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**PREPARATIONS FROM ST. JOHN'S WORT
(*HYPERICUM PERFORATUM L.*) ARE A NICHE FOR THE
DEVELOPMENT OF AGRO-INDUSTRIAL ACTIVITY**

Summary. *Agro-industries are having a significant global impact on economic development. Nonetheless, the full potential of agro-industries as an engine for economic development has not yet been realized in Ukraine, especially in Kherson region. The direction of cultivation and processing of medicinal plants is not very common in Ukraine, but on the contrary, in world practice the topics of healthy living, using the natural ingredients in medicine and the food industry are relevant. Used in work samples of St. John's wort (*Hypericum perforatum L.*) have been collected during spring-summer of 2021 within the territory of environmental research department «Burkuty» (hereinafter PNDV «Burkuty»), which is a territorial component of the national nature park «Oleshkivsky sands». The expediency of obtaining a preparation with antimicrobial and colouring properties based on St. John's wort, which grows in the Kherson region, has been substantiated. A technological scheme has been developed for obtaining a powdery final form of a preparation based on St. John's wort –*

preparation «Kh HP» for the food industry. The composition of the obtained preparation was identified by the methods of qualitative analytical analysis, thin layer chromatography and infrared spectroscopy.

Introduction

Proved the importance of such a theory by the existence of the dependence of the development of innovation processes on ensuring interaction in various forms, the need to develop it – changing the paradigm of the innovation process, the evolution of models of innovation processes, the development of «open innovation» [1]. The rapid and often unpredictable success of some herbal drugs within the medicinal and health food market is changing several criteria of management for botanical products and is posing challenges in diverse research fields. In fact, plants traditionally collected in the wild and used as traditional medicines by a scanty number of people (usually in a circumscribed area of the world) can nowadays become, within a half-dozen years, valuable sources of best-seller drugs marketed worldwide. Such «local to global» transitions can induce dramatic changes in plant collection, cultivation, supply, handling and marketing practices [2–3].

The peculiarities of Kherson region nature are determined by its geographical position in the south of Ukraine within the steppe zone of the Eastern European plain. In the south, the Kherson region is washed by the waters of the Black and Azov Seas. From west to east the region territory stretches from 31°46′ to 35°09′ east longitude for 258 km, and from south to north from 45°58′ to 47°05′ north latitude stretches for almost 180 km. The extreme points of the Kherson region are: Fedorivka village (Vysokopil district) in the north, the railway station Sivash on the Chongar Peninsula (Genichesk district) in the south, Cape Seredniy (on the peninsula Yahorlytsky Kut in Holoprystan district) in the west and Novy village in the east. Kherson region is located in the continental climate zone of temperate latitude and is characterized by a temperate-continental climate with mild snowless winters and hot dry summers. The area is within the temperate zone of illumination between about 46° and 47° north latitude, the total solar radiation is 4700–4900 MJ/m² and varies with the seasons, from north to south. The average annual amount of radiation balance is 2125 MJ/m [4, p. 29–33, p. 43, p. 44, p. 49].

The Kherson region vegetation consists of cenoses of zonal, extrazonal and intrazonal types. In this regard, the vegetation of the region is very diverse, composed of different types of cenoses, formed in the conditions of the diversity of the parent rock, soils, climate and moisture [5].

Complete integrated picture obtaining of the study objects should be determined on the basis of ecological and coenotic characteristics, biological productivity and energy value in natural steppe and artificial forest system [6; 7].

The complete optimization of agronomic conditions according to phytochemical production, actually, is a long and huge effort, needing years and long term financial support. Factors to be considered should emerge not only from in-field agronomic results, but also from physiological, genetical, biotic, abiotic and phytochemical data that could be scaled up to the application level. Moreover, being such approach financially burdensome, it should be wisely undertaken on economically sound plant, in the Kherson region.

The German Commission E designated St. John's wort as an approved herb in 1984, and it is currently one of the most widely consumed medicinal plants in the world [8]. The importance of St. John's wort as a dietary supplement has significantly increased in the last few years. The annual market for St. John's wort has reached \$210 million in the United States alone and over \$570 million worldwide [9].

St. John's worts grown in different regions have varying concentrations of bioactive compounds [10].

Thus, preparations with St. John's wort can be considered as a direction of development of agro-industrial activity of the Kherson region.

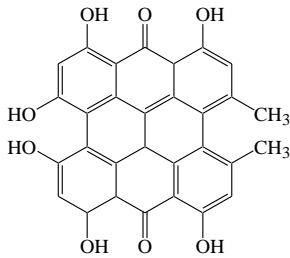
1. St. John's wort as the potentially suitable plant for obtaining a drug with antimicrobial and colouring properties for the food industry

Considering the plant raw materials from the standpoint of the presence of both antimicrobial and colouring properties, the analysis of known medicinal plants was carried out taking into account their chemical composition, information on pharmacological properties and potential suitability for food colouring. *Hypericum perforatum L.* should be singled out from medicinal plants with pronounced antimicrobial activity and known colouring properties.

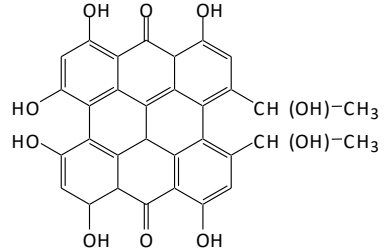
St. John's wort contains a variety of biologically active compounds that cause a manifold of pharmacological properties and is a valuable colouring plant.

The chemical composition includes:

Condensed anthracene derivatives – 0.1–0.5 %, which is accompanied by resinous substances (17 %);



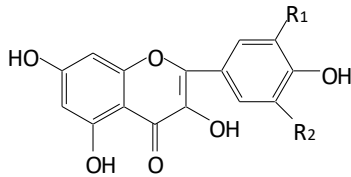
Hypericin



ψ-hypericin

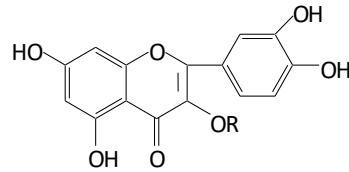
Flavonoids of the flavonol group – 5–6 %

aglycones – quercetin, myricetin; glycosides – hyperoside, rutin, quercitrin



R₁ = R₂ = OH – myricetin;

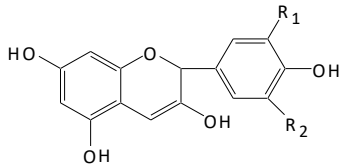
R₁ = OH, R₂ = H – quercetin



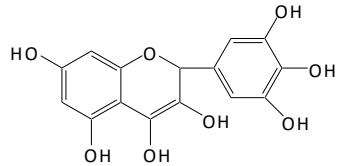
R = glucose -O- rhamnose – rutyn;

R = galactose – hyperoside

R = rhamnose – quercetin

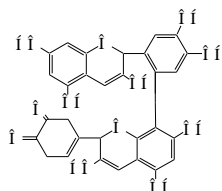


anthocyanins

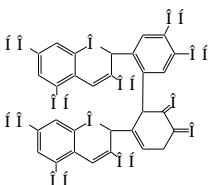


leucoanthocyanidins

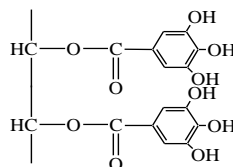
Tannins (10%);



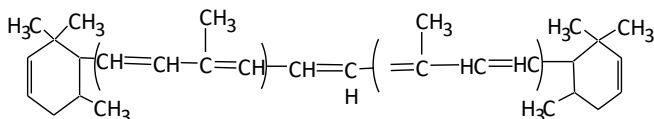
condensed poly phenols



hydrolysable polyphenols



Carotene (55mg/g);



In addition, St. John's wort contains essential oils, nicotinic and ascorbic acids, vitamins P and PP. The saponins presence and alkaloids traces are indicated by several researches. An in-depth study of the chemical composition of St. John's wort allowed us to isolate nine individual compounds related to flavonoids, phloroglucins, phenylpropanoids and sterols [11].

The genus *Hypericum* includes more than 450 species distributed in Europe, North America, North Africa and West Asia. These plants are widely used in folk medicine for the treatment of inflammation, bacterial and viral infections, burns and gastric disorders. The use for alleviating inflammation and promoting wound healing is well known for *H. Perforatum* L. (St. John's wort) and other species. Because of its pharmacological activity, *H. perforatum* L. is one of the most important species of this genus. This plant has been largely utilized for its efficacy in the treatment of mild to moderate depression. However, some other species have been utilized in traditional medicine and have been studied for their phytochemical composition and for their biological activities to date. *Hypericum* species contain biologically active secondary metabolites belonging to at least ten different classes, with prevalence of naphthodianthrones (hypericin and pseudohypericin), phloroglucinols (hyperforin), flavonoids (rutin, hyperoside, isoquercitrin, quercitrin,

quercetin, amentoflavone) and phenylpropanoids (chlorogenic acid). However, great variations in contents have been reported for wild populations worldwide [12]. Also, a number of studies of the biological activities of *Hypericum* species have shown that the most recognized species of this genus, *H. perforatum*, was not the most active. Comprehensive analysis of the published research on the chemical composition and biological activity, showed that *H. richeri* has a similar pharmacological potential as St. Jon's wort. The species, with high content of naphthodianthrone, which might be used against viruses and retroviruses, are: *H. androseamum*, *H. annulatum*, *H. barbatum*, *H. boissieri*, *H. elegans*, *H. hirsutum*, *H. hyssopifolium*, *H. humifusum*, *H. montanum*, *H. montbretii*, *H. triquetrifolium*, *H. richeri*, *H. rochelii*, *H. rumeliacum*, *H. thasium*, and *H. patulum*. Very few species (e.g. *H. inodorum* and *H. moseranum*) contained the similar amounts of hyperforin as *H. perforatum*. Since hyperforin was recognized as one of the most crucial components for the antidepressive activity, it seems that *H. perforatum* barely has an alternative for this purpose. Plant species containing considerable amounts of other acylphloroglucinol derivatives have the potential to demonstrate antibacterial and cytotoxic activity. Some of these species are: *H. sampsonii*, *H. ascyron*, *H. foliosum*, *H. geminiflorum* and *H. scabrum*. However, only a few studies concerning the activity of extracts and isolated compounds were done *in vivo*. Also, data on the safe usage of *Hypericum* constituents as phytotherapeutics are scarce. Since some of *Hypericum* species are scarcely distributed or endemic as well as some of the secondary metabolites are presented in very small amounts, bio-production, especially endophytes, could represent an abundant and reliable source of pharmacologically active metabolites of *Hypericum* species for exploitation in industry [13].

Study evaluated the *in vitro* antioxidant, antibacterial and phytochemical properties of essential oils of *Hypericum helianthemoides* (Spach) Boiss., *Hypericum perforatum* L. and *Hypericum scabrum* L. (Hypericaceae),

The essential oils obtained from dried flowering aerial parts of three *Hypericum* species were analyzed by gas chromatography and gas chromatography/mass spectrometry to determine chemical compositions. The antibacterial activity of essential oils within concentration ranges from 16 to 500 µg/mL was individually evaluated against *Bacillus cereus*, *Listeria monocytogenes*, *Proteus vulgaris* and *Salmonella typhimurium*. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of essential oils was determined using DPPH

assay. Essential oil yield of *H. helianthemoides*, *H. scabrum* and *H. perforatum* were 0.12, 0.20 and 0.21 mL/100 g dried material, respectively. The major constituents of the essential oils were α -pinene (12.52–49.96 %), β -pinene (6.34–9.70 %), (E)- β -ocimene (4.44–12.54 %), β -caryophyllene (1.19–5.67 %), and germacrene-D (2.34–6.92 %). The essential oils of three *Hypericum* species indicated moderate-to-good inhibitory activities against four bacteria, especially against *L. monocytogenes* [14].

The aim of this study was to assess the variability of chemical composition and biological activities of four *H. perforatum* samples, collected at different altitudes in the South Apennine of Italy. MTT assay was used to evaluate the antiproliferative activity of different samples concentrations (0.6–100 $\mu\text{g/mL}$) after irradiation at 365 nm. The inhibition of nitric oxide production was evaluated after 24 h of incubation using the macrophage cell line RAW 264.7 and sample solutions ranging from 12.5 to 1000 $\mu\text{g/mL}$. Antioxidant activities were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and β -carotene bleaching test (ranges were 12.5–1000 and 1–400 $\mu\text{g/mL}$, respectively). Chemical composition was evaluated through HPTLC, and different contents of hypericin and rutin have been observed. The most phototoxic sample was collected from Zumpano (no. 1 at 370 m), with IC₅₀ values of $24.61 \pm 0.02 \mu\text{g/mL}$. Sample no. 1 showed also the best radical scavenging activity (IC₅₀ value of $9.18 \pm 0.03 \mu\text{g/mL}$) and the best antioxidant activity (IC₅₀ value of $10.04 \pm 0.03 \mu\text{g/mL}$ after 30 min of incubation). Best activity of extract no. 1 was well in accordance with chemical data, including the phenolic total content and particular metabolome profile [15].

During the last two decades incidences of fungal infections dramatically increased and the often accompanying failure of available antifungal therapies represents a substantial clinical problem. The urgent need for novel antimycotics called particular attention to the study of natural products. The genus *Hypericum* includes many species that are used in the traditional medicine to treat pathological states like inflammations and infections caused by fungi. However, despite the diffused use of *Hypericum*-based products the antifungal potential of the genus is still poorly investigated. In this study five *Hypericum* species autochthonous of Central and Eastern Europe were evaluated regarding their polyphenolic content, their toxicological safety and their antifungal potential against a broad panel of clinical fungal isolates. LC-MS analysis led to the identification and quantification of 52 compounds, revealing

that *Hypericum* extracts are rich sources of flavonols, benzoates and cinnamates, and of flavan-3-ols. An in-depth screen of the biological activity of crude extracts clearly unveiled *H. hircinum* subsp. *majus* as a promising candidate species for the search of novel antifungals. *H. hircinum* is diffused in the Mediterranean basin from Spain to Turkey where it is traditionally used to prepare a herbal tea indicated for the treatment of respiratory tract disorders, several of which are caused by fungi. Noteworthy, the infusion of *H. hircinum* subsp. *majus* excreted broad antifungal activity against *Penicillium*, *Aspergillus* and non-albicans *Candida* isolates comprising strains both sensitive and resistant to fluconazole. Additionally, it showed no cytotoxicity on human cells and the chemical characterization of the *H. hircinum* subsp. *majus* infusion revealed high amounts of the metabolite hyperoside. These results scientifically support the traditional use of *H. hircinum* extracts for the treatment of respiratory tract infections and suggest the presence of exploitable antifungal principles for further investigations aimed at developing novel antifungal therapies [16].

Study was carried out to study elemental, nutritional, phytochemical and biological evaluation of *Hypericum perforatum*. The elemental analysis showed that Ca was highest (5600 µg/g) in leaves and lowest (2500 µg/g) in flowers. The potassium was highest (840 µg/g) in fruit and lowest (80 µg/g) in leaves. Magnesium was highest (260 µg/g) in stem and lowest (200 µg/g) in flowers. Sodium was highest (4900 µg/g) in stem and lowest (4700 µg/g) in leaves and flowers. Copper was highest (26 µg/g) in stem and lowest (10 µg/g) in leaves. Iron was highest (5000 µg/g) in flowers lowest (1200 µg/g) in stem. Zinc was highest (80 µg/g) in flowers and lowest (46 µg/g) in stem. Nickel, cadmium and Cobalt were < 5 µg/g for all plant parts. The nutritional analysis showed that the dry matter was in the range of (97.61 %) in stem and (96.38 %) in leaf, ash (5.43 %) in flowers and (1.90 %) in stem, crude protein (12.63 %) in leaf and (6.15 %) in stem, crude fibre (64.74 %) in flowers and (13.0 %) in leaf, ether extract (10.98 %) in fruit and (1.88 %) in stem and nitrogen free extract was (65.80 %) in leaf and (10.98 %) in flower, respectively. *Hypericum perforatum* did not show cytotoxic, insecticidal and antibacterial activity in vitro at different doses. The % activity was zero % in cytotoxic and insecticidal activities. However, *H. perforatum* plant parts revealed phytotoxic activity. The phytotoxic activity of leaf and fruit remained same (44.0 %) at highest dose (500 µg/ml). The phytochemical screening showed the presence of mucilage, tannins, anthraquinones, saponins, fats and oils and proteins

in all parts of the plant. Calcium oxalate was found in all parts except the fruit. Lignin and catechin was found in all parts except the leaf. Cutin was found only in stem and flower while chlorophyll was found only in stem and leaf [17].

Hypericum perforatum L., known as St. John's wort (SJW), has been thoroughly tested and is commonly used in the form of an oil, infusion, or diet supplements [18]. St. John's wort contains many bioactive compounds that have a positive effect on humans: hypericin (red dye), hyperoside, rutin, quercetin, tannins, and hyperforin [19]. Substances contained in *Hypericum perforatum* are especially known for their influence on mood alteration and anti-depressant effect via synergistic action, especially hypericin and hyperforin [20]. The therapeutic effect of St. John's wort is obtained through long-term use of substantial Foods amounts of the herb. Moreover, SJW has been described as a plant that may be potentially used in the treatment of many other diseases like AIDS or cancer, and exhibits antioxidant, antidiabetic, or anti-inflammatory properties [21; 22].

However, it is observed that nutritional decisions have an influence on human health and an increase in the incidence of chronic diseases associated with the development of civilization such as metabolic syndrome, type 2 diabetes, tumors, or autoimmune disease [23; 24]. Furthermore, obesity is an effect of imbalance between the amount of consumed calories and physical activity [25].

Although consumption of processed food is still high, customer awareness is increasing. Moreover, food processing may convert many ingredients into promoters of inflammation and cause an imbalance of gut microbiome factors. People select good-quality food more often and pay attention to food composition [26].

St. John's wort (*Hypericum perforatum* L.) is a medicinal plant that alleviates depression and other disorders due to its abundance of active ingredients. Hyperforin, rutin, and melatonin are the main active, and important, ingredients in St. John's wort that alleviate depression. In order to investigate the optimal conditions for accumulating these active ingredients, design of experiments and response surface methodology (RSM) was employed in this study. Two-month-old St John's wort plants were cultivated in growth chambers at varying temperatures, light intensities, and nutrient solution concentrations before analysis by HPLC, for determining differences in hyperforin, rutin, and melatonin content. The results showed that hyperforin and rutin contents were significantly influenced by temperature (18–23 °C) and light intensity

(49–147 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD)), whereas Hoagland's nutrient solution concentration (25–75 %) had little effect. The accumulation of melatonin might not be influenced by cultivation conditions. Light intensity and temperature are easily controlled environmental factors in artificial cultivation, both of which are related to secondary metabolite production in the plant. Based on RSM, the optimal conditions for the accumulation of hyperforin and rutin were obtained. The maximum content of hyperforin was 5.6 mg/g, obtained at a temperature of 19 °C, a nutrient solution concentration of 45 %, and a light intensity of 49 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. The maximum content of rutin was 3.8 mg/g obtained at a temperature of 18 °C, a nutrient solution concentration of 50 %, and a light intensity of 147 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. This evaluation of suitable conditions for the accumulation of bioactive compounds in St. John's wort can be applied to plant factories on a large scale [27].

Consequently, producers have been forced to design innovative products that meet customers' expectations, especially snacks such as cookies and sweets. This type of food is particularly desirable due to its easy availability and is a good basis for designing new products classified as a functional food, which is perceived as a healthier replacement for traditional foodstuffs [28]. Enrichment is one of the methods for functional food production. The composition of snacks can be improved using various ingredients (e.g., vitamins, microelements, fiber, or antioxidant substances) contained in numerous species of plants [29; 30]. Bakery products are a good food matrix to obtain products with increased bioactive properties as they are popular with consumers and constitute the main part of their daily diet [31].

The aim of the study was to assess the impact of *Hypericum perforatum* on the antioxidant activity, enzyme inhibitory effect, and antimicrobial properties of wheat flour cookies supplemented with St. John's wort. The aim of this study [32] was to characterize wheat cookies enriched with 0.5 % and 1.0 % of *Hypericum perforatum* L. (St. John's wort, SJW) and determine their pro-health properties in vitro after hydrolysis in simulated gastrointestinal conditions. The results indicated that 1.0 SJW was characterized by the highest content of polyphenols, flavonoids, and phenolic acids (2.32 mg mL⁻¹, 4.93 $\mu\text{g mL}^{-1}$, and 12.35 $\mu\text{g mL}^{-1}$, respectively). The enriching cookies had no effect on water absorption capacity (WAC) and oil absorption capacity (OAC). After in vitro hydrolysis, the highest peptide content was noted in 1.0 SJW (0.52 mg mL⁻¹), and the bioactive compounds were

characterized by high potential bioaccessibility (PAC), but poor bioavailability (PAV). The addition of SJW increased the ACE, α -amylase, and LOX inhibitory effect, but reduced the inhibition of pancreatic lipase. The highest antioxidant activity was noted for 1.0 SJW. The results showed that only 0.5 SJW and 1.0 SJW had slight antimicrobial activity against *E. coli* ATCC 25922 and *B. cereus* ATCC 14579 with MIC = 12.5 mg mL⁻¹. Fractions with molecular mass < 3.0 kDa were characterized with the highest p-coumaric acid content. The results show that SJW cookies had a higher content of bioactive compounds and more potent anti-metabolic syndrome effects.

Food enrichment is one of the effective methods of increasing the pro-health potential of products. This process is not only aimed at increasing the pro-health value of food, but can also influence the taste, smell, and texture. The study indicated that cookies enriched with St. John's wort had a higher content of bioactive compounds and antioxidant and anti-metabolic syndrome effects. These results showed that *Hypericum perforatum* L. has good potential to be used for the production of potential functional food with not only anti-depression properties.

The pharmacological properties of St. John's wort are widely covered in the medical studies. In particular, much attention is paid to the antimicrobial activity study. Thus, the high antibacterial ability of essential, alcohol, acetone and other extracts of St. John's wort to gram-positive and gram-negative bacteria has been established. It was found that tannins also have antimicrobial properties against a number of microorganisms. Highly active is water-alkaline extraction (pH = 9.0) against pathogenic bacteria. The antimicrobial activity of essential oil obtained from dried St. John's wort by steam distillation is shown in. Of complex compounds number isolated from St. John's wort, the fraction of phenolic substances has showed the highest antibacterial effect. Antimicrobial drugs such as imanin and novoimanin have been obtained, studied and introduced into medical practice on the basis of St. John's wort.

Thus, it is shown that biologically active compounds of St. John's wort (*Hypericum perforatum* L.) have high antimicrobial activity.

St. John's wort can be attributed to plants that can be used to colour food. Usually water decoctions are used, thus receiving violet-red, brown and olive scale of colours. At colouring only by flowers the compound red-violet, green-brown, tobacco dark colour turns out.

Of particular note is the fact that pharmacologically active compounds of St. John's wort chemical composition belong to groups of natural pigments, different in their chemical properties.

The composition and stability of food and pharmaceutical compositions of St. John's wort vary greatly depending on the origin of the plant material, the method of production, the lipophilicity of the solvents and the storage conditions, and this should be considered for both practical and scientific purposes.

2. Extraction features of biologically active substances with St. John's wort

One of the requirements for the successful drugs implementation made from natural raw materials is the release of biologically active and colouring substances from plant raw materials in a final form, convenient in usage.

Pharmacologically active (biologically active) substances are the substances that have the same effect on a living organism (etiotropic, symptomatic, selective, local, etc.), mainly natural substances of secondary synthesis (alkaloids, saponins, tannins, etc.), sometimes substances of basic synthesis, for example from the class of lipids, carbohydrates, vitamins.

Despite the many types of raw materials, physical and chemical properties of the released compounds, the creation of new technological techniques and the use of modern physico-chemical methods, the basis of the isolation process of biologically active substances are the following stages:

- grinding of raw materials;
- contact of the solvent with the raw material;
- separation of the extract from the raw material;
- extraction and regeneration of solvent from the extract and raw materials;
- isolation and purification of biologically active substance.

Today, extraction processes play a leading role in modern nutrition and pharmacognosy: the production of the main group of galenic preparations (extracts, tinctures), novogalenyh, production of individual phytopreparations, etc., due to which a fairly wide base of extraction methods has been accumulated. Since medicinal plants always contain a whole complex of pharmacologically active substances, their extraction can be carried out either by simultaneously isolating the whole complex of compounds, followed by separation into individual components, or by sequential extraction of individual compounds. However, most compounds in plants are biogenetically related, similar in chemical

structure and properties, which complicates sequential extraction. Therefore, most often the amount of biologically active substances is released together with impurities of concomitant compounds that are part of the starting material.

The implementation of this stage of the work is based on the task of developing a method of obtaining an antimicrobial preparation for colouring textile materials, the technological features of which would provide the possibility to obtain an antimicrobial effect of textiles while providing high quality dyes.

In essence, the main importance in the extraction process are diffusion phenomena (mass transfer) based on the equalization of the concentration between the solvent (extractant) and the compounds solution contained in the cell. Accordingly, the diffusion process that takes place during extraction can be characterized by the basic provisions of free molecular diffusion, i.e. when there are no obstacles between the adjacent solutions. There are molecular and convective diffusion.

Molecular diffusion is a process of gradual mutual impregnation of substances (liquid or gaseous) that border each other and are in macroscopic rest, due to the chaotic motion of molecules. The intensity of diffusion depends on the molecules kinetic energy (the larger it is, the more intense the diffusion). The driving force is the difference in the solutes concentrations in the adjacent liquids. As the difference in concentrations increases, the amount of substances that diffuses under equal conditions at the same time increases. In addition, the diffusion rate is also affected by the following factors:

- temperature (temperature increase increases the molecules mobility as a consequence of increasing the diffusion rate);
- molecular weight of the substance and the size of individual particles: the smaller the mass and radius of the diffusing parts, the faster the diffusion;
- density of the medium – with increasing density decreases the mobility of molecules;
- the size of the interface of substances, and the thickness of the layer through which diffusion occurs: the larger the interface, the more substance diffuses, the thicker the layer, the slower the diffusion;
- diffusion process takes a long time: the longer the diffusion, the more substance passes from one medium to another.

Convective diffusion occurs during of liquid and solute movement in a turbulent flow due to temperature changes, stirring, etc. The diffusion rate increases with increasing phase contact surface, concentration

difference and process duration. The main factors for the rate of convective transfer of substances are hydrodynamic conditions (speed and mode of fluid motion).

The process of extraction from medicinal raw materials is complicated by the presence of cell walls, which may have different physiological conditions. Usually for the preparation of drugs dry plant material with cells that have acquired the properties of a porous septum and allow bilateral diffusion are used. The selection process consists of separate moments: dialysis, desorption, dissolution and diffusion, which occur simultaneously as a general process. First, the extractant is impregnated into the middle of the plant material, diffuses through the intercellular passages through the cell walls (dialysis), which leads to swelling of its contents, and the transition to solution (desorption and dissolution). Further, due to the significant difference between the concentration in the solution, in the cell and externally, the process of transfer of solutes into the external volume of the extractant begins, the dialysis process is observed.

The chemical composition of the cell walls also has a significant effect, so the content of cerin, pectin, and lignin significantly impede the penetration of the extractant into the middle of the cell, as a result of dialysis proceeds slowly.

When extracting biologically active substances from medicinal raw materials, it is necessary to create optimal conditions for the diffusion process, taking into account the factors that affect the completeness and extraction rate: degree of grinding, concentration difference, temperature, extractant density, extraction time and hydrodynamic conditions.

The grinding degree of the raw material and the temperature of the extractant play an important role in ensuring the diffusion process. Grinding increases the contact surface of the raw material with the extractant, what reduces the diffusion distance of the extracted substances during extraction and increases their quantitative yield. However, excessively fine grinding could lead to a worsening of the extraction process, as the number of damaged cells increases sharply, what leads to leaching of concomitant substances and causes the transition of a significant number of suspended particles in the extract. The result is cloudy, poorly filtered extracts.

The temperature increasing accelerates the extraction process, yet in the production conditions of galenical preparations this factor should be used only for obtaining water extractions and taking into account the

thermolability of medicinal raw materials. Raising the temperature is desirable when extracting rhizomes, bark, and grass: hot water promotes better tissue separation and rupture of cell walls, thereby facilitating the diffusion process. Thus, the choice of the grinding degree and temperature is set taking into account the morphological and anatomical features of the raw materials used and the chemical nature of the compounds that are part of it and the choice of extractant.

As already mentioned, the difference in concentrations is the driving force of the diffusion process, so it is important throughout the extraction process to maintain the maximum concentration difference, which is often achieved by mixing the infused mass and changing the extractant (periodically or continuously).

The bulk of plant raw materials are fibre, proteins, chlorophyll, resins, mucus and other substances that significantly complicate the process of separation of biologically active natural compounds. In this aspect, the solvent used as the extractant has a strong influence on the quality of the extract. An important condition is the compliance of the extractant with a number of general requirements: selective solubility, high diffusion properties, chemical indifference to the selected substances, safety for humans, easy regeneration and reusability, cheapness and availability. Water is most often used as an extractant, due to the widest range of substances that can be extracted; has easy penetration into cell walls; causes hydrolysis of active substances, which increases when exposed to enzymes or heating. Typically, the extraction takes place in a neutral medium. If necessary, create narrower pH intervals of aqueous solutions (slightly alkaline, rarely slightly acidic).

There are methods based on the use of organic solvents as an extractant: ethanol, ether, chloroform, dichloroethane, acetone, gasoline. For some drugs use glycerin, fatty oils.

However, none of the used extractants satisfies all the requirements at the same time, so their choice is quite individual, for each case. It should be noted that a number of researchers are negative about the extraction of dyes from plant materials with organic solvents, as it loses the environmental friendliness of the whole direction.

The extraction with water and water-alcohol solutions under static conditions and with stirring was examined; the effect of ultrasonic treatment and extraction with water and water-alcohol mixtures under dynamic conditions at elevated temperature and pressure and the extraction with supercritical carbon dioxide were studied. It was established that, in the extraction of biologically active substances from

plant materials, the chemical affinity of an extractant the extracted component is of primary importance; an increase in the pressure under dynamic conditions is the second factor in importance, which increases the efficiency of extraction [33].

Plant Material:

Samples of St. John's wort (*Hypericum perforatum* L.) have been collected during spring-summer of 2021 in the environmental research department «Burkuty» (hereinafter PNDV «Burkuty»), which is a territorial component of the national nature park «Oleshkivsky sands» (hereinafter park or NNP). The territory of PNDV «Burkuty» is within Chalbas (Vynohradiv) Arena and occupies an area 1240.2 ha (15.5 % of the park territory), including the lands of Vynohradivska and Malokopanivska rural councils (Tsyurupynsky and Holoprystansky districts respectively, Kherson region). According to geobotanical zoning, this area is a part of the Lower Dnieper district sandy steppes, sands and floodplains (Didukh, ShelyagSosonko, 2003). According to the physical and geographical zoning, the research area is located in the Holoprystan-Dnipro geographical region of the Lower Dnieper terrace-delta lowland region, the Black Sea-Azov region.

Results:

The conditions option for extraction of the main active substances with St. John's wort was carried out taking into account the requirements for the choice of extractant and based on the above general principles of extraction. In addition, the previous positive experience of obtaining a number of drugs with St. John's wort used as pharmacological agents was taken into account: for a long time such antimicrobial drugs based on St. John's wort as imanin and novoimanin, herbal infusions of 40- and 70-degree alcohol, water decoctions, St. John's wort oil. Accordingly, a method based on successive five-fold water-alkaline extraction was chosen. The plant material of St. John's wort is extracted with a weak solution of alkali at boiling temperature; the extract is filtered, acidified with hydrochloric acid to a weakly acidic congo reaction. The precipitated former is decanted, centrifuged, washed with water until congo neutral, dried and ground to a powder. The obtained powder is a complex preparation containing biologically active compounds that determine various pharmacological properties, as well as dyes that can be used for colouring textile materials.

However, this method of production cannot provide a sufficient yield of biologically active substances necessary to obtain an antimicrobial

effect and saturated and pure shades during the colouring of textile materials.

The yield of active substances is significantly influenced by intensification using rapid changes in temperature and pressure. Therefore, for the most complete and fast extraction and reduction of dyes losses and active substances with St. John's wort, the diffusion process is intensified by short-term treatment of finely ground plant material with St. John's wort with superheated steam, followed by rapid pressure relief and subsequent stepwise extraction with 0.1 % and 1 % hydroxide sodium at boiling point. Activation is accompanied by the creation of a shock wave, which destroys the cellular structure of the processed raw material, with the breakdown of some weak bonds in the cellulose complex, and an increase in the phases contact surface. As a result, the speed of the adsorption process of the alkaline solution, which takes place in the first minutes of extraction, and wetting, facilitates the diffusion process. Thus, the subsequent extraction is more intense and proceeds to the complete leaching of dyes and biologically active substances, which provides high colour ability while maintaining antimicrobial properties.

The method is implemented as follows:

Air-dry plant material of St. John's wort (*Hypericum perforatum*) is crushed, activated by superheated steam in the following mode: temperature 170–240° C, steam pressure 1.0–3.4 MPa, activation time from 30 to 120 s, followed by a rapid decrease in pressure. Then extracted with an alkaline solution at boiling point taken in relation to the plant 1:10 by the method of fine maceration. This method involves an episodic change in the concentration difference at the interface at the expense of updating the extractant. The extractant (10-fold volume) is divided into portions, and the duration of extraction into periods, namely: first, the plant material is extracted for 10 min 3-fold volume of 0.1 % sodium hydroxide solution at boiling temperature and constant stirring; after this extraction is drained, and the residue is extracted 3 times with 1 % NaOH, 5 min, at boiling temperature and stirring; after extraction fusion, 5 min 2-fold 1 % NaOH, and similarly twice 5 min 1-fold 1 % NaOH. The residue is squeezed and the resulting extracts are combined, except for the first portion. After cooling, the obtained extract is acidified with sulphuric acid to a weakly acidic reaction in the congo, infused, the aqueous layer is decanted, the precipitate is centrifuged, washed from the acid residue, dried under dark ($T = 40\text{--}50^\circ\text{C}$) and grounded to a powder.

The result of this process is a dark brown powder. The resulting product was named «Preparation Kh HP»: Kh – the first letters of the Kherson city; HP – the first letters of the Latin name of St. John's wort (*Hypericum perforatum*).

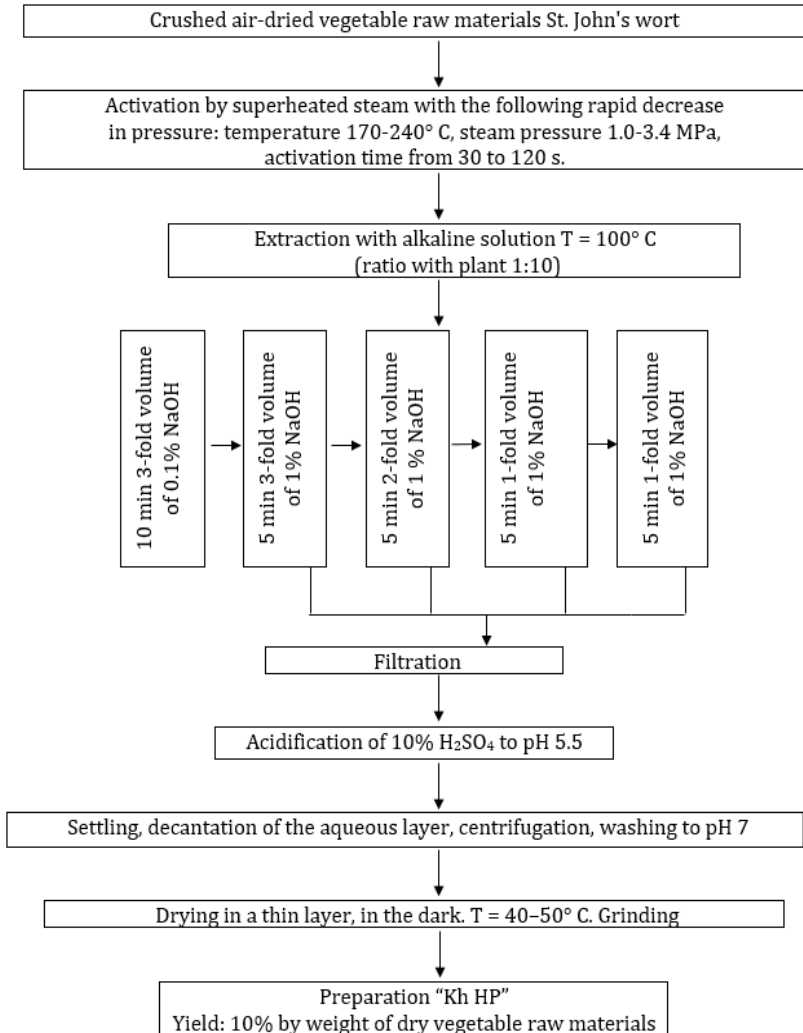


Fig. 1. The scheme of preparation «Kh HP» obtaining

3. Establishing the authenticity and quality of the preparation “Kh HP”

The paper presents methods for the criteria for quality control, authenticity and stability of preparations and raw materials based on *Hypericum perforatum* L. Various methods of extracting the most valuable components that make up the studied plants, as well as methods for their chromatographic determination, were proposed, metrological characteristics were obtained [34].

Before starting to develop the optimal mode of drug application, it is necessary to determine its overall quality.

The preparation “Kh HP” is slightly soluble in neutral water, completely soluble in 0.1 N aqueous sodium hydroxide solutions when heated.

Studies of qualitative composition were carried out according to generally accepted phytochemical analysis methods and techniques:

- qualitative reactions with appropriate reagents;
- chromatographic study;
- research using IR spectroscopy [11, 35].

The presence of flavonoids and tannins was detected by precipitation and colour reactions. Qualitative chemical reactions to anthracene derivatives and their glycosides were performed as follows. 0.2 g of «Kh HP» powder is boiled for 2 minutes with 0.5 ml of 10 % NaOH. After cooling, add 5 ml of water. 3 ml of the resulting solution is placed in a test tube; add 3 ml of 10 % HCl and 10 ml of benzene. Carefully mix and after stratification of the liquid drain the benzene layer, filtering it through a layer of cotton wool. The filtrate is shaken from 3 ml of 10 % ammonia solution. In the presence of anthracene derivatives, the ammonia layer acquires a cherry red (1,8-dioxyanthraquinones), purple (1,4-dioxyanthraquinones) or purple (1,2-dioxyanthraquinones) colour. Qualitative chemical reactions to flavonoids and their glycosides were performed as follows. Preparation of the extract: to 1 g of powder «Kh HP» add 20 ml of 0.1 NaOH, boil in a water bath for 5–10 minutes, then add 30 ml of distilled water and 50 ml of ethyl alcohol.

1. Cyanidin test (Chinoda test). To 2 ml of alcohol extract add 5–7 drops of concentrated HCl and 10–15 mg of metallic Mg or Zn, after 3–5 minutes red, orange, pink colours are observed. To accelerate the reaction and enhance the colour, it is recommended to heat the reaction mixture (2–3 minutes) in a boiling water bath.

2. To 1 ml of alcohol extract add 3–5 drops of 2 % basic lead (II) acetate. The formation of a yellow-orange colour indicates the presence of flavonoids.

3. To 1 ml of alcohol extract add 2 ml of 2 % solution of AlCl_3 in 95 % alcohol and 7 ml of 95 % alcohol; the solution turns greenish-yellow (flavonoids).

4. To 2–3 ml of alcohol extract add 2–3 drops of 3 % solution of ferrum (III) chloride. A brown or greenish-brown colour is formed.

Qualitative chemical reactions to tannins were performed as follows.

Extraction preparation: Add 1 ml of 0.1 N NaOH to 1 g of «Kh HP» powder, boil in water bath for 5–10 minutes, and then add 80 ml of distilled water.

1. To 2–3 ml of extract add 1 % gelatine solution and 1–2 drops of 10 % sodium chloride solution. A yellowish-white precipitate or turbidity of the solution is formed.

2. To 10 ml of the extract add 5 ml of a mixture (2 ml of HCl diluted in a ratio of 1:1 and 3 ml of 40 % formaldehyde solution). The resulting mixture is boiled for 30 minutes in a flask under reflux. In the presence of condensed tannins, they precipitate. The precipitate is filtered off. To 2 ml of filtrate add 10 drops of 1 % ammonium ferrum (III) sulphate dodecahydrate and 0.2 g of crystalline lead (II) acetate, the solution is stirred. In the presence of hydrolysable tanning substances, a blue or purple colour is observed.

3. To 1 ml of the extract add 2 ml of 10 % acetic acid and 1 ml of 10 % average salt of lead (II) acetate. A precipitate (hydrolysable tannins) is formed, which is filtered off. In the presence of condensed tannins, the filtrate turns dark green when 5 drops of 1 % ammonium ferrum (III) sulphate dodecahydrate and 0.1 g of lead (II) acetate are added to the filtrate.

The obtained data are confirmed by chromatographic analysis. Components separation of the preparation «Kh HP» was carried out by thin layer chromatography, which is as follows. The chromatographic process that continues during the mobile phase passage in a thin layer of sorbent (carrier) deposited on an inert surface is called chromatography in a thin layer of sorbent. The mechanism of chromatographic separation may be different, but most often it is adsorption. The movement of the mobile phase in the sorbent layer is carried out under the action of capillary forces.

Equipment. Usually use glass, aluminium or plastic plates of 10×10, 15×15, 20×20 cm², covered with a layer of sorbent (layer thickness is usually 0.25 mm). The chromatography process is carried out in rectangular or cylindrical glass vessels closed with a hermetically ground lid (chromatographic chambers). A solvent system is poured into the

bottom of the chamber, into which a chromatographic plate with the applied samples is immersed. Use micropipettes, microsyringes, calibrated capillaries or other devices suitable for applying solutions.

Immovable phase. Various modifications of aluminium oxide, cellulose, kieselguhr, silica gel with the addition of binders such as calcium sulphate or starch are used as sorbents. Tyrannized sorbents are also used.

The following brands of finished plates were used while the study:

- straight phase - «Silulfol» (Czech Republic), «Merck» (Germany) - ordinary or with a substrate that fluoresces at 254 or 365 nm;
- inverted phase - «Merck» (Germany) - ordinary or with a substrate that fluoresces at 254 or 365nm.

Before use, the finished plates with glass and aluminium substrates are usually activated by heating for 1 h at 100–105° C, to release moisture, which reduces the sorbent activity.

Movable phase. The choice of mobile phase in TLC (thin layer chromatography) should ensure the fulfilment of three main conditions:

- 1) good separation of the compounds under study;
- 2) high sensitivity of detection of these compounds;
- 3) good reproducibility of R_f .

Applying on plate. At a certain distance from the edge of the chromatographic plate, selected so that during immersion the mobile phase does not touch the applied substance, a graphite pencil is applied to the «start line», which marks the places of samples application. Samples are applied at a distance of at least 15 mm from the lower edge of the plate and at least 10 mm from the side edges. The distance between the samples at the starting line should be 20–30 mm, but not less than 10 mm.

R_f is the ratio of the speed of substance movement to the speed of eluent movement. In practice, R_f is calculated as the ratio of the distance from the starting line to the centre of the spot (a) to the distance travelled from the starting line by the front of the solvent system (b): $R_f = a/b$.

For the analysis of the preparation «Kh HP» as immovable phase used ready plates produced by «Silulfol» (Czech Republic); and as a movable - a mixture of solvents n-butanol-acetic acid-water (4:1:5).

The test solution is applied by capillary to the starting line of the chromatographic plate «Silulfol» (spot diameter at the starting line is not more than 5 mm). After drying, the plate is placed in a chromatographic chamber with a solvent system of n-butanol-acetic acid-water (4:1:5). After the front of the solvent has passed 10–12 cm, the plate is removed

from the chamber, dried in air until the solvent evaporates (make several blanks). The resulting chromatograms are viewed in visible and UV light.

On the chromatograms, flavonoids are detected by the characteristic fluorescence in UV light before and after the development of the chromatogram with 2–5 % alcohol solution of $AlCl_3$.

Tannins show a solution of 1 % vanillin in concentrated HC1 in the form of red-orange spots in visible light.

The experiment results are schematically presented in Fig. 2.

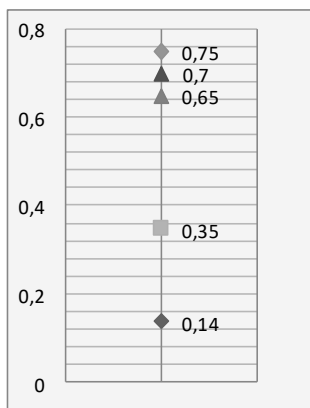


Fig. 2. Chromatographic analysis of the preparation «Kh HP»

The resulting chromatogram has three characteristic spots: 1) slightly collared with $R_f = 0.14$, 2) red spot ($R_f = 0.35$), 3) brown spot $R_f = 0.65$.

Yellow and dark brown fluorescence is observed in ultraviolet (UV) light, respectively, spots № 1 and № 3. When treated with a 1 % alcohol solution of aluminium chloride, a transition to yellow colour and yellow-green fluorescence in UV light is observed. Treatment of the chromatogram with a solution of 1 % vanillin shows characteristic red – orange spots № 4 ($R_f = 0.7$) and № 5 ($R_f = 0.75$). We can assume that the spot № 1 and № 3 corresponds to flavonoids (flavonoid glycosides, flavonols), № 2 – anthraglycosides, № 4, № 5 – tannins of catechin and leukoanthosis.

Tannins of the preparation «Kh HP» are represented by both compounds of hydrolysable and condensed groups, as evidenced by the positive reaction to their separation. To quantify the total amount of tannins used the method of Leventhal, which is based on the tannins ability much faster

than other phenolic compounds to be oxidized by potassium permanganate in the cold in the presence of indigo carmine and is as follows. 2 g of powder of the preparation «Kh HP» is placed in a conical flask with a capacity of 100 ml, pour 50 ml of boiling water and heat in water bath for 30 min with frequent stirring. The liquid is for a few minutes and carefully filtered through a layer of cotton wool into a volumetric flask with a capacity of 250 ml. The extraction is repeated several times until a negative reaction to tannins (test with a solution of ammonium ferrum (III) sulphate dodecahydrate). The liquid in the volumetric flask is cooled and the volume of the extract is adjusted to the mark with water. 25 ml of the obtained liquid is placed in a conical flask with a capacity of 1 l, 750 ml of water and 25 ml of indigosulfonic acid solution are added and titrated under constant stirring with 0.1 N potassium permanganate solutions until golden yellow. For the control experiment in a conical flask with a capacity of 750 ml pour 525 ml of distilled water, add 25 ml of indigosulfonic acid and titrate with constant stirring of 0.1 N potassium permanganate solutions to a golden-yellow colour.

The tannins percentage in a completely dry powder is calculated by the formula:

$$X = \frac{(V_1 - V_2) \times K \times D \times V \times 100 \times 100}{m \times V_0 \times (100 - \omega)}$$

V_1 – volume of 0.1 N potassium permanganate solution spent on extraction titration, ml;

V_2 – volume of 0.1 N potassium permanganate solution used for titration of the control experiment, ml;

K – titter correction (for oxalic acid);

D – tannin conversion factor: for hydrolysable tannins – 0.00415, for condensed – 0.00582;

V – the total volume of the extract, ml;

V_0 – volume of extract taken for titration, ml;

m – mass of powder, g;

ω – weight loss during drying of the powder, %.

From the experiments it was found that the total amount of tannins in the preparation «Kh HP» is 46 %. This exceeds the theoretical data of the total tannins amount in St. John's wort and may indicate condensation of tannins in the process of alkaline treatment of St. John's wort.

Infra-red spectroscopy data were used to determine the composition of the preparation «Kh HP» (Fig. 3).

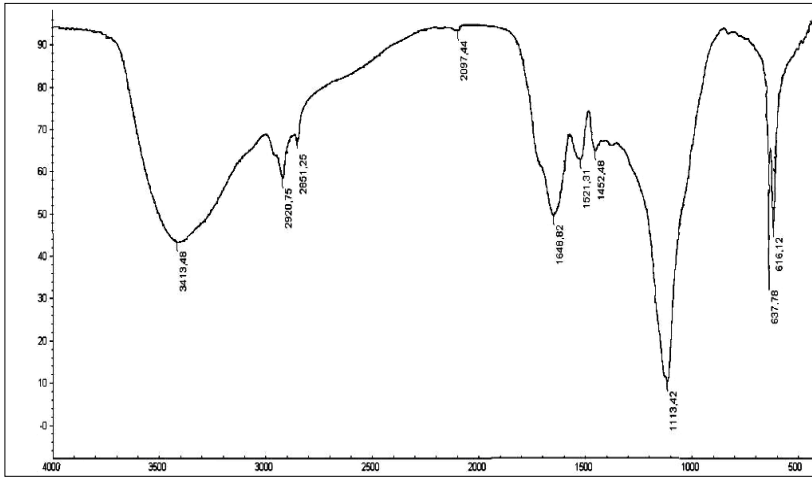


Fig. 3. Infra-Red – absorption spectra of the preparation «Kh HP»

The obtained characteristic absorption bands can be attributed to:

- 3413 cm^{-1} – valence vibrations -OH groups;
- 2920–2851 cm^{-1} indicate the presence of methyl and methoxyl groups;
- 1648 – carbonyl group of γ -pyrone;
- 1521–1425 – fluctuations of aromatic C = C, characteristic of condensed systems.

Thus, according to the obtained experimental data, the composition «Kh HP» is represented by monomeric and polymeric compounds of phenolic nature, which are part of the chemical composition of St. John's wort and have in their molecules groups C = O, -OH, CH, and fragments of molecules such as γ -pyron and quinoid cycles. The presence of these groups in the molecules determine the detection of these compounds of common chemical properties: weakly expressed acidic properties, the ability to enter into complexation reactions, oxidation, reduction, the possibility of forming internal and intermolecular bonds, and contribute to the possible effective use.

Conclusions

Complete integrated picture obtaining of the study St. John's wort should be determined on the basis of ecological and coenotic

characteristics, biological productivity and energy value in natural steppe and artificial forest system of Kherson region.

Hypericum perforatum L. has good potential to be used for the production of functional food. One of the requirements for the functional preparation and successful implementation of food from natural raw materials is the release of biologically active and colouring substances from plant raw materials in a final form, convenient in usage.

The process result of biologically active substances extraction of St. John's wort is a dark brown powder. The resulting product was named «Preparation Kh HP»: Kh – the first letters of the Kherson city; HP – the first letters of the Latin name of St. John's wort (*Hypericum perforatum*).

Studies of «Preparation Kh HP» qualitative were carried out according to generally accepted phytochemical analysis methods and techniques. Thus, according to the obtained experimental data, the composition «Kh HP» is represented by monomeric and polymeric compounds of phenolic nature. Weakly expressed acidic properties, oxidation, reduction, the possibility of forming internal and intermolecular bonds, the ability to enter into complexation reactions, and contribute to the possible effective use.

A result of this research corroborates the need to continue the study of *H. perforatum* in order to confirm its potential as a multi-purpose plant.

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