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THE STUDY OF THE EXTRACTION DYNAMICS OF BIOLOGICALLY ACTIVE SUBSTANCES FROM THE *BIDENS TRIPARTITA* L. HERB AND ANTIOXIDANT ACTIVITY OF THE OBTAINED EXTRACTS

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Abstract

The interest to study the genus Bidens and in particular *Bidens tripartita* L. (bur-marigold herb) is unmitigated due to its antioxidant, anti-allergic, antimicrobial, antifungal, hepatoprotective, immunostimulating and hypotensive activity. This pharmacological activities are determined by the presence of flavonoids, polyphenolic compounds, polysaccharides, components of essential oils, polyacetylenes, etc.

The aim of the research was to study the process of extracting various biologically active substances (polysaccharides, flavonoids, polyphenols) from bur-marigold herb with water-alcohol mixtures of various concentrations, to study their component composition, as well as to evaluate their effect on the oxidative effect of free radicals.

Materials and methods. The object of the research was the herb of *Bidens tripartita* L. Raw materials were collected and procured on the territory of Kharkiv, Zhytomyr and Poltava regions of Ukraine. All used methods for the quantitative determination of biologically active substances were pharmacopeial and described in various monographs in the European Pharmacopoeia and State Pharmacopoeia of Ukraine.

Results. The extracts of the bur-marigold herb were analyzed for the content of the extractable matter, flavonoids, polysaccharides and it was found that they are maximally extracted (about 80 %) in the 1st percolate with a drug/solvent ratio of 1:10 for all the extractants. It was found that with an increase of ethanol concentration in the extraction mixture, the content of polyphenols and flavonoids, is expectedly increased, as well as the value of the antioxidant activity of the corresponding extracts, but the amount of extracted polysaccharides is significantly reduced.

Conclusions. With the selected evaluation criteria, it was found that 40 % ethanol is optimal from the point of view of balanced extraction of flavonoids, polysaccharides and polyphenols. A strong positive and statistically significant correlation was found between the content of polyphenols in the obtained extracts and their antioxidant activity (Pearson correlation coefficient, $r = 0.9998$), slightly weaker for flavonoids ($r = 0.9886$), and an inverse correlation between polysaccharides content and TEAC-value.

Keywords: bur-marigold, extracts, flavonoids, polysaccharides, polyphenols, antioxidant activity.

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1. Introduction

Recently, the interest to study the genus Bidens as plants with a wide spectrum of pharmacological activity has increased significantly.

Trifid bur-marigold (*Bidens tripartita* L.) is a pharmacopeia species among more than 200 other Bidens species [1, 2]. It has been used in both folk and official medicine for many years. Herbal preparations of the bur-marigold herb have antioxidant, anti-allergic, antimicrobial, antifungal, hepatoprotective, immunostimulating, and hypotensive activity. This pharmacological activity is determined by the presence of flavonoids, polyacetylenes [3], polyphenolic compounds, polysaccharides [4, 5], components of essential oils [6], etc.

According to [7], the flowers and herb of *B. tripartita* are a source of antioxidants, which exhibit the properties of radical absorption. In this study, it was noted that although the total content of phenolic compounds in the herb extracts was higher than in the flower extracts, the latter were more active in absorbing radicals than the herb extracts. The authors suggested that not only the content of the sum of polyphenols, but also the total composition of the extract and other factors (solvent, antioxidant synergism) can play an important role in predicting its antioxidant activity.

According to [8], *B. tripartita* had potent antidiabetic activity and its active constituents (chlorogenic acid, luteolin and 7-O-glucoside of luteolin) could be beneficial for diabetes and its complications. In study [9], it was noted that aqueous *Bidens tripartita* L. extracts had a marked antibacterial activity against gram-positive cocci, gram-negative Escherichia coli and the alcoholic extracts from the raw material showed high antioxidative and anti-inflammatory activities.

70 % ethanol extract of the bur-marigold aerial parts and the ointment containing 2.5 % of this extract were used for experimental treatment of psoriasis patients in the study [10]. A gel based on bur-marigold extracts obtained by the extraction with 60 % ethanol showed good stable regenerative properties and accelerated the scarring process [11]. It was demonstrated that the anti-anaphylactic effect of the bur-marigold water infusions was not statistically significantly different from one of the reference drug, sodium cromoglycate, and subsequently, they were recommended as anti-allergic agents [12]. Same authors in another work [13] have showed that polysaccharides and flavonoids of bur-marigold statistically considerably ($p < 0.05$) reduced the number of degranulated mast cells as compared to that in the sample with an allergen, that indicating a stabilizing effect on mast cells of polysaccharides and flavonoids in the studied doses.

It has been shown that the sum of bur-marigold polysaccharides regenerates immune disorders and is not worse in its effectiveness than the drug «Immunal» for correction of immunodeficiency states [14]. Aqueous infusions of the aerial parts of *Bidens tripartita* L. showed significant anti-inflammatory activity compared to indomethacin [15].

It was found that bur-marigold extracts in vitro tend to protect DNA and the ability to inhibit enzymes involved in common pathologies such as diabetes and neurodegenerative diseases. It was also observed that bur-marigold has anti-proliferative activity, which can be further used in cancer treatment. The authors connect the observed biological properties to the presence of biologically active metabolites, such as chlorogenic acid, epicatechin, and luteolin-7-glucoside [16].

In our previous works, a systematic scientific studies were carried out with the aim of standardizing this type of herbal drug and its further introduction into State Pharmacopoeia of Ukraine (SPhU) [17, 18].

Analysis of the literature and our own research has been shown that the chemical composition of the herbal drug is quite diverse and it is not possible to mark out a single compound or even a certain class of natural compounds as a markers for bur-marigold. This type of herbal drug possesses a wide range of pharmacological activity, and not one specific substance (possibly the dominant one among others) is responsible for the action of the drug, but the total complex of biologically active substances. Taking this into account, to standardize the quality of possible herbal preparations, it is important to study the extraction process of the exactly sum of various biologically active substances from herbal drug with different solvents, to study their component composition, as well as to evaluate their effect on the oxidative effect of free radicals.

This work was aimed to study the extraction dynamics of extractive substances, flavonoids, polysaccharides and polyphenols from bur-marigold herb with water-alcohol mixtures of various concentrations and to determine the antioxidant activity of the obtained extracts as a stage in the pharmaceutical development of a herbal preparation based on the bur-marigold herb.

2. Materials and Methods

2.1. Samples of plant material

The object of research was the herb of *Bidens tripartita* L. Raw materials were collected and procured on the territory of Kharkiv, Zhytomyr and Poltava regions of Ukraine. Registration and analysis of samples were carried out at the base of the State Enterprise «Pharmacopoeial Center», Kharkiv, Ukraine. A macro- and microscopic analysis for the presence of characteristic features of the species was carried out, and tests were carried out to meet all the requirements of the national monograph of the State Pharmacopoeia of Ukraine «Bur-marigold herb» [19]. Based on the analysis carried out, the samples were combined into one sample, crushed, and sieved (mesh 500).

2.2. The choice of the extractant

One of the stages to study the completeness of extraction of biologically active substances is the choice of a water-alcohol mixture, as the degree of dissolution/extraction of a particular amount of biologically active substances depends on alcohol concentration. For example, it is known that flavonoid aglycones are extracted from herbal materials using water-alcohol mixtures with a high ethanol content (70–96 %), flavonoid glycosides, with a lower and medium ethanol content (30–70 %), polysaccharides, with a low ethanol content (0–30 %), etc. Taking this into account, in this work the extractants were aqueous-alcoholic solutions with the following ethanol content, %: Extractant 1–25 % ethanol, Extractant 2–40 % ethanol, and Extractant 3–60 % ethanol, which were optimal for extracting the sum of analyzed biologically active substances.

2.3. Obtaining extracts to study the extraction dynamics of biologically active substances and their assay

Three identical weighed portions of the powdered herbal drug were placed in the extractors and poured with prepared water-alcohol mixtures (25 %, 40 %, and 60 %, respectively) up to the «mirror». They were allowed to stay for 24 hours. Then, they were percolated slowly, obtaining the 1st percolate with a DSR of 1:10 (drug/solvent ratio), the 2nd percolate with a DSR of 1:15, and the 3rd percolate with a DSR of 1:20 for each water-alcohol mixture.

The criteria for evaluating the extraction degree of biologically active substances in the obtained extracts were as follows: the content of the extractable matter, flavonoids, polysaccharides, and polyphenols. All the methods for the quantitative determination of biologically active substances were pharmacopeial and described in various monographs in the European Pharmacopoeia and State Pharmacopoeia of Ukraine and has been previously described in our work [20].

2.4. Determination of antioxidant activity by the ABTS method

The method of the antioxidant activity determination is based on the reaction of the interaction of antioxidants with the radical cation 2,2′-azino-bis (3-ethylbenzothiazolin-6-sulfanol acid, ABTS) [21]. The activity of the test samples was evaluated expressed as Trolox standard, a water-soluble analogue of vitamin E. The study of antioxidant activity was carried out on percolates with a DSR of 1:10. For analysis, 2.5 g of the obtained percolates were taken, which were placed in a 25 ml volumetric flask, and the volume was adjusted to the mark with methanol. The results were expressed as Trolox equivalent antioxidant activity (TEAC) in mg per 1 g of the extract.

2.5. Statistical analysis

Pearson's correlation was used to calculate the relationship between content components in extracts bur-marigold and their antioxidant activity. The content of components and antioxidant activity is expressed as a mean of three replicates.

3. Results

The resulting extracts of the bur-marigold herb were analyzed for the content of the main biologically active substances (BAS), namely extractable matter, flavonoids, and polysaccharides. The results are shown in **Table 1** and **Fig. 1**; the results are given in % in terms of raw material; in brackets, % of the total content of specific BAS is indicated.

Table 1

The results of the content of BAS in bur-marigold herb when studying the dynamics of their extraction with different ethanol/water extractants

DSR	Flavonoids, %			Polysaccharides, %			Extractable matter, %		
	25 %	40 %	60 %	25 %	40 %	60 %	25 %	40 %	60 %
1:10	0.16 (71 %)	0.39 (74 %)	0.58 (80 %)	3.0 (82 %)	2.39 (83 %)	1.04 (84 %)	20.1 (79 %)	19.0 (83 %)	14.53 (82 %)
1:15	0.06 (26 %)	0.10 (19 %)	0.11 (15 %)	0.60 (16 %)	0.46 (16 %)	0.19 (15 %)	4.80 (19 %)	2.57 (11 %)	1.88 (10 %)
1:20	0.005 (3 %)	0.04 (7 %)	0.03 (4 %)	0.07 (2 %)	0.02 (1 %)	0.005 (0.5 %)	0.56 (2 %)	1.28 (6 %)	1.36 (8 %)

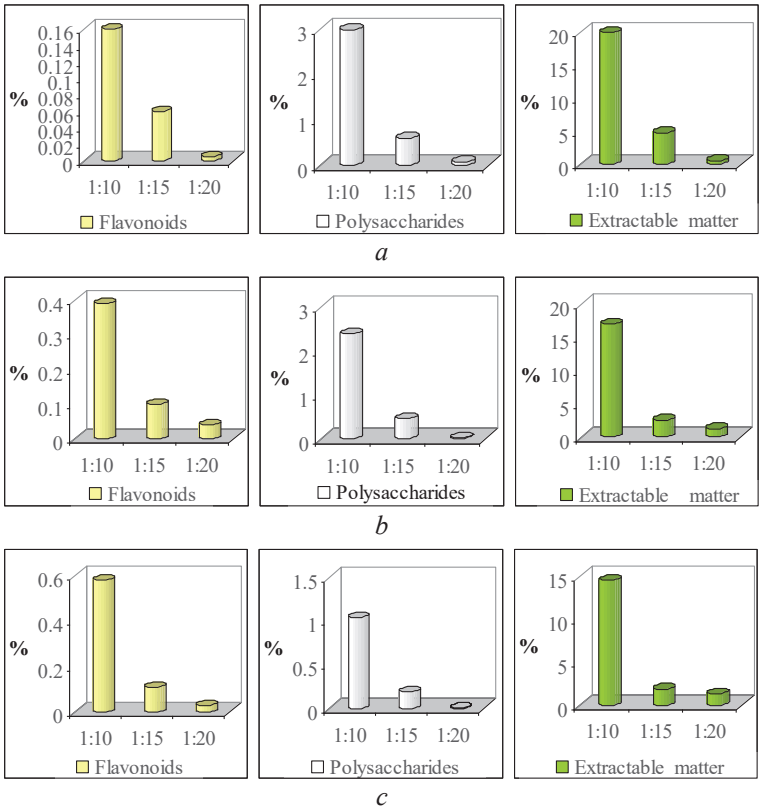

Fig. 1. Dependence of the BASs content of bur-marigold herb extracts on DSR (drug/solvent ratio) upon extraction with different extractants: *a* – 25 % ethanol; *b* – 40 % ethanol; *c* – 60 % ethanol; *X*-axis – content in %, *Y*-axis – DSR

From the above data, it is seen that the extractable matter, flavonoids, and polysaccharides are maximally extracted in the 1st percolate with a DSR of 1:10. Analyzing the dynamics of the extraction of substances, we can conclude that about 80 % (79–83 %) of the extractive matter is extracted in the first percolate for all the extractants, from 10 to 17 % in the second percolate, and from 4 to 8 % in the third one. Almost similar results were obtained when studying the dynamics of the extraction of flavonoids and polysaccharides: in the first percolate, flavonoids are extracted from 69 to 80 % depending on the concentration of water-alcohol extractant, and polysaccharides – not less than 80 % (82–84 %).

Taking into account the results obtained, further studies were carried out on extracts of DSR 1:10, in which polyphenolic compounds were additionally analyzed.

Table 2 shows the results of the determination of the content of polyphenols, flavonoids, and polysaccharides in the obtained extracts, as well as the results of the determination of their antioxidant activity. The results are given in % in terms of raw materials; the relative % content of

specific biologically active substances in relation to each other in different extractants are given in brackets, antioxidant activity (TEAC) in mg per 1 g of the extract.

Table 2

The results of determination of the BAS content in various aqueous-alcoholic bur-marigold extracts and their antioxidant activity

Extracts of bur-marigold DSR 1:10	Polyphenols, %	Flavonoids, %	Polysaccharides, %	TEAC (mg/g)
Extractant 1	0.43 (22 %)	0.16 (14 %)	3.0 (47 %)	0.878 (19.5 %)
Extractant 2	0.71 (36 %)	0.39 (35 %)	2.39 (37 %)	1.635 (36 %)
Extractant 3	0.83 (42 %)	0.58 (51 %)	1.04 (16 %)	1.990 (44 %)

4. Discussion

As can be seen from the above results, with an increase of ethanol concentration in the extraction mixture, the content of substances of phenolic nature, namely polyphenols and flavonoids, increases predictably, and the value of the antioxidant activity of the corresponding extracts also increases. On the other hand, with the transition from 40 % to 60 % ethanol, the amount of extracted polysaccharides decreases significantly (almost by half). **Fig. 2** shows the graphs of the dependence of changes in the content of these BASs and TEAC values (in relative percent) on the extractant concentration. As can be seen from **Fig. 2**, the intersection point of all four graphs is the concentration of the extractant – 40 % ethanol, which, in comparison with 2 other extractants, first of all, allows to extract in a balanced manner of all studied BASs, which are pharmacologically active in the bur-marigold, and on the other hand, it provides a sufficiently high TEAC value.

With regard to the results of antioxidant activity study of the obtained extracts, a strong positive and statistically significant correlation was found between the content of polyphenols in the obtained extracts and their antioxidant activity (Pearson correlation coefficient, $r=0.9998$, $p=0.0000001$), slightly weaker for flavonoids ($r=0.9886$, $p=0.0002$), and an inverse correlation between polysaccharides content and TEAC value ($r=-0.9538$, $p=0.0031$) (Correlation is significant at $r \geq 0.997$, at the 0.05 level [22]).

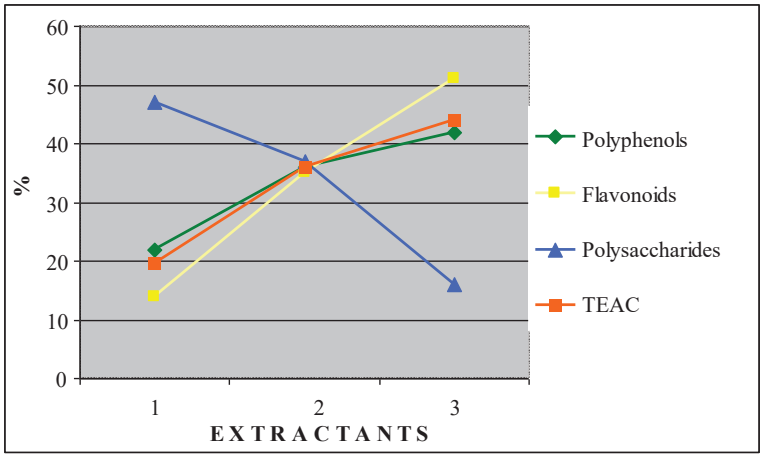

Fig. 2. The dependence of the BASs content and TEAC values (in relative percent) on the concentration of the selected extractant: extractant 1 – 25 % ethanol; extractant 2 – 40 % ethanol; extractant 3 – 60 % ethanol

In general, the selected conditions for the extraction of BAS from bur-marigold herb correspond to commonly used methods for obtaining extracts from herbal drugs containing phenolic compounds; however, the choice of 40 % ethanol, which provides a balanced extraction of different

classes of BASs, distinguishes our studies from studies [10, 11] and the obtained results are consistent with studies [7] that not only the content of the sum of polyphenols, but also the total composition of the extract play an important role in predicting its antioxidant activity.

Study limitations. Considering that these studies represent only one of the stages of the pharmaceutical development of the future herbal preparation, they should not be limited only to the study of the chemical composition of preparation components. It would be interesting to link these results, especially the studying of the antioxidant activity, with pharmacological studies to clarify the issue of the therapeutic dosage of the proposed preparation.

Prospects for further researches. Given that the proposed herbal medicine will be combined, i. e. contain several herbal extracts, in the future it is necessary to continue research data for other objects, components of the preparation, both from the point of view of studying the extraction dynamics of active substances and the choice of standardization criteria for remaining components, and from the point of view of studying the synergism of the action of various combinations of extracts for choosing the optimal composition of the future herbal preparation.

5. Conclusions

As a result of studying the extraction sum of biologically active substances from bur-marigold herb with various solvents, it was found that the extractable matter, polysaccharides, flavonoids and polyphenols are maximally extracted with water-alcohol mixtures with drug/solvent ratio of 1:10, and with the selected evaluation criteria, it was showed that 40 % ethanol is optimal from the point of view of balanced extraction of sum of biologically active substances.

A strong direct and statistically significant correlation was established between the results of the polyphenols content and the TEAC value of the aqueous-alcoholic bur-marigold extracts, a slightly weaker one for flavonoids, and an inverse correlation between the polysaccharides content and the TEAC value.

Conflict of interest

The authors declare that they have no conflicts of interest.

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