

# Mid Infrared Multispectral Analysis for Mental Health Effect of Mildew on Quality of *Gastrodia Elata* Blume

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**Abstract:** In order to systematically evaluate the impact of natural mildew process on the chemical quality of *Gastrodia elata*, especially its potential harm to mental health-related active ingredients and drug safety, the normal samples of Xiaocaoba homologous *Gastrodia elata* in Zhaotong, Yunnan Province and the samples of natural mildew in the laboratory for 27 days were selected as the objects in this study. The microscopic morphology of the mildew area was observed by biological microscope, and the infrared spectra of normal tissues, areas with different mildew types (such as lateral mildew, yellow pulp) and normal distal tissues were collected by Fourier transform infrared spectroscopy (FTIR) and attenuated total reflection (ATR) accessories, and analyzed by derivative spectroscopy and chemical imaging technology. The results showed that fungal hypha invasion caused tissue ulceration; The characteristic absorption of gastrodin (characteristic vibration of aromatic ring and glycosidic bond) and polysaccharides, which are closely related to mental health efficacy, was significantly weakened, and there was an obvious carbonyl absorption peak in the range of 1740-1700  $\text{cm}^{-1}$ , indicating that the mold metabolized and synthesized exogenous risk substances. It is worth noting that even if the visible mildew is removed, the chemical damage still exists, and even the recessive deterioration is detected in the distal tissue with normal appearance. Therefore, mildew not only destroys the physical structure of *Gastrodia elata*, but also leads to the degradation of its core active ingredients and the introduction of potential risk substances. The traditional "mold removal and retention" method has potential safety hazards.

**Keywords:** *Gastrodia Elata*; Mildew; Mid Infrared Spectrum; Chemical Quality; Mental Health; Quality Control

## 1. Introduction

*Gastrodia elata*, as a valuable traditional Chinese medicine, has a long history of medicine, especially in calming the liver and calming the wind, dredging collaterals and relieving pain, calming nerves and so on [1]. In recent years, with the increasing attention to the field of mental health, the potential of *Gastrodia elata* in relieving anxiety, depression, insomnia and adjuvant treatment of neurodegenerative diseases has been widely valued [2]. Its active ingredients, such as gastrodin and p-hydroxybenzyl alcohol, have been proved to have neuroprotective, anti-inflammatory and antioxidant effects, which are closely related to emotion regulation and cognitive function improvement. Therefore, the quality control and quality assurance of *Gastrodia elata* are directly related to the effectiveness and safety of its clinical application in the field of mental health [3].

Chinese medicinal materials are easily affected by environmental factors (especially temperature and humidity) during the process of harvesting, processing, storage and circulation. Mildew not only leads to the deterioration of the appearance, smell and texture of medicinal materials, but also more seriously, the growth and metabolism of mold may decompose the original active ingredients of medicinal materials and produce new secondary metabolites (such as mycotoxins), thus changing or even completely destroying their efficacy, and may introduce new health risks [4]. The influence of mildew on the chemical composition, pharmacological activity and final curative effect of *Gastrodia elata* has not been systematically clarified, which constitutes a key blind spot in the current quality control and safe drug use of *Gastrodia elata*. Therefore, it is of great theoretical significance and practical application value to develop rapid, accurate and in-depth technical means to identify the degree of mildew, analyze the chemical

changes caused by mildew, and evaluate its impact on the material basis of efficacy [5].

The traditional identification of mildewed medicinal materials mainly relies on sensory inspection (eye, hand, nose) and microbiological culture methods. These methods are subjective, time-consuming and laborious, and are difficult to reflect the changes in internal microstructure and chemical composition caused by mildewing [6]. As a classic means of morphological observation, biomicroscopy can directly reveal the microscopic morphological characteristics of the surface and section of medicinal materials, such as the destruction of tissue structure, the invasion and distribution of mycelium, spore morphology, etc., and provide direct evidence for the qualitative judgment of mildew location and degree [7]. In this paper, the samples of normal *Gastrodia elata* and different mildewed states (such as lateral mildewed spots, yellow mildewed pulp, black mildewed spots, etc.) were observed by biological microscope, which clearly showed the complete structure of normal *Gastrodia elata* tissue and the significant differences of mycelial infection and tissue decay in mildewed areas. This morphological comparison is the basis for evaluating the physical damage of mildew.

However, morphological observation alone cannot quantitatively or qualitatively reveal the complex chemical transformation in the process of mildew. This requires the help of modern spectral analysis technology. Mid infrared spectroscopy technology shows its unique advantages in this regard. The mid infrared spectral region (usually 4000-400  $\text{cm}^{-1}$ ) corresponds to the vibration and rotational energy level transition of molecular groups, which can provide rich "fingerprint" information of compounds. By analyzing the position, intensity and shape of the characteristic absorption peak in the spectrum, the organic chemical components in the sample, such as polysaccharides, proteins, phenols, alkaloids, etc., can be identified nondestructive and rapidly [8]. The multispectral imaging technology combining mid infrared spectroscopy with spatial resolution can better realize the visualization of the distribution of chemical components in the microscopic region of the sample [9].

The combination of morphological observation of biomicroscopy and chemical information acquisition of mid infrared multispectral analysis

constitutes a powerful analysis strategy. Morphological observation can accurately locate the front of mildew, colony aggregation area and possible histochemical abnormalities; While the mid infrared multi spectrum can scan the chemical composition of these specific areas synchronously, which is directly related to "where mildew" and "what chemical changes have taken place" [10]. For example, the mid infrared spectra of normal *Gastrodia elata* tissue at the junction of mildew and different color mildew spots can be compared and analyzed to find the band changes related to the reduction or disappearance of the characteristic active components of *Gastrodia elata* (such as gastrodin related groups), as well as the possible new characteristic bands related to the metabolites or decomposition products of mold. This correlation analysis is expected to reveal how the mildew process gradually degrades the effective components of *Gastrodia elata* and whether new compounds with potential neurotoxicity or other biological activities have been produced, so as to comprehensively evaluate the impact of mildew on the "quality" of *Gastrodia elata* - "quality" here not only refers to the appearance, but also refers to the chemical substance basis related to its mental health efficacy [11].

Based on the above background, the purpose of this study is to systematically study the effect of mildew on the quality of *Gastrodia elata* Blume by combining biomicroscopy and mid infrared multi spectral chemical analysis technology, especially focusing on the potential changes in its mental health-related chemical properties. According to the above experimental design ideas, this study will prepare comparative samples including normal *Gastrodia elata* (such as Xiaocaoba normal live *Gastrodia elata* in Zhaotong) and different types of mildew and different parts of mildew (such as meat, outside, mildew core and edge, etc.). Firstly, the high-resolution biological microscope was used to observe the local magnification of 100 times, and the histopathological changes caused by mildew were recorded in detail, providing accurate sampling and positioning guidance for chemical analysis. Then, the mid infrared multispectral imaging system is applied to scan the predetermined region of interest to obtain its chemical fingerprint. By comparing the spectral differences between normal and mildewed areas, we identified the key infrared spectral markers that could distinguish normal and mildewed

*Gastrodia elata*, analyzed the characteristic chemical changes corresponding to different mildewed forms (such as flavomyces and melanomyces), and discussed the potential correlation between the degree of mildewed *Gastrodia elata* and the content changes of specific chemical components (especially those related to neural activity).

## 2. Experimental Materials and Methods

### 2.1 Experimental Materials and Sample Preparation

The research object of this experiment is *Gastrodia elata* in Xiaocaoba production area of Zhaotong, Yunnan Province. The experimental samples are divided into two categories: one is the normal control sample, that is, the fresh black *Gastrodia elata* after cleaning and slicing; The other is the moldy test sample. Take the same batch of *Gastrodia elata* in the laboratory of Guoxue Road, Zhaoyang District, Zhaotong City, and store it naturally in the locker for 27 days (without any manual humidification), simulating the self moldy process caused by environmental humidity in the actual storage. During sample preparation, according to the observation requirements, slices were cut from typical parts such as normal tissue, moldy outer side, yellow moldy pulp, black mold spot and its junction with normal tissue for subsequent morphology observation and spectral analysis.

### 2.2 Instruments and Equipment

In this study, two kinds of instruments were used for joint analysis. The E5 biological microscope (serial number: 2653249-7) produced by Ningbo sunny Instrument Co., Ltd. was used for microscopic morphology observation, and the 100 fold magnification was used to characterize the mycelial distribution, spore morphology and tissue destruction on the surface and section of the sample. Tianjin Gangdong ftir-850 Fourier transform infrared spectrometer is used for chemical composition analysis, equipped with variable temperature horizontal attenuated total reflection (ATR) accessory (diamond crystal), which can realize nondestructive and rapid detection of samples in the spectral range of 4000 – 400  $\text{cm}^{-1}$ .

### 2.3 Experimental Methods and Data Analysis

Firstly, the morphology observation and image acquisition of different samples were carried out

by using the biological microscope to identify the microscopic characteristics of the mildew area, and locate the specific region of interest for spectral analysis. Then, place the sample on the crystal surface of ATR accessory, set the spectral resolution of 4  $\text{cm}^{-1}$ , and accumulate 32 scans to collect infrared spectral data. The key areas were scanned to obtain chemical imaging data. After baseline correction, ATR correction and normalization pretreatment of the original spectrum, the spectral differences between normal and mildewed samples were systematically compared by means of characteristic peak identification, principal component analysis (PCA) and chemical imaging, and the changes of chemical components and spatial distribution caused by mildewing were analyzed [12]. All experiments were completed in the Guoxue road laboratory, Zhaoyang District, Zhaotong City.

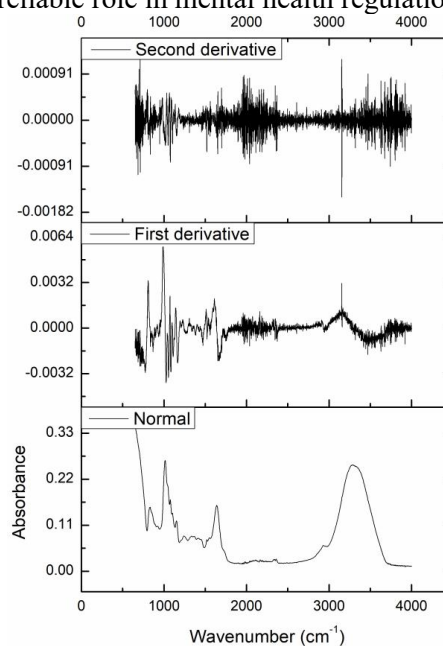
## 3. Combined Analysis of Mid Infrared Spectroscopy and Microscope

### 3.1 Normal Live *Gastrodia elata*

As shown in Figure 1, the mid infrared spectrum and its first and second derivative spectra of normal *Gastrodia elata* Blume in Xiaocaoba, Zhaotong, Yunnan, combined with the 100 times magnified image of the pulp tissue structure under the biological microscope, can systematically analyze its characteristics and correlate the chemical basis related to its mental health efficacy. The original spectrum showed the fingerprint characteristics of the typical organic components of *Gastrodia elata* in the range of 4000-400  $\text{cm}^{-1}$ : the wide and strong absorption band at 3600-3200  $\text{cm}^{-1}$  was attributed to the o-h stretching vibration, which was mainly due to the rich polysaccharides, phenolic compounds and bound water in *Gastrodia elata*. The polysaccharide component has been confirmed to have the potential of immune regulation and neuroprotection, which may indirectly support emotional stability and cognitive function through anti-inflammatory and antioxidant pathways; The C-H stretching vibration peak in the range of 3000-2800 $\text{cm}^{-1}$  corresponds to the alkyl and methylene structures, reflecting the side chain and glycosyl part of the active components of phenylpropanoid such as gastrodin and p-hydroxybenzyl alcohol, which are the core substances directly involved in sedation, anti

anxiety and neuroprotection; The absorption of amide I band near  $1650\text{-}1600\text{ cm}^{-1}$  suggests that there are proteins or peptides in the sample, which may be related to the activity of neuromodulatory peptides or enzymes; The absorption in the range of  $1500\text{-}1400\text{ cm}^{-1}$  comes from the vibration of aromatic ring C=C skeleton, which clearly indicates the existence of aromatic compounds such as gastrodin and its aglycones, which can play anti anxiety and neuroprotective roles by regulating GABAergic system, inhibiting glutamate excitotoxicity and reducing oxidative stress; The strong C-O – C and C-O stretching vibration at  $1100\text{-}1000\text{ cm}^{-1}$  directly corresponds to the glycosidic bond in polysaccharides and glycosides, especially the glucoside bond in gastrodin. This structure is the key chemical basis for its sedative effect. The first derivative spectrum further enhanced the spectral resolution. The significant inflection points at  $2920\text{ cm}^{-1}$  and  $2850\text{ cm}^{-1}$  enhanced the recognition of lipids and long-chain alkyl components, which may be involved in maintaining the integrity of neuronal cell membrane and affecting signal transduction; The extreme points near  $1650\text{ cm}^{-1}$  and  $1550\text{ cm}^{-1}$  clearly revealed the details of amide I and amide II bands of protein/peptide. The second derivative spectrum can separate more subtle hidden absorption. For example, the weak shoulder peak that may appear near  $1740\text{ cm}^{-1}$  suggests the existence of esters or carboxylic acids, and its antioxidant activity may assist in neuroprotection; The negative peaks at  $1070\text{ cm}^{-1}$  and  $1040\text{ cm}^{-1}$  were helpful to distinguish the vibration modes of different types of glycosidic bonds, which provided the basis for the specific recognition of gastrodin and its structural analogues. Based on the above spectral characteristics, the key chemical substances in normal *Gastrodia elata*, which are closely related to the efficacy of mental health, including gastrodin, polysaccharides, phenols and aldehydes, have been clearly identified. These components constitute the chemical basis for regulating mood, improving sleep and protecting nerve cells through synergistic action. At the same time, as shown in Figure 2, the biological microscope 100 times image shows that *Gastrodia elata* pulp cells are closely arranged and structurally complete, without hyphal erosion or tissue damage. This intact microscopic morphology ensures the uniformity and stability of the above active ingredients in

the spatial distribution from the histological level, providing important morphological support for *Gastrodia elata* to play a sustained and reliable role in mental health regulation.



**Figure 1. Mid Infrared Spectrum of Normal *Gastrodia Elata***

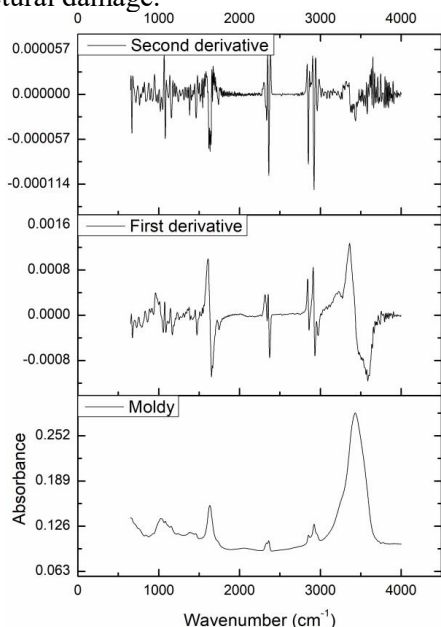


**Figure 2. Normal *Gastrodia Elata* Pulp Enlarged by 100 Times**

### 3.2 Outside of Moldy *Gastrodia Elata*

Combined with Figure 3 and Figure 4, from the original absorption spectrum, the O-H stretching vibration absorption band of the moldy sample in the  $3600\text{-}3200\text{ cm}^{-1}$  region shows a broader and more complex shape, and its strength and contour are significantly changed compared with the normal sample. This may be due to two reasons: first, a large number of metabolites (such as some hydroxylated toxins or secondary metabolites) produced by the growth and metabolism of mold contribute new hydroxyl signals; Second, the tissue corruption and hydrolysis reaction caused by mildew process may lead to the chain breaking of *Gastrodia elata*

inherent high molecular polysaccharide, producing more free hydroxyl or small molecular sugars, accompanied by the change of water distribution. The changes in this region indicate that *Gastrodia elata* polysaccharides with neuroprotective and immunomodulatory potential may undergo significant degradation or structural damage.



**Figure 3. Mid Infrared Spectrum of The Outer Side of Moldy *Gastrodia Elata***

In the C-H stretching vibration region of 3000-2800  $\text{cm}^{-1}$ , the relative intensity and fine structure of the absorption peak also changed. This suggests that the alkyl and methylene environment related to the core phenylpropanoid active ingredients such as gastrodin may be disturbed. The degradation activity of mold may act on the side chain or glycosyl part of these components, resulting in the change of characteristic C-H vibration mode, suggesting that the stability of gastrodin substances is challenged.



**Figure 4. Magnification of the Outer Side of Moldy *Gastrodia Elata* by 100 Times**

It is particularly noteworthy that in the

1740-1700  $\text{cm}^{-1}$  range, more significant or new absorption peaks may appear in the spectra of moldy samples than in normal samples. This region is the characteristic region of carbonyl (C=O) stretching vibration, especially pointing to esters, carboxylic acids or aldehydes. The appearance of the new peak strongly suggests that significant oxidation reaction has taken place in the process of mildew or that the mold has synthesized new carboxylic acid and ester metabolites. These newly generated carbonyl compounds may include fatty acid oxidation products or specific mycotoxin precursors. Their introduction may not only directly resist the pressure of oxidation system, thus weakening the neuroprotective ability of *Gastrodia elata*, but also bring unknown biological activity or toxicity, posing potential risks to the health of central nervous system.

The spectral changes in the 1650-1600  $\text{cm}^{-1}$  (amide I band) and 1550-1500  $\text{cm}^{-1}$  (amide II band and aromatic ring vibration) regions are equally critical. If the absorption intensity of these areas is weakened or the peak shape is dispersed, the endogenous proteins/peptides of *epilepis cannabina* may be decomposed by mycoprotease, and the benzene ring structure of aromatic active ingredients such as gastrodin may also be damaged or the concentration may decrease. The reduction of these two components is directly related to the weakening of the material basis of *Gastrodia elata* for regulating neurotransmitters, anti-inflammatory and direct neuroprotective functions.

The C-O-C and C-O stretching vibration absorption changes in the range of 1100-1000  $\text{cm}^{-1}$  directly reflect the structural integrity of glycosidic bonds and polysaccharides. The spectral band changes (such as intensity reduction, peak shift or broadening) of moldy samples in this region are the intuitive spectral evidence of gastrodin glycosidic bond breakage and structural polysaccharide utilization by mold enzymolysis. This indicates that gastrodin, the most critical active ingredient of *Gastrodia elata* Blume, which plays a calming and tranquilizing role, may be degraded, and its efficacy will be greatly reduced.

The first and second derivative spectra further magnify the above differences. In the derivative spectrum, some sharp inflection points or minima representing specific functional groups in normal samples may become flat, disappear or shift in the spectrum of moldy samples, which

confirms the change or loss of the original chemical composition microenvironment. At the same time, the derivative spectrum may reveal more new characteristic peaks that overlap in the original spectrum and belong to the metabolites of mold in the range of 1800-1700  $\text{cm}^{-1}$  or 1500-1400  $\text{cm}^{-1}$ , providing high-resolution clues for the identification of specific mold related compounds.

### 3.3 Remove Obvious Mildew Spots on the Moldy Side

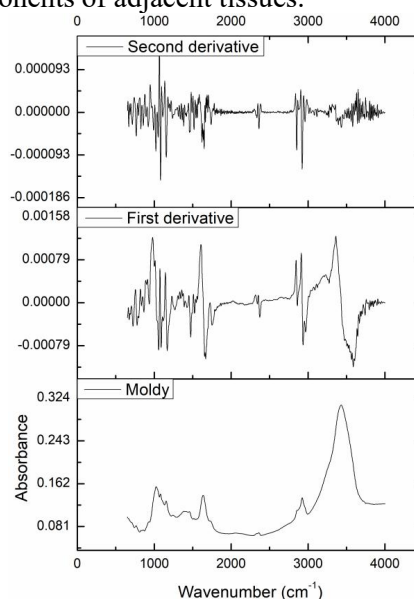
Combined with Figure 5 and Figure 6, the mid infrared spectrum analysis of the outer side of moldy *Gastrodia elata* after physical removal showed that the chemical spectrum of the sample showed a transition state between completely normal and severe mildew after partial removal of the visible mildew tissue, revealing the depth and incompleteness of the influence of mildew.

According to the original absorption spectrum, the O-H stretching vibration absorption band of the cut sample in the region of 3600-3200 $\text{cm}^{-1}$  was less extensive than that of the untreated severely moldy outer side, but still failed to return to the regular shape of normal *Gastrodia elata*. This indicates that although some moldy tissues have been removed, the water-soluble small molecular products produced by mold metabolism or the water distribution disorder caused by it may have penetrated into adjacent tissues. At the same time, the degradation process of the intrinsic structure polysaccharide of *Gastrodia elata* may have occurred in the mildew boundary region, resulting in the continuous change of the characteristics of the hydroxyl band in this region.

In the carbonyl region of 1740-1700 $\text{cm}^{-1}$ , the intensity of the new carbonyl absorption peak in the spectrum of the sample after removal treatment is generally weaker than that in the seriously moldy region, but the residual absorption higher than the normal background can still be observed. This strongly suggests that the oxidative metabolites (such as some organic acids, esters or oxidized lipids) produced by mold activities are not only limited to the surface of the mold spots visible to the naked eye, but also diffuse to the internal tissues to a certain extent or induce secondary oxidation reactions. These residual carbonyl compounds are potential chemical risk markers.

The absorption characteristics of 1650-1600  $\text{cm}^{-1}$  (amide I band) and 1550-1500  $\text{cm}^{-1}$  (aromatic

ring C=C vibration) regions are crucial for evaluating the retention of functional ingredients. If the spectrum after the removal treatment shows that the absorption intensity of mildewed samples in these areas has rebounded, but it is still lower than the normal level, it indicates that physical removal means can save some proteins and aromatic active ingredients (such as gastrodin) that have not been completely decomposed. However, the incompleteness of the recovery confirmed that the enzymes or active molecules secreted by the mold had caused perceptible chemical damage to these components of adjacent tissues.



**Figure 5. Spectrogram of Obvious Mildew Spots Removed from the Moldy Outer Side**



**Figure 6. Magnification of Obvious Mildew Spots Removed from the Moldy Outer Side by 100 Times**

The recovery degree of the spectral band profile of 1100-1000  $\text{cm}^{-1}$  glycosidic bond and polysaccharide characteristic region is the key to judge whether gastrodin and structural polysaccharide can be retained. If the absorption peak shape in this area is closer to normal than

that of untreated moldy samples, it indicates that the removal operation has effectively removed a large number of mold communities that directly decompose sugars. However, the slight shift of peak position or the slight increase of half peak width may indicate that the chemical environment of glycosidic bond or the structure of sugar chain has changed slightly and irreversibly.

### 3.4 Moldy Yellow Pulp

Combined with Figure 7 and Figure 8, from the original absorption spectrum, the overall absorption intensity and complexity of the yellow pulp area in the 1800-1500  $\text{cm}^{-1}$  "fingerprint area" increased significantly. In particular, a very prominent and sharp strong absorption peak appeared in the range of 1740-1710  $\text{cm}^{-1}$ . This feature clearly points to high concentrations of free carbonyl ( $\text{C}=\text{O}$ ) compounds, such as specific carboxylic acids, esters or aldehydes. This strongly indicates that in the Yellow mildew area, the metabolic activity of the mold (possibly *Aspergillus flavus* or related strains) is extremely vigorous, producing a large number of such small molecule acidic or esterified metabolites. These compounds are not only the signs of serious deterioration of components, but also some short chain fatty acids or specific organic acids may have direct or indirect stimulatory effects on the nervous system.

At the same time, the characteristic absorption band representing the intrinsic active components of *Gastrodia elata* was severely suppressed or changed: the absorption of amide I band near 1650-1600  $\text{cm}^{-1}$  was significantly weakened or masked, indicating that endogenous proteins were largely decomposed. The aromatic ring skeleton vibration peak (gastrodin, etc.) in the 1550-1500  $\text{cm}^{-1}$  region almost disappeared or severely deformed, suggesting that the core phenylpropanoid active ingredients have undergone large-scale chemical degradation or structural damage, and the direct drug effect material basis related to mental health has been nearly lost in this region. Although the glycosyl bond and polysaccharide characteristic region of 1100-1000  $\text{cm}^{-1}$  still showed signals due to the enhanced overall absorption, its fine structure had been seriously distorted, and its peak shape had widened and overlapped with the absorption of adjacent fungal metabolites, indicating that the enzymatic hydrolysis and glycosyl bond

breakage of polysaccharide chain were extremely serious.

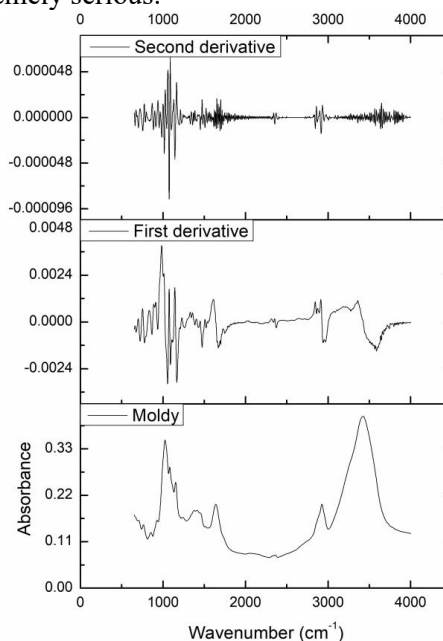


Figure 7. Spectrum of Moldy Yellow Pulp

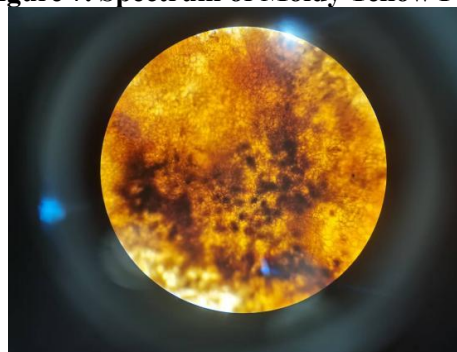


Figure 8. Magnification of Moldy Yellow Pulp by 100 Times

In the first derivative spectrum, the strong positive and negative peak pairs near 1740  $\text{cm}^{-1}$  and 1710  $\text{cm}^{-1}$  confirmed the existence of these new carbonyl compounds and their possible existence in a variety of forms with high sensitivity. The second derivative spectrum may reveal more hidden peaks representing quinones or other oxidation products after aromatic ring destruction in the range of 1600-1550  $\text{cm}^{-1}$ , which usually have stronger chemical reaction activity.

### 3.5 Mildewed Pulp at the Other End of *Gastrodia Elata*

Combined with Figure 9 and Figure 10, although the sample is no different from the normal pulp in appearance, its spectrum reveals chemical changes that cannot be ignored, indicating that the influence of mildew has exceeded the visible morphological boundary.

From the original absorption spectrum analysis, the region showed a systematic shift and disturbance different from the normal *Gastrodia elata* in several key bands: hydroxyl and water partition ( $3600\text{-}3200\text{ cm}^{-1}$ ), and the contour and relative intensity of the absorption band were significantly different from the normal sample. This may mean that even in the area without direct hyphal infection, the water migration and distribution changes caused by mildew process, or the infiltration of small molecular soluble metabolites, have changed the hydrogen bond network and chemical composition of the area, suggesting that the microenvironment inside the tissue has been affected.

Subtle changes in the key fingerprint area ( $1800\text{-}1000\text{ cm}^{-1}$ ):  $1740\text{-}1700\text{ cm}^{-1}$  although there is no carbonyl peak as strong as the Yellow mildew area, careful observation of the original spectrum or derivative spectrum may find that it is higher than the baseline of the normal sample or extremely weak shoulder peaks. This is a sensitive chemical marker that the oxidative metabolites of mold have diffused to the distal tissue, or caused slight oxidative stress in the distal tissue itself.  $1650\text{-}1550\text{ cm}^{-1}$  represents the absorption peak of protein/peptide (amide i/ii band) and aromatic components (gastrodin, etc.), and its absorption intensity may be slightly but uniformly weakened, or the peak shape may be slightly widened. This strongly suggests that extracellular enzymes (such as protease, phenol oxidase) or reactive oxygen species secreted by mold may have diffused or conducted through tissue fluid, resulting in slow and initial degradation of these active components in the distal region. The fine structure of  $1100\text{-}1000\text{ cm}^{-1}$  glycosidic bond and polysaccharide characteristic peak may change, such as the relative intensity ratio of specific peak position changes, or the resolution decreases. This suggests that the homogeneity of polysaccharides or the chemical environment of glycosidic bonds have changed microscopically, which may be the early signal of enzymatic hydrolysis.

#### **4. Analysis of the Influence of Mildew on the Quality of *Gastrodia elata***

Combined with the optical microscope observation image and the mid infrared spectrum analysis data, the influence of mildew on the quality of *Gastrodia elata* showed a systematic damage from macro to micro, from morphology

to chemistry, and posed a fundamental threat to its mental health related efficacy.

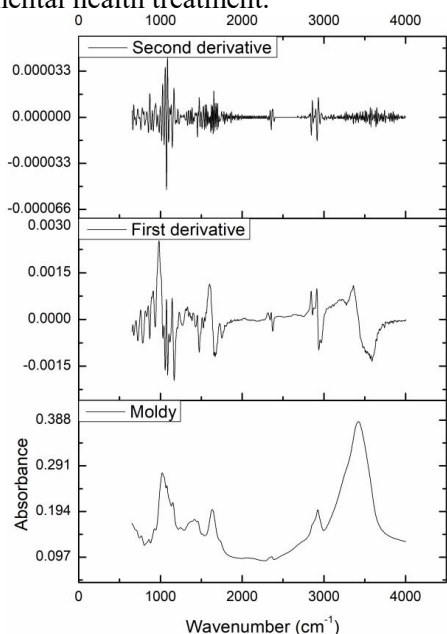
The direct destruction of morphology and structure and the beginning of chemical degradation. The microscope image clearly showed that the normal *Gastrodia elata* pulp tissue cells were orderly arranged and structurally intact. However, in the moldy area, a large number of mycelia invaded, and the tissues were ulcerated, loose or even disintegrated. The destruction of the physical structure provides conditions for the in-depth growth and enzymatic hydrolysis of the mold, which directly leads to the exposure of the active ingredients carried by the mold to the degradation environment.

The variation of chemical composition and the loss of functional material basis. Mid infrared spectrum analysis confirmed that the mildew process was accompanied by violent chemical transformation. In the moldy area, the spectral characteristics of the characteristic mental health components of *Gastrodia elata* (such as aromatic rings and glycosidic bonds in gastrodin, and specific absorption of polysaccharides) were significantly weakened or distorted, indicating that these core effective components were being enzymolysis or transformed by mold. At the same time, a new strong absorption peak appeared near  $1740\text{-}1700\text{ cm}^{-1}$  in the spectrum, which is the characteristic of carbonyl compounds (such as organic acids and esters) produced by mold metabolism. The introduction of these exogenous substances is not only not conducive to neural regulation, but also may bring unknown biological risks.

The specificity of mildew type and the severity of harm. Under the microscope, yellow and black mold spots showed different flora morphology. The mid infrared spectrum further revealed that the variation of chemical components in the Yellow mildew area was particularly intense, and its spectrum was almost dominated by the signals of fungal metabolites, indicating that this kind of mildew may lead to the complete replacement of the original chemical components of *Gastrodia elata*, the complete loss of efficacy and the highest potential safety hazard.

The concealment and spatial diffusion of influence. The most important thing is that the joint analysis of "the flesh without mildew at the other end" reveals the hidden danger. Under the microscope, the tissue in this area seems to be

intact, but the chemical composition has changed slightly but clearly (such as the weak shift or broadening of a specific absorption peak) detected by mid infrared spectroscopy. This proves that fungal metabolites or enzymes have spread to the distal end, resulting in the deterioration of the internal quality and safety of even normal looking tissues, which is unreliable for mental health treatment.



**Figure 9. Spectrum of the Flesh without Mildew at the Other End of the Moldy Gastrodia Elata**



**Figure 10. The Flesh without Mildew at the Other End of the Moldy Gastrodia Elata Enlarged by 100 Times**

## 5. Conclusions and Prospects

This study systematically revealed the profound impact of natural mildew process on the quality of *Gastrodia elata*, especially on the material basis and safety related to mental health, by integrating biological microscopic morphology observation and mid infrared multi spectral chemical analysis technology. The main conclusions are as follows:

The damage of mildew to *Gastrodia elata* Blume is a systematic process. Optical microscope observation directly confirmed the physical invasion and destruction of fungal hyphae on the tissue structure of medicinal materials, resulting in cell ulceration and texture deterioration. More importantly, the mid infrared spectrum analysis revealed from the chemical level that this morphological damage was accompanied by severe biochemical transformation: first, the significant degradation of the intrinsic active ingredients of *Gastrodia elata*, which showed that the characteristic infrared absorption of gastrodin (characteristic aromatic ring and glycosidic bond vibration) and neuroprotective polysaccharides directly related to mental health efficacy was significantly weakened or distorted, and the material basis of its core efficacy was seriously eroded; The second is the introduction of exogenous risk substances. New characteristic absorption peaks (especially in the 1740-1700  $\text{cm}^{-1}$  carbonyl region) belonging to the metabolites of mold generally appear in the spectrum, indicating the generation of organic acids, esters and other non *Gastrodia* inherent components, which may bring unknown pharmacological antagonistic effects or potential toxicity risks.

The study further revealed the irreversibility, concealment and spatial diffusion of mildew effect. The analysis of "cut part" samples showed that the chemical damage depth exceeded the boundary of visible mildew, and the quality could not be restored by physical removal alone. The detection of "non moldy pulp at the other end" issued a key warning: even inside the intact tissue, hidden deterioration of chemical components may occur due to the diffusion of small molecular metabolites or enzymes, making its safety and effectiveness impossible to guarantee. Therefore, local mildew means that the chemical integrity of the whole plant or batch of medicinal materials has been fundamentally threatened. The traditional "mold removal and retention" treatment is ineffective and dangerous at the chemical level, especially for *Gastrodia elata*, which is used in the sensitive field of mental health.

Based on the results of this study, further work can be carried out in the following directions in the future: further optimize the parameters of mid infrared multispectral imaging technology, establish the chemical fingerprint database and discrimination model of *Gastrodia elata* Blume

with different mildew types (such as Xanthomonas and melanomyces) and different mildew degrees, and achieve rapid and accurate qualitative and quantitative analysis of mildew. Combining mid infrared spectroscopy with metabolomics and microbiomics, we can more accurately identify the specific toxic metabolites produced in the process of mildew, and clarify the dynamic correlation mechanism between them and the degradation of key active ingredients, so as to provide a more comprehensive basis for safety evaluation. Empirical Study on the effect of mental health, using the chemical quality evaluation index established in this study, to guide cell or animal experiments, directly compare the biological activity differences between normal and different mildew degree *Gastrodia elata* extract in anti anxiety, anti depression, neuroprotection and other models, and demonstrate the harm of mildew from the pharmacodynamic level.

In conclusion, this study confirmed the unique value of mid infrared multispectral analysis technology in evaluating the impact of mildew on the quality of traditional Chinese medicine, provided innovative analysis strategies and a solid scientific foundation for ensuring the safety, effectiveness and stability of *Gastrodia elata* and its products in the field of mental health, and helped to promote the modernization and accuracy of quality control of traditional Chinese medicine.

### Acknowledgements

This work was supported by the Special Basic Cooperative Research Programs of Yunnan Provincial Undergraduate Universities' Association (Grant No. 202301BA07001-094, 202301BA070001-121).

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