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Research of *Morinda officinalis How's* oligosaccharide extraction and antidepressant effects

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Morinda officinalis How, as a traditional Chinese medicine aiming at nourishing kidney yang, not only can remove wind-dampness effects and strengthen muscles and bones, but, according to modern research, has also antidepression effect, enhances immunity and other functions. These functions are related to its complex chemical composition. *Morinda officinalis How*'s polysaccharide has been widely used in food, health care products and other fields. At present, in the field of medicine, oligosaccharides from *Morinda officinalis How* gain more attention. A number of experiments show that *Morinda officinalis How*'s oligosaccharides have a good antidepressant effect with less toxic side effects compared with Western medicines. However, the physical and chemical properties of oligosaccharide monomers are similar, so oligosaccharide monomers are hard to be separated and detected. This paper organized literature related to the chemical composition and pharmacological effects of *Morinda officinalis How* at home and abroad, and summarized the extraction method and antidepressant effect of *Morinda officinalis How*'s oligosaccharides. In addition, it provided scientific basis for further development of *Morinda officinalis How* medicine, and tried to improve the utilization of *Morinda officinalis How*.

Keywords: *Morinda officinalis How*, Oligosaccharides, Antidepressant effect, Extraction

INTRODUCTION

Morinda officinalis How, as dry roots of the *rubiaceae* family, is classified as a top grade in *Shennong's Classic of Materia Medica.* Together with bitter cardamon, fructus amomi and betel nut, they are known as *four southern medicines. Morinda officinalis How* is effective on nourishing kidney yang, removing wind-dampness effects and strengthening muscles and bones, so it is clinically used to treat impotence and emission, uterine cold with infertility, irregular menstruation, cold pain of the lower abdomen, arthralgia due to wind and dampness and limp wilting sinews and bones [1]. Research in recent years has found that *Morinda officinalis How* contains 11 compounds and 24 inorganic elements such as iridoids, inorganic acids, saccharides and anthraquinones [2]. *Morinda officinalis How* is clinically used to treat anti-depression, regulate immune and thyroid functions, enhance memory, and fight with tumor and other diseases [3,4]. The physical and chemical properties of oligosaccharide monomers are similar, so oligosaccharide monomers are hard to be separated and detected.

Morinda officinalis How has many saccharides which have similar chemical properties, so there are some difficulties in the processes of extraction, separation and examination. Optimizing the extraction method of chemical constituents of *Morinda officinalis* and exploring its biological activity are the hotspots of the present research.

EXPERIMENTAL

Studies on chemical compounds of Morinda officinalis How

Anthraquinone compounds. The anthraquinone compounds are effective ingredients showing the bioactivity of *Morinda officinalis How*. According to reports from home and abroad, the number of anthraquinone compounds which are extracted from the *Morinda officinalis How* is 34, with 7 groups of isomers [5]. Among them, 14 anthraquinone compounds are isolated from the root of *Morinda officinalis How* and their chemical structure is identified (Table 1).

Iridoids. The iridoids are widely distributed in *Morinda officinalis How* and have multiple biological activities [12]. 7 iridoids have been extracted and identified from *Morinda officinalis How* [5] (Table 1).The main features of iridoids in *Morinda officinalis How* are that in C_1 positions are mostly active –OH groups (occasionally keto appear), and iridoids mostly combine with D-glucose to form glycosides [13].

Saccharides. Saccharides account for a large part of the proportion of active ingredients in *Morinda officinalis How*. Shen [14] measured the content of several important components in *Morinda officinalis How*. The total content of water-soluble saccharides

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is 69.12%. therefore, water-soluble saccharides are the most active ingredients of *Morinda officinalis How* and have certain research and development value. Saccharides in *Morinda officinalis How* include monosaccharides, polysaccharides and oligosaccharides, the latter being also called inulin-type oligosaccharides.

Cui *et al.* [15] extracted four water-soluble oligosaccharide monomers from the roots of *Morinda officinalis How*. Feng *et al.* [16], using spectroscopy and chromatography with activated carbon and silica gel, isolated and identified 6 oligosaccharides from *Morinda officinalis How*. Three of the compounds were isolated from *Morinda officinalis How* for the first time.

According to *Chinese Pharmacopoeia* 2015, *Morinda officinalis How* should be calculated on the anhydrous substance, and nystose (C24H42O21) should not be less than 2.0%. Today, research at home and abroad pays lots of attention to the anti-depressive effect of oligosaccharides in *Morinda officinalis How*. How to optimize the extraction and isolation method of *Morinda officinalis How* oligosaccharides and find a high- efficient, rapid and accurate method for structural analysis is of significant interest to research.

Organic acids. Zhou *et al.* [17] isolated palmitic acid, while Cui *et al.* [15] isolated succinic acid from *Morinda officinalis How*.

Amino acids. Li *et al.* [18] isolated 11 kinds of free amino acids and 17 hydrolytic amino acids from the root of *Morinda officinalis How*, 7 of which are essential amino acids (Table 1).

Inorganic elements. Morinda officinalis How contains a vast number of inorganic elements, 11 of which are essential trace elements for humans [2]. It has been suggested that the biological activity of *Morinda officinalis How* may be the result of its chemical composition and content of inorganic elements, but this viewpoint needs to be proved.

It is well to be reminded that due to different origin, texture and processing methods of *Morinda officinalis How*, there are some variations in the chemical composition and element content of *Morinda officinalis How*.

Extraction and separation process of Morinda officinalis How's oligosaccharides

The chemical composition and pharmacological effects of *Morinda officinalis How* have been well studied, but there are few reports on the extraction and separation of active ingredients from *Morinda officinalis How*, especially for the *Morinda officinalis How*'s oligosaccharides. Natural oligosaccharides are generally extracted by traditional methods such as ethyl alcohol reflux. However, there are many uncertain factors like high extraction temperature, long time and solvent, which result in reducing the extract amount and affect clinical efficacy [19]. In addition, biochemical technology and enzyme reaction can also be used with starch and disaccharides (such as sucrose, etc.) to synthesize oligosaccharides. The isolation and purification of oligosaccharides are key steps in the study of oligosaccharide monomers, and silica gel column chromatography, ion exchange chromatography and other methods are commonly applied. This paper summarizes the various methods of extraction and isolation of oligosaccharides from *Morinda officinalis How*, and lays the foundation for finding a new effective, efficient and fast extraction method.

Extraction

Organic solvent methods. Oligosaccharides are often formed by 2-10 monosaccharides connected with glycosidic linkages. They are polyhydroxy, with a certain degree of hydrophilicity. According to the similarity-intermiscibility theory, many polar organic solvents can be used (such as ethanol, methanol, acetone, etc.) for extraction. Ethanol is most recommended, and oligosaccharides are generally insoluble in more than 90% ethanol.

Zhou *et al.* [20] chose 95% ethanol solution as the extracting solution, 8:1 liquid ratio, and extracted 3 times by reversed flow under heating, for 30 min each time. They got cinnamon oligosaccharides with 90.29% extraction rate, which was significantly higher than that of water extraction. Xin *et al.* [21] chose ethanol-methanol and ethanol to observe their effect on the extraction rate, and determined the optimum extraction conditions. The extraction rate of oligosaccharides was up to 3.6% by the phenol-sulfuric acid method.

Oligosaccharide extraction method mostly used in experiments was water solution and alcohol sedimentation method. 80% ethanol extracted polysaccharide precipitate in order to reduce the interference and be suitable to further purifying.

Ultrasonic extraction method. Ultrasonic extraction method mainly uses ultrasonic cavitation to destroy cell membranes, which contributes to the dissolution and release of active ingredients, reduces the extraction time, allowing to extract the maximum amount of ingredients in the shortest time. Ultrasonic extraction method has the advantages of high extraction speed, short time, high yield, no heating, etc. It has become an important means of many Chinese medicine composition analysis processes.

Microwave extraction method. Microwave extraction is a new technology that uses microwave to improve the extraction rate. Microwave-assisted extraction studies [22] have show that microwave irradiation-induced extraction has the characteristics of high selectivity, short operating time, low solvent consumption and high yield of active ingredients. In

the process of extraction, the medicine is nor agglomerated or gelatinized, which overcomes the characteristics of hot water extraction. Wang *et al.* [23] not only significantly increased the soybean oligosaccharide content, but made it easier to desalt through 500W microwave.

Biological methods

Traditional Chinese medicine enzymatic extraction is the main method of extracting the active ingredients in Chinese herbal medicine by biotechnology. Enzyme reactions with high specialization and other features are used to destroy the cell wall structure, so that the active ingredients can be fully exposed, which leads to improved extraction rate of the active ingredients. Shao [24] used *paenibacillus* WL strains with agarase to ferment and to find the optimum technology for oligosaccharides through agar enzyme degrading agar.

Isolation and purification

Absorbent activated carbon isolation method. Activated carbon is a non-polar adsorbent, and has a strong affinity for non-polar components. It is mainly used to separate water-soluble ingredients, such as glycosides, saccharides and amino acids in Chinese medicine. It is suitable for industrial large-scale preparation and separation because of its easy preparation and cheap price. Che *et al.* [25] used this method to separate *Crossostephium chinense* oligosaccharides and got oligosaccharides with different molecular weights.

Gel column chromatography. Gel chromatography is a separation and analysis technique developed in the 1960s with molecular sieve properties for the separation and purification of proteins and saccharides. Gels that are commonly used are Sephadex G and Sephadex LH-20. Harry *et al.* [26] got a kind of xylooligosaccharide with arabinosyl by Bio-Gel P-2 gel separation.

High performance liquid chromatography (HPLC). HPLC is a new type of rapid separation and analysis method developed on the basis of classical conventional chromatography. The principles include distribution chromatography, ion exchange chromatography and other methods. Nowadays the combination of HPLC-MS is commonly applied to qualitatively and quantitatively analyze high-purity samples.

Wang *et al.* [27] analyzed monosaccharides and oligosaccharides qualitatively and quantitatively with HPLC-MS in tea, such as rhamnose, arabinose, mannose, raffinose and others. The results show that rhamnose, xylose, arabinose and mannose were not detected in all tea samples.

PROGRESS IN PHARMACOLOGICAL EFFECTS OF *MORINDA OFFICINALIS HOW'S* OLIGOSACCHARIDES

Morinda officinalis How is a top grade in *Shennong's Classic of Materia Medica,* and a preferred medicine for tonifying the kidney Yang. There is an archaism, *Morinda officinalis How is a medicine for blood tier on kidney meridian.* As people enjoy improved living conditions with faster and faster pace of life, people's mental and psychological pressure are also virtually increased, and mental illness-patients tend to be younger than before. Domestic survey data show [28] that the suicide rate of depression patients is about 20 times higher than that of the average population. By 2020, depression is predicted to be the second major disability factor only to cancer. The commonly current clinical medicines for depression are TCAs, SNRIs, SSRIs and others. However, these drugs are only effective for a part of the depression patients, and may bring about delay in efficacy, adverse reactions and other issues [29]. Therefore, there is a great demand for the development of antidepressant drugs that have faster and effective features. In recent years, it has been found that oligosaccharides in *Morinda officinalis How* have an obvious therapeutic effect as antidepressants with little side effects, which is worth to be researched. This point of view provides foundation for the development of new antidepressant drug based on *Morinda officinalis How*'s oligosaccharides.

Antidepressant effect of oligosaccharides in Morinda officinalis How

Cui *et al.* [15] were the first to extract oligosaccharides from *Morinda officinalis How,* and gave the initial determination of oligosaccharides in *Morinda officinalis How* with antidepressant effect, which opened the door to the new world for scholars to study antidepressants.

Cai *et al.* [30] reported that oligosaccharides isolated from *Morinda officinalis How* could significantly shorten the immobility time of mouse tail suspension test without affecting the spontaneous activity of mice, demonstrating that oligosaccharides had certain antidepressant effect.

In Xu *et al.* [31] experiment, the depression male SD rats model was induced by chronic unpredictable stress methods. The behavioral changes of rats were observed by sugar-water preference test and forced swimming test with *Morinda officinalis How*'s oligosaccharides and fluoxetine as the reference substance. It was found that *Morinda officinalis How*'s oligosaccharides could increase the preference of rats for sugar and significantly reduce the time of forced swimming, indicating that oligosaccharides in *Morinda officinalis How* had antidepressant effect.

Zhang *et al.* [32] used rat lDRL 72s method and rats and mice forced swimming method [33,34]. The antidepressant effect of the alcohol extract of *Morinda officinalis How* was tested with ip desipramine as control. The results showed that the alcohol extract of *Morinda officinalis How* could significantly shorten the immobility time of the rats and mice, and greatly increased the number of rats intensified, with a dose-dependent manner. However, the alcohol extract did not affect the number of reaction rates and the effect ratio, indicating that *Morinda officinalis How*'s alcohol extract also had antidepressant effect.

Antidepressant mechanism of Morinda officinalis How's oligosaccharides

The main active ingredients in *Morinda officinalis How* are oligosaccharides, polysaccharides, DFM and so on. The antidepressant mechanism may consist in reduction of the oxidative damage of the brain tissue and neuronal damage in the hippocampus, increase in the expression of BD-NF, direct increase in the expression of 5-HT neurotransmitter, regulation of the hippocampal neural plasticity, etc. [35].

Cai *et al.* [30] induced mouse head-twitches test and other experiments with 5-HTP to determine the *Morinda officinalis How*'s oligosaccharides pharmacokinetic behavior. The results showed that the number of head twitches was increased in mice, and the contents of 5-HT and its metabolite 5-HIAA in the brain were significantly increased, but the ratio of 5-HT to 5-HIAA did not obviously change, indicating that *Morinda officinalis How* antidepressant effect may be related to increased 5-HT activity, but does not exclude the possibility of other neurotransmitter receptors.

With Western blot, Xu *et al.* [31] found that *Morinda officinalis How*'s oligosaccharides could significantly improve the expression of BDNF, GSK-3β and synapse proteins of hippocampus in depression male SD rat models, suggesting that the antidepressant effect of *Morinda officinalis How*'s oligosaccharides may be related to the regulation of key nodes in the neurotrophic pathway.

Li *et al*. [36] did further research with the RT-PCR method. In their study, *Morinda officinalis How*'s oligosaccharides (100 mg/kg) and desipramine (10 mg/kg) were chronically administrated in 21 d, which resulted in increasing expression of BDNF and BDNF mRNA in rat NGF. It demonstrates that the mechanism of *Morinda officinalis How*'s oligosaccharides in the treatment of depression may be related to the protection of corticosterone- induced injured neurons.

The current research shows that the mechanism of the antidepressant effect of *Morinda officinalis How*'s oligosaccharides is still not clear. It is just preliminarily proved, and needs further study to be confirmed.

Clinical applications of antidepressant effect of Morinda officinalis How's oligosaccharides

Liu *et al.* [37] observed 42 cases of kidney deficiency in depression patients, and treated them with *Morinda officinalis How*'s oligosaccharide capsules. They found that *Morinda officinalis How*'s oligosaccharide capsules were effective on mild to moderate depression.

The traditional Chinese medicine kidney deficiency scale [38] showed that the effectiveness of *Morinda officinalis How*'s oligosaccharide capsules was higher than that of the placebo control group. Within the therapeutic dose, comparing with fluoxetine hydrochloride, which was the control, the adverse reactions of *Morinda officinalis How*'s oligosaccharide capsules were lighter with better safety. This provides a basis for the promotion of clinical treatment of depression with *Morinda officinalis How*'s oligosaccharide capsules.

The study found that most depression patients had emotional depression, decreased interest in things, memory loss and other symptoms, which belong to "Yang deficiency" in traditional Chinese medicine. Warming kidney-yang can be considered to help with psychological disorders in depression patients, further treat depression [39]. Ren [40] found the effective rate of *Morinda officinalis How*'s oligosaccharide capsules for many symptoms in depression patients with kidney-yang deficiency was 85%, including depression mood, insomnia, fatigue, tinnitus, forgetfulness and slow statement. The results show that *Morinda officinalis How*'s oligosaccharides have a certain therapeutic effect for depression patients with kidney-yang deficiency.

RESULTS AND DISCUSSION

Morinda officinalis How contains complex chemical compounds and has a wide range of biological activities (Table 1), with no-hereditary little side effects. In clinical aspect it is effective and safe, therefore it has great research and development potential in the fields of diet, medical treatment and health care.

In recent years, the proportion of mental illness-patients is larger, with more and more young generation, but the mechanism of mental illness is still not clear. Most clinical medicines do not have notable curative effect but obvious side effects. The present study found that saccharide components accounted for more than half of the active ingredients in *Morinda officinalis How*, and *Morinda officinalis How*'s oligosaccharides had significant antidepressant effect.

Table 1. *Morinda officinalis Ho*w's compound names and pharmacological effects

Morinda officinalis How's compound name	Compound Name	Molecular formula	Pharmacological effects
Anthraquinones	l-Hydroxy-anthraquinone	$C_{14}H_{10}O_3$	Resists mutation and tumor
	1-Hydroxy-2-methylanthraquinone	$C_{15}H_{10}O_3$	
	1,6-Dihydroxy-2,4-dimethoxy-anthraquinone	$C_{16}H_{12}O_6$	
	1,6-Dihydroxy-2-methoxy-anthraquinone	$C_{15}H_{10}O_5$	
	1-Hydroxy-2-methoxy-anthraquinone	$C_{15}H_{10}O_4$	
	Physcion [6]	$C_{16}H_{12}O_5$	
	Rubiadin	$C_{14}H_6O_3$	
	Ruhiadin-1-methylether [7]	$C_{16}H_{12}O_4$	
	2-Hydroxy-3-hydroxymethyl-anthraquinone [8]	$C_{15}H_{10}O_4$	
	Tectoquinone [9]	$C_{15}H_{10}O_2$	
	3-Hydroxy-methoxy-2-methylanthraquinone	$C_{16}H_{12}O_4$	
	3-Hydroxy-1,2-dimethoxy-anthraquinone [10]	$C_{16}H_{12}O_3$	
	1,3,6-Trihydroxy-2-methoxy-anthraquinone	$C_{15}H_{10}O_6$	
	1,4-dihydroxy-2-methoxy-7-methylanthraquinone [11]	$C_{16}H_{13}O_5$	
Iridoids	Monotropein	$C_{16}H_{22}O_{11}$	Neuroprotective, antiinflammatory and analgesic
	Desacetylasperulosic acid		
	Asperuloside	$C_{18}H_{22}O_{11}$	
	Asperulosic acid	$C_{18}H_{24}O_{12}$	
	Asperuloside tetraacetate	$C_{26}H_{31}O_{15}$	
	Morindolide	$C_9H_{12}O_3$	
	Morofficinaloside	$C_{17}H_{26}O_{11}$	
Oligosaccharides	Nystose	$C_{24}H_{42}O_{21}$	A variety of biological activities (antidepressant, anti-stress)
	1F-Fructofuranosyl-nystose	$C_{30}H_{52}O_{26}$	
	Inulin-type hexasaccharide	$C_{36}H_{62}O_{31}$	
	Inulin-type heptasaccharide	$C_{42}H_{72}O_{36}$	
Polysaccharides	MOHP-I, MOHP-II, MOHP-III, MOHP-IV		Immune regulation, anti-osteoporosis
Organic acids	Palmitic acid	$C_{16}H_{32}O_2$	Antibiosis
	Succinic acid	$C_4H_6O_4$	
Amino acids	Isoleucine	$C_6H_{13}NO_2$	Ensures the normal operation of the body
	Methionine	$C_5H_{11}O_2NS$	
	Leucine	$C_6H_{13}NO_2$	
	Phenylalanine	$C_9H_{11}NO_2$	
	Valine	$C_5H_{11}NO_2$	
	Lysine	$C_6H_{14}N_2O_2$	
	Cystine	$C_6H_{12}N_2O_4S_2$	
Micro elements	Fe, Mn, Cu, Zn, Cr, Sn, Ni, Mo, Co, V, Sr		Tonify the kidney Yang, bone-invigorating

Therefore, the research and development of *Morinda officinalis How*'s oligosaccharides have a certain value for the treatment of modern depression. However, the commonly current extraction method of *Morinda officinalis How*'s oligosaccharides is water solution and alcohol sedimentation. The technology for isolation and determination of *Morinda officinalis How*'s oligosaccharides monomer is still not perfect. Besides, the understanding of *Morinda officinalis How*'s oligosaccharides is limited, and needs to be studied and improved at a higher level.

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