

Extraction and Analysis of *Hypericum perforatum* L. from Turkey

E. Moroydor Derun, Z. Eslek, and S. Piskin

Abstract—*Hypericum perforatum* L. is a member of the Hypericaceae (Guttiferae) family and commonly known as St. John's wort. There is a growing interest in this medicinal plant because of the constituents of this genus. A number of species have been shown to possess various biological activities such as antiviral, wound healing, analgesic, hepatoprotective, antimicrobial and antioxidant activities and also have therapeutic effects on burns, bruises, swelling, anxiety and mild to moderate depression.

In this study, the aerial parts of *Hypericum perforatum* L. are extracted and the main and effective constituents are determined. The analysis of the extracts was performed by GC-MS and LC-MS. As a next step, it is aimed to investigate the usage of the main constituents of the medicinal plant.

Keywords—Hypericaceae, *Hypericum perforatum* L., GC-MS, Guttiferae, LC-MS, Medicinal plant, St. John's wort.

I. INTRODUCTION

In recent years there has been growing interest in the usage of medicinal plants. This is due to several reasons, conventional medicine can have side effects and incorrect usage of synthetic drugs result in complications, and the large percentage of world's population do not have access to conventional pharmacological treatment.

A medicinal plant is any plant used in order to relieve, prevent or cure a disease or to alter psychological and pathological process, or any plant employed as a source of drugs or their precursors [1].

More than 300 species of the genus *Hypericum*, belonging to Hypericaceae (Guttiferae family), grow in the warm and temperate regions of the Earth. Among these species, *Hypericum perforatum* L. is traditionally used as a medicinal plant. It is a perennial herb that is often found in disturbed areas [2]. *Hypericum perforatum* L. (also named as St. John's wort) is a valuable, most frequently used and one of the best known herbs in recent years [3]. It has been used since Ancient Greece as a valuable folk remedy but has become one of the most important medicinal plant in the 20th century. Today, St. John's wort is best known for its use in the treatment of mild- to -moderately severe depressive disorders [4].

There is a growing interest in this herb because of its constituents. Most of the constituents in *Hypericum*

perforatum L. have shown various biological activities and have therapeutic effects on bruises, burns, swelling, anxiety and mild to moderate depression. The herb also has antidepressant, antiviral, antioxidant, antimicrobial wound healing, analgesic and hepatoprotective activity [5].

Also, this medicinal plant is a source of naphthodianthrone, flavonoids, xanthenes, tannins and essential oil [6].

The result of several studies showed that two of most active ingredients are hypericin and hyperforin. The structures of these ingredients are shown in Fig. 1 (a) and (b).

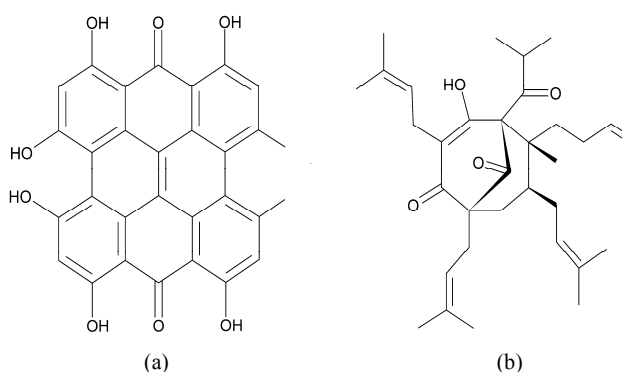


Fig. 1 The chemical structure of the two biologically active compounds of *Hypericum perforatum* L. (a) Hypericin (b) Hyperforin

Previous studies showed that the naphthodianthrone (hypericin) and pseudohypericin are responsible for the antidepressant activity of the plant. Also, some recent studies showed that phloroglucinol hyperforin and its derivatives inhibit various neurotransmitter receptors. Naphthodianthrone are generally stable because of their aromatic conjugation but phloroglucinol compounds, conversely, are unstable and sensitive to oxidation in explosion of air and light. Besides these constituents St. John's wort contains flavonoids (hyperoside, quercetin, rutine), essential oils (α -, β - pinene, humulene etc.) and other active components [7].

The essential oil of the herbs are also very important and contain a large variety of volatile secondary metabolites such as terpenes, terpenoids, phenolic and aliphatic derivatives. Generally they are characterized by a strong odor. The essential oil of *Hypericum perforatum* L. is liquid, strong odoured yellowish oil which is soluble in lipids or organic solvents and has a lower density than water [8].

There are several methods for extraction of essential oils of medicinal plants. Some of the best known are low or high-pressure distillation employing boiling water or hot steam,

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Soxhlet extraction, supercritical fluid extraction (by carbon dioxide) and microwave extraction. Hydrodistillation is done by an apparatus named Clevenger and recommended by several Pharmacopeias for the quality control of raw plant material in order to evaluate essential oil yield and the typical volatile constituents. Fig. 2 shows the Clevenger apparatus used for hydrodistillation of medicinal plants. Generally essential oils are characterized by gas chromatography (GC) and mass spectrometry (MS) techniques [8].

In this study, the medicinal plant *Hypericum perforatum* L. was extracted by 3 methods: 1) by hydrodistillation using a Clevenger apparatus, 2) by Soxhlet extraction method and 3) by distillation method. The extracted main and effective constituents were determined and analyzed with GC-MS and LC-MS.

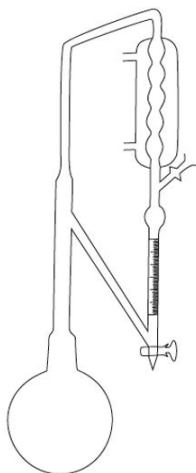


Fig. 2 Clevenger- type apparatus [9]

II. EXPERIMENTAL PROCEDURE

A. Plant Material

Aerial parts of the *Hypericum perforatum* L. (Fig. 3) were collected from Dörtyol, Hatay (Turkey) in August 2012 and dried in the shade at the room temperature. The voucher specimens of the plant were deposited in the Laboratory of Nanotechnology and Supercritical Fluid Technology, Faculty of Chemical and Metallurgical Engineering, Istanbul, Turkey.



Fig. 3 Aerial Parts of *Hypericum perforatum* L.

B. Extraction of Essential Oil by Hydrodistillation Method

The aerial parts of the plant were dried in shadow at room temperature and crushed into small particles with a grinder. 60 grams of powdered plant material were hydrodistilled with a Clevenger-type apparatus for 4 hours. Powdered plant material and Clevenger-type apparatus are shown in Figs. 4 and 5, respectively. The yellowish oil was dried over anhydrous sodium sulphate and stored at 4°C until it was analyzed. The analysis of essential oil extracted by hydrodistillation was done by GC-MS.



Fig. 4 Powdered plant material

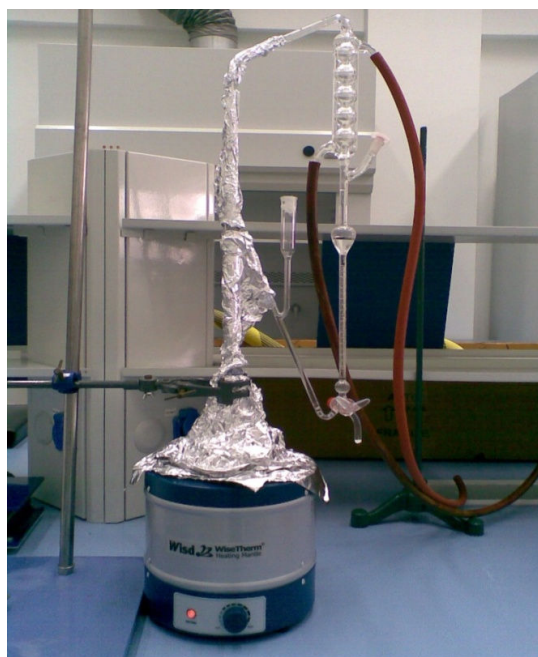


Fig. 5 Clevenger-type apparatus used in laboratory



Fig. 6 Soxhlet extractor

C. Extraction of Essential Oil by Soxhlet Extraction Method

10 grams of powdered plant material was extracted by the Soxhlet extractor shown in Fig. 6. The solvent used in the extraction process was ethanol and the temperature is 210°C. The extraction time was 2 hours and 20 minutes. In the first hour of the experiment, the plant material in the cartridge was immersed into solvent and in the second hour of the experiment, the plant material was washed by the solvent continuously. In the last 20 minutes, the solvent was recovered and extract was acquired purified from solvent. The extract was stored at 4°C until analysis. The analysis of the essential oil isolated was done by LC-MS.

D. Extraction of Essential Oil by Conventional Distillation Method

30 grams of powdered plant material was extracted using a conventional distillation process (Fig. 7). The extraction time was 4 hours. The yellowish oil was obtained in water, dried under anhydrous sodium sulphate and stored at 4°C until it was analyzed.

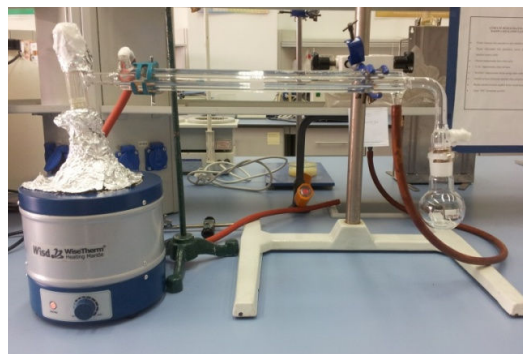


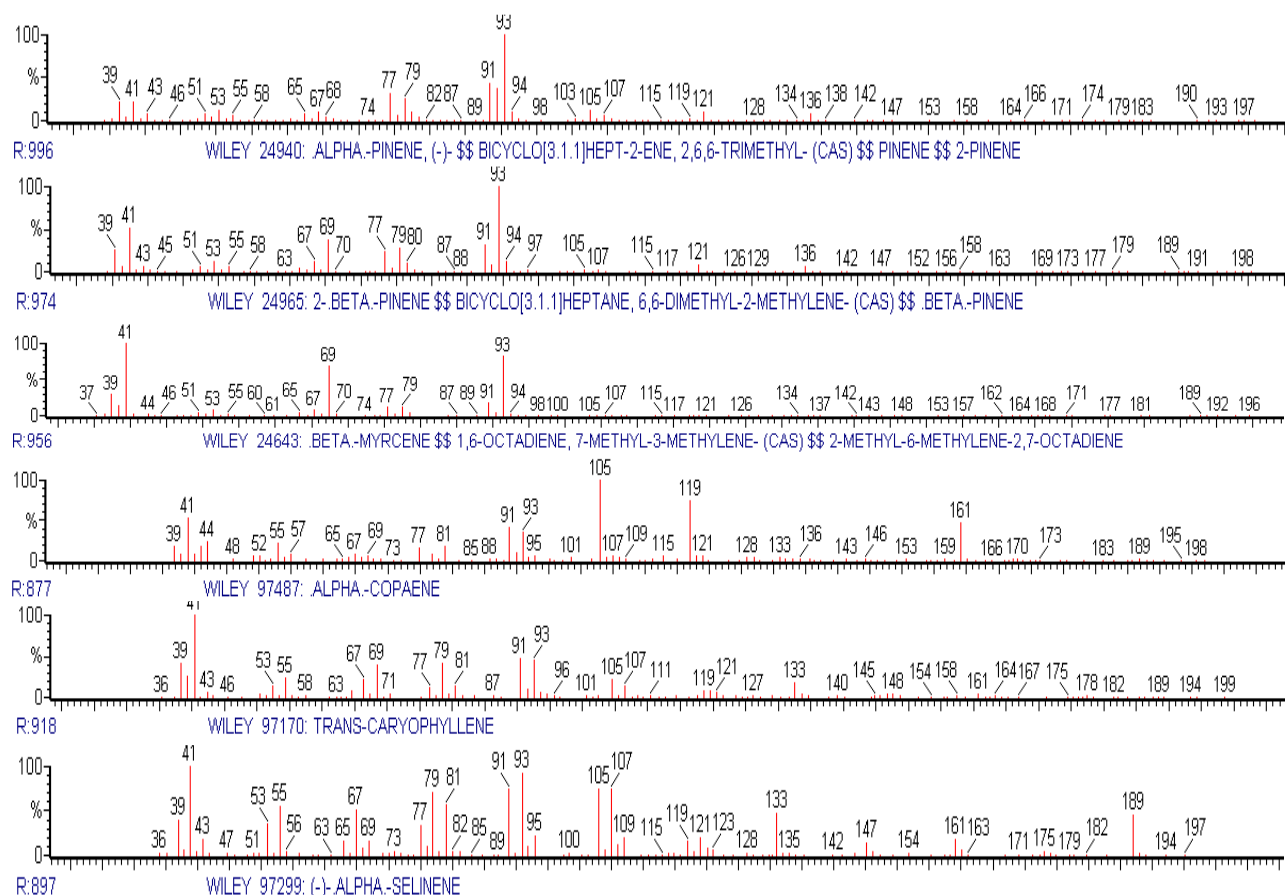
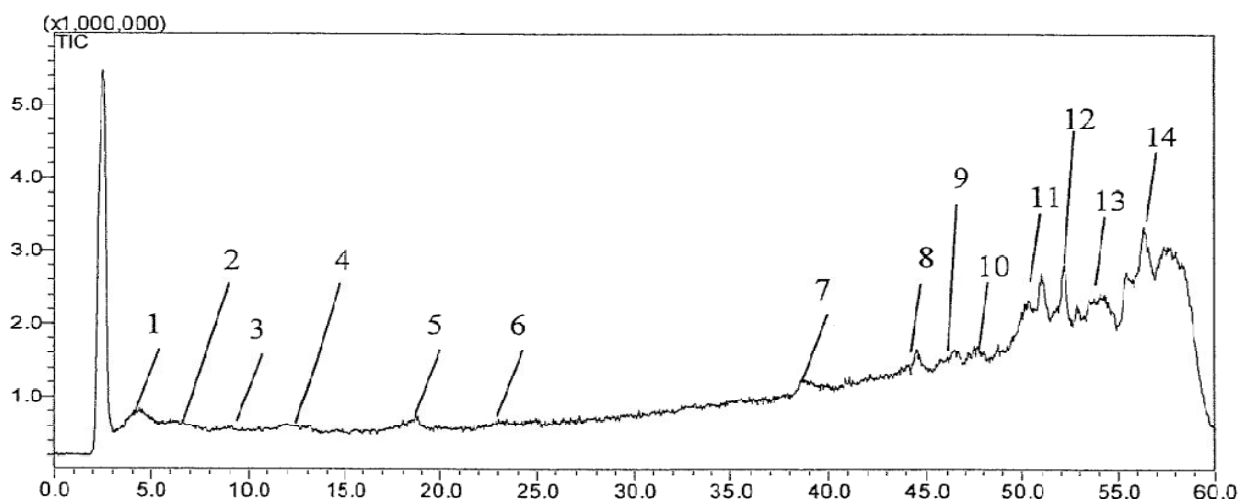
Fig. 7 Conventional distillation process

III. RESULTS AND DISCUSSION

A. Identification of Essential Oil Using GC-MS

GC-MS analyses were performed on a Perkin Elmer Clarus 500 MS instrument interfaced with electron ionization detector. A SGE ID-BPX5 capillary column of 0.25mm ID, 0.25µm film thickness and 30 m length was used with helium as the carrier gas. The initial temperature of the column was 50°C for 2 minutes, grew to 140°C with a 7°C/min rate and hold at this temperature for 2 minutes, then grew to 280°C with a 10°C/min rate, hold 10 minutes at this temperature. m/z interval was 35-400 and hold time was 40 minutes. The injection was performed in split mode (50:1) at 250°C. The identification was performed using the Wiley and Nist mass spectral library available on the data system and using literature.

GC-MS results showed that the main constituents of the essential oil of *Hypericum perforatum* L. were terpenes such as α -pinene, 2- β -pinene, β -mycrene, α -copaene, (*E*)-caryophyllene (sesquiterpene), α -selinene (sesquiterpene). The GC-MS graphics of these constituents are shown in Fig. 8. There are also hydrocarbons such as nonane, decane, 2,6-dimethyl decane, undecane and hexanal as an aldehyde in the GC-MS analysis of the essential oil.

Fig. 8 GC-MS graphics of the terpenes in *Hypericum perforatum* L.Fig. 9 LC-MS graphics of the active ingredients in *Hypericum perforatum* L.

B. Identification of Essential Oil Using LC-MS

LC-MS experiments were performed on a Teknokroma Tracer Excel ODS-A column. The mobile phase consisted of 10mM ammonium acetate (A) and a 55% methanol- 45% acetonitrile mixture (B). Flow rate, temperature and

retention time were 0.2mL/min, 30°C and 60min, respectively.

LC-MS results showed that the main constituents of the extract of *Hypericum perforatum* L. were quercetin, hyperoside, adhyperforin, coffeoylquinic acid, hyperforin

and quercitrin. The LC-MS graphics of these active constituents are shown in Fig. 9.

IV. CONCLUSION

Medicinal plants are recently used mostly because of their slight adverse effects than synthetic drugs. They have antimicrobial, antioxidant, antianalgesic effects and many like these. Therefore in this study, the active ingredients of *Hypericum perforatum* L., a valuable medicinal plant, were investigated. According to experiment results, it was seen that the most active and abundant ingredients of the plant were: α -pinene, 2- β -pinene, β -mycrene, α -copaene, (*E*)-caryophyllene, α -selinene, quercetin, hyperoside, adhyperforin, coffeoylquinic acid, hyperforin and quercitrin. The studies on this and some other medicinal plant are going on. These experimental studies will shed light on studies in medicine.

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