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 yin, not only can remove wind-dampness effects and strengthen muscles and bones, but, according to modern research, has also antidepressant 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 yin, 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 C₁ 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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Morinda officinalis How and separation of active ingredients from it have been well studied, but there are few reports on the extraction and isolation of its 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 phenolsulfuric 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
column 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
Shenong’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 IDRL 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 BDNF, 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’s compound names and pharmacological effects

<table>
<thead>
<tr>
<th>Morinda officinalis Ho’s compound name</th>
<th>Compound Name</th>
<th>Molecular formula</th>
<th>Pharmacological effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthraquinones</td>
<td>1-Hydroxy-anthraquinone</td>
<td>C_{14}H_{10}O_{3}</td>
<td>Resist mutation and tumor</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>1-Hydroxy-2-methylantraquinone</td>
<td>C_{15}H_{10}O_{3}</td>
<td></td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>1,6-Dihydroxy-2,4-dimethoxy-anthraquinone</td>
<td>C_{16}H_{12}O_{4}</td>
<td></td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>1,6-Dihydroxy-2-methoxy-anthraquinone</td>
<td>C_{15}H_{10}O_{4}</td>
<td></td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Physcion [6]</td>
<td>C_{16}H_{12}O_{4}</td>
<td></td>
</tr>
<tr>
<td>Rubiadin</td>
<td>C_{14}H_{6}O_{3}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Ruhadin-1-methylether [7]</td>
<td>C_{16}H_{12}O_{4}</td>
<td></td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>2-Hydroxy-3-hydroxymethyl-anthraquinone [8]</td>
<td>C_{15}H_{10}O_{4}</td>
<td></td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Tectoquinone [9]</td>
<td>C_{16}H_{12}O_{4}</td>
<td></td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>3-Hydroxy-methoxy-2-methylantraquinone</td>
<td>C_{16}H_{12}O_{4}</td>
<td></td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>3-Hydroxy-1,2-dimethoxy-anthraquinone [10]</td>
<td>C_{16}H_{12}O_{4}</td>
<td></td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>1,3,6-Trihydroxy-2-methoxy-anthraquinone</td>
<td>C_{16}H_{12}O_{4}</td>
<td></td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>1,4-dihydroxy-2-methoxy-7-methylantraquinone [11]</td>
<td>C_{16}H_{13}O_{3}</td>
<td></td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Monotropein</td>
<td>C_{16}H_{22}O_{11}</td>
<td>Neuroprotective, antiinflammatory and analgesic</td>
</tr>
<tr>
<td>Iridoids</td>
<td>Desacetylasperulosic acid</td>
<td>C_{16}H_{22}O_{11}</td>
<td></td>
</tr>
<tr>
<td>Iridoids</td>
<td>Asperuloside</td>
<td>C_{16}H_{22}O_{12}</td>
<td>A variety of biological activities (antidepressant, anti-stress)</td>
</tr>
<tr>
<td>Iridoids</td>
<td>Asperuloside tetraacetate</td>
<td>C_{26}H_{31}O_{15}</td>
<td></td>
</tr>
<tr>
<td>Iridoids</td>
<td>Morindolide</td>
<td>C_{8}H_{12}O_{3}</td>
<td></td>
</tr>
<tr>
<td>Iridoids</td>
<td>Morofficinaloside</td>
<td>C_{17}H_{26}O_{11}</td>
<td></td>
</tr>
<tr>
<td>Iridoids</td>
<td>Nystose</td>
<td>C_{24}H_{42}O_{21}</td>
<td></td>
</tr>
<tr>
<td>Oligosaccharides</td>
<td>1F-Fructofuranosyl-nystose</td>
<td>C_{30}H_{52}O_{26}</td>
<td></td>
</tr>
<tr>
<td>Oligosaccharides</td>
<td>Inulin-type hexasaccharide</td>
<td>C_{36}H_{62}O_{31}</td>
<td></td>
</tr>
<tr>
<td>Oligosaccharides</td>
<td>Inulin-type heptasaccharide</td>
<td>C_{42}H_{72}O_{36}</td>
<td></td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>MOHP-I, MOHP-II, MOHP-III, MOHP-IV</td>
<td>Immune regulation, anti-osteoporosis</td>
<td></td>
</tr>
<tr>
<td>Organic acids</td>
<td>Palmitic acid</td>
<td>C_{16}H_{32}O_{2}</td>
<td>Antibiosis</td>
</tr>
<tr>
<td>Organic acids</td>
<td>Succinic acid</td>
<td>C_{4}H_{6}O_{4}</td>
<td></td>
</tr>
<tr>
<td>Organic acids</td>
<td>Isoleucine</td>
<td>C_{6}H_{13}NO_{2}</td>
<td></td>
</tr>
<tr>
<td>Organic acids</td>
<td>Methionine</td>
<td>C_{6}H_{14}O_{2}NS</td>
<td></td>
</tr>
<tr>
<td>Organic acids</td>
<td>Leucine</td>
<td>C_{6}H_{13}NO_{2}</td>
<td></td>
</tr>
<tr>
<td>Amino acids</td>
<td>Phenylalanine</td>
<td>C_{9}H_{11}NO_{2}</td>
<td>Ensures the normal operation of the body</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Valine</td>
<td>C_{9}H_{11}NO_{2}</td>
<td></td>
</tr>
<tr>
<td>Amino acids</td>
<td>Lysine</td>
<td>C_{6}H_{14}N_{2}O_{2}</td>
<td></td>
</tr>
<tr>
<td>Amino acids</td>
<td>Cystine</td>
<td>C_{6}H_{12}N_{2}O_{4}S_{2}</td>
<td></td>
</tr>
<tr>
<td>Micro elements</td>
<td>Fe, Mn, Cu, Zn, Cr, Sn, Ni, Mo, Co, V, Sr</td>
<td>Tonify the kidney Yang, bone-invigorating</td>
<td></td>
</tr>
</tbody>
</table>

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