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BIOMEDICAL METHODS FOR TOTAL PHENOLIC CONTENT IN MEDICINAL PLANTS AND TINCTURES

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Abstract. ***Medicinal plants are plants that contain certain substances, certain healing compounds. Medicinal herbs are best used for prevention rather than treatment.This work aims to study the values of total phenolic content present in Calendula officinalis, Ginkgo biloba, Lycopodium clavatum, Equisetum arvense and in the tinctures. Their content will also be studied according to the storage period in order to signal any changes that may have occurred. The risks that can appear in the production of tinctures must be taken into acount. They could be of chemical nature, biological nature, physical nature. We mention here pieces of soil, plant debris, dust particles, pesticides, heavy metals, patogenic bacteria, fungi. Preventing the contamination of major tinctures relies on several methods: maintaining proper sanitation, using a high concentration of alcohol, and ensuring optimal humidity.***

Keywords: Calendula officinalis, Ginkgo biloba, Lycopodium clavatum, Equisetum arvense, total phenolic content, tinctures

1. INTRODUCTION IN MEDICINAL PLANTS AND TINCTURES

Medicinal plants have high antioxidant activity, and oxidative stress produced by reactive oxygen species that can cause serious deficiencies and lead to most diseases is reduced with their help according to [1]. Also the phenols protect the human body against the oxidative stress.

According to the source [2] Lycopodium clavatum contain important phenols as vanillic acid, coumaric acid, ferulic acid, syringic acid. Another source of phenols is Calendula officinalis. This plant contains flavonoids as quercentin, luteolin, kaempferol which protect the cells against oxidative stress [3].

Ginkgo biloba presents flavonoids, phenolic acids, organic acids, terpenoids [4]. Equisetum arvense present phenolic components which protect the plants againts pathogenic agents and UV radiation [5].

Tinctures are pharmaceutical products. Tinctures are obtained from hydro-alcoholic solutions and medicinal plants left to macerate in them according to [6].

Calendula officinalis is a plant that is predominantly found in the Mediterranean area. Being considered a medicinal plant, it is particularly effective against skin diseases and inflammation, like it is mentioned in the research presented in [7]. An important fact to mention is that this plant can be used in teas, creams, compresses, oils [8].

Lycopodium clavatum belongs to the Lycopodiaceae family, its spores are used in the treatment of rheumatic diseases, gastrointestinal diseases and neurological diseases such as Alzheimer's disease [9]. An important fact to mention is that this plant sustains the urinary sistem. Using Lycopodium clavatum was observed to eliminate kidney stones, like it is mentioned in the research presented in [10]. Lycopodium clavatum has anticancerogenic action. The studies show that extracts from this plant act against colon cancer [11].

Ginkgo biloba is considered a living fossil with important antioxidant properties, with a role in ensuring optimal blood circulation and improving memory [4].

In the study presented in [12] the mice which were treated with Ginkgo biloba leaves have improved their memory. This fact is very important for the treatment against aging.

Equisetum arvense is used to treat rheumatism, urinary system diseases, against alopecia [13]. The plant has significant amounts of silicon, which increase the production of collagen [14].

Tinctures obtained from these plants can also prevent and treat diseases of the oral cavity, diseases of the bone, muscular system [3].

2. FOLIN - CIOCALTEU METHOD APPLIED ON MEDICINAL PLANTS

Plants that contain specific chemicals or healing compounds are known as medicinal plants. It is better to utilize medicinal herbs for prevention than for therapy.

The purpose of this study is to determine the amounts of total phenolic content found in tinctures and in Calendula officinalis, Ginkgo biloba, Lycopodium clavatum, and Equisetum arvense. In order to indicate any potential modifications, their content will also be examined in relation to the storage time. It is necessary to

consider the potential hazards associated with the manufacture of tinctures. They may be physical, biological, or chemical in nature. Here, we discuss soil fragments, plant debris, dust particles, heavy metals, pesticides, fungus, and pathogenic bacteria.

Using a high alcohol concentration, maintaining good hygiene, and making sure there is enough humidity are all important ways to keep significant tinctures from becoming contaminated.

Like it is mentioned in literature, each experimental method has its first step the preparation of samples. Firstly, we let dry at room temperature for 14 days the following plants:

- Calendula officinalis,
- Lycopodium clavatum,
- Ginkgo biloba,
- Equisetum arvense

After the plants are well dried, we grind the samples.

The next step is to weigh 0,3 g of each sample on an analytical balance. Also, 30 ml of 80° ethanol was poured over each sample. After that, the samples are filtered. In the following figure are presented ethanolic plants extract:

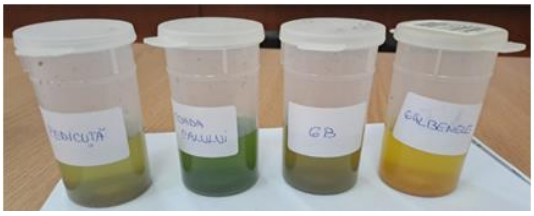

Figure 1. Ethanolic plants extract

The Folin - Ciocalteu Method has the following description: It is based on the reaction of the extract with the Folin-Ciocyanate reagent. The reagent is a mixture of phosphomolybdic acid and phosphotungstic acid. The acids are reduced in the presence of phenols to a complex of blue tungsten and molybdenum blue oxides [15].

Over the 25µl of extracts, 125 µl of 0.2N Folin-Ciocalteu reagent is added. For 5 minutes the mixture is incubated at room temperature.

Subsequently make up with 100 µl sodium carbonate solution and leave for 2 hours at room temperature. The absorbance of the reaction mixture is read at 760 nm using a Biotek Sinergy 2 multichannel spectrophotometer. A solution of methanolic gallic acid (0,001- 0,15 mg/mL) was used to make the calibration curve. The expression of the total phenolic content was performed in milligrams of gallic acid equivalent (GAE) per gram of plant dry weight. Each determination was performed three times [16].

The tinctures are made from Calendula officinalis, Lycopodium clavatum, Ginkgo biloba, Equisetum arvense and the alcoholic substrat, wine brandy or cherries brandy. We used 20 grams of each dry plant and 100 ml alcohol. The period of macerations are one month and six months. The same method, Folin - Ciocalteu is used for tinctures.

In the following figure is represented the Flowchart of tincture with wine with Calendula officinalis, Ginkgo biloba, Equisetum arvense, Lycopodium clavatum:

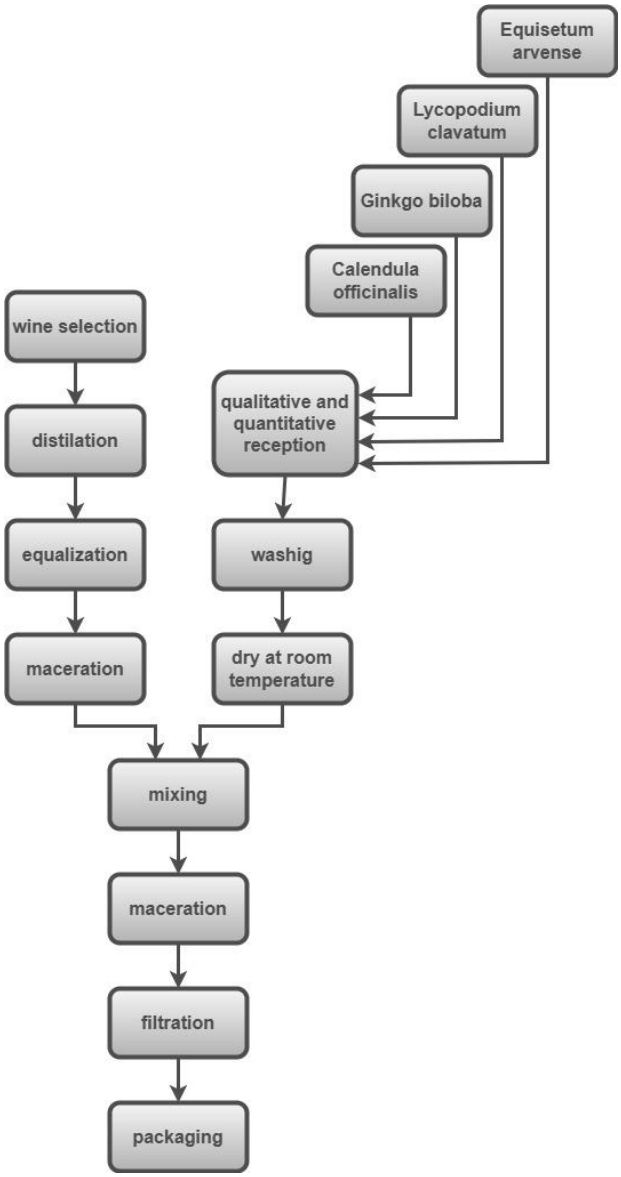

Figure 2. Flowchart of tincture with wine brandy with Calendula officinalis, Ginkgo biloba, Equisetum arvense, Lycopodium clavatum

In the following figure is represented the Flowchart of tincture with cherries bramdy with Calendula officinalis, Ginkgo biloba, Equisetum arvense, Lycopodium clavatum:

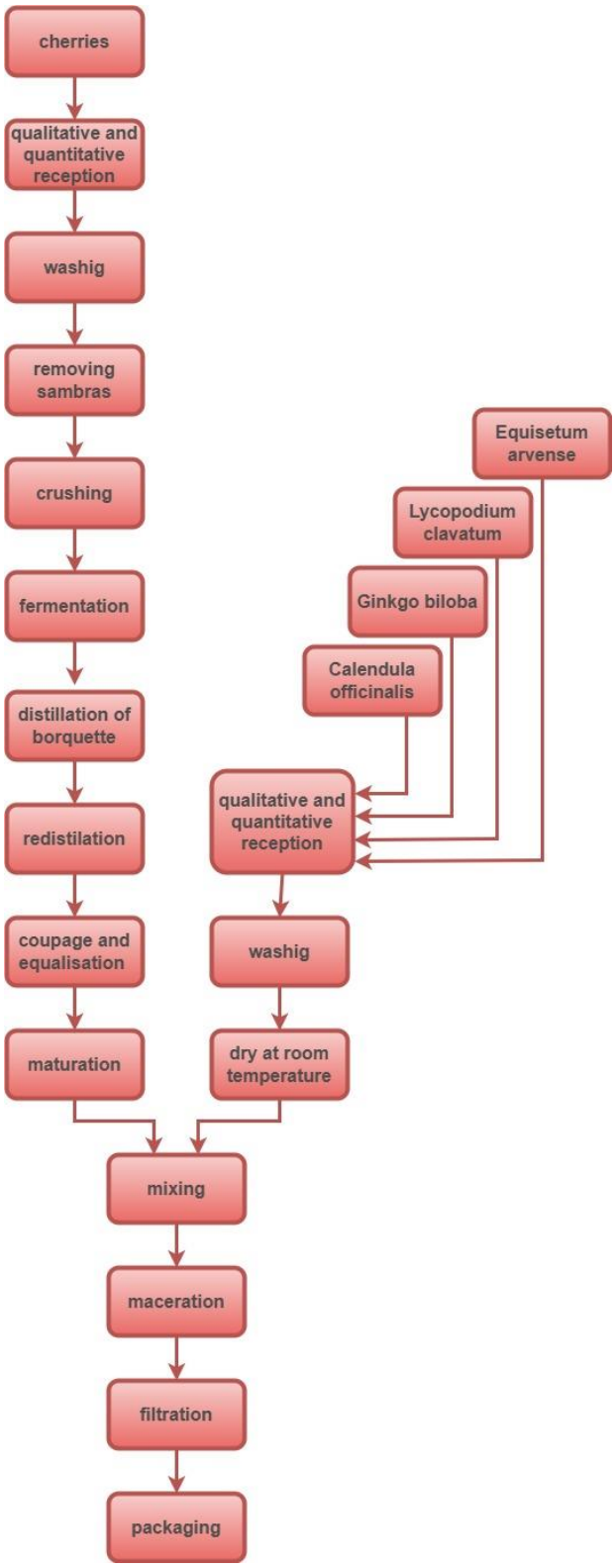

Figure 3. Flowchart of tincture with cherries brandy with Calendula officinalis, Ginkgo biloba, Equisetum arvense, Lycopodium clavatum

3. MEDICINAL PROPERTIES RESULTED FOR TINCTURES

According to Table 1, the highest values of total phenolic content were present in Ginkgo biloba extract, 1.707 mg GAE/ml, and the lowest in Equisetum arvense , 0.75 mg GAE/ml. Intermediate values were detected in Calendula officinalis, 1.484 mg GAE/ml and in Lycopodium clavatum extract, 0.758 mg GAE/ml.

The study presented in [17] showed an increased value for Calendula officinalis on dry matter of 21.23 ± 0.74 mg GAE/g SU. An average of 2,194 mg GAE/g DM was expressed in Lycopodium clavatum species [18]. A phenolic content of 1 .50 ± 0.13 mg GAE/g was measured in ethanolic extract of Lycopodium [19].

Equisetum arvense was distinguished by a total polyphenols value of 1.46%, [20]. Significant values were present in the study presented in [5] of 82.63 mg GAE/100 DW, in the case of Equisetum arvense plant. Total phenolic content in Ginkgo biloba sample marked values of 2.803 mg GAE/g FM, [21] slightly higher value than that obtained in the present study.

The study described in [22] presents a phenolic content for a methanolic extract of Ginkgo biloba of 76.0 ± 5.2 mg GAE/g dry weight. A value of 355.8 mg of GAE/g of the dried ethanol extract was also measured [23].

Table 1. Total phenolic content - extract.

Extract	Total phenolic content (mg GAE/ ml)
Calendula officinalis	1,484
Lycopodium clavatum	0,758
Ginkgo biloba	1,707
Equisetum arvense	0,75

Table 2. Total phenolic content - tinctures.

Tincture	Total phenolic content (mg GAE/ ml)
Tincture with wine brandy (one month maceration	2,408
Tincture with wine brandy (six months maceration	2,591
Tincture with cherries brandy (one month maceration)	2,181
Tincture with cherries brandy (six months maceration)	2,241

Table 2 shows the total phenolic content values of the tinctures. It is well known that storage period can influence certain chemical parameters. Thus a slight increase in phenolic content from 2,408 mg GAE/ml to 2,591 mg GAE/ml is observed in the tincture obtained with cherry brandy. The amount of phenolic content also changes in the case of the tincture made with wine brandy. A value of 2,181 mg GAE/ml at one month and 2,241 mg GAE/ml at six months is observed.

4. CONCLUSIONS

All the medicinal plants used in this study have an important phenolic content and also the tincture with cherry brandy and the tincture with wine brandy. Phenolic content and human health are closely linked. The purpose of this study was to determine the amounts of total phenolic content found in tinctures and in Calendula officinalis, Ginkgo biloba, Lycopodium clavatum, and Equisetum arvense. The period of maceration doesn't affect significantly the content. A good practice in the tincture's fabrication is necessarily for an increased content of phenols.

In order to be sure that we obtain these results, we must pay particular attention to the control measures used in the process of obtaining tinctures. Each step in this process must be treated with attention and the risks will be closely monitored.

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