Metabolomics research on Tibetan medicinal substances

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Abstract Background: Tibetan medicinal substances (TMS) has a complete theory that is based on five source doctrines. The theories of "Liuwei", "Baxing", "Shiqi Gongxiao", and "Sanhuawei" constitute the prominent core components of the five source doctrines with significant Tibetan characteristics.

Objective: This review summarizes the existing literature on the metabolomics and its use in TMS and offers recommendations for future research.

Methods: Studies on TMS and the application of metabolomics on TMS are reviewed and summarized.

The shortcomings of existing studies and recommendations for future studies are discussed.

Conclusions: The recent researches have studied on the theory of TMS. Metabolomics will be increasingly applied to the identification of compounds in TMS, and the resultant detection of biomarkers will help elucidate the metabolic pathways of these substances.

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Introduction

With thousands of years of clinical practice, Tibetan medicinal substances (TMS) is important modality of traditional medicine in China and protect the health of Tibetan people effectively. Because of the limitations of past analytical technologies, we have not been able to scientifically explain the theories of TMS (such as Liuwei, Baxing,
Shiqi Gongxiao, and Sanhuawei). However, the rapid development of new analytical techniques that are high throughput and highly sensitive and accurate have enabled scientific research capable of providing evidence for TMS theories. Metabolomics is a scientific methodology that simultaneously monitors metabolic networks through the analysis of tissues and other biological samples, such as blood and urine, to elucidate the complex physiologic and pathologic states of organisms using endogenous and exogenous metabolic changes. Metabolomics involves a systematic and comprehensive evaluation, thus it is consistent with TMS, which involves multi-components, multi-targets, multi-levels, and multi-metabolic pathways. Therefore, by using metabolomics technologies, not only can the chemical constituents in TMS be detected but also a comprehensive analysis and understanding of TMS theory can be derived.

### Tibetan medicinal substances theory

The theories of Liuwei, Baxing and Shiqi Gongxiao form the basic components of TMS. These theories are based on the five source doctrines and expound the characteristics and properties of TMS. The five elements in the five source doctrine refer to water, earth, fire, gas (wind), and emptiness. The Four Medical Tantras states that all living things, including medicines, are sourced from the five elements. This statement expresses the dialectical relationship between herb growth and the natural environment and provides a theoretical basis for the Liuwei and Baxing theories. According to Liuwei, Tibetan medicinals have six tastes—acidic, bitter, sweet, acrid, salty, astringent—that are closely related to their pharmacologic effects. The six tastes are also related to the five sources (Fig. 1). Generally, the taste of a medicine is sweet when the property of the medicine is earth combined with water; acidic when earth and fire are combined; salty when water and fire are combined; bitter when water and wind are combined; and astringent when earth and wind are combined.

The theory of Sanhuawei states that the taste of a medicine changes when the medicine is metabolized, and Liuwei transforms into three end tastes. After being metabolized, sweet and salty medicines become sweet. Astringent, bitter, and acrid tastes transform into a bitter taste, while acidic remains acidic. Thus, the tastes of Tibetan medicines stay sweet, bitter, or acidic. Sanhuawei is an important part of the modernization of TMS research. The Baxing theory describes the property of a medicinal substance. There are eight properties: heavy, moist, cool, blunt, light, acerbic, hot, and sharp. The theory of Shiqi Gongxiao refers to the 17 different effects that medicinal substances have on disease. These effects are eight pairs of opposing properties: cold—hot, warm—cool, dry—thick, smooth—rough, light—heavy, stable—dynamic, blunt—sharp, soft—dry and—mild. Shiqi Gongxiao stems from Liuwei, thus, when the taste of a medicine is consistent with Sanhuawei, the clinical effect is best. Although the theories of Liuwei, Baxing, Shiqi Gongxiao, and Sanhuawei are derived from the five source doctrines, the material basis and mechanism of the theories remain unknown.

### Recent research on Tibetan medicinal substances

TMS has received increasing scientific attention, resulting in studies on multiple compounds, multiple targets, and its unique theory, but few studies have focused on other aspects of TMS. TMS is a holistic healing modality whose efficacy is based on the interaction of several ingredients. Thus, detection and validation of relative concentrations of chemical compounds in medicinal substances should be based on the Liuwei theory. Doing so will establish quality standards and ensure the safety and effectiveness of medicines. As an integrated and dynamic system, the pharmacologic actions of TMS are based on the Shiqi Gongxiao theory. As such, systematic studies should be conducted to verify their mechanisms of action.

Recently, sporadic achievements have been made in exploring the chemical characterization and quality control of Tibetan medicinal substances. Fei et al established a stable and repeatable method to identify the Tibetan herb, Songdi (Saxifraga umbellulata var. pectinata), used to treat inflammation and toxic heat, and based on HPLC fingerprints. They found that plant species and altitude affected the cluster analysis of the samples. An interesting medicinal substance in Tibetan medicine is Zuota. Zhao et al used multiple techniques, including scanning electron microscope and high performance liquid chromatography, to characterize Zuota from four different sources. They found that all four samples of Zuota had a common inorganic mixture of HgS, sulfur, and graphite.

Only a small portion of TMS have undergone scientific research and even fewer studies have focused on their metabolism and therapeutic effect. Hence, there is an urgent need to apply advanced technologies to detect chemical components, determine potential biomarkers, and explore the probable metabolic pathways that may aid in explaining the theories of Liuwei and Sanhuawei. Therefore, we suggest that the application of metabolomics be applied to the exploration of Tibetan medicines. In comparison with conventional research tools, metabolomics is a systematic and high throughput field of study, which may aid in understanding as-yet-unknown aspects of...
Tibetan medicinal substances that contribute to their pharmacologic effects and chemical characteristics.

Metabolomics and its use in studying Tibetan medicinal substances

Overview of metabolomics

Metabolomics is the quantitative and qualitative analyses of all small molecule metabolites in a biological cellular system at a given time and condition performed to quantitatively describe its overall endogenous metabolic response to internal and external changes. Analytical platforms currently used for metabolomics studies mainly include nuclear magnetic resonance (NMR) and mass spectrometry (MS). Chromatography technologies are often coupled with MS. Metabolomics analysis generally includes sample collection, preparation, chemical analysis, and data processing (Fig. 2).

Application of metabolomics in Tibetan medicinal substances research

Similar to herbs used in traditional Chinese medicine, the many species, plant growth conditions, and processing procedures have made the scientific study of Tibetan herbs just as difficult. However, metabolomics has greatly mitigated this difficulty in recent years (Fig. 3).

For example, Liao et al developed an ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) method for qualitative and quantitative analysis of major compounds in the snow lotus herb (*Saussurea eopygmaea* Hand.-Mazz.), used to treat rheumatic diseases. They found that this approach had the potential to characterize *S. eopygmaea* and other Tibetan herbs. Zhou et al developed a method that coupled rapid, sensitive, reproducible UPLC-QTOF-MS for qualitative and quantitative analysis of phenolic compounds in Dianxinghao (*Artemisia minor*), demonstrating that this approach had the prospect for characterization of *A. minor* and other Tibetan herbs. Fan et al developed a rapid comprehensive $^1$H NMR-based metabolomics method to differentiate the two species of herba swertiae mussotii (*Swertia mussotii*) and herba swertiae chirayita (*Swertia chirayita*). The primary biomarkers responsible for their differences were screened and findings revealed significant differences between the two species. Thus, $^1$H NMR-based metabolomics appears to be reliable and effective for the metabolic profiling and discrimination of Tibetan herbs and could be used to verify their genuine origin. La et al developed a method to study the Tibetan herb of Duyiwei (*Lamiophlomis rotate*), which used to treat swelling pain, by ultrahigh performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-TOF/MS) metabolomics compared with high performance liquid chromatography coupled to a UV

![Figure 2](image-url)

**Figure 2** Metabolomics analyses workflow for targeted and untargeted metabolomes. Note: GC is an abbreviation for gas chromatography, MS is for mass spectrum, TOF is for time-of-flight, LC is for liquid chromatography, NMR is for nuclear magnetic resonance, PCA is for principal component analysis, PLS-DA is for partial least squares discriminant analysis, OPLS-DA is for orthogonal partial least squares discriminant analysis, and HCA is for hierarchical cluster analysis.
detector (HPLC-UV).\textsuperscript{14} Based on their results, they explained the reason of the replacement of the whole plant with its aerial parts in the 2010 edition of Chinese Pharmacopoeia.\textsuperscript{15} Their study aimed to establish a new powerful method to protect Lamiophlomis rotata and control its quality.

Liu et al used HPLC-QTOF-MS to identify 40 compounds (including 17 flavonoids, 15 alkaloids, and 8 phenylpropanoids) in herb of spiny meconopsis (Meconopsis horridula Hook.f. Thoms) (used to treat traumatic injury).\textsuperscript{16} The study provided a comprehensive method for understanding this herbal medicine using metabolomics technologies. Yang et al employed a Fourier transform infrared spectroscopy method and found that the signal intensity of gentiamarin (1611 and 1075 cm\textsuperscript{-1}) positively correlated with the content change in different extracts of Zangyinchen (S. mussotii Franch) (used to treat icteric viral hepatitis).\textsuperscript{17} Twelve compounds were identified from total iridoids and xanthones extracted from S. mussotii by HPLC-MS.\textsuperscript{18} Jiang et al extracted single compounds with high purity from the Tibetan animal medicine of plateau zokor (Myospalax baileyi), the skeleton of a blind underground mole rat, which used for treating wind cold damp bi, using a new two-dimensional liquid preparative chromatographic method.\textsuperscript{19}

To elucidate the anti-hypoxia and anti-anxiety effects of Fu Fang Jin Jing oral liquid (FJJOL), Liu et al applied \textsuperscript{1}H NMR metabolomics to explore the changes in metabolites and the mechanism of anti-hypoxia and anti-anxiety. The results indicated that FJJOL ameliorated hypobaric hypoxia effects by regulating energy metabolism, choline metabolism, and improving the symptoms of anxiety.\textsuperscript{20} Lang et al combined HPLC-MS and NMR to evaluate the antitumor effects of Zimoli (Mirabilis himalaica), which used in Tibetan medicine to treat uterine cancer and kidney stones. One new rotenoid compound, mirabijalone E, and eight known rotenoids, were isolated from this herb. Some compounds exhibited cytotoxicity against A 549 and HeLa cells, and have potential as anti-cancer agents.\textsuperscript{21} Saussurea laniceps Hand.-Mazz. is known as snow lotus. It is used for rheumatoid arthritis, stomach ailments, and menstrual cramps in Tibetan folk medicine. Yi et al developed an integrated strategy based on ultra-performance liquid chromatography diode array detection quadrupole time-of-flight mass spectrometry for metabolic and pharmacokinetic studies of S. laniceps in rats. The biomarkers, umbelliferone, scopoletin, and their metabolites were the primary components in the metabolic process.\textsuperscript{22}

Conclusions

Tibetan medicine has a complete theory based on the five source doctrines. Among the theories of Liuwei, Baxing, Shiqi Gongxiao, and Sanhuawei, Liuwei and Baxing reflect the properties and effectiveness of TMS. The active constituents in medicinal substances can be considered the basis of the Liuwei and Baxing theories. Metabolomics studies, including the aforementioned, have detected changes in those constituents after being metabolized, a process akin to that of Sanhuawei theory. The aim of metabolomics is comprehensive characterization of the total metabolites in a biological system (such as plants and humans) and qualitatively and quantitatively identifying chemical compounds, monitoring dynamic changes \textit{in vivo}, and screening for potential biomarkers.\textsuperscript{23} As metabolomics is increasingly applied to the identification of compounds in TMS, the resultant detection of biomarkers will help elucidate the metabolic pathways of these substances. This in turn will help give credence to Tibetan medicine and its theories.

Declaration of interest

The authors report no declarations of interest.

Acknowledgments

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References