Research Article
CODEN (USA): IJPLCP

ISSN: 0976-7126
Saleh, 11(9):6953-6958, 2020

International Journal of Pharmacy & Life Sciences
Open Access to Researcher

FT-Raman phytochemical analysis of *Hypericum perforatum* L. (Hypericaceae) species

Basel Saleh*

Department of Molecular Biology and Biotechnology

Atomic Energy Commission, P.O.Box 6091, Damascus, Syria

Article info

Received: 03/07/2020

Revised: 09/09/2020

Accepted: 16/09/2020

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Abstract

Hypericum perforatum L.(St. John's wort) is a perennial herbaceous plant displayed an important role in pharmaceutical and medicine applications. Fourier transform raman spectroscopy (FT-Raman) technique has been employed to determine the chemical constituents of four wild *H. perforatum*(HP)genotypes collected from rural regions of Lattakia-Syria. FT-Raman analysis highlighted 7, 5, 6 and 6 distinguished peaks for HP1, HP2, HP3 and HP4 genotypes, respectively. Of which three peaks were common for the four studied *H. perforatum* genotypes: peak of 1250cm^{-1}assigned to C–O stretch-Carboxylic acids group, peak of 1600cm^{-1}assigned to C=C stretch aromatic-Aromatics group and peak of 2850 cm^{-1}assigned to C–H stretch-Alkanes group. These functional groups known to exhibited different biological activities worldwide. This report could be considerd as the first study of *H. perforatum* FT-Raman.

Keywords: *Hypericum perforatum*, Fourier transform raman spectroscopy (FT-Raman),Chemical constituents, Functional groups

Introduction

Hypericum perforatum L. species belonged to *Hypericum* genus and Hypericaceae family which included approximately 500 species of flowering plants (Franklin et al., 2017). In Syria, it has been reported the occurrence of 21 species belonged to this genus (Mouterde,1970). *H. perforatum* L. commonly known as St. John's wort, is a perennial herb native to relatively dry temperature zones of Europe and North America (Çırak et al., 2010).

Hypericum species have a critical role in pharmacy and medicine researches and applications due to their bioactive constituents (flavonoids, xanthones, biflavonoids, proanthocyanins, naphthodianthrones and phenylpropanes) (Crockett, 2010). Of which, different secondary metabolites including tannins, essential oils, naphthodianthrones, flavonoids, phloroglucinols, xanthones, procyanidins, phenylpropanes, amino acids and other water-soluble components were occurred in *H. perforatum* (Çırak et al., 2010; Saleh, 2019).

Hypericum species were considered as a potent candidate for application as an antioxidant (Silva et al., 2005);antiviral, antiretroviral, and antitumor (Çırak et al., 2010); nematicidal, antibacterial and insecticidal (Crockett, 2010);

***Corresponding Author**
E.mai: ascientific@aec.org.sy

antidepressant, antiviral, and antibacterial (Barnes et al., 2001); antibacterial and antioxidants (Pirbalouti et al., 2014);anticancer, anti-AIDS and anti-tumour (Rout and Swain, 2016);antioxidant and antimicrobial (Heydarian et al., 2017) and anti- leukemia cell lines NB4 and HL-60 (Guo et al., 2019).

Due to *H. perforatum* medicinal value beside its traditional applications in folk medicine and its importance as an ornamental plant; it became one of the most commercially plants used (Saleh, 2019). Thereby, many researches focused on its chemical composition variability all over the world (Silva et al., 2005; Tatsis et al., 2007; Çırak et al., 2010; Pirbalouti et al., 2014;Agapouda et al., 2019; Guo et al., 2019; Saleh, 2019; Lou et al., 2020).

Different physical, chemical and microscopic approaches have been successfully applied for decades to determine structure, chemical composition and properties of plant cells, tissues and organs. Of which, Raman spectroscopy was restricted for a long time primarily to academic researches. Then it has been considered as an efficacy tool in quality control in various industrial applications as well as in structure elucidation of plant substances (Gierlinger and Schwanninger, 2007).

Recently, Agapouda et al. (2019) applied different analytical methods for *H. perforatum* extracts composition identifying, *e.g.* gas chromatography-mass spectrometry (GC–MS), high-performance layer chromatography (HPLC), high-performance thin-layer chromatography (HPTLC) and thin-layer chromatography (TLC)analyses.

For decades, the consumption of *H. perforatum* -derived products has dramatically increased and presently it is considered as one of the most consumed medicinal plants worldwide (Silva et al., 2005).

Due to lack information regarding *H. perforatum* L. phytochemical analysis in Syria, the current study focused on determination its chemical constituents using FT-Raman analysis.

Material and Methods

Plant materials

Aerial parts of four wild *H. perforatum* genotypes (10 plants/genotype) were collected from rural regions of Lattakia-Syria (Table 1). Sampling has been performed during flowering stage. Plant samples were shade dried for two weeks, milled to fine powder by special electric mill and stored separately in glass bowls until used.

FT-Raman assay

The fine powder for each sample was used as template for FT-Raman analysis in the wave number range of 3500-500 cm^{-1}. IR measurement has been performed using NXR FT-Raman (Thermo, USA) instrument for FT-Raman analysis.

Results and Discussion

FT-Raman spectra of the aerial parts for the four studied *H. perforatum* genotypes was presented in Figure 1. FT-Raman analysis highlighted 7, 5, 6 and 6 distinguished peaks for HP1, HP2, HP3 and HP4 genotypes, respectively (Table 2). Of which three peaks were common for the four studied *H. perforatum* genotypes: peak of 1250cm^{-1}assigned to C–O stretch-Carboxylic acids group, peak of 1600cm^{-1}assigned to C=C stretch aromatic-Aromatics group and peak of 2850 cm^{-1}assigned to C–H stretch-Alkanes group (Table 2).

Verotta et al. (2000) isolated prenylated phloroglucinol hyperforin (3−5) from *H. perforatum* aerial parts and investigated their structures elucidated using spectroscopic approaches. Whereas, Silva et al. (2005) reported that the Kaempferol 3-rutinoside and rutinacetyl were identified for the first time in *H. perforatum* total ethanolic extract using high-performance layer chromatography (HPLC) with a diode-array detector (DAD)- mass spectrometry (MS) - (MS) coupling (HPLC–DAD–MS–MS) technique.

Tatsis et al. (2007) reported that the phenolic acids (chlorogenic acid, 3-O-coumaroylquinic acid); flavonoids (quercetin, quercitrin, isoquercitrin, hyperoside, astilbin, miquelianin & I3,II8-biapigenin) and naphtodianthrones (hypericin, pseudohypericin, protohypericin & protopseudohypericin), phloroglucinols (hyperforin & adhyperforin) were mainly detected in Greek *H. perforatum* using liquid chromatography (LC)-solid-phase extraction (SPE)-nuclear magnetic resonance (NMR) coupling (LC/SPE/NMR) and/or liquid chromatography -mass spectrometry (MS) coupling (LC/MS) techniques. Moreover, they reported the occurrence of two phloroglucinols (hyperfirin and adhyperfirin) for the first time.

Çırak et al. (2010) reported that the hydrocarbon and oxygenated sesquiterpenes such as caryophyllene oxide (6.01–12.18%), β-selinene (5.08–19.63%), α-selinene (4.12–10.42%), γ-muurolene (5.00–9.56%), β-caryophyllene (4.08–5.93%), spathulenol (2.34–5.14%) and d-cadinene (3.02–4.94%) were mainly presented in *H. perforatum* (collected from northern Turkey) essential oil using GC-FID and GC-MS techniques. Whereas, monoterpenes, both hydrocarbon and oxygenated, were occurred in scarce amounts of α- and β-pinene, myrcene, linalool, cis- and trans-linalool oxide, and α-terpineol. Whereas,

Pirbalouti et al. (2014) reported that the major constituents of three *Hypericum* species (*H. helianthemoides*, *H. scabrum* and *H. perforatum* collected from Southwest Iran) were a-pinene (12.52–49.96%), (E)-b-ocimene (4.44–12.54%), b-pinene (6.34–9.70%), germacrene-D (2.34–6.92%) and b-caryophyllene (1.19–5.67%) using GC and GC/MS techniques. Moreover, Chatzopoulou et al. (2006) reported Greece *H. perforatum* essential oil phytochemical analysis using GC and GC/MS techniques. They reported that germacrene D was the main constituent presented in wild-grown (22.8%) and cultivated plants (16.9%), followed by 2-methyloctane (10.8–17.8%), β-caryophyllene (6.6–10.3%), α-pinene (5.2–10.1%) and bicyclogermacrene (4.1–4.8%). Whereas, 14 constituents characterized the wild-grown plants, were not presented in cultivated once.

Heydarian et al. (2017) applied fourier transform-infrared spectroscopy (FT-IR) technique to identify polysaccharide extracted structure's of *H. perforatum*. Recently, Guo et al. (2019) reported Spirohypolactones A (1) and B (2) and hyperhexanones C–E (3–5), five degraded cyclohexanone-monocyclic polyprenylated acylphloroglucinol (C-MPAP) derivatives were identified in *H. perforatum* stems and leaves, and their structures were elucidated by extensive spectroscopic analyses and electronic circular dichroism (ECD) calculations. Moreover, Saleh (2019) recently reported that n-Hexadecanoic acid (28.58%), Octadecane (10.42%) and Tricosane (9.66%) for *H. triquetrifolium*, whereas, Isooctyl phthalate (30.39%), Tetracosane (28.18%) and Nonacosane (9.12%) were presented in *H. thymifolium;* while they were ß -Selinenol (18.13%), Elemol (12.77%) and ß-Elemene (10.73%) for *H. perforatum* were identified as a major constituents presented in their aerial parts essential oils using GC/MS technique.

More recently, Lou et al. (2020) reported that six new polycyclic polyprenylated acylphloroglucinols, hyperfols C-H (1–6) and seven known ones (7–13), were isolated from *H. perforatum* aerial parts and their structures were elucidated using 1D and 2D NMR techniques.

Other reports focused on other *Hypericum* species phytochemical analysis; *e.g.* Rout and Swain (2016) applied HPTLC & FTIR techniques to identify methanolic hypericin leaves content in *H. gaitii* Haines. More recently, Wang et al. (2020) reported that hookerianones A – E (1–5), five new polyprenylated acylphloroglucinols (PPAPs) and six known ones, were isolated from *H. hookerianum* aerial parts and that their structures were elucidated using MS and NMR techniques.

Overall, *H. perforatum* studied genotypes showed some differences in their chemical constituents using FT-Raman analysis. These observed differences could be related to geographical distribution differ in their altitude and annual rainfall, where the four studied *H. perforatum* genotypes were collected(Saleh, 2019).

In conclusion, chemical constituents of four wild *H. perforatum* genotypes collected from Lattakia-Syria have been determined by FT-Raman technique. Data showed genotyping variation in chemical constituents could be related to *H. perforatum* geographical distribution. Of which three functional groups(Carboxylic acids, Aromatics and Alkanes) were commonly identified for the four studied *H. perforatum* genotypes. Application of other analytical performance methods like GC-MS, HPLC or others to identify the chemical compounds for each functional group and to investigate their potential biological role is requested. This report could be considered as the first study of *H. perforatum* FT-Raman.

Acknowledgements

I thank Dr. I. Othman (Director General of AECS) and Dr. N. Mirali (Head of Molecular Biology and Biotechnology Department in AECS) for their support, and also the Plant Biotechnology group for technical assistance.

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Table 1:Description of the studied *H. perforatum* genotypes in the current study

Genotype	Code	Altitude (m)	Annual rainfall (mm)
H. perforatum1	HP1	80	750
H. perforatum2	HP2	420	850
H. perforatum3	HP3	546	1200
H. perforatum4	HP4	680	1250

Table 2:FT-Raman analysis of the studied *H. perforatum* genotypes.

Genotype	Peak No	IR frequency (cm^{-1})	Observed IR (cm^{-1})	Bond	Functional groups
HP1	1	680-610	650	C-H bend	Alkyne
	2	900-690	880	=C-H oop bend	Aromatics
	3	2000-1000	1100	C–O secondary alcohol stretch C–O stretch	Ethers
	4	1300-1200	1250	C–O stretch	Carboxylic acids
	5	1600-1400	1600	C=C stretch aromatic	Aromatics
	6	2140-2100	2127	C≡C terminal alkyne	Acetylenic (alkyne)
	7	2970-2850	2850	C–H stretch	Alkanes
HP2	1	680-610	650	C-H bend	Alkyne
	2	1300-1200	1250	C–O stretch	Carboxylic acids
	3	1600-1400	1600	C=C stretch aromatic	Aromatics
	4	2140-2100	2127	C≡C terminal alkyne	Acetylenic (alkyne)
	5	2970-2850	2850	C–H stretch	Alkanes
HP3	1	2000-1000	1100	C–O secondary alcohol stretch C–O stretch	Ethers
	2	1300-1200	1250	C–O stretch	Carboxylic acids
	3	1600-1400	1600	C=C stretch aromatic	Aromatics
	4	2140-2100	2127	C ≡C terminal alkyne	Acetylenic (alkyne)
	5	2970-2850	2850	C–H stretch	Alkanes
	6	3600-3200	3430	O–H stretch	Hydrogen bonded alcohols, phenols
	1	680-610	650	C-H bend	Alkyne C-H

					bend
				C–O secondary alcohol stretch	
	2	2000-1000	1100	C–O stretch	Ethers
	3	1300-1200	1200	C–O stretch	Carboxylic acids
HP4	4	1300-1200	1250	C–O stretch	Carboxylic acids
	5	1600-1400	1600	C=C stretch aromatic	Aromatics
	6	2970-2850	2850	C–H stretch	Alkanes

HP1-HP4: *H. perforatum1* genotype - *H. perforatum4* genotype

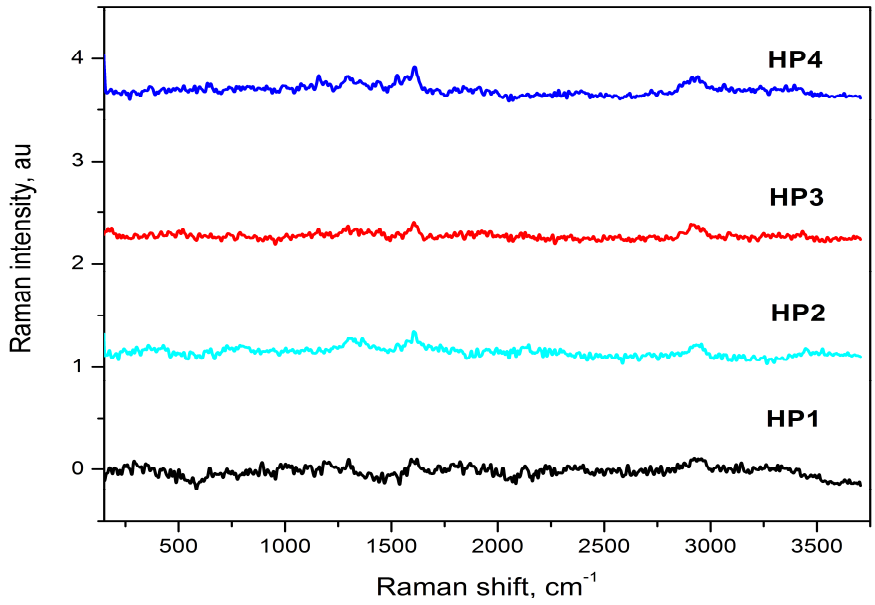

Fig. 1: Observed FT-Raman vibration wave numbers of the studied *H. perforatum* genotypes, aerial parts. HP1-HP4: *H. perforatum1* genotype - *H. perforatum4* genotype

Cite this article as:
Saleh B. (2020). FT-Raman phytochemical analysis of *Hypericum perforatum* L. (Hypericaceae) species, *Int. J. of Pharm. & Life Sci.*, 11(9): 6953-6958.
Source of Support: Nil
Conflict of Interest: Not declared
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