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FULL PAPER

Comparison of heavy metals, secondary metabolites, and total polyphenols in hypericum perforatum l. and althaea officinalis L

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Hypericum perforatum L. and *Althaea officinalis L.* are medicinal plants of interest to the pharmaceutical, health, and food industry, as well as the study of their chemical composition is very important to evaluate the most influential components against their importance in the treatment of diseases. The purpose of this study was the comparison of the secondary metabolites, heavy metals, and total polyphenols. The heavy metals are determined by inductively coupled plasma mass spectrometry. The obtained extracts were analyzed using Gas Chromatography Flame Ionization Detector. Based on the result, it can be seen that the element that has the most difference in value is lead and cadmium, respectively 0.2025 mg/kg (Pb) and 0.375 mg/kg (Cd) for *H. perforatum L.*;0.41 mg/kg (Pb) and 0.142 mg/kg for *A.officinalis L.* The compounds that have the most differences compared to *Hypericum perforatum L.* and *Althaea officinalis L.* extracts are: Tricyclene (26.706%; 0.142%); alpha-Pinene (15.613%; 0.044%); beta-Pinene (2.262%; 0.132%); cis-beta-Terpineol (0.826%; 0.334%); Caryophyllene-E (9.398%; 11.122%); alpha-Humulene (5.920%; 10.480%); and Ledol (0.674%; 1.356%). The total polyphenol (TP) content of *Hypericum perforatum L.* is 7.25% (w/w) Gallic acid and 0.56% (w/w) Gallic acid for *Althaea officinalis L.*. As we mentioned, *H. perforatum L.* has a higher level of total polyphenols. Our results demonstrated that the two medicinal plants had a variety of chemical compounds with promising qualities that could lead to in-depth investigation into their possible uses in cosmetic and pharmaceutical fields. Further trials pertaining to the application of these two medicinal plants' essential oils and extracts may originate from this research.

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KEYWORDS

Althaea officinalis L.; chemical composition; heavy metals; Hypericum perforatum L.

Introduction

Hypericum perforatum L. - Chemical components Hypericum perforatum L., a perennial plant that grows worldwide, is referred to as "St. John's wort". Traditional medicine has been used it for many years to treat a wide range of conditions, such as sciatica, superficial wounds, mild to moderate depression, anxiety, and minor burns. The two primary active ingredients have been identified as hypericin, a naphtodianthrone,

and hyperforin, a phloroglucinol [1]. Various preparations (alcoholic or aqueous extracts) have identified seven types of bioactive natural components (phytochemicals) in the aerial sections of H. perforatum L. Phloroglucinols, anthraquinone derivatives, and naphthodianthrones According to Hadzhiiliev and Dimov (2015), flavonol glycosides include flavonoids, flavonols, glycosides, and flavones, as well as biflavones, amentoflavone (I3′, II8-biapigenin), catechins, phenylpropanes, proanthocyanidins, tannins, and xanthones [2,3].

About 65 percent of the steam distillates are composed of 2-methyloctane and α-pinene, according to a list of 29 components provided by Roth (1990). St. John's wort essential oils also contain typical terpenes, like monoterpenes (α-pinene and β-pinene, limonene, B-caryophyllene, myrcene, geraniol, germacrene D, B-farnesene, humulene), as well as larger amounts of hydrocarbons, alkanols, and undecane, n-undecane, n-nonane, n-tetradecanol, 2-methyloctane and 2-methyl-dodecane, C16 and C29 alkanes, C24, C26, and C28 alkanols, and 2-methylbutenol [4,5].

Althaea officinalis L. - chemical components

A. officinalis is commonly used to treat moderate gastritis, insect bites, skin burns, and irritation of the mucous membranes of the mouth, throat, and related dry cough. In addition, it treats burns, ulcers, abscesses, diarrhea, constipation, and inflammations. It is also used to treat catarrh of the mouth, throat, gastrointestinal tract, and urinary tract. Although it is native to Asia, Eastern Europe, and Northern Africa, *Althaea officinalis L.* is a member of the Malvaceae family, which is distributed throughout the globe. According to Ali Shah SM. *et al.* (2011), this plant thrives in humid and semi-humid environments and blooms pinkish-white from July to September [6]. The flower *Althaea officinalis L.* (ALOF) contains the following: starch (25–35%), mucilage (5%), saccharose (10%), pectin (11%), and tannins, scopoletin, asparagine, coumarins, and phytosterols; polyphenols (p-coumaric acid, hypolaetin-8glucoside, caffeic acid, isoquercitrin, kaempferol, genistein, daidzein, rutin, quercetin, and catechin) and tannins [7,8].
Knowing that the presence of heavy metals and the determination of total polyphenols have a high importance in the quality and safety of plant-based products, the purpose of this study was the comparison of the secondary metabolites, heavy metals, and total polyphenols found in *Hypericum perforatum L.* and *Althaea officinalis L.* [9].

Experimental

Materials

The following reagents were obtained from Sigma-Aldrich: gallic acid, nitric acid, hydrogen peroxide, hexane, and Folin-Ciocalteau (Sigma-Aldrich). The reagents were utilized for both oil extraction and microwave digestion. Inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies model 7800), analytical balance (Oahu's Corporation, USA), and digestion microwave (BERGHOF type Speedwave XPERT). Equipment made by Clevenger (Isolab Labogerate GmbH). AnalyticJena SPECORD 50 spectrophotometer with Nexis Gas Chromatography Flame Ionization Detector (GC-FID -2030).

Methods

The sample was chosen at random from Tirana's pharmacies. The aerial components of the plant (leaves) were selected for analysis. Samples were gathered, shed, and dried without exposure to sunshine. A grinder was then used to powder it. Before analysis, the powdered materials were stored at room temperature in a dry, airtight container. ICP-MS is used to determine the heavy metals. Steam distillation was used to remove the

essential oil from the leaves. Utilizing GC/FID, the extracted materials were examined. According to ISO 14502-1:2005, the Folin-Ciocalteau method was used to calculate the total amount of polyphenols [10].

Analysis of sample preparation by microwave digestion

The digestion of 0.5 grams of material (plant leaves) was the basis for this analysis. The sample was ground, and then put in a container with 6 milliliters of concentrated HNO_3 and 1 milliliter of 30% H_2O_2. After that, the container was put in the microwave, which used three running stages to carry out the heating plan.

After being digested, the samples were put into PTFE tubes and diluted with 2% HNO_3 to a final volume of 50 ml. Since nitric acid is the most effective oxidant for arsenic during microwave digestion, it is most commonly used. Nevertheless, some materials are better suited for hydrochloric acid. The indirect approach begins with a closed vessel's microwave digestion phase, which is followed by an instrument analysis [11].

Oil extraction

Utilizing Clevenger equipment, the oil was extracted from the leaves. Two plants' worth of leaves was cleared of dirt, rocks, and other foreign plants before being extracted. After 50 g of the plant was weighed and dried at 40 °C to a constant weight, the plant was removed for analysis. The plant was ground after it was dried and then it was exposed to steam. Using a ratio of 10:1 (ml/g) water/dry herb, steam distillation extraction was performed in a Clevenger apparatus. This involved placing 50 g of the sample into a 500-volumetric flask and adding water until the flask was filled [12].

Following the construction of the condenser-equipped Clevenger apparatus, they were put inside the circular flask to be heated in a heating bowl. To facilitate the cooling process as much as possible, the Clevenger system was linked to an ongoing water supply. The temperature is adjusted such that the water-vapor-containing oil flows into the graded distillation tube and the extra water falls back into the flask. After that, the water-plant mixture was subjected to distillation for the ideal amount of time- three hours, it turned out [13].

The Instrument optimization for GC-FID and ICP-MS

Analysis of essential oil was carried out in Nexis Gas Chromatograph GC-2030 with a data handling software Lab solution, equipped with a Split/Split less injector SPL 2030, On–Column injector unit OCI 2030, and Flame ionization detector FID-2030 [14]. The essential oil was extracted from the leaves of *Hypericum perforatum L.* and *Althaea officinalis L.* by steam distillation and chemically analyzed using GC/FID. 10 µl of essential oil was accurately weighed in a vial and 1000 µl of Hexane. Temperature program of the column: 60 °C for 1 min, 4 °C/1 min till 180 °C and 20 °C/1 min till 250 °C, total 40 min. A carrier gas, Helium column flow 1ml/min constant speed, total flow 14 ml/min split ratio 100:1. Injection volume 1 µl (10 µl syringe) [15].

For heavy metals analysis, a conventional Agilent 7800 ICP-MS was employed. An Agilent SPS 4 autosampler was used for sampling. The typical sample introduction system was set up in the 7800 ICP-MS (Inductively coupled plasma mass spectrometry) [16].The optimization of the instrument is done every day according to the conditions recommended by the manufacturer [17,18].

Determination of total polyphenols

Total polyphenols were determined using the Folin-Ciocalteau method refers to ISO 14502-

1:2005 [19]. Tea extract (0.5 ml) was diluted with distilled water up to 50 ml, and then 1 ml was taken and put into a test tube. Add 5 ml of Folin-Ciocalteau 10% (diluted using distilled water) followed by vortexing for 5 minutes, and then add 4 ml of 7.5% sodium carbonate solution. The sample was then stored in a dark room for 1 hour. The absorbance of the sample at a wavelength of 740 nm was measured using a UV-Vis Spectrophotometer. Total polyphenols were determined from the standard curve equation for Gallic acid with a concentration range of 10-100 mg/L [20].

Results and discussion

Data analytical methods for heavy metal analyses with ICP-MS

Four toxic heavy metals, including Pb, Hg, Sn, and Cd, were examined in total. These days, therapeutic plants are thought to be a source of heavy metal toxicity in both humans and animals. Tin, lead, cadmium, and mercury are the heavy metals most commonly associated with human harm [21]. The World Health Organization (WHO) sets limits for the presence of heavy metals in raw medicinal plants. However, most users do not check for heavy metal accumulation before using these plants. Heavy metals of the samples were analyzed using extracted oil samples by ICP-MS.

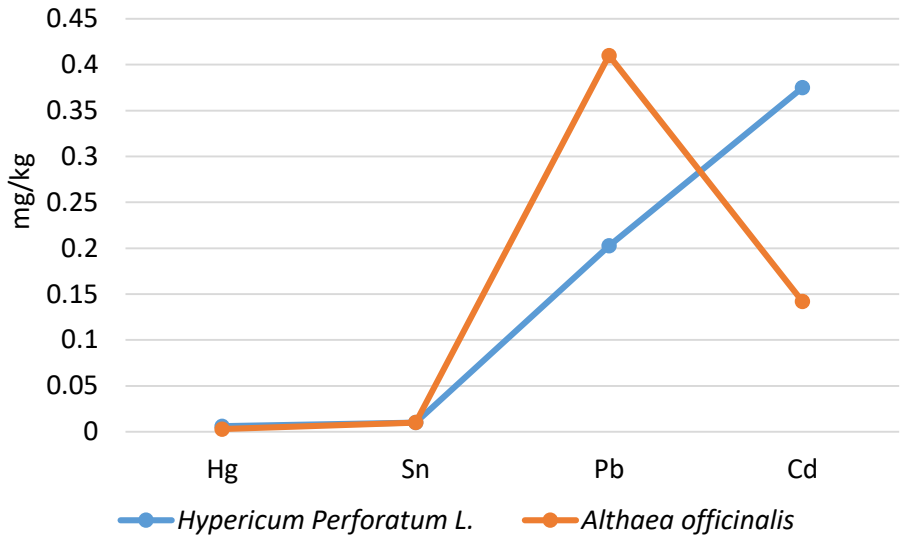

FIGURE 1 Distribution of the presence of heavy metals in Hypericum Perforatum L. and Althaea officinalis L. leaves

This study is designed to evaluate also four heavy metals by ICP-MS technics. According to the graph (Figure 1), you can see that the element that has the most difference in value is lead and cadmium, respectively:

a) Lead 0.2025 mg/kg for Hypericum perforatum L. and 0.41 mg/kg for Althaea officinalis L.

b) Cadmium 0.375 mg/kg for Hypericum perforatum L. and 0.142 mg/kg for Althaea officinalis L.

Mercury and tin values range from Hg (0.006 mg/kg) and Sn (<0.01 mg/kg) for H. perforatum L. while Hg (0.0029 mg/kg) and Sn (<0.01 mg/kg) for A. officinalis. All values are within standards determined by FAO/WHO.

Data analytical methods for profile analyses with GC-FID

The identification of secondary metabolites (Figures 2 and 3) was made by a comparing

their relative retention time and mass spectra with those of compounds published in literature and databases. The identified components were listed according to their retention time. The percentage composition of compounds was calculated according to their chromatographic peak area. The percentage of oil after extraction was 0.2 % for Hypericum perforatum L. and 0.4% for Althaea officinalis L.

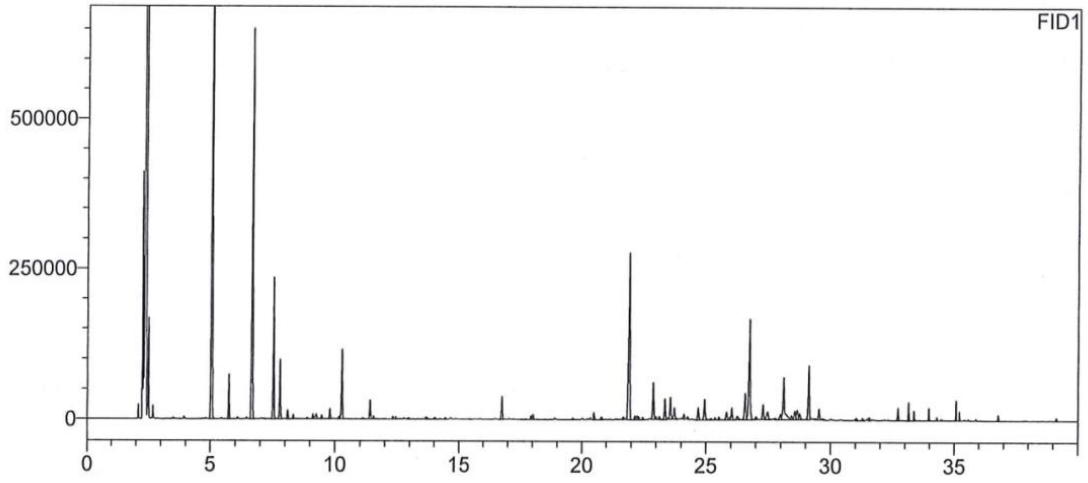

FIGURE 2 Hypericum perforatum L. chromatogram graph

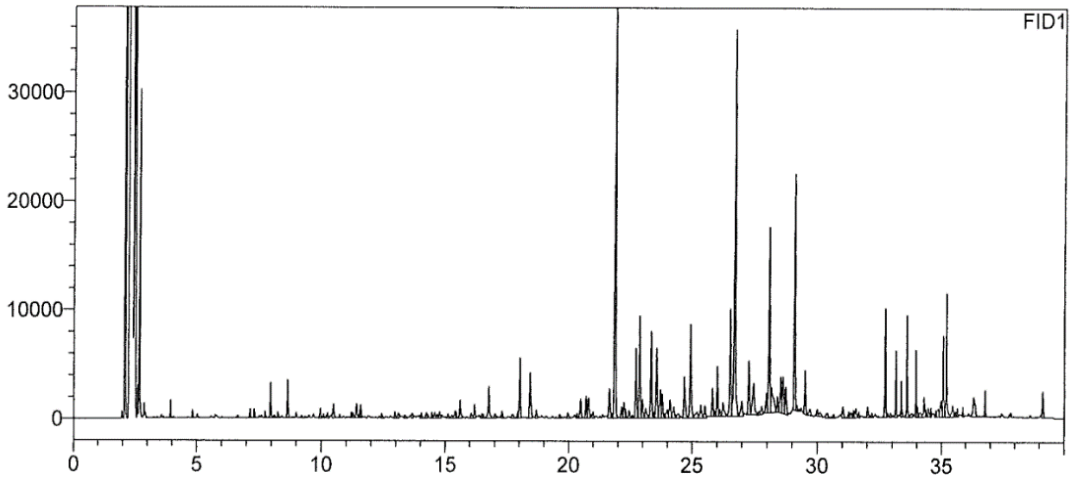

FIGURE 3 Althaea officinalis L. chromatogram graph

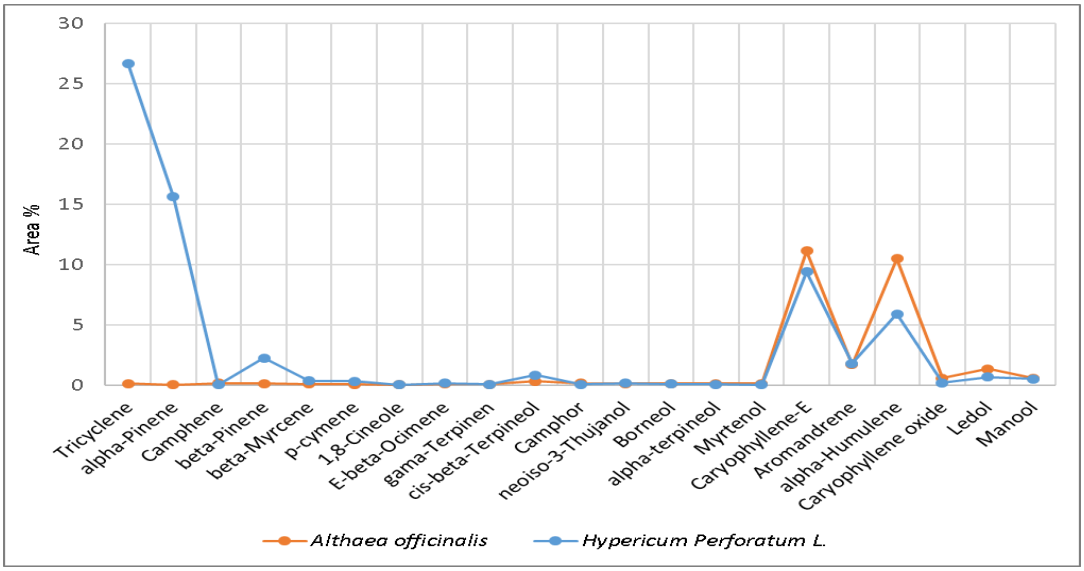

FIGURE 4 Distribution according to the area in percentage in the comparison between the same chemical compounds found in Hypericum Perforatum L. and Althaea officinalis L. extracts
From the analyses carried out it is noted that the compounds that have more differences

compared in both extracts are:
a) tricyclene: 26.706% for *H. perforatum L.* compared to 0.142% for *A. officinalis.*
b) alpha-Pinene: 15.613% for *H. perforatum L.* compared to 0.044% for *A. officinalis.*
c) beta-Pinene: 2.262% for *H. perforatum L.* compared to 0.132% for *A. officinalis.*
d) cis-beta-Terpineol: 0.826% for *H. perforatum L.* compared to 0.334% for *A. officinalis.*
e) Caryophyllene-E: 9.398% for *H. perforatum L*. compared to 11.122% for *A. officinalis.*
f) alpha-Humulene: 5.920% for *H. perforatum L*. compared to 10.480% for *A. officinalis.*
g) Ledol: 0.674% for *H. perforatum L.* compared to 1.356% for *A. officinalis*.
From these analyses (Figure 4), it emerged that the main components that were most abundant in *Hypericum perforatum L.* were tricyclene, alpha-Pinene, beta-Pinene and cis-beta-Terpineol. While in the *Althaea officinalis L.* Caryophyllene-E, alpha-Humulene, and Ledol dominated compared to H. perforatum. The main components of our study, β-caryophyllene and α-pinene, are similar to the main components of this study [14].

Total polyphenols of Hypericum perforatum L. and Althaea officinalis L.

As we mentioned above, the total polyphenols were determined using the Folin-Ciocalteau method refers to ISO 14502-1:2005. The total polyphenol (TP) content is 7.25% (w/w) Gallic for *Hypericum perforatum L.* acid and 0.56% (w/w) Gallic acid for *Althaea officinalis L.* As we mention, *H. perforatum L.* has a higher level of total polyphenols. The content of polyphenols in tea is always associated with good health benefits and has potential application in food, cosmetics, and pharmaceutical industries.

Conclusion

From result of two medical plant (*Hypericum perforatum L.* and *Althaea officinalis L.*) analysis we conduct:
- All values of heavy metals (lead, cadmium, mercury, and tin) analyzed are within standards determined by FAO/WHO. But the elements that has the most difference in value is lead and cadmium, where lead is more presented in *Althaea officinalis L.* and

cadmium is more presented in *Hypericum perforatum L*.

- The analyzed chemical compounds that have more differences in these plants are: Tricyclene, alpha-Pinene, beta-Pinene, and cis-beta-Terpineol which are mostly in *H. perforatum L.* while Caryophyllene, alpha-Humulene, and Ledol have the higher value in *A. officinalis*.
- The total polyphenol (TP) content is higher in *Hypericum perforatum L.* than in *Althaea officinalis L.*

Our findings demonstrated that the two medicinal plants under investigation had a variety of chemical compounds with promising qualities that could lead to in-depth investigation into their possible uses in cosmetic and pharmaceutical fields. Further trials pertaining to the application of these two medicinal plants' essential oils and extracts may originate from this study.

Limitation

The plants have been randomly selected in pharmacies in Tirana, being convinced that they are Albanian production. The limitation of this study is that the results are not comprehensive and that there is no data if the cultivation of the plants was done in the same habitat and in the same climatic conditions.

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Authors' Contributions

All authors are included in:

1- Data collection for oil extraction and Instrument optimization for GC-FID and ICP-MS.

2- Sample selection and processing, sample extraction and application of methods to identify the chemical profile, heavy metals, and total polyphenols in the laboratory.

3- The final version of the manuscript was approved by all authors.

Conflict of Interest

The authors declare no conflict of interest in this study.

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