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Review Article

Modern Approaches to the Analysis of Kelp (*Laminaria* sp.) as Pharmacopoeial Herbal Drugs and Food Products

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ABSTRACT

Background: Currently, the chemical composition of *Laminaria* J.V. Lamour. species is well studied; they have found applications in the food, cosmeceutical and pharmaceutical industries. The main groups of biologically active compounds are polysaccharides (alginic acid, laminarin, mannitol, fucoidan, and others) and minerals (iodine compounds, magnesium, potassium, calcium, iron) that are determined according to pharmacopoeial and All-Union State Standards requirements. **Materials and Methods:** For data obtaining various types of search tools and engines such as Google, Google scholar, scientific literature (including Russian sources), normative documentation of Russian Federation (State Pharmacopoeia of Russian Federation IV edition, All-Union State Standards, and others) electronic databases such as e-Library, Scopus, Web of Science, Pubmed were used. **Results:** In the course of this review study, a modern characteristic of the kelp thallus as a pharmaceutical, cosmeceutical, food substance of plant origin is presented. The data on the chemical composition, harvesting, and processing of raw materials are summarized. The standardization and safety issues of kelp thallus are considered taking into account modern pharmacopoeial and food international requirements. The approaches to the qualitative and quantitative analysis of biologically active compounds (polysaccharides, iodine) and the determination of safety indicators are studied. **Conclusions:** The regulatory documentation that is used in the quality control of kelp needs to be finalized and updated. For pharmacopoeial analysis, all possible physicochemical methods (gravimetric, titrimetric, spectrophotometric) should be presented in the newly approved monograph. In this case, modern procedures should be developed, including HPLC with various types of detection (determination of the carbohydrates profile and polysaccharides, including methods with acid and enzyme hydrolysis). This will ensure the required level of quality, the safety of kelp (Laminaria) raw materials.

Key words: Kelp, *Laminaria japonica*, *Laminaria saccharina*, Alginic acid, Laminarin, Mannitol, Fucoidan, Iodine.

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INTRODUCTION

Laminaria J.V. Lamour. species are well-known brown algae. They are widely used in the food, cosmeceutical and pharmaceutical industries. The name of the *Laminaria* plants genus comes from the Latin word "*lamina*", which means "plate" and indicates the lamellar form of the algae thallus. Laminaria (seaweed) belongs to the class of brown algae (lat. *Phaeophyceae*), the *Laminariaceae* family. There are 30 species of kelp, three of which grow in the seas of the southern hemisphere, and the rest – in the seas of the northern hemisphere. The most of kelp species grow in the Pacific Ocean. Japanese kelp (*Laminaria japonica* Aresch.) and saccharine kelp (*Laminaria saccharina* (L.) J.V.Lamour.) are producing plants of kelp thallus according to the pharmacopoeial monograph (PM) of State Pharmacopoeia of the Russian Federation XIV edition (SPRF XIV) "*Laminariae thalli*".[1]

The following drugs based on Laminaria are listed on the State Register of Medicines of the Russian Federation: thallus kelp (from various Russian manufacturing companies), "Mamoclam ®" for mastopathy treatment (film-coated tablets with kelp extract), general tonic agent "Limanovit®" E (alcohol drops for oral administration with Vitamin E and Laminaria thallus extract), keratoprotective agent "Okovidit®" (gel for external use with Laminaria thallus extract).[2] Kelp thallus has vitamin, tonic (especially in the elderly and weakened people), anti-sclerotic, mild laxative effect, regulates the activity of the gastrointestinal tract and normalizes metabolism.[3]

Dried kelp thallus of brown seaweed (Japanese and saccharine kelp) collected from June to October is used as crude herbal drugs (medicinal plant raw materials, MPRM).[1] Kelp is widely cultivated in Japan, Korea, China and the Far East of Russia as a valuable food and medicinal plant. Kelp thallus is used as a mild laxative for chronic atonic constipation (including in patients with hyperlipidemia). Laminaria is prescribed as an additional remedy for atherosclerosis, for the treatment and prevention of endemic goiter, hyperthyroidism, mild forms of Graves' disease, chronic and acute enterocolitis,

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proctitis. The issues of standardization of kelp CHD are debatable. In this regard, this review was prepared. It defines the main criteria for standardization, indicates the shortcomings of existing methods and presents further prospects.

MATERIALS AND METHODS

For data obtaining various types of search tools and engines such as Google, Google scholar, scientific literature (including Russian sources), normative documentation (ND) of Russian Federation (State Pharmacopoeia of Russian Federation IV edition and others) electronic databases such as e-Library, Scopus, Web of Science, Pubmed were used.

RESULTS AND DISCUSSION

Botanical characteristic

Species *Laminariaceae* family are algae with a ribbon-like thallus from 1 to 12 m long and 10-35 cm wide. The thallus (thallome) near the base tapers into a trunk, which at the bottom branches into rhizoids – root-shaped formations; rhizoids help the algae attaching to stony soil. The kelp plate is linear, mucous, soft, with wavy edges, greenish-brown. Every year, in the late autumn, it dies, and in the winter it grows again. All algae are penetrated by mucous passages and gaps. Laminaria propagates by moving zoospores, which are formed in sporangia on the surface of the plate. The kelp life expectancy is from 2 to 4 years, depending on climatic conditions. Japanese kelp grows along the southern shores of the Sea of Japan and the Sea of Okhotsk, in the Pacific Ocean along the shores of the southern Kuril Islands and Sakhalin. Saccharine kelp forms significant thickets along the shores of the White, Barents and Kara Seas, in the Arctic Ocean. Laminaria forms thickets on stones and rocks in the coastal zones of the seas and oceans at a depth of 2 to 25 meters in places with the constant movement of water. Laminaria reserves fluctuate depending on climatic factors in the coastal zone (from several tens of thousands to hundreds of thousands of tons). Saccharine kelp is found in massive quantities, forms large underwater meadows in the coastal zone at depth. Laminaria is cultivated on underwater plantations in China and Japan.[4,5]

Kelp CHD harvesting

Laminaria thallus (*Laminariae thalli*) used in medicine. For the most part, a two-year thallus is prepared, since it is larger in size, accumulates a maximum of biologically active compounds (BAC) and contains less water. Algae is collected at a depth of 5-6 m, catching it with the help of special poles with a fork-shaped spring at the end, on which the thallus is wound. At the same time, the pole is lowered into the water, the algae is rotated and then pulled out. Less commonly, thalli are cut from the bottom with special braids. In addition, a fresh thallus is also harvested, which is carried ashore by the tides. Only large, biennial thalli are collected. To ensure the renewal of kelp, the thickets are operated every three years. The collected CHD are washed from sand and silt, cleaned from impurities of marine plants, shells and other contaminants, dried in the sun, laying a thin layer on a cloth, tarpaulin or cardboard.[4,5]

External and microscopic signs

Whole CHD are thalli of Japanese kelp – dense, leathery, ribbon-like plates folded in length, without trunks or pieces of plates with a length of at least 15 cm and a width of at least 7 cm (Figure 1A).

Plate thickness should be not less than 0.03 cm; the edges of the plates are solid, wavy. Saccharine kelp thallus is dense, leathery, wrinkled leaf-shaped plates without trunks or pieces of them at least 10 cm long and at least 5 cm wide. Plate thickness should be not less than 0.03 cm. The edges of the plates are wavy. The presence of plates with tears along the edges and the middle is allowed. The color of the whole thallus is from light green to dark green; tan, red tan, sometimes tan, light brown, tan; outside the thallus is covered with a white coating of salts. Shredded CHD are strips of thallus with a width of 0.2-0.4 cm, a thickness of at least 0.03 cm. Color from light green to dark green; greenish-brown, light brown, red-brown, sometimes tawny, light brown, greenish-black; outside the stripes of thallus are covered with a white coating of salts. The crushed kelp CHD are pieces of thallus of various shapes passing through a sieve with 3 mm holes (Figure 1B). Color from light green to dark green; tan, light brown, red-brown, sometimes tan, greenish-black; outside, thallus slices are covered with a white coating of salts. All types of CHD have a characteristic odor and salty taste.

The following anatomical and diagnostic microscopic features are characteristic for the whole, shredded and crushed CHD. The epidermis should be visible when examining thallus from the surface. The epidermis of kelp thallus consists of small, almost square cells with thick walls, through which numerous roundish mucous receptacles are visible (Figure 2).[1]

Nutritional and energy value of Laminaria

On a par with, 100 g of the dry Laminaria kelp includes 17.1-32.0 g of alginic acid, 8.46-28.48 g of mannitol, 5.97-18.99 g of crude protein, and 19.35-45.29 g total ash, including 0.13-0.69 g iodine and 4.35-12.65 g of potassium, energy value is 262 kcal. Laminaria kelp stores not only large quantities of the various micro- and macronutrients but also many significant vitamins.[4]

Laminaria biologically active compounds

The main active compound in kelp is the polysaccharide – alginic acid, which is a linear polymer consisting of residues of the linked β-(1→4)-glycosidic bonds of D-mannuronic and α-(1→4)-glycosidic bonds of L-guluronic acids (molecular weight 200 kDa). The content of L-guluronic acid in the molecule is 30-60%. The ratio between mannuronic and guluronic acids in the Norwegian algae *Laminaria*

Figure 1: Kelp thallus: whole CHD (1), crushed CHD (2).

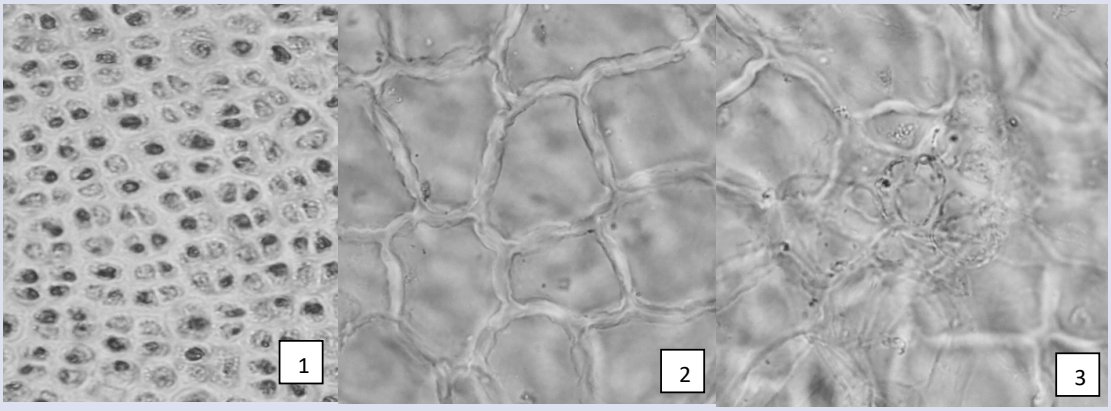

Figure 2: Laminaria (seaweed, kelp) thallus. 1 – fragment of the epidermis (400 ×); 2 – parenchyma cells (200 ×); 3 – receptacle with mucus in the parenchyma (200 ×).

digitata (Hunds.) Lamour is 3.1: 1, in *Laminaria cloustoni* Edm. (*Laminaria hyperborea*) – 1.6: 1. Alginic acid is a heterogeneous substance, the ratio between mannuronic and guluronic acids in its various fractions ranges from 3: 1 to 1: 1. Alginic acid molecules contain fragments of alternately bonded mannuronic and guluronic acids and blocks containing only mannuronic and only guluronic acids. The latter components are relatively resistant to hydrolytic influences, which allows enriching the alginic acid fraction with L-guluronide by combining hydrolysis and fractionation. As a result of such enrichment, a product is formed that has a pronounced ability to selectively bind divalent ions as a polyelectrolyte. [4,6,7] Alginic acid is an intercellular substance and one of the components of the cell walls of algae. Polymannuronic acid usually passes into the solution during extraction, while polyguluronic acid remains in the cell walls and is masked by cellulose. Alginic acid is contained in algae in the form of salts – alginates in an amount up to 30% of dry weight. It is slightly soluble in water, forms a viscous colloidal solution. Alginic acid can absorb 200-300 times the amount of water (by weight), which leads to the widespread use of alginates in the industry. The content of alginic acid in kelp thallus is experiencing seasonal fluctuations.[4]

Laminaria contains up to 21% of the laminarin polysaccharide (laminaran), consisting of β-D-glucopyranose residues with ligaments 1→3 (less often – 1→6) in linear chains and 1→6 in branched chains. Laminaribiosis disaccharide is formed by incomplete hydrolysis of laminarin. [8,9-11] The residues of D-mannitol polyhydric alcohol can be attached to a part of laminarin molecules by β-(1→1)-bonds. [12] The concentration of mannitol in the thallus of kelp ranges from 15-21% (on a dry basis) in summer to 3-4% in winter.[13] Brown algae are considered to be a laminarin reserve nutrient. Two forms of laminarin are known which differ in molecular weight and solubility in water.[4] Other polysaccharides are found in kelp besides alginic acid and laminarin. *L. saccharina* contains cellulose (5.7%); sulfated polysaccharide fucoidan is found. Fucoidan is a sulfated polysaccharide of plant origin, containing L-fucose as the main component, as well as galactose, mannose, xylose, glucuronic acid in small quantities. Fucoidans have an extremely wide spectrum of biological effects on organisms. However, the structural heterogeneity of this class of polysaccharides still does not completely establish the relationship of their structure with activity. Fucoidans, the non-toxic[14] sulfated polysaccharides extracted from brown algae, have been extensively studied for their diverse biological activities (hypolipidemic effect[15], antioxidant[16], antitumor[17] activities).

A variety of sterols have been identified in the saccharine kelp. Their content is 0.2%. Fucosterol predominates in the composition of sterols (87%), 24-methylene cholesterol (11%), cholesterol (0.05%), 24-ketocholesterol (0.05%), saringosterol (1.8%) are also present. Oxylypines – monohydroxy unsaturated fatty acids and 13(S)-hydroxy-6(Z),9(Z),11(E),15(Z)-octadecatetraenoic acid, were found in various types of kelp, including sugar kelp. This indicates the presence of active lipoxygenases with ω-6 specificity in algae.[4] In addition to these compounds, the kelp thallus contains a significant amount of L-fructose (up to 2%), proteins (up to 9%), vitamins C (up to 111 mg%), B12 (0.04-0.05 μg/g in dry matter), B1, B2, D, carotene, violaxanthin, as well as brown pigments – fucoxanthin[18], neoxanthin, neofucoxanthin, etc. that mask chlorophyll. Brown algae contain, in addition to chlorophyll A, chlorofucin (chlorophyll C, or γ-chlorophyll).[19,20] Phlorotannins found in Laminaria are oligomers of phloroglucinol (polyphloroglucinols) that have anti-proliferative activity, anti-hyperlipidaemic and anti-hyperglycemic effects.[21,22] *L. japonica* contains essential oil (EO), characterized by the GC-MS method, usually applied for EO analysis.[23] EO main components are tetradeconoic, hexadecanoic, (9Z,12Z)-9,12-octadecadienoic, and (9Z)-hexadec-9-enoic acids; EO has antioxidant and antibacterial activities.[24] Traces of fatty oil is found in *L. japonica*; polyunsaturated fatty acids, phospholipids, glycolipids are characterized.[25] A new alkaloid type amino acid – laminin (trimethyl-(5-amino-5-carboxyl)-ammonium dioxalate) was isolated from laminaria *Laminaria japonica* Aresch. Kelp contains minerals: macro- and microelements (manganese, copper, iron, arsenic, cobalt, bromine, boron) This, as a rule, a significant amount of iodine (0.15–0.54%, sometimes up to 1.5%), most of which is in the form of iodides, as well as in the form of iodine compounds, in particular, diiodotyrosine are presented. It was established that the iodine content in kelp from the northern regions is greater than in kelp, which grows south.[26] Structural formulas of Laminaria BAC are presented in Figure 3. A method for producing biologically active substances from Laminaria kelp has been developed by Russian researchers. It includes sequential extraction of crushed kelp thallus to obtain mannitol, a water-soluble polysaccharide complex (12.6%), laminaran, fucoidan and sodium alginate. Thus, several products are produced simultaneously.[27]

Standardization of Laminaria raw materials

According to PM "*Laminariae thalli*" determination of the main groups of biologically active substances is carried out by qualitative reactions for iodine, polysaccharides, reducing sugars. 0.04 g of crushed raw materials (by the section "Quantitative determination. Determination of iodine content") is poured onto a piece of cellophane 2 × 2 cm in size, which is folded in the form of a sachet. It is placed in a fixative and burned in a 300-400 ml flask with oxygen (by the General Pharmacopoeia Monograph "Method of combustion in a flask with oxygen"). 10 ml of 0.5% starch solution containing 0.2% sulfamic acid solution is used as the absorbing liquid, a blue color should be observed (if the iodine

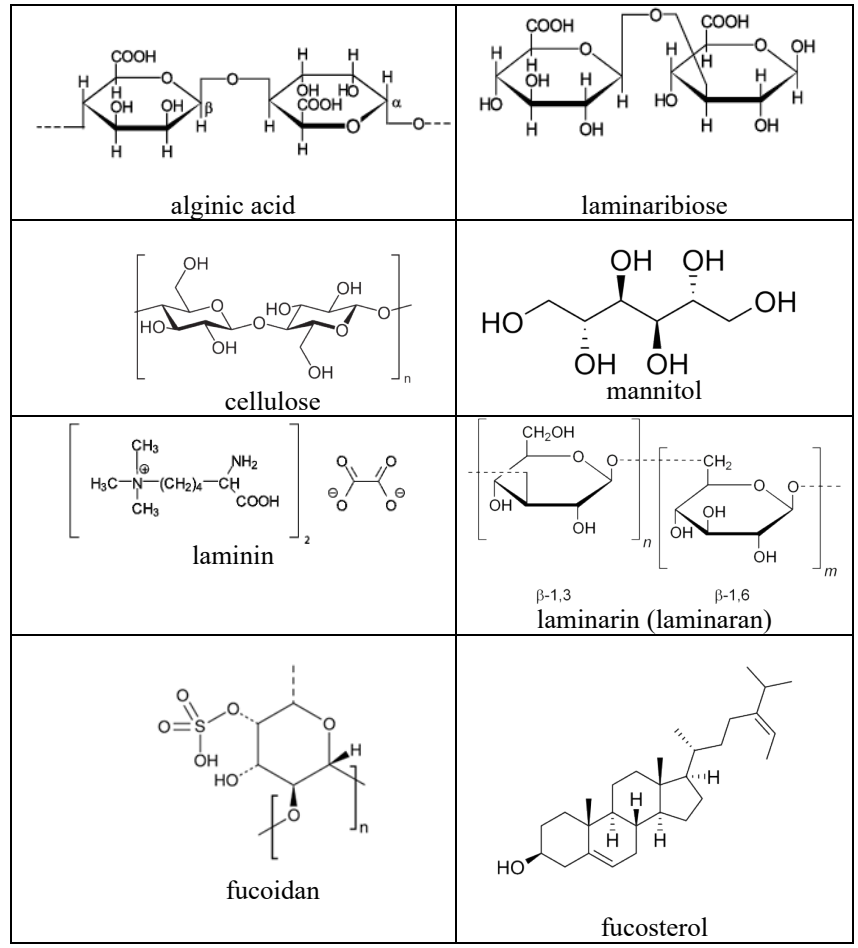

Figure 3: Structural formulas of Laminaria biologically active compounds.

content is at least 0.1%). To 10 ml of solution A (according to the section "Quantitative determination. Determination of the content of polysaccharides") 10 ml of alcohol 96% is added, mixed; flocculent clots should be observed precipitated during standing (polysaccharides). The solution with the precipitate is filtered through a glass filter (pore diameter 5-15 μm), the precipitate on filter is transferred to a test tube, 2 ml of diluted hydrochloric acid is added, heated, then 10 ml of copper tartrate reagent is added and heated again; an orange-red precipitate should be observed (reducing sugars).[1]

The "Tests" section contains indicators for the whole, shredded and crushed raw materials, established "no more than": humidity (15%); common ash (40%); ash, insoluble in hydrochloric acid (20%); foreign matter – thallus with a thickness of less than 0.03 cm (15%), thallus with yellowed edges (10%); organic impurity (not allowed); mineral impurity – shells, pebbles (0.5%); sand (0,2%). The finesse of raw materials should be as follows; shredded raw materials: particles not passing through a sieve with holes 3 mm in size, – not more than 5%; particles passing through a sieve with holes of 0.2 mm in size, – no more than 5%. Safety indicators include heavy metals and arsenic, radionuclides, residual amounts of pesticides, microbiological purity. These indicators are determined according to the General Pharmacopoeia Monographs. The maximum allowable arsenic content is 90 mg/kg.[1]

The quantitation section includes determination of iodine (not less than 0.1%) titrimetrically and polysaccharides (not less than 8%) gravimetrically in whole, shredded and crushed raw materials of Laminaria.

Determination of iodine content. An analytical sample of the raw material is crushed to a particle size passing through a 0.5 mm sieve. About 0.06 g (accurately weighed) of the crushed raw material is placed on a piece of cellophane (20 × 20 mm), which is folded in the form of a bag, placed in a retainer and burned in a 1000 ml flask with oxygen (in accordance with the General Pharmacopoeia Monograph "Method of burning in a flask with oxygen"). The mixture of 2 ml of a 0.1 M sodium hydroxide solution and 50 ml of water is used as the absorbing liquid. After burning, the flask is vigorously shaken for 3 minutes, then the ground joint of a flask, holder and inner walls of the flask are washed with 100 ml of water. The mixture (consisting of 25 ml of 10% potassium acetate solution in glacial acetic acid, 15 drops of bromine) is added dropwise to a slightly yellow color. The flask is left for 2 minutes, 85% formic acid is added dropwise until the solution is discolored, and after 1 minute 3 ml of 3% sulfamic acid is added. The contents of the flask are shaken periodically for 3 minutes, then 1.0 g of potassium iodide is added and the liberated iodine is titrated with a 0.005 M sodium thiosulfate solution (starch is an indicator). The iodine content in absolutely dry raw materials in percent (X) is calculated by the formula:

$$X = \frac{0{,}0001058 \cdot \mathrm{V} \cdot 100 \cdot K \cdot 100}{a \cdot (100 - W)}$$

where 0,0001058 – the amount of iodine corresponding to 1 ml of a 0.005 M sodium thiosulfate solution, g;

V – volume of a 0.005 M sodium thiosulfate solution spent on titration, ml;

K – correction factor to the titer of 0.005 M thiosulfate solution;

a – sample mass of raw materials, g;

W – moisture content in the raw material,%.[1]

Determination of polysaccharide content. An analytical sample of the raw material is crushed to the size of the particles passing through a 3 mm sieve. About 10.0 g (accurately weighed) of the crushed raw material is placed in a 250 ml conical flask with a thin section, 100 ml of water is added and heated under reflux on an electric stove for 30 minutes, maintaining a slight boiling. The extract is decanted into a 500 ml volumetric flask through 5 layers of gauze, inserted into a glass funnel with a diameter of 55 mm and previously moistened with water. Extraction is carried out another 4 times in 100 ml (each time for 30 minutes). The filter is washed with water, the volume of the solution is adjusted to the mark with water and stirred (solution A). 25.0 ml of solution A is placed in a 150 ml centrifuge tube, 25 ml of alcohol 96% is added, stirred, heated in a water bath at 30 ° C for 5 minutes. After 30 minutes, the contents of the tube are centrifuged at 5000 rpm for 30 minutes. The supernatant is filtered through a glass filter (pore diameter 5-15 μm), dried to constant weight, with a diameter of 40 mm under vacuum at a residual pressure of 13-16 kPa. Then the precipitate is quantitatively transferred to the same filter using 10 ml of a 95% water-alcohol mixture (1: 1) and washed with 10 ml of 96% alcohol. The filter with the precipitate is dried in the air, adjusted to constant weight at a temperature of 102 - 105 ° C and weighed. The content of polysaccharides in absolutely dry raw materials in percent (*X*) is calculated by the formula:

$$X = \frac{(m_2 - m_1) \cdot 500 \cdot 100 \cdot 100}{a \cdot 25 \cdot (100 - W)}$$

where m_1 – filter weight, g;

m_2– filter weight with sediment, g;

a – sample mass of raw materials, g;

W – moisture content in the raw material,%.[1]

Determination of sand content. An analytical sample of the raw material is crushed to the size of the particles passing through a 1 mm sieve. About 20.0 g (accurately weighed) of the crushed raw material is placed in a 150 ml beaker, 60 ml of 10% hydrochloric acid solution is added and heated to boiling, stirring continuously until the swelling of the mass ceases. Then the beaker is covered with a watch glass and continued to heat for 15 minutes. At the end of heating, the beaker is poured to the top with water, the contents are vigorously stirred with a glass rod and left for 3 to 5 minutes, after which sand is levigated. To do this, a 5 - 10 ml pipette is connected to a water tap or a large water bottle with a rubber hose and a water flow is set at a speed of 160 - 170 ml/min. Then a beaker with the analyzed mass is substituted under its stream and the tube is immersed into it at a distance of 1-2 cm from the bottom. Water is drained over the edge of the beaker. The duration of the levigating is about 20 minutes, after which the water from the beaker is decanted. To remove the remaining particles of the raw material, the sediment in a beaker is poured into 25-30 ml of a saturated solution of sodium chloride, stirred and, having allowed the sand to settle to the bottom, carefully drain the liquid together with suspended particles of the raw material. Processing with a sodium chloride solution is repeated 3-4 times until the suspended particles are removed completely. Then the precipitate in a beaker is washed 2 to 3 times with water and quantitatively transferred to an ashless filter. The precipitate, together with the filter, is ashed in a weighed crucible, placed in a muffle furnace, calcined for 15 minutes at a temperature of 550 - 650 ° C and weighed after cooling.

The sand content in absolutely dry raw materials in percent (X) is calculated by the formula:

$$X = \frac{(m_2 - m_1) \cdot 100 \cdot 100}{a \cdot (100 - W)}$$

Where m_1 – empty crucible weight, g;

m_2 –crucible weight with ash, g;

a – sample mass of raw materials, g;

W – moisture content of the raw material,%.[1]

Laminaria pharmacopoeial safety control

According to SPRF XIV safety indicators include heavy metals and arsenic, radionuclides, residual amounts of pesticides, microbiological purity. The maximum allowable arsenic content is 90 mg/kg. This indicators are determined according General Pharmacopoeial Monographs.[1]

The "Kelp" monograph of European Pharmacopoeia (EP) regulates the quality control of Fucus algae.[28] The different objects are used due to the growth specifics of brown algae various types. According to SPRF XIV, the requirements for the content of mercury, cadmium, and lead in kelp thallus coincide with the standards set for all herbal medicines (HD): 0.1 mg/kg for mercury, 1.0 mg/kg for cadmium and 6 mg/kg for the lead. At the same time, domestic requirements for the content of the element in kelp thallus coincide with the requirements of the EP for Fucus algae for mercury and arsenic but differ for cadmium (4.0 mg/kg) and lead (5.0 mg/kg). It should be noted that the harmonization of domestic and European standards for the content of arsenic in brown algae was achieved only in the latest version of the SPRF: from 0.5 mg/kg (a single norm for all HD in the SPRF XIII) to 90 mg/kg. It is important to keep in mind that inorganic forms of arsenic are the most toxic; they are hundreds of times more toxic than organic ones.[29] At the same time, approximately half of the arsenic contained in the algae is bioavailable.[30] It was previously believed that in marine biota the proportion of inorganic form of arsenic is extremely small, despite a large amount of total arsenic. In this regard, EP and the SPRF normalize the content of general arsenic only. Meanwhile, studies in recent years have shown that in some algae the content of toxic inorganic arsenic is increased: more than 100 mg/kg in brown algae of Hijiki (*Sargassum fusiforme* (Harv.) Setch.)[31] and up to 23 mg/kg in some samples of Laminaria.[32,33] The developers of the American Pharmacopoeia (USP)[34] normalize the content of exclusively inorganic arsenic, based on the results of studies of the content of various forms of arsenic in marine biota and taking into account their toxicity. The European Food Safety Authority also draws attention to the potential of large amounts of inorganic arsenic to be ingested when algae are consumed as food and as a dietary supplement.[35,36]

To analyze the content of heavy metals and arsenic in all HD, including the kelp thallus, starting from the 13th edition of the SPRF, spectral methods are used instead of the colorimetric analysis method for the selective determination of elements. Atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectroscopy (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) are now used in the pharmacopeial analysis.[1] In the EP, the use of the same analysis methods as in the SPRF is provided. In USP[34], starting from 2014, for the analysis of elemental toxicants, including in HD, the use of only spectral methods with inductively coupled plasma is recommended. Since AAS has several irreparable disadvantages: in the flame, there is insufficient sensitivity and a limited set of detectable elements due to the low ionization temperature, when using a graphite furnace, it has a narrow range of measured concentrations, a limited set of detectable elements and low analysis speed.[37] Special equipment is required to determine arsenic and mercury by the AAS,

as well as mercury by the ICP-AES method. According to the USP, the hydrochloric acid extraction procedure has been used to determine the inorganic arsenic content since the early 80s.[38] But at present, this method has been replaced by a selective method based on the chromatographic separation of various forms of arsenic, followed by ICP-MS analysis.[39-41] For sample preparation, the SPRF recommends using methods of sample ashing in open and closed vessels. Microwave digestion in pressure vessels is provided as a reference method. The sample preparation is the most difficult stage in the analysis of elemental toxicants; it contributes 90% of the time and forms the total error in the results of quantification of elements in the sample. [42] USP and EP excluded open vessel digestion methods due to the possible loss of target elements and the significant influence of researchers' competence on the analysis.[43,44]

Thus, there are a number of issues that need to be adjusted when performing pharmacopoeial safety control.

Determination of quality indicators of Laminaria food products

Russian national standard GOST (All-Union State Standard) 31583-2012 is approved for frozen Laminaria kelp (seaweed) made from algae of the *Laminaria* family and intended for food purposes. 11 *Laminaria* species (*L. bongardiana*, *L. gurjanovae*, *L. longipes*, *L. dentigera*, *L. digitata*, *L. yezoensis*, *L. inclinatorhiza*, *L. appressirhiza*, *L. saccharina*, *L. angustata*, *L. japonica*,) and 3 *Cymathaere* species (*C. japonica*, *C. fibrosa*, *C. triplicate*) can be harvested for production.[45]

According to this ND, organoleptic and physical indicators, chemical indicators, toxic elements, microbiological indicators must be determined, there are links to advising GOSTs. So, chemical indicators are determined according to GOST 26185-84.[46] This ND includes "Analysis methods for seaweeds (raw, frozen and dried) and dried seaweed", namely the determination of the mass fraction of water, ash, sand, total nitrogen, impurities. The method for determining total nitrogen is based on the oxidation of organic matter by burning it in sulfuric acid in the presence of a catalyst (copper(II) sulfate, potassium sulfate), distilling off the ammonia formed and trapping it with a titrated sulfuric acid solution, followed by back-titration of its excess. The mass fraction of nitrogen in the sample is calculated by the amount of ammonia-bound acid. The strength of a dried Laminaria plate is also determined by measuring the force required to tear a plate. The mass fraction of alginic acid is determined by back titration. It is carried out with sulfuric acid of the excess sodium hydroxide remaining after its interaction with alginic acid. The mass fraction of mannitol is determined by photoelectrocolorimetry method; mannitol is extracted with water, a complex compound with copper sulfate is formed, its amount is determined by measuring the optical density. Iodine is determined qualitatively by the interaction of iodine with starch (formation of a complex compound colored in blue). The quantitative determination of iodine (titrometric method) is based on the formation of a colored complex compound of iodine with sodium nitrate in an acidic medium and titrometric determination (KI – titrant). A colorimetric determination of this complex compound is also possible.[46]

CONCLUSION

Confirmation of safety and effectiveness is the basis of modern approach to the development and addition of quality indicators list and their norms for MPRM, pharmaceutical substances of plant origin (PSPO) and herbal drugs (HD), including kelp thallus. The revision of the existing quality standards for PSPO, drugs produced on their basis, taking into account the principle of MPRM-PSPO-HD standardization, remains relevant; modern methods of determination should be the same for every stage of HD production. One of the important parameters in the standardization is the qualitative and quantitative determination of the BAC main groups possessing pharmacological effect of drugs that are made on the basis of this MPRM.[47] Laminaria HD are used, as a rule, as a laxative, as a source of iodine. To confirm the quality of the MPRM, its identity (qualitative reactions, TLC) and the content of BAC are determined. In PM "Laminariae thalli" there are only qualitative reactions in the section "Determination of the main groups of BAC"; it is advisable to include TLC analysis for monosaccharides after hydrolysis in this section.

Modern methods of analysis, such as LC-MC, allow more precise characterization of a number of polysaccharides, for example, laminarine (to determine the molecular weight, chain length). However, in routine pharmacopeia analysis, polysaccharides quantification is more relevant to carry out by acidic hydrolysis and subsequent HPLC measurement of resulting monosaccharide concentrations along with spectrophotometric quantification procedure.[48] PM requires several methods for quality control; each method has its advantages and disadvantages. Titrimetry and gravimetry are those methods that can be carried out in any laboratory. Nevertheless, the laboratory base must be updated to meet the current level of technological development. This allows to ensure and maintain the current level of safety and quality of HD based on kelp thallus (Table 1).

The principle of MPRM-PSPO-HD standardization should be taken into account when developing approaches to assessing the content of BAC in the *Laminaria* MPRM. The analysis should be carried out in PSPO and in a HD based on it for the same main groups of BAC using the same methods.

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CONFLICTS OF INTEREST

None.

Table 1: Standardization issues of kelp thallus for SPRF further development.

Index	Quality tests		Quantification	
	Current tests according to monograph	Additional tests	Current procedure(s)	Additional procedure(s)
Iodine	forming blue complex with starch after mineralization	the same	Titrimetry	potentiometric determination
Polysaccharides	precipitation with 96% alcohol	TLC of monosaccharides after hydrolysis	Gravimetry	spectrophotometry, HPLC-RID, HPLC-MS
Reducing sugar	copper tartrate reagent in acidic media		–	–

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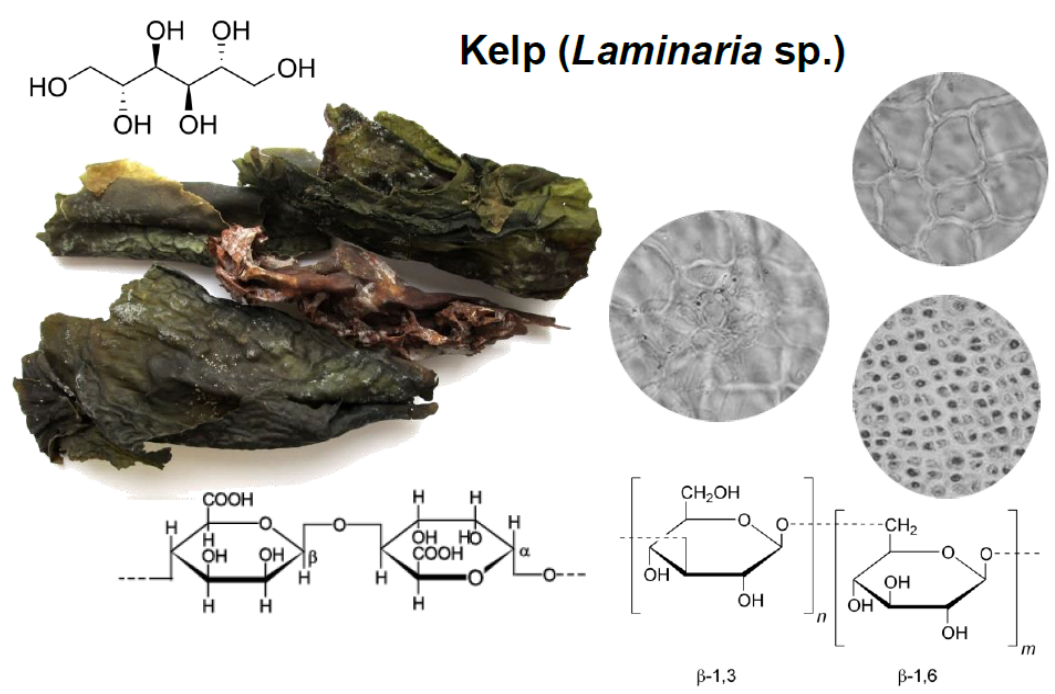
GRAPHICAL ABSTRACT



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