GC-MS Profiling of Essential Oils from Aerial Parts of Hypericum triquetrifolium Turra using ITEX and HD Methods

Hoshyar A. Azeez

1Department of Medical Laboratory Science, Sulaimani Polytechnic University, Sulaimani, Kurdistan Region, Iraq.

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ABSTRACT

Hypericum (H.) triquetrifolium contains numerous bioactive molecules in the aerial parts. Such molecules have numerous biological activities, including antioxidant, anti-inflammatory, antidepressant, antimicrobial, antiviral, and anticonvulsant compounds. Despite the presence of H. triquetrifolium in Iraq, no previous studies have examined the biochemical contents of plant in the region. This study is carried out to evaluate the variability of the essential oil profile of aerial parts of Hypericum triquetrifolium Turra. Samples were also analyzed using gas chromatography-mass spectrometry (GC-MS) with two different extraction methods, namely in-tube extraction (ITEX) dynamic headspace and hydrodistillation (HD). A total of thirty-three, forty-three, and thirty-nine compounds were identified in stem, leaves, and flowers, respectively. Monoterpene hydrocarbons were the main compounds in the essential oil extracted from different parts. The highest constituent detected in the ITEX/GC-MS in stem parts was alpha-pinene (20.47%). On the other hand, the predominant compound of the essential oil detected by the HD method was cubenol (26.72%). The essential oil extracted from leaves by ITEX and HD methods showed 43 compounds, ITEX/GC-MS detected 32 products in the essential oil of leaves; vubenol was the highest (23.64%), and Using ITEX and HD/GC–MS, a subset of 39 chemical components in H. triquetrifolium essential oil were detected in the flower. Twenty-five out of 39 compounds, were analyzed by ITEX/GC-MS. The main constituent was 3-methyl-nonane (27.76%). HD/GC-MS method was characterized by detecting 23 components in flower oil. The major chemical structure identified in the essential oil in flowe by HD/GC-MS was the monoterpene hydrocarbons represented by alpha-pinene (18.39%). Our data indicates that the ITEX method is more accurate than HD in the separation of essential oil components in this plant.

Keywords: Hypericum triquetrifolium, ITEX/GC-MS, HD/GC-MS, Essential oil

1. Introduction

Several species belong to the H. genus is recognized to have a valuable worldwide effect as traditional medicinal plants. In particular, the species H. triquetrifolium is defined as a perennial herb, which is widely branched along most of the length, the inflorescence is yellow, pyramidal shape, and all aerial parts are covered with typical dark glands[1]. This species (H. triquetrifolium) is also characterized with a wide range of ecological amplitude that enable them to grow under various environmental conditions. This species is found in abundance along roads, as a weed in fields and meadows[3]. H. triquetrifolium is a very common plant In Iraq. It usually grows in the lower forest zone; in the moist steppe zone, and it is found on rocky mountain slopes, stony hillsides, and stepping plains, sometimes near streams, in vineyards, gardens, and fields, on sandstone, clay, loam, gravel[3]. The traditional medical uses of H. triquetrifolium are consequences of their contents with various bioactive molecules, including; naphthodianthrones, hypericin and pseudohypericin[4], hyperforin and adhyperforin[5], essential oils[6], xanthones[7], phenolic compounds (tannic acid, p-OH-benzoic acid, chlorogenic acid, and caffeic acid), procyanidins, and other water-soluble compounds[8], which have various biological values; namely antioxidant, anti-inflammatory, antidepressant, antimicrobial, antifungal, antiviral, and anticonvulsant activities.

Essential oils in various plant parts are the most important bioactive compounds characterized by their odorous principles. Such oils evaporate when they are exposed to the air. Essential oils are usually present in specialized secretory structures, including glandular hair, modified parenchyma cells and oil tube called vittae. These oils are mostly produced by either a direct function of the protoplasm through the decomposition of the resinogenous layer of the cell wall, or an indirect way by the hydrolysis of certain glycolysis[9].
In several species, the variability of the essential oil profile occurs due to the effect of different factors, including developmental stages and plant organs maturation[10, 11], climate conditions[12], and soil texture[13]. Contents and compositions of essential oils in Hypericum plants are also affected by harvesting time[14, 15], the organ type, and the condition of biomass growth[16].

Several methods, such as hydrodistillation (HD), Solid Phase Microextraction (SPME) and The In-Tube Extraction Technique (ITEX), are used to extract essential oils from different parts of the plant. The quantitative and qualitative of extracting essential oils differ according to the extraction technique. The ITEX dynamic headspace has become a good alternative extraction method for a small amount of plant material in comparison with the classical one HD. The ITEX separates volatile compounds from a liquid or solid composition prior to GC analysis; and this feature is of importance for those samples that cannot be directly injected into the GC[17]. The main objective of the current study is to compare and evaluate variations of essential oils in H. triquetrifolium stems, leaves and flowers collected by ITEX and HD methods using GC-MS.

2. Materials and Methods

2.1 Plant Material

Stem, leaves, and flowers from 8-10 plants of H. triquetrifolium Turra were randomly collected during plant development from July – October in an experimental field located on the road in the Peramagrun mountain district of Sulaimania province in the North – East of Iraq at an altitude of (1150 m.a.s.l.). Stems, leaves, and flowers were individually separated for the biochemical studies.

In light of the morphological patterns described in Flora of Iraq, the plant was botanically identified by Dr. Saman A. Ahmad., Filed Crops Dept., College of Agricultural Engineering Sciences, Sulaimani University. Voucher specimens are kept at the Kurdistan Botanical Foundation Herbarium (ESSE 14682). Following the harvest of plant materials; they were air-dried at room temperature (22 ± 2 °C) for seven days, and followed by the identification of essential oil contents using ITEX / GC-MS and HD/GC-MS.

2.2 Isolation of the Essential Oil

2.2.1 ITEX Method

The current study was conducted at the Institute of Life Sciences (Institutul De Stiintele Vietii) at the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca-Romania. All other parameters were constantly maintained for all samples: syringe temperature was set at 60°C; agitation speed was set at 500 RPM; the final extraction volume was 1000 µL; extraction speed was set at 100 µL/second; temperatures of desorption and trap cleaning were at 200°C and 250°C respectively; and trap cleaning time was set at two minutes. The ITEX fiber used in this study was the ITEX-II Trap (G23) -SilicoNert 2000. Tenax TA 80/100 mesh, fiber. Following optimal incubation, a dose of 250 µL headspace sample was injected into the GC-MS.

2.2.2 Hydrodistillation method

The essential oil of the stems, leaves, and flowers of H. triquetrifolium was determined via hydrodistillation using a Cleverenger-type apparatus. A quantity of 30 grams of dried samples were placed in 450 mL distilled water, and then placed on a heater adjusted at 60°C for 180 minutes since the distillation began. The volatile oil was dried over anhydrous sodium sulfate to remove water from the oil and stored in a sealed vial at 4°C until analysis[18].

2.3 Identification and Quantification by GC-MS

The current study was investigated at the Food Science and Technology Dept., USAMV-Cluj-Napoca-Romania. The analyses of samples were carried out using GC-MS (Shimadzu GC-MS QP-2010 model) equipped with an AOC-5000 autosampler (Combi PAL). A Zebrone ZB-5ms column of 50m x 0.32 mm i.d. and 0.25 µm film thickness was used for the analyses. The setup of the analysis was made by using injector temperature at 250 °C; pressure at 93.1 kPa; linear velocity of 44.0 cm/second; split ratio 1:200; carrier gas-helium; detector MS; ion source temperature 250.0 °C; interface temperature 250.0°C; MS mode EI, scan range 50-400u; scan rate 2000u/second. The program for column oven temperature was: 60 °C (3 minutes) to 160 °C at 3 °C/minute to 150 °C (10 minutes). Separated compounds were identified by comparing the detected mass spectra of our samples with their equivalent mass spectra libraries of NIST27 and NIST147.

3. Results and Discussion

In the present research, different parts (stems, leaves and flowers) of H. triquetrifolium Turra with native distribution in Iraq were gathered and subjected to ITEX and HD/GC-MS analysis of their compositions of essential oils (Tables 1, 2 and 3 in the supplementary file). Our data exhibited that the separation and identification of the essential oil compounds analyzed by the ITEX/ GC-MS method is more sensitive than HD in all plant parts.

3.1 Essential oils profile in leaves

The essential oil extracted from leaves of H. triquetrifolium by ITEX and HD methods revealed the presence of 43 compounds (Figure 1). The ITEX/GC-MS detected 32 products, including the essential oil of leaves, which were categorized into five groups: three monoterpane aldehydes, twenty-four monoterpane hydrocarbons, one monoterpane alcohol, two sesquiterpenes and one monoterpane acetate while HD/GS-MS analyzed 16 compounds which distributed into four class: one monoterpane aldehydes, twelve monoterpane hydrocarbons, one monoterpane alcohol, two sesquiterpenes, one monoterpane hydrocarbon and one monoterpane alcohol.
alcohol and two sesquiterpenes (Figure 2). Nineteen components were detected in leaves, and considered as unique compounds. The considerable components of the essential oil of leaves collected by ITEX/GC-MS included (E)-2-hexenal (12.63%), 2,3,3-trimethyl-octane (11.36%), 2,2,3,3-tetramethyl-hexane (6.42%), alpha-pinene (5.75%), 7-methyl-pentadecane (9.79%) and undecane (6.15%). Cumene (7.5%), 3-methyl-nonane (6.85%), vubenol (23.64%), beta-humulene (6.70%) and cadalene (5.85%) were better extracted by the HD method. The insolubility of these compounds could explain their absence or presence in the extraction methods. Only; 2,3,3-trimethyl-octane, alpha-pinene, gamma-muurolene, delta cadinene, calamenene and 2,5-diphenyl-1,4-benzoquinone were obtained and detected by the two methods. The lowest component, namely cis-linalool oxide is possibly produced by the Maillard reaction that leads to the thermal degradation of carbohydrate[19]. Nevertheless; the availability of hydrocarbon compounds is considered as the characteristic product of Hypericum triquetrifolium, which confirms the results of[20-22]. The leaves of H. triquetrifolium collected from Calabria were analyzed for their composition of essential oil, and they found that nonane (8%), beta-pinene (8%), alpha-pinene (13%), myrcene (16%), beta-caryophyllene (5%), germacrene-D (10%), sabine (13%) and caryophyllene oxide (5%) were predominated compounds which were partially different from our results. It has been identified that two compounds of essential oils extracted from leaves by the HD method, namely n-tetradecane (25.70%) and alpha-himachalene (27%) as major components, which is not in concurrence with our results[23]. The difference between our results and researchers; may be related to the condition of cultivation, time of sample collection and genotypical patterns of samples. The influence of environmental factors on the oil composition contents has already been reported and suggested that the variations in the essential oils profile of plant species can be affected by seasonal alteration, geographic sharing, and growth cycle[24].

![Figure 1: Chemical components of the essential oils in leaves of H. triquetrifolium extracted and detected by ITEX-GC/MS and HD/GS-MS.](image1)

![Figure 2: Class concentration of compounds extracted and detected by ITEX-GC/MS and HD/GS-MS of H. triquetrifolium leaves.](image2)

### 3.2 Essential Oils Composition in Stems

A total (of 21 and 15) oil products were separated from the stem by ITEX and HD methods, respectively (Figure 3). In the ITEX/GC-MS analysis, the 21 identified components were stored in four groups, including one monoterpenic aldehyde, sixteen monoterpenic hydrocarbons, one monoterpenic ketone, and three monoterpenic alcohols (Figure 4). Therefore, most compounds detected in the Hypericum volatile oil were monoterpenic hydrocarbon compounds (89.81%). The main compounds detected by this method on the stems were alpha-pinene (20.47%), 2,4-dimethyl-hexane (11.52%), 2,3,3-trimethyl-octane (10.74%), camphene (9.79%) and undecane (9.28%) 1,3-dimethyl-benzene (5.62%). On the other hand, the number of compounds found in the HD/GC-MS was 15 in the stem. The identified products in this method were classified into three classes: monoterpenic hydrocarbon (13 compounds), monoterpenic alcohol (1 compound) and monoterpenic ketone (1 compound) (Figure 2). The monoterpenic hydrocarbon fractions were existing with a large amount of the essential oil (49.70%). Major compounds of the essential oil detected by this method were cubenol (26.72%), cumene (16.56%), 3-methyl-Nonane (7.87%), humulene (7.23%), and cadalene (6.67%). Also, the major oil components detected by the ITEX method in the stem are not present in oil products identified by the HD/GC-MS. The essential oil of H. triquetrifolium contains compounds of interesting biological properties. Previous studies stated that alpha-pinene is used as an anti-inflammatory, and in the treatment
of arthritic diseases\cite{25,26}. Camphene exerted antitumor activity\cite{27}. Therefore, humulene has anti-inflammatory activity\cite{28}.

Comparing the essential oil composition in this study and previous researches revealed some qualitative and quantitative differences. It is concluded that some notable compounds such as n-tetradecane (21.30%), alpha-himachalene (14.20%), tricyclene (10.70%) and cyclogeraniol acetate (7.60%) that were characteristics of a previous study (Jaimand K, Rezaee MB, Naderi M & Azadi R, Karimi SH 2012) were not identified in the present work. The flower of \textit{H. triquetrifolium} from Calabria was studied for its composition of essential oil, and they found that nonane (15%), germacrene-D (13%), caryophyllene oxide (12%), beta-caryophyllene (11%), alpha-pinene (10%), myrcene (5%), beta-pinene (4%), sabine (3%) were the predominant constituents\cite{29}. These differences between our results and previous studies with regards to the composition of essential oil may be due to the condition of growth, time of sample collection and genotypes of underlying samples. Moreover, some authors stated that the difference of chemical compositions of essential oils observed in \textit{H. perforatum} depending on the plant organs examined\cite{29}. The essential oil of \textit{H. triquetrifolium} contains some compounds owning interesting biological properties. Tricyclene was found to be useful in the treatment of temporomandibular\cite{30}. Caryophyllene oxide is considered an antifungal agent\cite{31}.

Previous studies communicated that the volatile oil having a large amount of \(\alpha\)-humulene and germacrene D exhibited an exceptional cytotoxic influence to the human tumor cell line\cite{32,33}. 

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Figure3}
\caption{Constituents of the essential oils in stem of \textit{H. triquetrifolium} Turra detected by ITEX-GC/MS and HD/GC-MS.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Figure4}
\caption{Class concentration of constituents isolated and detected by ITEX/GC-MS and HD/GC-MS of \textit{H. triquetrifolium} stems.}
\end{figure}

\subsection*{3.3 Essential Oil Composition in Flowers}

A subset of 39 chemical components in \textit{H. triquetrifolium} essential oil were identified by ITEX and HD/GC–MS. Twenty-five out of 39 compounds were analyzed by ITEX/GC-MS (Figure 5). The chemical classes of compounds present in the oil of flowers were monoterpenes aldehyde (2 compounds), monoterpenes hydrocarbon (16 compounds), monoterpenes ketone (2 compounds), monoterpenes alcohol (3 compounds) and sesquiterpenes (4 compounds) (Figure 6). The constituents identified in the essential oil of flowers by ITEX/GC–MS, being the principal components, are 3-methyl-nonane (27.76%), 2,3,3-trimethyl-octane (20.94%), 7-methyl-pentadecane (15.42%), alpha-pinene (14.44%), 2,4,6-trimethyl-octane (8.61%), and dodecane (6.99%) while the minor constituents in oil were delta-cadiene (0.10%), ylangene (0.11%), 2,3,5-trimethyl-decane (0.11) and 2-methyl-5-(1-methylethenyl)-2-cyclohexene-1-ol (0.11%). The HD/GC-MS method characterized 23 chemical components in oil of the flower (Table 2 and Figure 2). The major chemical structures identified in the essential oil of the flowers by HD/GC-MS were the monoterpenes hydrocarbons represented by alpha-pinene (18.39%), 7-methyl-pentadecane (6.26%) and gamma-murolene (6.25%) and sesquiterpene represented by caryophyllene oxide (10.84%) and germacrene D (4.17%). Two major compounds, namely alpha-pinene and 7-methyl-pentadecane were identified by two methods. The composition of the essential oil in the flowers showed 25 unique compounds.
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**References**


