Assessment of Authenticity of Market Samples of *Hypericum* using Phytochemical Fingerprinting Tools

Mohammad Ali Farboodniay Jahromi a, Mohammad M. Zarshenas a,b† and Nastaran Babaei Rizvandi b

a Medicinal Plants Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran. 
b Department of Phytopharmaceuticals (Traditional Pharmacy), School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

Authors’ contributions

This work was carried out in collaboration among all authors. The study conception and design were performed by authors MMZ and MAFJ. Acquisition of data was carried out by author NBR. Analysis and interpretation of data were done by authors MAFJ, MMZ and NBR. Drafting of manuscript was carried out by authors MAFJ and MMZ. The critical revision of the manuscript was made and completed by author MAFJ. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2022/v33i230448

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/82573

Received 20 November 2021
Accepted 25 January 2022
Published 27 January 2022

Original Research Article

ABSTRACT

Aims: *Hypericum perforatum* L., known as “Hofarighun” is a widely used herbal drug in Traditional Persian Medicine (TPM). Detection of non-relevant plants, instead of this species, in the herbal market encourages the need for the establishment of its chemical authentication and standardization, through implying rapid and efficient phytochemical techniques.

Study Design: Twelve *Hypericum* samples were acquired from traditional medicine markets of different regions of Iran (Tehran, Sanandaj, Mashhad, Kerman, Bandar Abbas, Ahvaz, Yazd, Babol, Yasuj, Shiraz (Chehel Giah), Shiraz (Kazerun Gate), and Shiraz (Adloo Zerehi), based on microscopic characterization. Positive control was taken in the form of cultivated specimen of *H. perforatum*.

Place and Duration of Study: Study was performed in Medicinal plants processing Research Center, SUMS, Shiraz in the months between February to December 2021.
Methodology: Essential oil samples were injected into a gas chromatograph (GC) and compounds were identified as per the spectra obtained. Total phenol, flavonoid and HPTLC analysis of samples were also done.

Results: α-pinene was found in highest proportion in majority of samples i.e. 35.55-63.69%. However other compounds such as 1-dodecanol (10.82%), caryophyllene (15.87%) and β-cubebene (15.14%) were also analyzed in samples and the cultivated sample respectively. Total phenol and flavonoid content among the Hypericum extracts were found to be between 50.31±3.22 to 262.76±8.12 mg Gallic Acid Equivalent (GAE)/g of Ext. and 13.47±1.68 to 79.26±5.78 mg Quercetin Equivalent (QE)/g of Ext., respectively.

Conclusion: The noticeable findings of present study can be used as a framework for authentication of Hypericum perforatum samples. The methods used were found to be feasible and efficient in detection of adultrations and may contribute to minimize the safety and efficacy concerns over the samples available in the traditional herbal pharmacies.

Keywords: Hypericum perforatum; essential oil; phenol; flavonoid; HPTLC; antioxidant.

1. INTRODUCTION

Physiological disorders including depressive disorders may serve as one of the leading cause of disability throughout the world, involving amelioration through central neurotransmitters reuptake inhibitors viz. non-selective serotonin and noradrenaline reuptake inhibitors, monoamine oxidase enzyme (MAO) inhibitors and GABA-mimetic agents [1]. One of the most popular medicinal plants used in Iranian traditional and folk medicine for the treatment of these diseases is “Hofarighun” which has been well documented in many reference sources of medicine and pharmacy [2]. Hypericum perforatum L., known as St. John’s Wort, from the family Hypericaceae has been the subject of numerous scientific and clinical research studies. The medicinal parts of the plant are flowers and twigs [3]. The extracts, products and chemical components of this plant have shown anti-epileptic, anti-schizophrenic, anti-migraine, analgesic, antidiabetic, antimicrobial, wound healing and antioxidant effects in various clinical studies [4,5]. Additionally, new research have interestingly revealed its encouraging effects in the treatment of nicotine and alcohol addiction [6]. Phytochemical screening of Hypericum species have revealed the presence of phytocompounds such as phenolics and their aliphatic derivatives, naphthodiantrons, flavonoids, xanthones, pyrones and terpenes [7-9]. The essential oil of this plant is commonly used as a preservative in food and health products [10]. Hofarighun is widely administered by traditional and folk healers and is being supplied in the Iranian medicinal plants market [11]. But so far little research has been done on the authenticity of the species of the genus, Hypericum in the market that are sometimes mistakenly prescribed by local and traditional vendors and therapists in place of the main genus or species. Therefore, authentication of the samples available in the medicinal herbal market was found to be an absolutely logical need. In the present study, 12 Hypericum samples were collected from the herbal medicine market, regardless of the place of planting or the time of collection of the samples. During this study, the morphological characteristics, botanical features and phytochemical contents of various Hypericum samples were examined in order to provide a comparative model, relevant to compounds profiles and the overall differences between the samples supplied in the market. In order to compare the chemotaxonomic and morphological characteristics of these species, a sample of H. perforatum was grown under the standard condition and used as a control in the present study.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Gallic acid, ferric chloride, methanol, potassium ferricyanide, ethanol, Folin-Ciocalteu reagent and TLC silica gel 60 F254 aluminum plates were obtained from Merck, Darmstadt, Germany. 1,1-diphenyl, 2-picryl hydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) and quercetin were purchased from Sigma Aldrich Chemical Co USA. All other chemicals and solvents used were of analytical grade from Merck.

2.2 Plant Collection and Identification

Various samples of Hofarighun from the pharmaceutical market have been collected from
Tehran, Sanandaj, Mashhad, Kerman, Bandar Abbas, Ahvaz, Yazd, Babol, Yasuj, Shiraz (Chehel Giah), Shiraz (Kazerun Gate), and Shiraz (Adloo Zerehi). The collected samples, were deposited to the herbarium of department of pharmacognosy, school of pharmacy, Shiraz University of Medical Sciences and characterised by plant taxonomist and ultimately, each specimen was allocated a herbarium number. Moreover, a standard sample of *H. perforatum* was used in this study. All samples were ground using an electric mill. Details of samples including place of collection and herbarium voucher numbers are given in Table 1.

### 2.3 Taxonomic and Morphological Screening

The initial step in implementing fingerprint analysis of plants is the morphological analyses. This process has an important role in identifying the macroscopic and microscopic characteristics of plant species and their relevant pharmacobotanical properties. Different parts of the *Hypericum* samples, including the stem, leaf and flower were powdered by a Chinese mortar and the powders were separately passed through a 70-mesh sieve. Each plant sample was weighed (5 g) and transferred to a test tube and 5 ml solution of 60% hydrated chlorine was added to it and then heated on a flame and centrifuged after boiling. Then the top layer was discarded and 50 ml of distilled water was added to the residue and again centrifuged. Subsequently, the bottom layer was transferred to a petri dish, and a few drops of ethanolic solution of phloroglucinol-hydrochloric acid were added followed by addition of glycerin to prevent the samples from drying out and to increase their stabilities. The slides prepared from various parts of *Hypericum* samples were then digitally photographed using a Ceti Magnum-PH Trinocular Compound Microscope. The relevant specifications observed were recorded and examined thoroughly.

### 2.4 Extraction of Essential Oil

Essential oil was isolated according to a method given in the European Pharmacopoeia [12]. Each *Hypericum* Sample (50 g) was separately crushed in a grinder. The powdered plant material was transferred into a round-bottomed flask and 500 mL distilled water was then added. The mixture was further subjected to hydrodistillation for 4 h using a Clevenger type apparatus. The essential oil samples were separately collected and dried over anhydrous sodium sulphate and stored at 4 ºC until GC/MS analysis and antioxidant assay.

### 2.5 Screening the Essential Oil Composition

#### 2.5.1 GC/MS analysis

The analysis was performed on a gas chromatograph 7890A system coupled with a mass detector 5975 C, Agilent technologies, USA. HP-5MS capillary column (5% phenyl methyl siloxane, 30 m × 0.25 mm× 0.25 µm) was used [13]. Oven temperature was adjusted to rise from 60 to 280 ºC at a rate of 10 ºC/min and held at 280 ºC for 10 min. Helium was used as the carrier gas with a flow rate of 1 mL/min. The interface temperature was 280 ºC. A volume of 1 µL of the essential oil was injected in split mode (1:50) and mass spectra were acquired in EI mode (70 eV) in a mass range of 30–600 m/z.

| Samples | Scientific name | Herbarium No. | Place of collection |
|---------|-----------------|---------------|---------------------|
| S1      | Hypericum scabrum L. | PM 1067       | Ahvaz               |
| S2      | Hypericum elongatum L. | PM 1068       | Bandar Abbas        |
| S3      | Hypericum elongatum | PM 1069       | Tehran              |
| S4      | Hypericum perforatum L. | PM 1070       | Kerman              |
| S5      | Hypericum scabrum L. | PM 1071       | Yazd                |
| S6      | Hypericum helianthemoides (Spach) Boiss. | PM 1072 | Sanandaj            |
| S7      | Hypericum scabrum L. | PM 1073       | Yasuj               |
| S8      | Hypericum scabrum L. | PM 1074       | Babol               |
| S9      | Hypericum scabrum L. | PM 1075       | Mashhad             |
| S10     | Hypericum elongatum L. | PM 1076       | Shiraz (Chehel Giah) |
| S11     | Hypericum elongatum L. | PM 1077       | Shiraz (Kazerun Gate) |
| S12     | Hypericum perforatum L. | PM 1078       | Shiraz (Adloo Zerehi) |
| S13     | Hypericum perforatum L. | PM 1079       | Control             |
2.5.1.1 Identification of volatile compounds and GC/MS fingerprints

Each sample was diluted 1:5 with dichloromethane before injection. The samples were dried over sodium sulfate prior to injection and 1 μL of diluted essential oil sample was injected into the gas chromatograph. Identification and quantification of essential oil components was performed by calculating Kovats Index (KI) for each constituent. Comparison of data were made, using the information given in Wiley nl 7 library, Adams [14], NIST [15] and Pherobase [16] mass spectral sources as well as the values reported in the literature. In order to confirm the structure of each oil component, inspection of mass spectral fragmentation pattern of each compound was also performed and the results were compared with the reported values.

2.6 Preparation of Ethanolic Extract

The ethanolic extracts were prepared by adding 25 g each of powdered Hypericum samples to 250 ml of 96% ethanol, in separate Erlenmeyer flasks. The flasks were capped and agitated in the dark at 25 °C for 3 hours on a magnetic stirrer (IKA, Germany). The extract were concentrated under reduced pressure at 40 °C on a rotary evaporator and finally freeze-dried in a vacuum freeze dryer (Christ Alpha 1- 4 LD, Martin Christ, Germany) and stored at 2 °C pending analysis.

2.6.1 Determination of total phenolic content of extracts

Measurement of total phenolic content was performed according to the Folin-Ciocalteu method. In this assay, gallic acid was used as a standard. To prepare a calibration curve, 2 mg of gallic acid was dissolved in 10 ml of methanol, to get 200 μg/mL stock solution. From this solution, a serial dilutions were made to provide solutions containing 200, 160, 80, 40 and 20 μg/mL of gallic acid. To 500 μL of each concentration, 5 mL of Folin-Ciocalteu reagent and 4 mL of sodium carbonate solution (105.9 g/L) were added respectively. The samples were vortexed and the absorbance were recorded after 15 minutes against the blank at 765 nm using a UV/VIS Spectrophotometer (PG Instrument Ltd.). Methanol was used as the blank. A calibration plot was made of absorbance versus concentration and the equation line so obtained, was used to calculate the concentration of unknown samples. Assessment of total phenolic content of extracts was performed using a methanolic solution of 0.5 g/L of ethanol extract obtained from each Hypericum sample [17]. All results are presented as mean±standard deviation and the phenolic content was expressed as mg gallic acid equivalent per gram of dry extract (mg GAE/g).

2.6.2 Determination of total flavonoid content of extracts

Determination of total flavonoid content of various Hypericum extracts were conducted using the aluminum chloride spectrophotometric method [18]. A stock solution of quercetin as a standard, consisting of 0.5 mg/mL of quercetin in methanol was prepared and then a calibration curve was generated with solutions containing 100, 80, 60, 40, 20 μg/mL of quercetin. To 3 ml of each concentration, 3 ml of 2% aluminum chloride (AlCl₃.6H₂O) solution was added which turns the solution yellow. To make 2% aluminum chloride solution, 5 g of aluminum chloride was dissolved in 250 ml of methanol. To measure the total flavonoid content of the samples, solutions of Hypericum extracts were prepared at a concentration of 0.5 mg/mL. To 3 ml of each concentration, 3 ml of 2% aluminum chloride solution was added. The mixture were vortexed and allowed to stand for 15 minutes, and the absorbance were read at 415 nm using a UV Spectrophotometer. All the tests were conducted in triplicate and their mean values were reported. Total flavonoid content of the extracts were calculated using regression equation derived from the quercetin calibration curve. Methanol was used as a blank.

2.7 High Performance Thin-Layer Chromatography Fingerprints

High-Performance Thin Layer Chromatography (HPTLC) includes four main components called Automatic TLC Sampler 4 (ATS4), TLC tank or Automatic Developing Chamber (ADC2), and Visualizer 2, which display spots in UV wavelengths (254 and 366 nm), and TLC scanner. In order to perform thin-layer chromatography, a CAMAG HPTLC system (Camag, Muttenz, Switzerland) was used in the present study [19]. Solutions of 3 mg/mL in methanol of each Hypericum extract was prepared, and 25 μl of each sample was loaded
onto a silica gel 60 F\textsubscript{254}, aluminum plate (10×20 cm). The loaded TLC plate was developed in ethyl acetate-acetic acid-formic acid-water (50:11:11:2) as the eluting solvent. The developed TLC plate was dried and then sprayed with anisaldehyde-sulfuric acid reagent and heated at 110 °C for 10 min until the spots were visualized. Anisaldehyde-sulfuric acid reagent was freshly prepared by dissolving 0.5 ml anisaldehyde in 10 ml of pure acetic acid. The solution was diluted to a volume of 85 mL by addition of methanol. Finally, 5 ml concentrated sulfuric acid was added and the resulting solution was mixed thoroughly.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Taxonomic and morphological characteristics

Various vegetative and reproductive parts of each 
Hypericum sample, including flowers, leaves and stems were prepared and powdered separately. The images from each plant part were carefully obtained and their microscopic features were thoroughly examined. Major variations in microscopic characteristics among various samples were observed, particularly in fruit endocarp tissues, stem and petal textures, exocarp tissue and pollen grains. Assessment of the secretory structures of vegetative organs showed the presence of type A canals in stem tissues of all Hypericum species, whereas the type B secretory canals were observed in subepidermic tissues of 
Hypericum perforatum, which are in agreement with the previous reports [20,21]. All examined species including 
Hypericum perforatum declared 1–2 layered palisade parenchyma in leaf mesophyll, as indicated in the earlier studies [22].

The outstanding anatomical characteristics such as equifacial leaves, anomocytic stomata and 1–2 layered palisade parenchyma were observed in 
Hypericum perforatum as reported earlier [23]. Whereas dorsiventral leaves, anisocytic stomata and 2–3 layers of palisade parenchyma were distinctive features in other studied Hypericum samples.

3.1.2 Essential oil components

The detailed results of GC/MS analysis of the essential oil samples and the types of compounds present in various 
Hypericum samples are given in Table 2. Moreover, the calculated KI values for each compound, derived from the GC spectrum, are presented. In order to identify the chemical composition of each 
Hypericum essential oil sample, the retention index and the mass spectral fragmentation pattern of each compound were analyzed simultaneously. Fig. 1 shows the GC chromatogram of essential oil of the aerial parts of a cultivated sample of 
Hypericum perforatum, used as a control in the present study.

3.1.3 Phenolic content

The ethanolic extracts of 12 
Hypericum samples and a field-grown sample of 
Hypericum perforatum as control were assayed for the total phenolic content. The highest phenolic content in the samples of S4, S9, S10 and S6 were found to be 262.76±8.12, 153.41±8.31, 132.64±8.5 and 113.87±4.92 mg GAE/g of dried extract respectively as listed in Table 3. Lower values of phenolic content were detected to be 98.80±10.83, 80.88±4.18, 74.72±2.06 and 70.5±2.99 in a descending order for S8, S2, S5 and S1 respectively [Table 3]. The lowest phenolic content was noticed in the samples were found to be 62.97±1.37, 62.54±2.46, 58.98±2.29 and 50.31±3.22 mg GAE/g of dried extracts for S3, S7, S11 and S12 respectively [Table 3].

3.1.4 Flavonoid content

Determination of total flavonoid content of all 
Hypericum samples indicated the greatest flavonoid content in the samples S4, S10, S5, S9, and S2 (79.26±5.78, 42.37±0.3, 38.67±3.36, 38.22±2.78 and 37.83±5.11 QE/g of dried extracts respectively), while the lowest values were detected for S3, S12 and S1 (18.94±0.17, 14.97±0.62 and 13.47±1.68 mg QE/g of dried extracts respectively) as presented in Table 3. The total flavonoid content recorded for the sample S4 (79.26±5.78 mg QE/g) were the highest among all the investigated samples [Table 3]. In general, some of the extracts exhibited almost close values in terms of flavonoid contents [Table 3].

3.1.5 HPTLC profile of 
Hypericum extracts

All samples of ethanolic extracts showed almost similar thin-layer chromatographic patterns and no remarkable difference was observed between the samples. Inspection of the high performance thin-layer chromatoplate obtained from various ethanolic extracts of 
Hypericum samples and the

Faroodniyaj Jahromi et al.; EJMP, 33(2): 1-13, 2022; Article no.EJMP.82573
control, declared an efficient separation of their chemical components. Comparing the profiles and the $R_f$ values of compounds of various Hypericum samples with those of the control (H. perforatum), indicated the similarity of their chemical composition as illustrated by the chromatogram in Fig. 2. However, samples S1, S4, S9 and S10, slightly differed from others and the control, in terms of number, size and intensity of the spots [Fig. 2].

3.2 Discussion

The aim of this study was to evaluate the herbal samples presented as Hypericum in the Iranian herbal medicine market. In practice, various specimens of this plant with close macroscopic features are available under the name of Hypericum in Iranian herbal pharmacies which are prescribed to control or treat inflammatory and infectious diseases, depression and mental disorders. However, lack of careful monitoring of the sources, quality or mode of cultivation could sometime hamper the selection of the right specimens in the pharmaceutical market. It is therefore mandatory to conduct regular screening of medicinal herbs available in the market. Evidence have revealed that medicinal plants are sometimes contaminated with non active or toxic plants which are sometimes intentional, but in many cases, this is supposed to be due to the incompetence of the traditional sellers or therapists in the correct recognition of the genus or species.

![Fig. 1. GC spectrum of the control sample (Hypericum perforatum)](image-url)
Table 2. Essential oil composition of various *Hypericum* Samples

| Component        | S_1 | S_2 | S_3 | S_4 | S_5 | S_6 | S_7 | S_8 | S_9 | S_{10} | S_{11} | S_{12} | C     | Kf^{Cal} | Kf^{Rep} | Ref. |
|------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|--------|--------|--------|-------|---------|----------|------|
| Isononane       | -   | -   | -   | -   | -   | -   | -   | -   | -   | -      | -      | -      | 5.32  | 860     | 864      | 22.23|
| Nonane          | 0.63| 0.58| 0.88| 0.2 | 0.83| 1.58| 0.75| 1.44| 0.71| 1.16   | 1.06   | 1.77   | 1.33  | 899     | 900      | 22.23|
| α- Thujene      | -   | -   | 0.64| 1.03| -   | -   | -   | -   | -   | -      | -      | -      | 0.29  | 925     | 924      | 24   |
| α- Pinene       | 52.08| 54.48| 50.1| 0.86| 35.55| 49.39| 51.77| 50.7| 38.81| 63.69  | 63.68  | 37.48  | 2.37  | 935     | 938      | 22   |
| Sabinene        | -   | -   | -   | -   | -   | -   | -   | -   | -   | -      | -      | -      | 0.19  | 960     | 961      | 25   |
| Camphene        | 1.33| 0.78| 1.28| -   | 0.49| 0.61| 0.55| -   | 0.67| 0.7    | -      | -      | 0.52  | 966     | 964      | 27   |
| Verbenene       | 0.94| -   | 0.55| -   | -   | -   | -   | 0.59| 0.63| -      | -      | -      | -     | 976     | 975      | 24   |
| β- Pinene       | 1.55| 2.94| 1.71| 0.17| 1.83| 2.75| 2.59| 3.96| 3.55| 3.63   | 1.83   | 0.92   | 989   | 982     | 25     |      |
| β- Myrcene      | -   | 0.67| -   | -   | 0.5 | 0.78| -   | -   | -   | 0.94   | 0.95   | 0.5    | 0.26  | 995     | 995      | 23   |
| Decane          | 0.95| 0.99| 1.36| 1.84| 3.08| 0.94| 1.02| 1.1 | 1.25| -      | 0.85   | -      | 0.48  | 999     | 1000     | 27   |
| p- Cymene       | 2.61| 1.69| 1.69| 3.67| 3.89| 1.2 | 5.18| -   | -   | 1.2    | 1.41   | 3.89   | -     | 1025    | 1027     | 23   |
| Limonene        | 2.53| 1.89| 2.91| -   | 1.03| 1.67| 1.69| 1.62| 1.12| 1.9    | 2.14   | 1.03   | -     | 1034    | 1031     | 27   |
| β- Ocumene      | -   | -   | -   | -   | -   | -   | -   | -   | -   | -      | -      | -      | 3.37  | 1048    | 1050     | 27   |
| γ- Terpinene    | 0.6 | -   | -   | 1.3 | 0.71| 2   | 1.42| 2.33| -   | 1.3    | -      | -      | 1.06  | 1060    | 1060     | 28   |
| Acetophenone    | 0.53| 0.96| -   | -   | 0.76| 0.92| -   | -   | -   | 0.46   | -      | -      | -     | 1068    | 1068     | 29   |
| 2-Methyldecane  | -   | -   | -   | -   | -   | -   | -   | -   | -   | -      | 0.24   | 0.24   | 0.24  | 1076    | 1076     | 24   |
| Undecane        | 1.17| 1.03| 1.39| 1.54| 1.08| 1.24| 1.03| 1.24| -   | 0.93   | 0.91   | 1.38   | 0.23  | 1099    | 1100     | 26   |
| Linalool        | 0.61| -   | 0.59| -   | -   | -   | -   | -   | -   | 2.28   | -      | -      | -     | 1100    | 1101     | 29   |
| α-Camphelenol   | 3.27| -   | 2.77| -   | 0.67| 1.65| 3.18| 3.6 | 1.49| 2.23   | -      | 0.67   | -     | 1130    | 1130     | 30   |
| trans-Pinocarveol| 1.57| 1.77| 1.68| -   | 0.45| 1.16| 2.01| 1.12| 0.92| 1.13   | 1.08   | 0.45   | -     | 1141    | 1142     | 25   |
| Camphor         | 1.07| 0.84| -   | 0.28| 0.41| -   | 1.7 | 0.44| -   | -      | 0.41   | -      | -     | 1146    | 1148     | 24   |
| Borneol         | 2.69| 0.77| 2.98| -   | -   | 1.12| 1.45| 0.53| -   | 0.81   | -      | -      | -     | 1170    | 1169     | 29   |
| 4-Terpinion     | -   | -   | -   | 0.29| 0.58| -   | 0.61| 0.41| -   | 0.58   | -      | -      | -     | 1179    | 1179     | 26   |
| Cymen-8-ol      | -   | -   | 0.96| -   | 0.43| -   | 0.55| -   | 0.55   | -      | 0.43   | -     | 1186    | 1187     | 27   |
| α-Terpinion     | 0.55| 0.79| 0.28| -   | -   | 0.63| 0.88| -   | -   | -      | -      | -      | -     | 1192    | 1190     | 28   |
| Dodecane        | -   | -   | 1.17| -   | -   | -   | 0.49| -   | 0.28   | 1.20   | 1.20   | 0.31  | 1200    | 1200     |      |
| Verbenone       | 1.04| 2.03| 2   | 0.43| 0.73| 2.95| 1.08| 1.15| 0.92| 0.43   | -      | -      | -     | 1214    | 1214     | 32   |
| trans-Carveol   | 0.86| 0.88| 1.41| -   | 0.56| 1.65| 0.74| 0.84| 0.78| -      | -      | -      | -     | 1220    | 1220     | 27   |
| Linyl acetate   | 0.77| -   | 1   | -   | -   | -   | -   | 4.15| -   | -      | -      | -      | -     | 1256    | 1257     | 30   |
| Thymol          | 1.23| 0.61| 3.67| 0.73| 0.55| 0.97| -   | 3.81| 0.73| 0.64   | -      | -      | -     | 1293    | 1292     | 25   |
| Carvacrol       | 1.37| 0.94| 2.9 | 0.64| 0.57| 0.86| -   | 2.17| 1.18| 0.64   | 0.27   | 0.27   | -     | 1301    | 1299     | 22   |
| Methyl caprate  | -   | -   | -   | -   | -   | -   | -   | -   | -   | -      | 0.27   | 1327   | 1328   | 32    |
| Component                       | \( S_1 \) | \( S_2 \) | \( S_3 \) | \( S_4 \) | \( S_5 \) | \( S_6 \) | \( S_7 \) | \( S_8 \) | \( S_9 \) | \( S_{10} \) | \( S_{11} \) | \( S_{12} \) | \( C \) | \( KI^{\text{Cal}} \) | \( KI^{\text{Exp}} \) | Ref. |
|--------------------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|-----|----------------|----------------|-----|
| Bicycloelemene                 |          |          |          |          |          |          |          |          |          |          |          |          | 0.39| 1335           | 1336           | 28  |
| Eugenol                        |          |          |          |          |          |          |          |          |          |          |          |          |      | 1350           | 1351           | 33  |
| \( \alpha \)-Copaeene          | 0.6      | 1.14     | 0.77     | 1.03     | 1.17     | 1        | 0.96     | 0.57     | 0.73     | 0.78     | 0.87     | 1.17     | 0.17| 1379           | 1376           | 23  |
| \( \beta \)-Cubebene           |          |          |          |          |          |          |          |          |          |          |          |          |      | 15.14          | 1391           | 30  |
| \( \beta \)-Bourbonene         |          |          |          |          |          |          |          |          |          |          |          |          | 0.98| 1405           | 1406           | 27  |
| \( \beta \)-Elemene            |          |          |          |          |          |          |          |          |          |          |          |          | 2.45| 1411           | 1410           | 25  |
| (+)-\( \beta \)-Funebrene      |          |          |          |          |          |          |          |          |          |          |          |          |      | 3.05           | 1414           | 25  |
| Caryophyllene                  | 0.91     | 0.95     | 0.96     | 1.89     | 1.36     | 2.43     | 0.75     | 0.52     | 1.58     | 2.72     | 2.87     | 1.36     | 15.87| 1424           | 1423           | 25  |
| Aromadendrene                  | 0.76     | 1.36     | 0.6      | 0.8      | 1.05     | 0.74     | 0.59     | 0.6      | 0.91     | 0.79     | 0.8      |          |      | 1444           | 1443           | 24  |
| 1-Dodecanol                    |          |          |          |          |          |          |          |          |          |          |          |          |      | 1471           | 1472           | 31  |
| \( \alpha \)-Murolene          | 1.45     |          |          |          | 6.17     | 3.29     | 1.52     | 1.97     |          | 2.84     |          | 6.17     | 1.02| 1484           | 1484           | 29  |
| \( \beta \)-Selinene           | 0.83     | 1.5      |          | 5.43     | 5.07     | 0.79     |          |          |          |          |          | 5.07     | 0.82| 1493           | 1489           | 32  |
| Pentadecane                    | 0.88     |          | 0.56     | 3.97     |          |          |          |          |          |          |          |          |      | 1498           | 1500           | 23  |
| Zingiberene                    |          | 2.54     |          |          |          |          |          |          |          |          |          |          | 0.23| 1500           | 1501           | 24  |
| Bicyclogermacrene              |          |          |          |          |          |          |          |          |          |          |          |          | 10.76| 1505           | 1505           | 26  |
| \( \beta \)-Farnesene          |          |          |          |          |          |          |          |          |          |          |          |          |      | 1510           | 1509           | 31  |
| \( \alpha \)-Amorphene         | 1.43     | 2.81     | 1.17     | 1.94     |          |          |          |          | 1.51     | 1.81     | 1.71     | 1.94     |      | 1518           | 1516           | 24  |
| Nerolidol                      |          |          |          |          |          |          |          |          |          |          |          |          | 1.53| 1527           | 1527           | 33  |
| \( \delta \)-Cadinene          | 2.53     | 4.2      | 1.74     | 2.34     | 4.24     | 2.36     | 1.92     | 3.28     | 3.61     | 3.58     | 2.34     |      | 1531           | 1530           | 30  |
| \( \alpha \)-Calacorene        | 0.52     | 0.67     | 0.52     | 0.45     | 0.76     |          |          |          |          |          |          | 0.45     |      | 1549           | 1548           | 28  |
| Spathulenol                    | 1.05     | 2.36     | 0.69     | 2.23     | 1.33     | 2.08     | 0.86     | 0.94     | 1.17     | 1.55     | 1.33     |      | 1582           | 1585           | 27  |
| Caryophyllene oxide            | 0.91     | 0.78     | 4.57     | 2.21     | 0.95     | 0.57     |          | 1.28     | 1.46     | 2.21     |          |      | 1591           | 1589           | 29  |
| \( \tau \)-Murolol             | 0.55     | 0.55     | 0.55     | 1        |          |          |          |          |          |          |          | 1        |      | 1648           | 1648           | 24  |
| \( \alpha \)-Cadinol           |          |          |          | 0.88     |          |          |          |          |          |          |          |          | 2.29| 1661           | 1660           | 27  |
| \( \alpha \)-Bisabolol         |          |          |          |          |          |          |          |          |          |          |          | 0.86     | 1703 | 1704           | 1704           | 33  |
| Myristic acid                  |          |          |          |          |          |          |          |          |          |          |          | 0.42     | 1766 | 1765           | 1765           | 25  |
| Pentadecanol                   |          |          |          |          |          |          |          |          |          |          |          | 7.36     | 1771 | 1772           | 1772           | 30  |
| Hexahydrofarnesyl acetone      | 0.66     |          | 2.19     |          | 0.66     |          |          |          |          |          |          | 0.75     |      | 1842           | 1843           | 26  |
| Cyclohexadecane                |          |          |          |          |          |          |          |          |          |          |          |          |      | 1883           | 1883           | 24  |
| Palmitic acid                  |          |          |          |          | 0.8      |          |          |          |          |          |          |          | 1.05| 1864           | 1964           | 22  |
| Heptadecanol                   |          |          |          | 2.65     |          |          |          |          |          |          |          |          | 0.81| 1970           | 1969           | 33  |
| Linoleic acid                  |          |          |          |          |          |          |          |          |          |          |          | 0.52     |      | 2094           | 2095           | 30  |
| Identification (%)             | 93.03    | 89.83    | 82.03    | 56.08    | 78.42    | 86.24    | 88.35    | 78.63    | 74.6     | 95.41    | 90.93    | 78.92    |      | 85.09          |              |     |
Table 3. Total phenolic and flavonoid content of *Hypericum* samples

| Sample | Total phenol mg GAE/g of Ext. (Mean±SD) | Total flavonoid mg QE/g of Ext. (Mean±SD) |
|--------|-----------------------------------------|-------------------------------------------|
| S1     | 70.5±2.99                               | 13.47±1.68                                |
| S2     | 80.88±4.18                              | 37.83±5.11                                |
| S3     | 62.97±1.37                              | 18.94±0.17                                |
| S4     | 262.76±8.12                             | 79.26±5.78                                |
| S5     | 74.72±2.06                              | 38.67±3.36                                |
| S6     | 113.87±4.92                             | 36.31±1.08                                |
| S7     | 62.54±2.46                              | 23.35±1.28                                |
| S8     | 98.8±10.83                              | 36.47±1.99                                |
| S9     | 153.41±8.31                             | 38.22±2.78                                |
| S10    | 132.64±8.5                              | 42.37±0.30                                |
| S11    | 58.98±2.29                              | 28.67±2.12                                |
| S12    | 50.31±3.22                              | 14.97±0.62                                |
| C      | 227.49±8.27                             | 64.39±7.47                                |

S1: Ahvaz  S2: Bandar Abbas  S3: Tehran  S4: Kerman  S5: Yazd  S6: Sanandaj  S7: Yasuj  S8: Babol  S9: Mashhad  S10: Shiraz Chehel Giah  S11: Shiraz Kazerun Gate  S12: Shiraz Adloo Zerehi  C: Control

Fig. 2. HPTLC of methanolic extracts visualized with anisaldehyde-sulfuric acid

Considering the prescription and sales of unauthenticated or uncharacterized samples of medicinal plants, 12 samples, presented as *Hypericum* in the Iranian medicinal plant markets were collected from various cities. Each sample was then identified and their essential oil analysis and HPTLC fingerprinting were performed and finally all the samples examined for their total phenolic and flavonoid contents.

The most important approach in the systematic characterization of *Hypericum* species is their secretory tissues. These structures can be found in the stem, bark, petals, sepals and pistil. But part of these organs are lacking or show varied distribution in different species. Secretory glands are the specific microscopic features present in all species, but they are different in terms of number, type and position. Secretory vesicles are also present in all species, but they differ in terms of diameter, cavity and the location among the species and different varieties. The dark nodules were observed in certain organs of some *Hypericum* species. This feature is considered as a specific microscopic characteristic among *Hypericum* species [23].

The results of present study confirmed that all samples collected from different markets, belong to the genus *Hypericum*. Based on the taxonomic characterisation, samples S1, S5, S7, S8, and S9, were confirmed to be *H. scabrum*, while S2, S3, S10 and S11 were identified as *H. elongatum*. The sample S4, was characterized as *H. perforatum*, whereas, S6 was identified as *H. helianthemoides*. 
The results of essential oil analysis showed a significant correlation between the volatile components of different Hypericum samples [Table 2]. α-Pinene, a bicyclic monoterpene showed the contribution of greater than 5% and was the dominant constituent in all samples of essential oil, except S4 and control. This finding is consistent with the results of previously reported studies, that introduced α-pinene, as the major component of the essential oil of Hypericum species in southern France, Turkey and Italy [23-25]. Caryophyllene oxide and β-cubebene were the dominant constituents of the control. This is in close agreement with the results of earlier studies, conducted on different Hypericum samples in Lithuania and Croatia, which showed the oxygenated sesquiterpenes, such as caryophyllene oxide and β-cubebene, as the most dominant constituents of essential oil in all tested samples, while a small contribution of oxygenated monoterpenes were detected [26,27]. The main component of the essential oil of S4 sample was recognized to be 1-dodecanol. This compound has been detected as one of the major components of the essential oil of French H. perforatum var. perforatum, which corroborates the results of previous studies [28].

As can be seen in Table 2, most of the essential oil samples revealed high content of α-pinene, which is consistent with the values given in the Iranian herbal pharmacopoeia for H. perforatum [29]. While the sample S4, was found to be rich in 1-dodecanol, which is different from other specimens in terms of major components of essential oil. It is noteworthy to point out that, factors such as contamination with other plants, methods of drying and storage, moisture and light, phenological stages, collection area, method of processing and seasonal changes and particularly the genetic characteristics of the species might have affected the essential oil yield and composition, [30,31]. Consequently, the variations observed in volatile constituents of some of the Hypericum samples may thus in part or totally be attributed to the above mentioned factors.

Beside essential oil, Hypericum species contain a non volatile group of phenolic constituents including hypericin, a natural polyphenolic polycyclic quinone, and hyperforin a terpene ketone. These compounds and their structural analogs have already shown synergistic antioxidant effects and anti lipid peroxy radical properties [32,33]. The phenolic and flavonoid content of Hypericum specimens undergo significant quantitative alterations during the vegetative stages [34-36]. The total phenolic content of the samples S6, S9 and S10 were found to be in close agreement with the average phenolic content previously reported for Hypericum species (150.44 mg GAE/g of dried extract), using ethanol as extraction solvent. As given in Table 3, higher values of total phenol were found in S4 and control samples, which are consistent with the research of Öztürk et al. on H. perforatum in Turkey [37,38]. As presented in Table 3, the phenolic content of the remaining samples were found to be in agreement with the results of another research, reported earlier [39].
The flavonoid content of all tested *Hypericum* samples were also consistent with the results of Öztürk et al. [37]. Table 3 shows that, the samples S1, S2, 3, 5, 7, S11 and S12 were comparable in terms of phenolic and flavonoid contents, whereas the samples S6 and S8 were close in terms of their flavonoid content. The samples S9 and S10 showed similar values for flavonoid content, while S4 (*H. perforatum*) revealed a close similarity to that of the control in terms of both phenol and flavonoid content, which could be due to their species resemblance (Table 3, Figure 3). Considering the proof of the relationship between soil salinity and increase in total phenols and flavonoids, it is also likely that, S4 sample might have collected from a place with higher degree of soil salinity, compare to other samples [40,41]. Determination of flavonoid content of *Hypericum* samples indicated that our results are consistent with those of previous studies [37,42]. In general, the extracts of *Hypericum* samples S1, S2, S3, S5, S7, S11 and S12 showed closer distribution range of phenolics and flavonoids compare to other samples [Table 3, Fig. 3]. While almost close ranges of phenolic and flavonoid concentrations were detected between the samples S6 and S8, S9 and S10 and the S4 and control. In order to investigate the profile of phytochemical markers in various *Hypericum* specimens, high performance thin-layer chromatography were employed. Comparison of HPTLC profiles of *Hypericum* samples, indicated similarities among the samples in terms of their polar and non polar components [Fig. 2]. However, the differences observed between the chemical profile of various *Hypericum* extracts with those of the control and their specific markers can be considered as a benchmark in differentiation and diagnostic characterization of various *Hypericum* samples [43]. Therefore, HPTLC fingerprinting may be considered as an efficient diagnostic tool for authentication and quality assessment of *Hypericum* species and the related herbal samples available in the market.

4. STATISTICAL ANALYSIS

Statistical analysis was carried out using SPSS software version 22.0 (IBM, Armonk, NY, USA) and the values expressed as mean±SD. Independent t test was carried to compare the data and their significance of the difference was assessed at $P < 0.05$ level.

5. CONCLUSION

To ensure the major safety and efficacy concerns arising from the use of herbal medicines available in the traditional pharmacies, there is always a need for efficient herbal authentication methods. The results of this study clearly indicate that the use of instrumental techniques of analysis can greatly help in establishing quality assurance and the management of adultrations in the market samples of *Hypericum*. These techniques could be used in detecting the variations in the chemical constituents of *Hypericum* samples obtained from different geographic regions. The reliability and simplicity of these methods also encourage their potential use in careful quality assessment of the highly traded plants like *Hypericum* species, prior to their use in drug, food and cosmetic formulations.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The research proposal was approved by the Ethics Committee of Shiraz University of Medical Sciences, under the registration code; IR.SUMS.REC.1397.297 on June 23, 2018.

ACKNOWLEDGEMENT

This work is part of the research conducted by Nastaran Babaei Rizvandi for the award of the Pharm. D. degree. The authors wish to gratefully acknowledge the financial support from the deputy for Research and Technology, Shiraz University of Medical Sciences (IR.Sums.Rec.1397.297).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Yoon BE, Woo J, Chun YE, Chun H, Jo S, Bae JY, An H, Min JO, Oh SJ, Han KS, Kim HY. Glial GABA, synthesized by monoamine oxidase B, mediates tonic inhibition. The J physiol. 2014:592(22):4951-68.

2. Ghasemi Y, Khalaj A, Mohagheghzadeh A, Khosravi AR, Morowvat MH. Composition and antimicrobial activity of the essential
The determinations of the total hyperforin as a standard purity analysis and stability of Hypericum perforatum L. Investigation of secretory structures in species of Hypericum genera from Bihor county, Romania. Note I. Vegetative organs. Farmacia. 2011;59:424–431.

Ciccarelli D, Andreucci AC, Pagni AM. Translucent glands and secretory canals in Hypericum perforatum L.(Hypericaceae): Morphological, anatomical and histochemical studies during the course of ontogenesis. Ann Bot. 2001;88(4):637–644.

Bayat M, Rahiminejad M, Ghaemmaghamy L. Investigation of secretory structures in some of the Hypericum species in Iran. J Plant Res (Iran J Biol). 2016;29(1):15-30.

Gitea D, Şipoş MO, Tamaș MI, Pasca BI. Secretory structures at species of Hypericum genera from Bihor county, Romania. Note I. Vegetative organs. Farmacia. 2011;59:424-431.

Tekin M. Pharmacobotanical study of Hypericum thyrsus. Rev Bras Farmacogn. 2017;27(2):143-152.

Cakir A, Duru M, Harmandar M, Cirimmina R, Passannanti S, Plozzi F. Comparison of the volatile oils of Hypericum scabrum L. and Hypericum perforatum L. from Turkey. Flavour Fragr J. 1997;12(6):285-287.

Pintore G, Mario C, GianPiero B, Riccardo C. Essential oil composition of Hypericum perforatum L. var. angustifolium DC growing wild in Sardinia (Italy). J Essent Oil Res. 2005;17:120–127.

Shrivastava M, Dwivedi LK. Therapeutic potential of Hypericum perforatum: A review. Int J Pharm Sci Res. 2015;6(12):4982-4988.

Finney-Brown T. Hypericum perforatum and substance dependence. Aust J Med Herb. 2008;20(3):121-122.

Barnes J, Anderson LA, Phillipson JD. St John's wort (Hypericum perforatum L.): A review of its chemistry, pharmacology and clinical properties. J Pharm Pharmacol. 2001;53(5):583-600.

Erdelmeier C. Hyperforin, possibly the major non-nitrogenous secondary metabolite of hypericum perforatum L. Pharmacopsychiatry. 1998;31(S1):2-6.

Bisset N, Wichtl M. Herbal drugs and phytopharmaceuticals medpharm GmbH scientific publishers: Stuttgart, Boca Raton: CRC Press; 1994.

Orth H, Rentel C, Schmidt P. Isolation, purity analysis and stability of hyperforin as a standard material from Hypericum perforatum L. J Pharm Pharmacol. 1999;51(2):193-200.

Bahmani M, Khaksarian M, Rafieian-Kopaei M, Abbasi N. Overview of the therapeutic effects of Origanum vulgare and Hypericum perforatum based on Iran’s ethnopharmacological documents. J Clin Diagn Res. 2018;12(7):FE01-FE04.

EDQM (Council of Europe). European Pharmacopoeia 6th edn: EDQM; 2008. Available: https://www.edqm.eu/medias/fichiers/Annual_Report_Activite.pdf.

Farboodniai Jahromi MA, Etemadfarh F, Zebarjaz Z. Chemical characterization and antimicrobial activity of essential oil from the leaves of bienertia cycloptera. Chem. Nat. Compd. 2016;52(5):936-938.

Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry, Allured publishing corporation, Carol Stream, IL; 2007.

NIST Chemistry WebBook, Chemical Name Search. Available: https://webbook.nist.gov/chemistry/ available online: (Accessed on 6.6.2020).

Pherobase, Kovats Retention Index. Available: https://www.pherobase.com/krvts/available online: (Accessed on 6.6.2020).

Meda A, Lamien CE, Romito M, Millogo J, Nacoumla OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food chem. 2005;91:571-577.

Pourmorad F, Hosseinimehr S, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr J Biotechnol. 2006;5(11):1142-1145.

Hemmalakshmi SU, Priyanga SU, Devaki KA. Phytochemical screening and HPTLC fingerprinting analysis of ethanolic extract of Erythrina variegata L. Flowers. Int J Pharm Sci. 2016;8:210-217.

Ciccarelli D, Andreucci AC, Pagni AM. Translucent glands and secretory canals in Hypericum perforatum L.(Hypericaceae): Morphological, anatomical and histochemical studies during the course of ontogenesis. Ann Bot. 2001;88(4):637-644.

Bayat M, Rahiminejad M, Ghaemmaghamy L. Investigation of secretory structures in some of the Hypericum species in Iran. J Plant Res (Iran J Biol). 2016;29(1):15-30.

Gitea D, Şipoş MO, Tamaș MI, Pasca BI. Secretory structures at species of Hypericum genera from Bihor county, Romania. Note I. Vegetative organs. Farmacia. 2011;59:424-431.

Tekin M. Pharmacobotanical study of Hypericum thyrsus. Rev Bras Farmacogn. 2017;27(2):143-152.

Cakir A, Duru M, Harmandar M, Cirimmina R, Passannanti S, Plozzi F. Comparison of the volatile oils of Hypericum scabrum L. and Hypericum perforatum L. from Turkey. Flavour Fragr J. 1997;12(6):285-287.
26. Radusiene J, Judzentiene A, Bernotiene G. Essential oil composition and variability of *Hypericum* perforatum L. Growing in Lithuania. Biochem Syst Ecol. 2005;33(5):113–124.

27. Vuko E, Dunkić V, Ruščić M, Nazlić M, Mandić N, Soldo B, Sprung M, Fredotović Ž. Chemical composition and New Biological Activities of Essential Oil and Hydrosol of *Hypericum* perforatum L. ssp. veronense (Schrank) H. Lindb. Plants. 2021;10(5):1014.

28. Schwob I, Bessiere JM, Masotti VR, Viano J. Changes in essential oil composition in Saint John's wort (*Hypericum* perforatum L.) aerial parts during its phenological cycle. Biochem. Syst. Ecol. 2004;32:735–745.

29. Iranian Herbal Pharmacopoeia. Tehran: Ministry of Health and Medical Education of Iran. Food and Drug Administration. 2002;541-549.

30. Li Y, Zidorn C. Seasonal variations of natural products in European herbs. Phytochem Rev 2022;10:1-27.

31. Sun P, Kang T, Xing H, Zhang Z, Yang D, Zhang J, Paré PW, Li M. Phytochemical Changes in Aerial Parts of *Hypericum* perforatum at Different Harvest Stages. Rec Nat Prod. 2019;13(1):1-9.

32. Orčić DZ, Mimica-Dukić NM, Francišković MM, Petrović SS, Jovin ED. Antioxidant activity relationship of phenolic compounds in *Hypericum* perforatum L. Chem Cent J. 2011;5(1):1-8.

33. Lü JM, Lin PH, Yao Q, Chen C. Chemical and molecular mechanisms of antioxidants: Experimental approaches and model systems. J Cell Mol Med. 2010;14(4):840-860.

34. Bruni R, Sacchetti G. Factors affecting polyphenol biosynthesis in wild and field grown St. John's Wort (*Hypericum perforatum L.* Hypericaceae/Guttiferae). Molecules. 2009;14(2):682-725.

35. Zdunic G, Godjevac D, Savikin K, Petrovic S. Comparative analysis of phenolic compounds in seven *Hypericum* species and their antioxidant properties. Nat prod commun. 2017;12(11):1934578X1701201140.

36. Makarova K, Sajkowski-Kozielewicz JJ, Zawada K, Olchowik-Grabarek E, Ciach MA, Gogolewski K, Dobros N, Ciechowicz P, Freichels H, Gambin A. Harvest time affects antioxidant capacity, total polyphenol and flavonoid content of Polish St John's wort’s (*Hypericum* perforatum L.) flowers. Sci Rep. 2021;11(1):1-2.

37. Öztürk N, Tunçel M, Potoğlu-Erkara İ. Phenolic compounds and antioxidant activities of some *Hypericum* species: A comparative study with *H. perforatum*. Pharm Biol. 2009;47(2):120-127.

38. Çelen G, Ozkan S, Ahyan F. The phenolic compounds from *Hypericum* perforatum and their antimicrobial activities. Hacettepe J Biol Chem. 2008;36(4):339-345.

39. Morshedloo MR, Moghadam MR, Ebadi A, Yazdani D. Genetic relationships of Iranian *Hypericum* perforatum L. wild populations as evaluated by ISSR markers. Plant Syst Evol. 2015;1(2):657-665.

40. Rezazadeh A, Ghasemnezhad A, Barani M, Telmadarrehei T. Effect of salinity on phenolic composition and antioxidant activity of artichoke (*Cynara scolymus L.*) leaves. Res J Med Plant. 2012;6(3):245-252.

41. Rizzo P, Altschmied L, Stark P, Rutten T, Gündel A, Scharfenberg S, Franke K, Bäumlein H, Wessjohann L, Koch M, Borisjuk L. Discovery of key regulators of dark gland development and hypericin biosynthesis in St. John’s Wort (*Hypericum perforatum*). Plant Biotechnol J. 2019;17(12):3299-3312.

42. Kurkin VA, Pravdivtseva OE. Flavonoids from the aerial parts of *Hypericum perforatum*. Chem Nat Compd. 2007;43(5):620-621.

43. Agapouda A, Booker A, Kiss T, Hohmann J, Heinrich M, Csupor D. Quality control of *Hypericum* perforatum L. Analytical challenges and recent progress. J Pharm Pharmacol. 2019;71(1):15-37.

© 2022 Farhoodniay Jahromi et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history: The peer review history for this paper can be accessed here: https://www.sdiarticle5.com/review-history/82573