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PHYTOCHEMICAL SCREENING OF THE BIOACTIVE COMPOUNDS IN THE MOST WIDESPREAD MEDICINAL PLANTS FROM CALARASI - SILISTRA CROSS - BORDER AREA

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Abstract: *The cultivation of medicinal and aromatic plants has a long tradition in the Calarasi – Silistra region. In this area, 81 species of medicinal and aromatic plants – from the cultivated and spontaneous flora – were identified as a result of a study we performed. Of those, 16 species were experimentally cultivated in two pilot cultures, one in Calarasi County and one in Silistra District, and were analyzed from the point of view of the active principles they contain. The following compound categories were identified during the study: aminoacids, proteins, alkaloids, saponins, tannins, triterpenoids, glycosides, polysaccharides, vitamins A and E. The results of the study allowed the identification of species with a high content of active principles and led to recommendations for local cultivators of medicinal plants. A database was also created, containing information about the most common medicinal plants found in the area and their active principles content (www.medplanet.dbioro.eu).*

Key words: *bioactive compounds, medicinal plants, phtochemical screening.*

1. Introduction

Medicinal plants are a group of species that accumulate different active principles, useful in treating various human or animal diseases. The long term use of herbs in medicine is a sure indication of their value and usefulness in the future.

Phytoterapy is a source of treating and improving certain diseases by using the beneficial effects of medicinal plants. An important amount of therapeutic products are derived from medicinal plants (77% for cardiovascular diseases and 74% for digestive diseases). There are over 1700 medicinal plant species, of which more than 500 are cultivated.

In modern medicine, the importance of medicinal plants is increasing [7]. Nowadays, pharmaceutical and cosmetic industries are increasingly using plant resources form rural or unpolluted areas. It

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is a well known fact that large Western pharmaceutical and cosmetical companies buy various plant products, especially medicinal plants harvested from the wild flora in countries that have not yet applied intensive chemicalization in agriculture, such as some areas in Romania or Bulgaria.

Some of the species from the wild flora can not provide sufficient material for the pharmaceutical, cosmetic and food industries. There are various active principles of spontaneous medicinal plants depending on concentration, quality, climate, area and other characteristics, that make difficult their quantitative determination in phytoterapy.

Advanced studies regarding the medicinal plants from Calarasi - Silistra area (performed within a cross - border project from the Romania - Bulgaria Cooperation Programme) show that, in this area, there are over 80 species of cultivated and spontaneous medicinal and aromatic plants.

The aim of this paper is to present the phytochemical screening of bioactive compounds found in the most widespread herbs in the area. The obtained results were used as important key aspects in making recommendations concerning the cultivation of certain species, whose active principles can be valued as phytoterapeutical products, food supplements and cosmetics.

2. Objectives

In order to perform phytochemical screening of bioactive compounds found in the medicinal plants from Calarasi – Silistra area, the following objectives were established:

- Identify the wide-spread medicinal plants in the target area;
- Establish the possible types of bioactive compounds to be identified in medicinal plants;
- Establish optimal methods to perform the phytochemical screening;
- Obtain herbal extracts from the plant material selected for analysis;
- Perform the phytochemycal screening;
- Interpret the results and give recommendations;
- Create a database with information about medicinal plants in the border area.

3. Material and Methods

An inventory comprising 80 of the most common species of medicinal plants identified in Calarasi – Silistra border area was made and the obtained results were published on the website: www.medplanet.dbioro.eu. From these, a total of 8 species were selected and analyzed in terms of their active principles: *Cynara scolymus* (artichoke), *Salvia officinalis* (sage), *Calendula officinalis* (marigold), *Lavandula angustifolia* (lavander), *Mentha piperita* (mint), *Plantago lanceolata* (plantain), *Matricaria chamomila* (chamomile), *Hypericum perforatum* (St. John's wort).

Two species of medicinal plants were from Bulgaria (*Lavandula angustifolia* and *Mentha piperita*) and the other species were from the Calarasi area, Romania.

The bioactive compounds analyzed in the mentioned plants were: amino acids, proteins, alkaloids, saponins, tannins, triterpenoids, glycosides, polysaccharides.

The following methods were used to identify the bioactive compounds in medicinal plants:

Amino acids identification method (Ninhydrin method) [2].

Protein identification method (Xantoproteic reaction)

Alkaloids identification method (Dragendorff method) [2].

Saponins identification method (Foam test and Lieberman – Bourchard method [5], [18].

Tannins identification method (Ferric chloride test) [21].

Tripenoids identification method (Salkowski test) [21].

Glycosides identification method (Borntrager test) [21].

Polysaccharides identification method (Molisch reaction) [6].

Obtaining extracts from the medicinal plants selected for analysis:

Herbal extracts were obtained through two methods: aqueous and hydroalcoholic extraction. A known quantity of plant material was mechanically crushed, weighen and subjected to aqueous extraction for 24 hours, at low temperature. The same amount of plant material was extracted in 70% ethanol, at low temperature, holded for 3 days. After filtering, the obtained aqueous and hydoalcoholic extracts were concentraded and stored at 4^0C until the moment of utilisation.

4. Results and Discussions

After performing the analysis of bioactive compounds of the studied medicinal plants, the following results were obtained:

Cynara scolymus (artichoke) extracts compounds Table 1

Compounds	Test	Aqueous extract	Hydroalcoholic extract
Amino acids	0.25% Ninhydrin	++	+
Proteins	Xantoproteica	+	±
Alkaloids	Dragendorff	+	+
Saponins	a) Foam test	++	+
	b) Lieberman-Bourchard	++	+
Tannins	Ferric chloride	+	++
Triterpenoids	Salkowski	+	+
Glycosides	Borntrager	+	++
Polysaccarides	Molisch reaction	+	+

Mentha piperita (mint) extracts compounds Table 2

Compounds	Test	Aqueous extract	Hydroalcoholic extract
Amino acids	0.25% Ninhydrin	++	+
Proteins	a) Xantoproteica	+	+
	b) Biuret	+	+
Alkaloids	a) Dragendorff	+	+
	b) Hager	+	+
Saponins	a) Foam test	++	++
	b) Lieberman-Bourchard	++	++
Tannins	Ferric chloride	+	+
Triterpenoids	Salkowski	+	+
Glycosides	Borntrager	+	+
Polysaccarides	Molisch reaction	+	++

Calendula officinalis (marigold) extracts compounds Table 3

Compounds	Test	Aqueous extract	Hydroalcoholic extract
Amino acids	0.25% Ninhydrin	+	+
Proteins	Xantoproteica	++	+
Alkaloids	Dragendorff	+	+
Saponins	a) Foam test	+	+
	b) Lieberman-Bourchard	+	+
Tannins	Ferric chloride	-	-
Triterpenoids	Salkowski	+	+
Glycosides	Borntrager	+	+
Polysaccarides	Molisch reaction	+	+

Plantago lanceolata (plantain) extracts compounds Table 4

Compounds	Test	Aqueous extract	Hydroalcoholic extract
Amino acids	0.25% Ninhydrin	+	+
Proteins	Xantoproteica	+	+
Alkaloids	Dragendorff	-	-
Saponins	a) Foam test	-	-
	b) Lieberman-Bourchard	-	-
Tannins	Ferric chloride	+	+
Triterpenoids	Salkowski	+	+
Glycosides	Borntrager	+	++
Polysaccarides	Molisch reaction	+	++

Matricaria chamomila (chamomile) extracts compounds Table 5

Compounds	Test	Aqueous extract	Hydroalcoholic extract
Amino acids	0.25% Ninhydrin	+	+
Proteins	a) Xantoproteica	-	-
	b) Biuret	-	-
Alkaloids	a) Dragendorff	-	-
	b) Hager	-	-
Saponins	a) Foam test	-	-
	b) Lieberman-Bourchard	-	-
Tannins	Ferric chloride	+	+
Triterpenoids	Salkowski	-	-
Glycosides	Borntrager	+	+
Polysaccarides	Molisch reaction	+	+

Hypericum perforatum (St. John's wort) extracts compounds Table 6

Compounds	Test	Aqueous extract	Hydroalcoholic extract
Amino acids	0.25% Ninhydrin	+	+
Proteins	a) Xantoproteica	±	-
	b) Biuret	±	-
Alkaloids	a) Dragendorff	++	++
	b) Hager	++	++
Saponins	a) Foam test	++	++
	b) Lieberman-Bourchard	++	++
Tannins	Ferric chloride	+	+
Triterpenoids	Salkowski	+	++
Glycosides	Borntrager	+	++
Polysaccarides	Molisch reaction	+	+

Salvia officinalis (sage) extracts compounds Table 7

Compounds	Test	Aqueous extract	Hydroalcoholic extract
Amino acids	0.25% Ninhydrin	+	+
Proteins	a) Xantoproteica	+	+
	b) Biuret	+	+
Alkaloids	a) Dragendorff	±	±
	b) Hager	±	+
Saponins	a) Foam test	±	±
	b) Lieberman-Bourchard	±	±
Tannins	Ferric chloride	+	++
Triterpenoids	Salkowski	+	++
Glycosides	Borntrager	+	+
Polysaccarides	Molisch reaction	++	++

Lavandula angustifolia (lavander) extracts compounds Table 8

Compounds	Test	Aqueous extract	Hydroalcoholic extract
Amino acids	0.25% Ninhydrin	+	+
Proteins	a) Xantoproteica	++	+
	b) Biuret	+	+
Alkaloids	a) Dragendorff	-	-
	b) Hager	-	-
Saponins	a) Foam test	±	-
	b) Lieberman-Bourchard	±	-
Tannins	Ferric chloride	+	++
Triterpenoids	Salkowski	+	++
Glycosides	Borntrager	-	-
Polysaccarides	Molisch reaction	-	-

Legend: „++" abundant; „+" moderate; „±" low/reduced; „-" absent

The results from the phytochemical screening of the studied medicinal plants extracts have shown that amino acids are found in all these plants, the mint and artichoke aqueous extracts being very rich in these compounds.

Proteins are found in most herbal extracts, except for those obtained from chamomile.

In contrast, the aqueous extract from marigold is very rich in proteins. The highest content of Alkaloids is found in St. John's wort, while plantain and lavender do not contain this type of compound [23].

Saponins are present in almost all studied herbs, except for plantain and chamomile.

Hydroalcoholic extracts of sage, artichoke and lavender are rich in tannins, while marigold do not contain these compounds.

Triterpenoids were found in all the analyzed extracts, except for chamomile.

Glucosides are present in all studied plants, except for lavender and lavender extracts are the only ones that do not contain polysaccarides.

Analyzing the results, it can be observed that the studied medicinal plants containing the largest number of bioactive compounds are sage, St. John's wort, mint and artichoke.

The studied bioactive compounds have a broad range of biological activities. For example, phytochemicals such as saponins have anti-inflammatory effects [20], hemolytic activity, and cholesterol binding properties [9], glycosides are known to lower blood pressure [8] and tannins exhibit antioxidant, antimicrobial and antiviral effects [16]. The plant extracts were also revealed to contain triterpenoids, which are known to produce an inhibitory effect on inflammation [15] and alkaloids that have been reported to exert analgesic, antispasmodic and antibacterial activities [9].

The phytochemical screening results of the artichoke extract (*Cynara scolymus* L.) are consistent with the results found in [1], where authors mentioned the presence of tannins, alkaloids, saponin and terpenoids in this plant. The results shown in Table 1 for sage (*Salvia officinalis* L.), lemon balm (*Melissa officinalis* L.), peppermint (Mentha piperita L.) and St John's wort (*Hypericum perforatum* L.) extracts are in partial agreement with previous studies [12], [17], [19], [22].

It is difficult to compare the data with the literature because several variables influence the results. According to some authors, the quantity and the composition of bioactive compounds present in plants are influenced by the genotype, extraction procedure, geographic and climatic conditions, and the growth phase of the plants [3], [19].

Plant cells produce two types of metabolites. Primary metabolites are involved directly in growth and metabolism (carbohydrates, lipids and proteins). Most natural products are compounds derived from primary metabolites such as amino acids, carbohydrates and fatty acids and are generally categorized as secondary metabolites. Secondary metabolites are considered products of primary metabolism and are generally not involved in metabolic activity (alkaloids, phenolics, essential oils and terpenes, sterols, flavonoids, lignins, tannins, etc.) [11]. These secondary metabolites are the major source of pharmaceuticals, food additives, fragrances and pesticides [10], [12], [13].

Similar analyses were conducted in areas that have a long tradition in the cultivation and utilization of medicinal plants, such as Pakistan [4] and India [14].

Phytochemical screening results can be found in a database with the most important medicinal and aromatic plants in Calarasi – Silistra border region. The database can be accessed at: www.medplanet.dbioro.eu. There are other

websites/databases with information about herbs, but they only contain information about the medicinal plants found in Romania (www.plante-medicinale.ro).

5. Conclusions

A phytochemical screening, including qualitative analyses of bioactive compounds, was performed for a total of 10 medicinal plant species: 2 species from Bulgaria (Silistra) and 8 species from Romania (Calarasi). From the obtained results, recommendations were made concerning the cultivation of certain medicinal plant species, according to their high amount of active principles and their potential in medications, dietary supplements or cosmetics industries. Among the medicinal plants that were studied in this paper, sage, St. John's wort, lemon balm and artichoke (from Romania) and mint (from Bulgaria) have the highest content of the analyzed active principles.

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