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## MADDER RHIZOMES AND ROOTS, SUBSTANTIATION OF CHOICE AND APPROACHES TO STANDARTIZATION

*The article presents an overview of the genus madder, its chemical composition, pharmacological activity, drugs and medicinal use of madder rhizomes and roots in medicine. Substantiated is the necessity of improving the existing regulatory framework for standardization of raw materials and the development of the national monograph, to meet modern requirements and comply with the European Pharmacopoeia. A method of identification of anthracene derivatives in madder rhizomes and roots by thin-layer chromatography has been developed. This method could be used in the development of national monograph of State Pharmacopoeia of Ukraine (SPU), Madder Rhizomes and Roots.*

*Key words:* standardization; medicinal raw materials, madder rhizomes and roots.

### ASSIGNMENT FORMULATION

The market of herbal medicines is constantly expanding. Nowadays, the European market is regarded as one of the largest commercial markets of medicinal plants in the world, medicinal raw materials (MRM) and phytomedications. Harmonisation of requirements to quality and safety of medicines is a general trend in modern pharmacopoeia standards worldwide [17]. The EU countries pay considerable attention to the quality control program of herbal medicines. Numerous countries are conducting research to develop their own national monographs on medicinal raw materials and herbal drugs within national pharmacopoeias [8]. In Ukraine, the MRM quality is regulated by the corresponding monographs of the State Pharmacopoeia of Ukraine (SPU). Therefore, the development and introduction to SPU of the monographs 'harmonized' with the requirements of the European Pharmacopoeia is an urgent task of the national pharmacy [4-6].

### ANALYSIS OF PREVIOUS STUDIES AND PUBLICATIONS

The genus *madder* (*Rubia* L.) of madder familia *Rubiaceae* consists of about 60 species distributed in the temperate climate zones of Asia and the

Mediterranean, with separate species spread in Central Europe and in Africa, and about 15 species in Central and South America. In the territory of the former Soviet Union grow about 20 species, most of which are spread in Central Asia [1,9,11]. *Flora of the URSR* refers to three species, i. e. *R. tinctorum* L. (dyer's madder), found predominantly in a cultivated or feral state on the Black Sea coast, Crimea, as well as in the wild and cultivated – in the Caucasus and Central Asia; *R. iberica* (Fisch. Et DC.) C. Koch (*R. tinctorum* var. *iberica* Fisch. Et DC.) found on the Black Sea coast, Crimea, and the Caucasus; whereas *R. tatarica* (Trev.) Fr. Schmidt, growing in Eastern and Southern Ukraine, is a pontychno-Caspian endemic [9].

These grow as perennial herbs or short subshrubs or shrubs. Leaves are simple and entire, crosswise opposite, with petiole stipules, in shape and size similar to the leaves, with which they form together 4-8 (10) – member whirls. Flowers are in the apical or axillary cymes, often collected in a multiple panicle or, rarely, a racemose inflorescence, flowers small, monoecious, rarely polygamous-dioecious. The calyx is marked by an invisible edge, without denticles. The Corolla is flat, with a shortened tube, star-shaped or cupped, rarely short funnelform or calyciform, quinquepartite, rarely quadripartite or hexapartite, yellow, greenish or whitish. The number of stamens is equal to

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the number of petals with straight or curved anthers; styles two, separate. The fruit is drupaceous, rather fleshy and juicy, than dryish, one lobe (through reduction of one of the lobes) or, more infrequently, dithecal, at the top deeply grooved but not divided into pericarps; seeds marked by hard, hornlike endosperm [9].

Raw materials from wild plants are picked in early spring (March — early April) or in the late vegetation phase (from early August up to early winter frosts) by means of manually digging out the rhizomes and roots 20-30 cm deep, rhizomes prevailing in the raw material collection. With the view to preserving the undergrowth, collecting is carried out once every 2-3 years on the given plot. In orchards and vineyards, where madder is viewed as weed, it is collected yearly during re-ploughing. Collecting wild madder is labour-consuming and, therefore, not economically feasible, thus necessitating cultivation instead. Indeed, as a medicinal plant dyer's madder was cultivated in the Krasnodar region of Russia, the Crimea and the Poltava region.

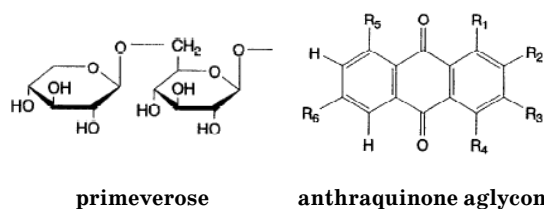
When cultivated, 3-year-old plants are subject to digging out the entire underground part, with an average yields from a 2-3-year-old plantation ranging from 0.9 to 1.2 tonnes per hectare, roots prevailing. The collected raw material is shaken off the soil, aerial parts removed, and roots sliced and spread out for drying without rinsing. The raw material is dried in a single thin layer in the shade or in an attic, adequate ventilation provided. If chamber dried, the temperature should not exceed 45 °C. While drying the raw material is continually turned over to prevent molding. The yield of the dried crude material constitutes 25-30 % of the feed. Upon packing in bales of 50-75 kg, the crude material is subject to shelf-storage in a dry, properly ventilated store room, with the shelf life of 3 years.

To comply with the specifications provided by article 76 SPh, USSR, 11<sup>th</sup> edition, raw material should be whole or crushed. The whole raw material should consist of dried longitudinally wrinkled cylindrical rhizomes and roots of a varying length and thickness from 2 to 18 mm. The rhizomes are normally hollow. Maroon on the outside, rhizomes develop a visible red-and-brownish bark with a peeling cork in cross section, and vermeil red pulp, odourless, sweetish to taste with a marked bitter aftertaste. When chewing, the saliva acquires a reddish colouring. Crushed raw material is maroon or vermeil red.

The standardized allowance for the dried raw material provides for anthracene-associated derivatives of at least 3 %; moisture under 13 %;

total ash content of no more than 10 %; other parts of madder of no more than 1.5 %; other organic matter below 1%; and mineral impurities below 1 % [2].

Identified in rhizomes were organic acids, i. e. citric, malic, tartaric; triterpenoids; vitamin C; anthraquinones (Table 1), i.e. alizarin, rubiadin, purpurin, lucidin, pseudo-purpurin, ruberythric acid, lucidin-3-O-primeveroside, rubiadin-3-O-primeveroside, rubiadin-3-glucoside, purpurin-3-carbonic acid, nordamnacantol, galiosin, purpuroxanthin, munjistin; iridoid asperuloside.



In aerial parts have been determined: iridoids – asperuloside (0,16 %) and deacetyl-asperuloside; pectines; phenolcarbonic and hydroxycinnamic acids – chlorogenic, neochlorogenic, caffeic acids; coumarins (0,16 %); flavonoids such as quercetin, kaempferol, apigenin, luteolin, hyperoside, quercetin-3-arabinoside and rutin [10, 13, 21].

In conventional medicine the drugs derived from *Rubia tinctorum* are used as spasmolytic or diuretic due to reduced smooth muscle tone followed by an increased peristalsis of urinary tracts, contributing to a painless excretion of uroliths, particularly of those containing calcium and magnesium phosphates and oxalates and renal sand. The pharmacological effect is achieved through the ability of anthracene, especially alizarin group, to form chelate complexes with ions of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , thus causing the loosening of urinary calculi [3, 7, 12, 14-16, 19].

Madder preparations in production include herbal tea «Marena», a dietary supplement in 50 g packs (OLR NPO «Phytobiotechnolohiyi», Kyiv, Ukraine); herbal tea «Marena» — dietary supplement, 2g sachets, № 25 (composed of 55 % madder + 45 % of green tea) (OLR «NPO Phytobiotechnolohiyi», Ltd., Kyiv, Ukraine); 0,3 g sachets № 100 (PE «Parapharmatsiya», Zheleznovodsk, Russian Federation); dyer's madder, rhizomes and roots («Phytosyrovyna», Zhytomyr, Ukraine); madder, rhizomes and roots in capsules № 30 (PE «Naturalis - Ukraine»); dyer's madder, rhizomes and roots (RPE «Axion», Simferopol, Ukraine); dyer's madder in 50 ml drops of 60 % aqueous-alcoholic extract of rhizomes and roots of dyer's madder and 40 % purified water («Rodnyk Zdrovovya», Ukraine); phytourolit tincture of 50 ml in

Table 1

## THE MAIN ANTHRAQUINONES FOUND IN RHIZOMES AND ROOTS OF DYER'S MADDER

№	Substance	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	M <sub>r</sub>
1	Anthraquinone	H	H	H	H	H	H	208
2	Alizarin	OH	OH	H	H	H	H	240
3	Ruberythric acid	OH	primeverose	H	H	H	H	534
4	Alizarin glucoside	OH	glucose	H	H	H	H	402
5	Munjistin	OH	COOH	OH	H	H	H	284
6	Lucidin	OH	CH <sub>2</sub> OH	OH	H	H	H	270
7	Lucidin primeveroside	OH	CH <sub>2</sub> OH	primeverose	H	H	H	564
8	Lucidin glucoside	OH	CH <sub>2</sub> OH	glucose	H	H	H	432
9	Purpurin	OH	H	OH	OH	H	H	256
10	Pseudopurpurin	OH	COOH	OH	OH	H	H	300
11	Purpurin-3-carbonic acid	OH	CH <sub>3</sub>	COOH	H	H	H	283
12	Xanthopurpurin	OH	H	OH	H	H	H	240
13	Quinizarin	OH	H	H	OH	H	H	240
14	Rubiadin	OH	CH <sub>3</sub>	OH	H	H	H	254
15	Rubiadin primeveroside	OH	CH <sub>3</sub>	primeverose	H	H	H	548
16	Rubiadin glucoside	OH	CH <sub>3</sub>	glucose	H	H	H	416
17	Galiosin	OH	OH	COOH	OH	H	H	300
18	2-Hydroxy-methylanthraquinones	H	CH <sub>2</sub> OH	H	H	H	H	238
19	1,8-Dihydroxyanthraquinones	OH	H	H	H	OH	H	240
20	2,6-Dihydroxyanthraquinones	H	OH	H	H	H	OH	240

flasks № 1 (Luhansk PhP), with 100 ml tincture containing biologically-active substances of 3 g chamomile blossoms, 3 g field horsetail herb, 3 g field restharrow roots, 3 g birch leaves, 3 g dyer's madder rhizomes and roots, 3g orthosiphon leaves, 2g fennel fruit and 70% ethanol excipients; herbal tea № 27 «Kidney Purifying», sachets of 1,5 g № 20 («Likpharm «Adonis», Donetsk, Ukraine), consisting of dyer's madder roots, chamomile blossom, nettle leaves, peppermint leaves, water piper herb, tickseed herb, juniper fruit, rose cinnamon fruit; dry extract of dyer's madder in tablets of 0,25g (ZAO «Vifiteh», ZAO «Pharmtcenter VILAR», Russian Federation); cystenal drops (Galena/Norton Healthcare (Czech Republic/UK) — until 2005.) litovit-U, tablets of 0,5g №180, 140 g (RPE «Nov», Novosibirsk, Russian Federation), consisting 60 % rhizomes and roots of dyer's madder and 40 % natural zeolite [3,7,12].

In homeopathy, both powdered, raw material and tincture are commonly used for anemia and spleen disorders.

In Tibetan traditional medicine, *R. cordifolia* L. is used in complex prescriptions for maladies as tonsillitis and diphtheria; in India's traditional medicine it is included into pharmacopoeia of *Ayurveda* where it is referred to as *Manjistha* — as an anti-inflammatory and haemostatic agent in amenorrhea and anuria conditions; and in Korean

traditional medicine (as part of multicomponent decoction) — in cardiovascular disease. Apart from these traditional medicine makes wide use of madder preparations in powder, infusion, decoction, and extract for a number of disorders including those of the kidney, urinary tract, liver, spleen, respiratory organs, intestinal and bone tuberculosis as well as rickets, osteomyelitis, anemia with amenorrhea, ascites, dysentery, scrofulosis, gout, sciatica (lumbosacral radiculitis); and externally for ulcers, dermatomycosis and pigment spots. A decoction can also be used externally for skin cancer [18, 20].

Contraindications for application of madder-derived drugs include acute or chronic glomerulonephritis, urolithiasis with a renal impairment and gastric ulcer [7, 12].

#### IDENTIFICATION OF UNRESOLVED ISSUES OF THE GENERAL PROBLEM

Rhizomes and roots of dyer's madder were included into the USSR State Pharmacopoeia, 11<sup>th</sup> edition. Following the 24 years since its publication, the requirements to the quality of herbal raw materials and herbal preparations have undergone significant amendments, necessitating the development of new approaches to the analysis and standardisation in compliance with the Eph framework of standards.

## FORMULATION OF THE PURPOSES OF THE ARTICLE

The purpose of this work was to substantiate the choice of medicinal material, i. e. dyer's madder rhizomes and roots to confirm the necessity for the development of standardized analytical record to meet the current requirements to the quality of herbal preparations and study the possibility of 'harmonizing' the national requirements with the identification C EP monograph requirements to the given raw material.

In the course of the present study a chromatographic analysis of the raw material samples was carried out. The analysis was performed according to the requirements to the medicinal plant monograph development (MRM) promoted by the «Ukrainian Pharmacopoeial Research Centre for Quality of Medicines» [4].

## OUTLINE OF RESEARCH SUBJECT MATTER

### *Study of Raw Materials*

The object of the study were eight samples of madder's rhizomes and roots collected in 2012-2013 in the Crimea, the Russian Federation, in the vicinity of Melitopol and in Kharkiv.

In compliance with the Eph identification requirements of plant material by thin-layer chromatography (TLC) was viewed as compulsory.

### *Identification C. Thin-layer Chromatography.*

Top of the plate	
An alizarin : the purple zone	An Orange zone A purple zone (alizarin) An Orange zone
Reference solution	Test solution

**Fig. 1.** Scheme fingerprint of the reference solution and the test solution

Identification was carried out with the use of *Silicagel 60 F<sub>254</sub>* plates, Merck company, in the solvent system of *toluol R* — *acetone R* — *icy acetic acid R* (35:5:4). To prepare the test solution 0,5 g of powder (180) (2.9.12) of raw materials added 5 ml alcohol (96 per cent) was added, then brought to the boiling point and allowed to cool and centrifuged. The supernatant solution was decanted and used for chromatography. The applied volume was — 10 mcl. To prepare the reference solution, 1 mg of alizarin was dissolved in 10 ml of 96 % ethanol. The distance held by the mobile phase constituted 10 cm from the starting line. The resulting chromatograms were dried in the air and then sprayed

by an alcoholic solution of potassium hydroxide *R*, dried and viewed in daylight. The sequence of zones on chromatograms of the test solution and reference solution are presented in Figure 1.

Chromatogram of reference solution has a band that fits alizarin. The chromatographic analysis of all samples of raw material displays several spots, one of which coincided with alizarin (Fig. 1).

## CONCLUSIONS AND PROSPECTS FOR FURTHER RESEARCH

1. The analysis of preparations of rhizomes and roots of dyer's madder indicates the relevance of this raw material in the pharmaceutical market.

2. Consideration of the current requirements for quality of medicinal raw materials and herbal drugs testifies for the necessity of developing a national monograph on the rhizomes and roots of dyer's madder to meet current requirements harmonized with the European Pharmacopoeia.

3. We hereby suggest the next statements — including a national monograph section “*Identification C*” and identification of anthracene derivatives in raw material to be carried using TLC.

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**МАРЕНИ КОРНЕВИЩА І КОРЕНІ, ОБҐРУНТУВАННЯ  
ВИБОРУ ТА ПІДХОДИ ДО СТАНДАРТИЗАЦІЇ**

У статті наведено загальну характеристику роду марена, хімічний склад, фармакологічну активність, препарати та застосування кореневищ і коренів марени в медицині. Обґрунтовано необхідність удосконалення існуючої нормативної бази стандартизації сировини та розробки національної монографії, яка б відповідала сучасним вимогам та була гармонізована з Європейською фармакопеею. Розроблено методику виявлення антраценпохідних у кореневищах і коренях марени методом тонкошарової хроматографії, яку буде використано при розробці проекту національної монографії у ДФУ «Марени кореневища і корені».

**Ключові слова:** стандартизація; лікарська рослинна сировина, марени кореневища і корені.

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**МАРЕНА КОРНЕВИЩА И КОРНИ, ОБОСНОВАНИЕ ВЫБОРА  
И ПОДХОДЫ К СТАНДАРТИЗАЦИИ**

В статье приведена общая характеристика рода марена, химический состав, фармакологическая активность, препараты и применение корневиков и корней марены в медицине. Обоснована необходимость усовершенствования существующей нормативной базы стандартизации сырья и разработки национальной монографии, которая соответствовала бы современным требованиям и была гармонизирована с Европейской фармакопеей. Разработана методика выявления антраценпроизводных в корневиках и корнях марены методом тонкослойной хроматографии, которая будет использована при разработке проекта национальной монографии в ГФУ «Марены корневиков и корни».

**Ключевые слова:** стандартизация; лекарственное растительное сырье, марены корневиков и корни.

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