of moxa wool. Vertical arrows indicate absorption peaks of moxa wool. Error bars have been labeled on each data. (b) PLS prediction model of moxa wool in different purities based on terahertz absorption spectrum. Characteristic absorption peak intensities on different purities of moxa wool at (c) 4.22THz, (d) 4.57THz, (e) 5.09THz and (f) 6.28THz.

To go a step further analyze the cause for variation tendency of the intensity of absorption peaks different purity of moxa wool, as shown in Fig. 2(c,d,e,f), we can clearly find that with the increase of the purities of moxa wool, the intensity of absorption peaks shows an increasing trend. For the different purities of moxa wool, with the increase of the purity of moxa wool, the relative content of terpineol increases regularly. Through the analysis, the relative content of terpineol increases with the increase of moxa wool purities. Hence, we can consider that the change of relative terpineol content leads to the change of absorption peak intensity of different purities of moxa wool.

In addition, according to the correspondence between terpineol and the peak positions of moxa wool, we selected three characteristic peak positions of moxa wool as 4.22, 5.09 and 6.28 for further modeling and analysis.

3.2.1 Linear fitting of moxa wool with different purities

Linear fitting is used to model and analyze moxa wool with different purities. Moxa wool prediction model is established according to set calibration and prediction set of 2:1. A total of 60 samples (20 samples for each variety) were applied to calibrate and 30 samples (10 samples for each variety) were applied to predict and the purities of 10:1, 15:1 and 30:1 are numbered as "1", "2" and "3" respectively. We use the characteristic peak intensity and characteristic peak area as two parameters to linear fitting the peak height (area). Three absorption peaks of 4.22, 5.09 and 6.28THz are regarded as characteristic absorption peak intensities, and three integral regions of 4.10-4.48THz, 4.76-5.89THz and 6.09-6.56THz are taken as peak areas. Such as Table.2, linear fitting results of different purity moxa wool; Fig. 3, linear fitting prediction results of different purity moxa wool.

Table. 2 Properties of models for prediction of different purities in moxa wool using characteristic absorption intensities or areas.

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The characteristic absorption peaks, absorption intensities, absorption areas, and determination coefficient (R²) are as follows:

<table>
<thead>
<tr>
<th>Characteristic absorption peaks</th>
<th>Determination coefficient (R²)</th>
<th>Absorption intensities (Y)</th>
<th>Determination coefficient (R²)</th>
<th>Absorption areas (Y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.22THz</td>
<td>0.9753</td>
<td>Y=0.00956x-0.00212</td>
<td>0.95286</td>
<td>Y=(9.0875E-4)x+0.0019</td>
</tr>
<tr>
<td>5.09THz</td>
<td>0.97549</td>
<td>Y=0.10499x-0.03821</td>
<td>0.98362</td>
<td>Y=0.01929x+0.02842</td>
</tr>
<tr>
<td>6.28THz</td>
<td>0.99055</td>
<td>Y=0.02891x-0.011036</td>
<td>0.96025</td>
<td>Y=0.00309x+0.00535</td>
</tr>
</tbody>
</table>

Fig. 3. Linear fitting prediction consequences of characteristic absorption peak intensities on different purities of moxa wool at (a) 4.22THz, (b) 5.09THz and (c) 6.28THz. Linear fitting prediction results of characteristic absorption peak areas on different purities of moxa wool at (d) 4.22THz, (e) 5.09THz and (f) 6.28THz.

As shown in Table 2, where x, Y and R² are the purity of the sample, the absorption peak intensity (or area) of the sample and the determination coefficient, respectively. From Fig. 3, for the prediction of characteristic absorption peaks heights, we can find that the coefficient of determination at 5.09THz can reach 0.983 is the highest among three characteristic absorption peaks; for the prediction of characteristic absorption peak areas, integral region of 6.09-6.56THz is the highest which the coefficient of determination can reach 0.962. The determination coefficient R² in the modeling results of different purity moxa wool are greater than 0.95, which indicates that terahertz technology has the ability to quantitatively detect the purity of moxa wool.

3.2.2 Partial least squares modeling of moxa wool with different purities based on terahertz spectrum

Firstly, we analyze and obtain the terahertz spectrum of moxa wool, remove the thickness of the obtained spectrum during processing, and then smooth and remove the baseline, which can correct the baseline fluctuation and enhance the signal-to-noise ratio (SNR).

Moreover, PLS regression prediction model was established by us to predict different purity of moxa wool, in order to enhance the precision of the model. The characteristic peak intensities of the same three characteristic peaks at 4.22, 5.09 and 6.28THz are used as the input of PLS model, outputs correspond to “1”, “2” and “3” respectively. A total of 60 samples (20 samples for each variety) were applied to calibrate and 30 samples (10 samples for each variety) were applied to predict.

In the end, the precision of the model is assessed by the determination coefficient (R²) and root mean square (RMSE). The verification includes correction of correlation coefficient and full cross-validation of RMSE. In order to verify the correction model, the model is applied to the spectral data of the prediction set to obtain the prediction root.
mean square (RMSEP) and determination coefficient (R²). The purity of PLS modeling and prediction samples is consistent with that of linear model. It can be found out from Fig. 2b that the R² of prediction model with different purity of moxa wool is all overstep 0.990, and the RMSE is under 0.05. In contrast to the linear fitting outcomes, the outcomes show that the R² of PLS modeling prediction is go beyond 0.990, which is overstep than that of linear fitting 0.950.

3.3 Study on different growing years of moxa wool

We have studied different growing purity of moxa wool, and want to further study different growing years of moxa wool (7-year growth period 2014 moxa wool). As shown in Fig. 4a, there are four characteristic absorption peaks at 4.22, 4.57, 5.09, and 6.28THz of different growing years of moxa wool. The characteristic absorption peaks of moxa wool in different growing years are the same as those of moxa wool in different purities.

We further analyze the reason for variation tendency of the intensity of absorption peaks different growing years of moxa wool, as shown in Fig. 4(c,d,e,f), we can clearly find that with the increase of the years of moxa wool, the absorption peaks intensity of moxa wool increased at first and then decreased. Through the analysis, the relative content of terpineol increases first and then decreases with the increase of moxa wool years. And in 2014, the terpineol content of moxa wool was the highest. Therefore, the change of terpineol relative content leads to the change of absorption peaks intensities of moxa wool in different purities.

![Graphs showing absorption peaks and characteristic intensities](image)

**Fig. 4.** (a) THz absorption spectral on basis of different growing years of moxa wool. Vertical arrows indicate absorption peaks of moxa wool. Error bars have been labeled on each data. (b) PLS prediction model of moxa wool in different growing years based on terahertz absorption spectrum. Changes of characteristic absorption peak intensities on different growing years of moxa wool at (c) 4.22THz, (d) 4.57THz, (e) 5.09THz and (f) 6.28THz.

In addition, according to the correspondence between terpineol and the peak positions of moxa wool, we selected three characteristic peak positions of moxa wool as 4.22, 5.09 and 6.28THz for further modeling analysis.
3.4 Partial least squares modeling of moxa wool with different growing years based on terahertz spectrum

We set up a partial least square regression prediction model for different growing years to predict. The characteristic peak intensities of the three characteristic peaks at 4.22, 5.09 and 6.28THz are regarded as the input of PLS model, outputs correspond to “1”, “2” and “3” respectively. It is constructed according to the ratio of calibrated set to predicted set of 2: 1. A total of 60 samples were applied to calibrate and 30 samples were applied to predict. It will be obtained from Fig. 4b that the $R^2$ of predictable model with different growing years of moxa wool is all above 0.990, and the RMSE is less than 0.05.

3.5 Analysis of Raman spectra

3.5.1 Analysis of Raman spectra in terpineol

After using THz technology to analyze the different growing years and different purities of moxa wool through the change of terpineol. We try to use the Raman spectroscopy to analyze moxa wool in different growing years and different purities through the change of terpineol. Ultimately, the results of the two technologies are compared.

From Fig. 5a, it is the Raman spectroscopy of terpineol, which have two obvious characteristic absorption peaks in the band of 1400cm$^{-1}$-600cm$^{-1}$, which are located at 1455cm$^{-1}$ and 1518cm$^{-1}$ respectively. It is found that the characteristic absorption peak at 1455cm$^{-1}$ may be due to asymmetric angular vibration of CH$_3$. The absorption peak at 1518cm$^{-1}$ may be the variable angle of CH [42].

3.5.2 Analysis of Raman spectra in different purities of moxa wool and different growing years of moxa wool

In order to use Raman spectroscopy to analyze moxa wool in different growing purities and different growing years through the change of terpineol. The Raman spectroscopy of moxa wool were collected.
(a) 

![Graph showing Raman shift and intensity for terpineol](image)

(b) 

![Graph showing Intensity vs. Raman shift for different concentrations](image)

(c) 

![Graph showing Intensity vs. Raman shift for different years](image)
Fig. 5. Raman spectrum curves based on the (a) terpineol; (b) different growing purities of moxa wool; (c) different growing years of moxa wool. Vertical arrows indicate absorption peaks of moxa wool. Error bars have been labeled on each data.

From Fig. 5b and Fig. 5c, it is the Raman spectra of moxa wool, which have two obvious characteristic peaks in the band of 1400cm⁻¹-600cm⁻¹, which are located at 1461cm⁻¹ and 1522cm⁻¹ respectively. Comparing the Raman spectra of Fig. 5a in terpineol, we can find that the peak positions of the Raman spectra of moxa wool are 1455cm⁻¹ and 1518cm⁻¹, which correspond to the characteristic peaks of terpineol 1455cm⁻¹ and 1518cm⁻¹ respectively. Because the characteristic frequency of small groups bound atoms is not completely independent of other parts of the molecule, the vibration of each group is generally in a range. The vibration frequency of CH₃ is 1455cm⁻¹ -1465cm⁻¹; the vibration frequency of CH is 1515cm⁻¹-1525cm⁻¹ [43,44]. It is explained that the characteristic absorption peaks of moxa wool at 1461cm⁻¹ and 1518cm⁻¹ correspond to the characteristic peaks position of terpineol at 1455cm⁻¹ and 1522cm⁻¹ with a frequency shift. For this reason, we can obtain that these two absorption peaks are resulted in terpineol.

For the different purities of moxa wool, From Fig. 5b, with the increase of moxa wool purities, the absorption peaks intensity of moxa wool increase. Moreover, with the purities of moxa wool, the terpineol content increases regularly. We can get a conclusion that the change of characteristic absorption peaks intensity of different purities moxa wool is caused by the change of terpineol content.

We further analyze the reason for variation tendency of the intensity of absorption peaks different growing years of moxa wool, we can clearly find from Fig. 5c that with the increase of moxa wool years, the absorption peaks intensity of moxa wool increases at first then decrease. The truth is with the increase of moxa wool growing years, the terpineol content increase at first then decrease, in the 2014, the terpineol content is the highest. Hence, the change of terpineol content gives rise to the increase of characteristic absorption peaks intensity.

3.5.4 PLS modeling of moxa wool with different purities based on Raman spectroscopy.

We also wanted to make a contrast to the precision of these two techniques for moxa wool in different purities. The characteristic absorption peaks intensities at 1461cm⁻¹ and 1522cm⁻¹ were selected to establish PLS model. A total of 60 samples were applied to calibrate, 30 samples were applied to prediction. The analysis results are shown in the Fig. 6a the $R^2$ of the model is 0.852, which is far from the terahertz model ($R^2>0.95$). Hence, THz spectroscopy has palpable superiorities in measurable analysis of moxa wool purities.

3.5.3 PLS modeling of moxa wool with different growing years based on Raman spectroscopy

We choose moxa wool in different growing year corresponding to the characteristic peak intensities of terpineol at 1461cm⁻¹ and 1522cm⁻¹ to establish PLS model to compare the prediction accuracy of terahertz technology and Raman spectroscopy. A total of 60 samples were applied to calibrate, 30 samples were applied to predict. The analysis results are shown in the Fig. 6b the $R^2$ of the model is 0.815, which is far below from the terahertz fitting model ($R^2>0.95$). Therefore, THz technology has evident preponderances in quantitative analysis of moxa wool growing years.
3.6 Different growing years and different purities of moxa wool in the market are predicted, based on established PLS theoretical model.

For the sake of testing and verifying the prediction accuracy and reliability of the model of moxa wool in different purities and different growing years in section 3.3, 3.4 and 3.5, we bought different purities of moxa sticks in different wormwood -to-moxa ratios (10:1, 15:1 and 30:1) were 8 years from the market, and different growing years of moxa wool which are numbered as "2017", "2014" and "2012" respectively was 15:1. For different purities of moxa wool samples, each variety suppressed 15 samples, with a total of 45 samples. For different growing years of moxa wool, each variety will also prepare 15 samples. Then we respectively collect terahertz spectra and Raman spectroscopy at specific bands. The spectral data were substituted into the PLS model for prediction, in which 5 samples were selected for each growing years (purities) for prediction. Consistent with the classification in section 3.3, 3.4 and 3.5, we assigned "1", "2" and "3" to the three growing years (three purities) in turn. After the assignment, it was determined as "1", corresponding to the “2012”, and set the thresholds of the prediction model as 0.5 to assess the prediction precision of the moxa wool model. As shown in Table.3, It is concluded that in this experiment, the prediction accuracy in terahertz spectroscopy of different purities of moxa wool is 93.9%, and that of different growing years is 95.3%. Conversely, the Raman spectroscopy prediction accuracy is lower than THz technology. The terahertz spectroscopy model can well predict the different growth years and different concentrations of moxa wool in the market.

<table>
<thead>
<tr>
<th>Component</th>
<th>(THz) Accuracy (%)</th>
<th>(Raman) Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:1</td>
<td>90.5</td>
<td>75.6</td>
</tr>
<tr>
<td>15:1</td>
<td>91.2</td>
<td>81.2</td>
</tr>
<tr>
<td>30:1</td>
<td>100.0</td>
<td>77.2</td>
</tr>
<tr>
<td>2012</td>
<td>94.3</td>
<td>70.9</td>
</tr>
<tr>
<td>2014</td>
<td>100.0</td>
<td>80.0</td>
</tr>
<tr>
<td>2017</td>
<td>92.8</td>
<td>73.4</td>
</tr>
</tbody>
</table>

4. Discussion

In this research, we reckoned, tested and verified the practicality of fast lossless testing of the purity and growing years of moxa wool based on terahertz spectroscopy.
Firstly, linear fitting was set by using the peak height (area) of the above-mentioned three characteristic peaks with different purities of moxa wool, and the $R^2$ in the model was outdo 0.95. Besides, we obtained a more accurate prediction model of different purity moxa wool by PLS model, and the determination coefficient $R^2$ was greater than 0.99. Next in importance, the PLS combined the growing years of moxa wool with intensities of the terpineol characteristic peaks were established, where it demonstrated an accurate model with high prediction coefficient of determination (greater than 0.98). Before we get a conclusion of the predicted accuracy of moxa wool is beyond 0.95, we try a lot of sample preparation methods, among them, grinding fineness of sample and thickness of sample, we have found that the moxa wool was grinded twice (After twice grinding, the sample can be ground more fully.) which is more beneficial to tabletting and detection of spectral information. After grinding moxa wool, through controlling the quality of the moxa wool, we collected the best spectroscopy of moxa wool. Through improving sample preparation methods, we have got the high prediction accuracy of moxa wool. After using the THz spectroscopy to analyze the purities and growing years of moxa wool, we repeated the above experiments by using Raman technology spectroscopy, trying to compare the quantitative accuracy for the purities and growing years of moxa wool between these two technologies. The $R^2$ of the Raman technology spectroscopy model is above 0.80, which is far from the THz technology ($R^2>0.95$). Finally, we predicted the real samples in the market, and compared the prediction accuracy of terahertz technology and Raman technology. It is concluded that in this experiment, the prediction accuracy in terahertz spectroscopy of different purities is 93.9%, and that of different growing years is 95.3%. Conversely, the Raman spectroscopy prediction accuracy is lower than THz technology. Therefore, the THz spectroscopy provides a new approach to detect the purities and growing years of moxa wool and also offers a strong technical support on nondestructive monitoring of terpineol content. It provides a new idea for the identification of inferior moxa wool in the market and a new method for identifying the quality of moxa wool in traditional Chinese medicine.

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Author contributions: Di Zhu did data collection, mathematical modeling and writing articles. Zhi Zhu did the calculation of molecular simulation. Wenchao Tang provided Chinese herbal medicine moxa wool. Yutian Wang Zhengan Tian, Yan Peng, and Yiming Zhu participated in revising the thesis. All authors have read and approved the manuscript.

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