separations

Supplementary Materials

Hypericum spp.—An Overview of the Extraction Methods and Analysis of Compounds

Valentin Ion [1], Irina Ielciu [2,*], Anca-Gabriela Cârje [1], Daniela Lucia Muntean [1], Gianina Crişan [2,†] and Ramona Păltinean [2,†]

1 Department of Analytical Chemistry and Drug Analysis, Faculty of Pharmacy, "George Emil Palade" University of Medicine, Pharmacy, Science and Technology, 540142 Târgu Mureş, Romania; valentin.ion@umfst.ro (V.I.); anca.carje@umfst.ro (A.-G.C.); daniela.muntean@umfst.ro (D.L.M.)
2 Department of Pharmaceutical Botany, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy, 400010 Cluj-Napoca, Romania; gcrisan@umfcluj.ro (G.C.); rpaltinean@umfcluj.ro (R.P.)
* Correspondence: irina.ielciu@umfcluj.ro
† These authors contributed equally to this work.

Supplementary Tables S1–S3.

Citation: Ion, V.; Ielciu, I.; Cârje, A.-G.; Muntean, D.L.; Crişan, G.; Păltinean, R. *Hypericum* spp.—An Overview of the Extraction Methods and Analysis of Compounds. *Separations* **2022**, *9*, 17. https://doi.org/10.3390/separations9010017

Academic Editors: Daniele Naviglio and Federica Bianchi

Received: 11 December 2021
Accepted: 11 January 2022
Published: 14 January 2022

Table S1. HPLC and UHPLC techniques for the analysis of Hypericum Species.

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
1.	*H. aciculare* *H. andinum* *H. brevistylum* *H. decandrum* *H. laricifolium* *H. silenoides* (flowering aerial parts)	acylphloroglucinol derivatives	uliginosin A isouliginosin B isohyperbrasilol uliginosin B hyperbrasilol B	Assay the antichemotactic activity, in the in vitro lipopoly-saccharide-induced chemotaxis on rat polymorphonuclear neutrophils (PMNs) cells, of lipophilic extracts of six Peruvian *Hypericum* species	C18, 150 X 3.9, 4	A-Water B-Acetonitrile and Methanol 8:2	25	UV-VIS, DAD detector, scan between 190-400 nm, 350 nm	[59]
2.	*H. foliosum* *H. androsaemum* *H. perforatum* (aerial parts)	phenolic acids flavonoids phloroglulinol derivatives	quinic scid chloro- genic acid miquelianin amentoflavones 3,4 dimethylbenzoic acid (+) catechin quercetin-3-O-sulp hate	*In vivo* evaluation of antidepressant activity of a *H. foliosum H. androsaemum* and *H. perforatum* extracts, the pre-clinical safety data and the major marker compounds identified on it	Method A – RP, 150 X 3.9, 5 Method B – Phenyl 150 X 3.9, 5	Method A - trifluoroacetic acid acid 0.05 % in water, acetonitrile, methanol Method B - trifluoroacetic acid acid 0.05 % in water,	Method A 90 Method B 70	UV-VIS, DAD detector, scan between 210 and 450 nm	[36]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			phloroglucinols derivatives			acetonitrile,			
3.	*H. perforatum* (roots)	acylphloroglucinol naphthodianthrone	hyperforin hypericin	Hyperforin and Hypericin extraction method development with extraction efficiency evaluation	C18, 150 X 4.6, 3.5	A-Formic acid 1 mM in water B-Acetonitrile	-	UV-VIS, DAD detector, 272 nm hyperforin 600 nm for hypericin	[71,62]
4.	*H. perforatum* *H. maculatum* *H. hirsutum* *H. tetrapterum* (stems, leaves, flowers)	polyphenolic compounds flavonoids and hypericin	p-coumaric acid feurlic acid hyperoside izoqurcitrozide rutosite quercitrozide quercetol kaempferol luteolin hypericin	HPLC screening of bioactives compounds and antioxidant capacity of different hypericum species	-	A-0.1 % Acetic acid in water B-methanol	35	UV-VIS 330 nm for polyphenolic compounds 370 nm for flavanoids	[46]
5.	*H. ascyreia* *H. androsaemum* *H. inodora* *H. coridium*	anthraquinones phloroglucinols flavonoids	emodin pseudohypericin protopseudohypericin	Phytochemical profiling of several *Hypericum* species identified using genetic markers	anthraquinones and phloroglucinols C18, 50 X 3, 2.7 and	A-10 % acetonitrile in water, pH=2.7 adjusted with	38 and 100	UV-VIS DAD and UV-VIS detector	[2]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
	H. myriandra *H. adenosepalum* (leaves and stems)		hypericin protohypericin total hypericins phloroglucinols chlorogenic acid catechin naringenin naringin rutin hyperoside isoquercetin quercitrin kaempferol-3-O-gl ucoside		chlorogenic acid and flavonoid content C18, 150 X 4.6, 5	trifluoroacetic acid B-acetonitrile or A-5 % acetonitrile in water, pH=2.7 adjusted with trifluoroacetic acid B-80 % acetonitrile pH=2.7		440 nm for emodin 590 nm for hypericin 270 nm for phloroglucinol s 229 nm for (+)-catechin, naringenin, and naringin 254 nm for chlorogenic acid and flavonols	
6.	*H. perforatum* *H. perfoliatum* *H. tomentosum* *H. ericoides* (aerial parts)	tocopherols	α-tocopherol γ-tocopherol δ-tocopherol	Investigation and quantification of fatty acid composition and tocopherol content in four Tunisian *Hypericum* species	C18, 250 X 4.6, 5	A-methanol B-acetonitrile	-	UV-VIS DAD detection 292 nm	[66]
7.	*H. perforatum* (dried tissue	phenolic acids flavonoids	chlorogenic acid rutin	Genetic diversity of *Hypericum* perforatum in	C18, 250 X 4.6, 5	A-0.01 % (V/V) phosphoric acid	55	UV-VIS DAD detector	[39,88]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
	plant ground to powder)		hyperoside quercitroside quercetin	Qinling mountains-China		B-methanol C-acetonitrile		270-700 nm	
8.	*H. perforatum* (top of 2/3 plants-air-dried plant material)	phenolic acids flavonoids naphtodianthrones	chlorogenic acid caffeic acid 2,4 dihydroxybenzoic acid neochlorogenic acid isoquercitrin quercitrin avicularin hyperoside rutin (+)-catechin (-)-epicatechin pseudohypericin Hypericin Hyperforin	Altitudinal impacts on chemical content and composition of *H. perforatum* in northern Turkey	phenolic acids and flavonoids C18, 250 X 4, 5 naphtodiantrones C18, 250 X 4, 5	phenolic acids and flavonoids A-0.3 % phosphoric acid in water B-0.3 % phosphoric acid in acetonitrile naphtodiantrones: A-ethyl acetate B-0.1 M natrium dihydrogen phosphate pH=2.0 C-methanol	45 and - no time analysis indicated	UV-VIS DAD detector 203, 280, 320, 360 nm and 207, 589 nm	[79]
9.	*H. perforatum, H. rumeliacum H. tetrapterum*	anthraquinones and phloroglucinols	pseudohypericin hypericin and their	Evaluation of the elicitation potential of cryogenic	C18, 50 X 3, 2.7	A-5mM ammonium acetate	12	UV-VIS 270 nm for phloroglucinol	[52]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
	(vegetative parts of *in vitro* grown species)		protoforms hyperforin, adhyperforin and other unidentified phloroglucinol derivatives HP-phl-1 HP-phl-2 HR-phl-1, HR-phl-2	treatment on secondary metabolism of some *Hypericum* species.		B-acetonitrile		s 590 nm for napthdianthro nes	
10.	*H. calycinum* (cells cultures)	xanthones	-	Involvement of cytosolic aromatic aldehyde dehydrogenase for xanthone biosynthesis starting from benzoic acid derivatives	C18, 100 X 4.6, 3.5	A-1mM formic acid B-Methanol	36	UV-VIS 254 and 292 nm	[54,92, 93]
11.	*H. perforatum* (air-dried aerial parts)	naphthodianthron e	hypericin	The effects of temperatures on growth and Hypericin Biosynthesis and antioxidant capacity	C8, 150 X 4.6, 5	A-0.03 M KH2PO4 (adjusted to pH=7.0 with 0.5 mol/L K2HPO4) B-methanol	14	UV-VIS 588 nm	[81]
12.	*H. perforatum*	naphtodianthrone	hypericin	Simultaneous	C18, 100 X 2.1, 1.7	A-10 mM of	13	UV-VIS	[32]

HPLC and UHPLC Techniques									
No.	Plant (plant parts)	Class of Compounds	Compounds Specific	Application	Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)	Mobile Phase	Analysis time (min.)	Detection	Ref.
	(*in vitro* shoot cultures)	s bisanthrones skyrin derivatives	pseudohypericin protohypericin protopseudohyper icin emodin and skyrin oxyskyrin, iridoskyrin, rubroskyrin and luteoskyrin	determination of naphtodianthrones, emodin, skyrin and new bisanthrones in Hypericum perforatum L. in vitro shoot cultures		ammonium acetate acidified with 0.1 % formic acid B-acetonitrile/me thanol (80:20)		439 nm and 590 nm FLD excitation 236 nm emission 590 nm	
13.	*H. scruglii* *H. hircinum* *H. perforatum* (aerial parts)	phenolic acids flavonoid/flavonol s cyclitols naphtodianthrone s	shikimic acid chlorogenic acid hyperoside quercetin quercitrin hypericin	Phytochemical profile and α-glucosidase inhibitory activity of Sardinian *H. scruglii* and *H. hircinum*	C18, 150 X 4.6, 5	A-Water B-methanol C-acetonitrile D-phosphoric acid For hypericin: A-acetonitrile B-0.3 % (V/V) phosphoric acid	25	UV-VIS DAD detector Chlorogenic acid, quercetin, hyperoside, quercitrin-340 nm Shikimic acid-210 nm FLD Excitation 315 nm and emission 590	[51]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
								nm	
14.	*H. perforatum* (aerial parts)	phenolic acids flavonols naphtodianthrones	chlorogenic acid, quercetin, rutin, pseudohypericin hypericin	The effects of abiotic stressors and signal molecules on phenolic composition and antioxidant activities of in vitro regenerated *H. perforatum*	C18, 100 X 4.6, 5	A-0.5 % trifluoroacetic acid B-0.5 % trifluoroacetic acid in acetonitrile	30	UV-VIS DAD detector 280 nm, 320 nm, 370 and 520 nm	[80]
15.	*H. perforatum* (raw material)	phenolic acids flavonoids diterpenes	hyperforin hypericin method 1 protocatechuic acid neochlorogenic acid nhlorogenic acid (-)-epicatechin rutin hyperoside isoquercitrin quercitrin	Methods of establishing the authenticity and quality of *H. perforatum* Criteria for quality control, authenticity and stability of preparations.	phenolic acids, flavonoids and diterpenes: C18, 250 X 2, 5 hyperforin and hypericin C18, 50 X 2, monolith	phenolic acids, flavonoids A-acetonitrile B-0.1 % formic acid naphthodianthrones and phloroglucinols A-acetonitrile B-0.1 % formic acid	30 and 25	UV-VIS DAD detector Scan 1 190-800 nm Scan 2 190-800 nm	[20,89]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			quercetin I3,II8-biapigenin method 2 furohyperforin hyperforin adhyperforin pseudohypericin hypericin						
16.	*H. perforatum* *H. canariense* (entire plants)	multiple classes	gallic acid protocatechuic acid protocatechuic aldehyde p-OH-benzoic acid p-OH-benzoic aldehyde vanillic acid vanillin syringic acid salicylic acid chlorogenic acid caffeic acid	Alteration of phenolic metabolism differed species-specifically via PAL - (phenylalanine ammonia-lyase) Quantitative changes of free amino acids	measurement of *Hypericum*-specific metabolites (naphtodiantrones and phloroglucinols) C18, 30 X 2.1, 1.8 amino-acids zorbax eclipse for amino acids-150 X 4. 6 3.5	hypericum-specific metabolites (naphtodiantrones and phloroglucinols) A-water B-acetonitrile amino-acids A-40 mM Na2HPO4, pH=7.8 B-acetonitrile/MeO	3.3 and 26	MS UV-VIS FLD for amino-acids with o-phthalaldehyde and 9-fluorenylmethyl chloroformate	[90,91]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			ferulic acid p-coumaric acid sinapic acid quercitrin hyperoside astragalin rutin luteolin-7-O-glucoside apigenin-7-O-glucoside eriodictyol-chalcone aspartic acid glutamic acid serine histidine glycine threonine arginine alanine			H/water 45/45/10 (v/v)			

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			tyrosine cysteine valine methionine Phenylalanine isoleucine leucine lysine proline						
17.	*H. perforatum* (the top 6 cm)	flavonoids acylphloroglucinol derivatives tocopherols	quercetin kaempferol I3, II8-biapigenin hyperforin adhyperforin α, β, δ, σ-tocopherols	Characterization of *H. perforatum* macerates prepared with different fatty oils	C18, 50 X 2.1, 1.9	flavonoids and acylphloroglucinol derivatives: A-0.1 % formic acid B-0.1 % formic acid in acetonitrile tocopherols A-methanol B-acetonitrile	21.3 -	UV-VIS DAD detector 270 nm, 370 nm 295 nm, 299 nm	[63]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
18.	*H. andinum* *H. brevistylum* *H. silenoides* *H. laricifolium* (aerial parts)	flavonoids naphtodianthrones dimeric acylphloroglucinols	chlorogenic acid rutin hyperoside guaijaverin quercetin phloroglucinols uliginosin isouliginosin isohyperbrasilol uliginosin B hyperbrasilol B *No naphtodiantrones detected*	Phytochemical profiles and antidepressant-like activity- four Peruvian *Hypericum* species	RP, 150 X 2.1	A-10 mM ammonium acetate buffer adjusted to pH=5.0 with glacial acetic acid B-9:1 mixture of acetonitrile and methanol	50	UV-VIS DAD detector multiple wavelengths	[26]
19.	*H. laricifolium,* *H. quitense* *H. decandrum* (plant parts)	multiple compounds	Chromatographic fingerprinting	Developing a predictive model for the antibacterial potential of the genus *Hypericum* using chromatographic fingerprinting with three *Hypericum* species	C18, 100 X 4.6, monolithic column	A-0.1 % phosphoric acid in water B-acetonitrile	50 min	UV-VIS DAD 254, 280 and 360 nm	[77]
20.	*H. perforatum* *H. maculatum*	phenolic compounds	phenolic acids: chlorogenic	Targeted metabolomic in the genus	phenolic acids flavonoids:	phenolic acids flavonoids:	40 and	UV-VIS DAD detectors	[37]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
	H. tetrapterum *H. erectum* *H. humifusum* *H. monogynum* *H. kouytchense* *H. canariense* (*in vitro* grown plants)	anthraquinones phloroglucinols flavonoids	caffeic ferulic cinnamic and gallic acid flavonoids: naringenin apigenin amentoflavone kaempferol kaempferol-3-O-gl ucoside catechin quercetin rutin hy-peroside quercitrin and isoquercetin anthraquinones: hypericin pseudohypericin	*Hypericum* in response to biotic elicitors	C18, 150×4.6 mm, 5 anthraquinones and phloroglucinols: C18, 50 × 3, 2.7	A-5 % acetonitrile pH=2.7 B-80 % acetonitrile pH=2.7 anthraquinones and phloroglucinols: A-10 % acetonitrile, pH adjusted to 2.7 with trifluoroacetic acid B-acetonitrile	20	multiple wavelengths	

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			phloroglucinol						
21.	*H. androsaemum* *H. ericoides*, *H. moserianum* *H. olympicum* (fresh aerial parts)	flavonoid content	flavonoid aglycones: quercetin myricetin isorhamnetin rhamnetin kaempferol luteolin and apigenin	Phytochemical profile, antioxidant and antibacterial activity of four *Hypericum* species from the UK	C18, 250 X 4.6, 5	A-water B-acetonitrile acidified with 1 % acetic acid	-	UV-VIS DAD, 254 nm	[16]
22.	*H. caprifoliatum* *H. carinatum* *H. linoides* *H. myrianthum* *H. polyanthemum* (aerial parts of the plants)	dimeric phloroglucinols benzophenones benzopyrans	uliginosin B hyperbrasilol japonicin cariphenone A cariphenone B 6-isobutyryl-5,7-dimethoxy-2,2-dimethyl-benzopyran (HP1) 7-hydroxy-6-isobutyryl-5-methoxy-2,2-dimethyl-benzopyran (HP2)	Antifungal and antichemotactic activities and quantification of phenolic compounds in lipophilic extracts of *Hypericum* spp. native to South Brazil	C18, 150 X 3.9, 4	Dimeric phloroglucinols: A-acetonitrile B-0.1 % trifluoroacetic acid in water, benzopyrans and benzophenone:s A-60 % acetonitrile, B-40 % water	10 and 20	UV-VIS 220 nm 270 nm	[58]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			5-hydroxy-6-isobutyryl-7-methoxy-2,2-dimethyl-benzopyran (HP3)						
23.	*H. perforatum* (aerial parts)	naphthodianthrones	hypericin	Enhanced hypericin extraction xylanase-assisted extraction	C18, 250 X 4. 6, 5	Different mobile phases	40	UV-VIS 590 and 284 nm	[41]
24.	*H. perforatum*	naphthodianthrones	hypericin	Molecularly imprinted polymer for specific extraction of hypericin	C18, 250X4.6, 5	A-0.50 M, pH= 3.7 ammonium acetate-acetic acid buffer B-methanol	8	UV-VIS 590 nm	[82]
25.	*H. perforatum* *H. annulatul* *H. calycinum* *H. hirsutum* *H. hookerianum* *H. humifusum* *H. maculatum* *H. olympicum* *H. pseudohenryi* (aerial parts)	acylphloroglucinols naphthodianthrones flavonoids phenolic acids	hyperforin pseudohypericin hypericin rutin hyperoside amentoflavone (-)-epicatechin (+)-catechin chlorogenic acid	Phytochemical profile of fingerprinting and finding suitable markers for *Hypericum* species.	flavnoids: C18, 100 X 4.6, 2.6 hyperforin, pseudohypericin, hypericin: C18, 100 X 4.6, 2.6	A-0.025 % trifluoroacetic acid in acetonitrile B-0.025 % trifluoroacetic acid in water A-0.025 % trifluoroacetic	flavonoids: 50	UV-VIS DAD detector multiple wavelengths	[69]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			neochlorogenic acid quercitrin isoquercetin			acid in acetonitrile B-200 mM ammonium acetate			
26.	*H. aviculariifolium* *H. pruinatum* (flowering parts parts)	naphthodiantrones phloroglucinols flavonoids phenolic acids polyphenols	pseudohypericin hyperforin adhyperforin chlorogenic acid neochlorogenic acid (-)-epicatechin rutin	Determining the elicitation effect of jasmonic acid (JA) on the accumulation of secondary metabolites in vitro regenerated flowering plants.	flavonoids and phloroglucinols: RP, 150 X 4.6, 3 catechin and phenolic acids: C18, 250 X 4.6, 5 naphtodianthrones: C18, 150 X 4.6, 5	flavonoids, epicatechin and hyperforin: A-0.3 % phophoric acid in water B-0.3 % phosphoric acid in acetonitrile phenolic acids and catechins: A-0.5 % acetic acid in water B-methanol naphtodianthrones:	30 and 37	UV-VIS DAD detector 324 nm catechin and phenolic acids naphtodianthrones 590 nm	[5,44]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
						A-ethyl acetate B-0.1 M sodium dihydrogen phosphate solution adjusted to pH 2.0 using phosphoric acid C-methanol			
27.	*H. perforatum* (aerial parts)	naphthodianthrones phloroglucine derivatives	hypericine hyperforine pseudohypericine	Comparative analysis of naphthodianthrone and phloroglucine derivatives in St. John's Wort extracts	C18, 250 X 4,5 tested to allow for separation with highest possible peak shape at best resolution.	optimised buffer systems A-aquous with phosphoric acid and triethylamine B-organic, different acetonitrile and methanol mixtures with fosphoric acid and triethylamine	60	UV-VIS UV detection 292 nm 591 nm	[87]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
						additives			
28.	*H. androsaemum* *H. polyphyllum* (flowering air-dried plant material)	naphthodianthrones phloroglucinol derivatives phenolic acids biflavone flavonols	hypericin pseudohypericin hyperforin adhyperforin chlorogenic acid neochlorogenic acid caffeic acid 2,4-dihydroxybenzoic acid biflavone, 13,II8-biapigenin hyperoside isoquercitrin quercitrin quercetin avicularin rutin (+)-catechin (-)-epicatechin	Altitudinal changes in secondary metabolite contents of *H. androsaemum* and *H. polyphyllum*	separation of flavonoids, epicatechin hyperforin: C18, 150 X 3, 3.5 dihydroxybenzoic acid, and caffeic acid derivatives” C18, 250 X 4.6, 5 naphthodiantrones: C18, 150 X 4.6, 5	separation of flavonoids, epicatechin hyperforin: A-0.3 % phosphoric acid B-0.3 % phosphoric acid in acetonitrile catechin, catechin, dihydroxybenzoic acid, and caffeic acid derivatives: A-0.5 % glacial acetic acid in water) B-acetonitrile for separation of	30 37 15	UV-VIS PDA/DAD detector, multiple wavelengths	[45]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
						dihydroxybenzoic acid and catechin; 100% methanol for elution of caffeic acid derivatives naphthodiantrones: A-ethyl acetate B-buffer sodium dihydrogen phosphateadjusted to pH=2.0 with phosphoric acid C-methanol			
29.	*H. perforatum*	naphthodianthrones	hypericin	Combined effect of elevated CO2 and temperature on growth, biomass and secondary metabolite of *H. perforatum*	C18, 250 X 4.6, 5	A-5mM ammonium acetate in water B-acetonitrile B-methanol	20	UV-VIS PDA detector 589 nm	[43]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
30.	*H. andinum* *H. brevistylum* *H. caprifoliatum* *H. carinatum* *H. linoides* *H. myrianthum* *H. polyanthemum* *H. silenoides* (aerial parts)	benzophenones benzopyrans dimeric phloroglucinols	benzophenones cariphenone A cariphenone B benzopyrans 6-isobutyryl-5,7-dimethoxy-2,2-di methyl-benzopyra n 7-hydroxy-6-isobutyryl-5-meth oxy-2,2-dimethyl-benzopyran 5-dimethoxy-2,2-d imethyl-benzopyr an dimeric phloroglu-cinols hyperbrasilol B aponicin uliginosin	Leishmanicidal activity of lipophilic extracts of some *Hypericum* species	C18, 150 X 3.9, 4	A-60 % acetonitrile B-40 % water A-95 %, 5 % water 0.01 % trifluoroacetic acid	24	UV-VIS 270 nm 220 nm	[61]
31.	*H. reflexum*, *H. canariense*	phenolic acids flavonoids	chlorogenic acid rutin	Phytochemical analysis and in vitro biological	C18, 250×4.6mm, 5	A-0.02 % phosphoric acid	130 min.	UV-VIS DAD detector	[19]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
	H. grandifolium (top flowering aerial parts)		hyperoside, isoquercitrin quercetin	activity of three *Hypericum* species from the Canary Islands		pH=2,7) B-acetonitrile/methanol C-ethyl acetate/mix of eluent A/B		quercitrin-210 nm hyperforin-270 nm hypericin-590 nm	
32.	*H. perforatum* *H. angustifolium* (roots)	xanthones prenylated xanthones	biyouxanthone D 1,3,5,6-tetraoxyxanthone 1,3,6,7-tetraoxyxanthone 1,7-dihydroxyxanthone toxyloxanthone B paxanthone 5-O-methyl-2-deprenylrheediaxanthone B	Influence of *Hypericum* roots elicitation with chitosan derivatives	-	A-0.1 % phosphoric acid in water B-methanol	70 min	UV-VIS 260 nm 320 nm	[72]
33.	*H. perforatum ssp. veronense* *H. perfoliatum* *H. empetrifolium* ssp.	phenolic acids, flavonoids, naphthodianthrones phloroglucinols	multiple 3–O–caffeoylquinic acid quercetin–3–O–gal	Metabolomic fingerprinting and genetic discrimination of four *Hypericum* taxa from Greece	C18 250 × 4.6, 5,	A-10 mMM ammonium acetate, pH= 4.5 B-acetonitrile C–methanol	-	UV-VIS DAD detector 270 nm 350 nm 590 nm	[48]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
	empetrifolium *H. triquetrifolium* (aerial parts		actoside quercetin–3–O–glycoside quercetin hypericin						
34.	*H. perforatum* (dried flowers)	naphthodianthrones phloroglucinols polyphenols	naphthodianthrones: hypericin and pseudohypericin phloroglucinols hyperforin and adhyperforin flavonols: myricitrin myricetin derivatives quercetin-3-O-rutinoside quercetin-3-O-galactoside quercetin-3-O-gluc	Analysis of bioactive compounds among different *H. perforatum* provenances with the sole effect of year climatic pattern	C18, 250 X 4.6, 5	polyphenols: B-2.5 % formic acid solution in acetonitrile A-2.5 % solution of formic acid in water naphthodianthrones and phloroglucinols: B-acetonitrile A-20 mM ammonium acetate in water	50	UV-VIS Polyphenols: 330, 350 nm Phloroglucinols: 290 nm Naph-thodianthrones: 590 nm	[34]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			oside quercetin quercitrin cinnamic acids: 3-O-caffeoylquinic acid p-coumaroilquinic acid 5-O-caffeoylquinic acid and p-coumaric acid 3-O-caffeoylquinic acid and 5-O-caffeoylquinic acid biflavonoids: biapigenin amento-flavone						
35.	*H. perforatum* (apical shoots seedlings and reproductive	naphthodianthrones flavonoids and flavonols	hypericin hyperforin amentoflavone chlorogenic acid	The objective was to assess the possible role of O3 as an elicitor of antioxidant	C18, 150 X 4.6, 5	hypericin A-0.1 M triethylammonium	28 55	UV-VIS 270 nm 590 nm	[84]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
	tops)	phenolic acids	hypericin hyperforin hyperoside isoquercitrin kaempferol quercetin quercitrin rutin	compounds in *H. perforatum*		B-acetonitrile hyperforin and other phenols: A-85 % phosphoric acid B-acetonitrile C-methanol			
36.	*H. capitatum var. capitatum* *H. capitatum var. luteum* *H. elongatum var. elongatum* *H. olympicum* *H. polyphyllum* *H. retusum* *H. salsolifolium* *H. spectabile* (aerial parts)	naphthodianthrones acylphloroglucinol flavonoids and flavonols phenolic acids	hypericin pseudohypericin hyperforin, adhyperforin chlorogenic,neochlorogenic, caffeic 2,4-dihydroxybenzoic acids hyperoside isoquercitrin quercitrin quercetin avicularin	Secondary metabolites of Hypericum species from the Drosanthe and Olympia sections	flavonoids, epicatechin, and hyperforin: RP, 150 X 4.6, 3 phenolic acids, catechin, and mangiferin: C18, 250×4.6, 5	flavonoids, epicatechin, and hyperforin: A-0.3 % phosphoric acid as eluent B-0.3 % phosphoric acid in acetonitrile phenolic acids, catechin, and mangiferin: A-0.5 % acetic	30 37 15	UV-VIS flavonoids, epicatechin, and hyperforin: 270-360 nm phenolic acids, catechin, and mangiferin: 275-360 nm naphthodiantrones:	[5]

HPLC and UHPLC Techniques									
No.	**Plant (plant parts)**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			rutin (+)-catechin (−)-epicatechin mangiferin I3, II8-biapigenin amentoflavone			acid in water B-100 % methanol naphthodiantrones: A-ethyl acetate, B-aqueous 0.1 M sodium dihydrogen phosphate solution adjusted to pH=2.0 using phosphoric acid C-methanol		560 nm	
37.	*H. perforatum* (aerial parts)	flavonoids	rutin hyperoside quercitrin quercetin biapigenin	Variation in concentrations of major bioactive compounds in *H. perforatum* from Lithuania	C18, 150 X 4, 7	A-acetonitrile:water:H3PO4 (19:80:1) B-acetonitrile	30	UV-VIS 254 nm	[10]

Table S2. LC-MS and LC-MS/MS Techniques for the analysis of Hypericum Species.

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
1.	*H. aciculare* *H. andinum* *H. brevistylum* *H. decandrum* *H. laricifolium* *H. silenoides* (flowering aerial parts)	acylphloroglucinol derivatives	uliginosin A isouliginosin B isohyperbrasilol uliginosin B hyperbrasilol B	Assay the antichemotactic activity, in the in vitro lipopoly- saccharide-induced chemotaxis on rat polymorphonuclear neutrophils (PMNs) cells, of lipophilic extracts of six Peruvian *Hypericum*, qualitatively characterize the acylphloroglucinol	C18, 150 X 3.9, 4	A-water B-Acetonitrile and Methanol 8:2	25	ESI + ESI - single quadrupole	[59]
2.	*H. perforatum*	acylphloroglucinol derivatives	hyperforin adsecohyperforin secohyperforin adhyperforin	Hyperforin production in *H. perforatum* root cultures	Direct infusion	-	-	ESI - QTrap	[62]
3.	*H. perforatum* *H. maculatum* *H. hirsutum* *H tetrapterum* (stem leaves flowers)	polyphenolic compounds and hypericin	hypericin	Screening of bioactives compounds and antioxidant capacity of different *Hypericum* species		A-Acetic acid 0.1 % in water B-methanol	35	-	[46]

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
4.	*H. perforatum* (in vitro shoot cultures)	naphthodianthrones, bisanthrones precursors skyrin derivatives	hypericin, pseudohypericin, protohypericin and protopseudohypericin emodin and skyrin oxyskyrin, iridoskyrin, rubroskyrin and luteoskyrin	Simultaneous determination of naphtodianthrones, emodin, skyrin and new bisanthrones in *H. perforatum* in vitro shoot cultures	C18, 100 X 2.1, 1.7	A-10 mM of ammonium acetate acidified with 0.1 % formic acid B-acetonitrile/methanol (80:20)	13	ESI - Orbitrap	[32]
5.	*H. scruglii* *H. hircinum* *H. perforatum* (aerial parts)	phloroglucinol derivatives	3-geranyl-1-(2′-methylbutanoyl)-phloroglucinol 3-geranyl-1-(2′-methylpropanoyl)-phloroglucinol	Phytochemical profile and α-glucosidase inhibitory activity of Sardinian *H. scruglii* and *H. hircinum*	RP, 250 X 4,6, 4	A-0.1 % formic acid in water B-0.1 % acetonitrile	-	ESI - QQQ	[51]
6.	*H. perforatum*	phenolic acids	chlorogenic acid	The effects of abiotic stressors	C18, 100 X 4.6, 5	A-0.5 %	30	ESI –	[80]

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
	(aerial parts)	flavonoids naphthodianthrones	quercetin rutin pseudohypericin hypericin	and signal molecules on phenolic composition and antioxidant activities of in vitro regenerated *H. perforatum*	C18, 150 X 2.1, 5	trifluoroacetic acid in water B-0.5 % trifluoroacetic acid in acetonitrile A-0.5 % formic acid in water B-0.5 % formic acid in acetonitrile	21	ESI + QQQ	
7.	*H. perforatum* (shoot cultures)	naphthodianthrones	hypericin and pseudohypericin	Biological studies confirmed that nano-perlite and MnO2/perlite-NCs effect to H. perforatum showing positive effects Determination of hypericin and pseudohypericin using LC-MS/MS analysis	RP, 40 X 2, 5	A-0.01 M, pH=7 triethylammonium acetate buffer B-methanol and acetonitrile (1:1)	10.5	ESI -	[55]
8.	*H. perforatum*	phenolic acids	hyperforin	Method of establishing the	phenolic acids,	phenolic acids and	30	phenolic	[20,89]

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
	(raw plant)	flavonoid diterpenes polyprenylated acylphloroglucinol derivative naphthodianthrones	hypericin method 1 protocatechuic acid neochlorogenic acid chlorogenic acid (-)-epicatechin rutin hyperoside -soquercitrin quercitrin quercetin I3, II8-biapigenin method 2 furohyperforin hyperforin adhyperforin pseudohypericin	authenticity and quality of *H. perforatum*	flavonoids and diterpenes: C18, 250 X 2, 5 hyperforin and hypericin derivatives C18. 50 X 2 monolithic	flavonoids A- Acetonitrile B-0.1 % formic acids in water naphthodianthrones and phloroglucinols A-acetonitrile B-0.1 % formic acid in water	25	acids and flavonoids ESI- Scan mode m/z 120-650 naphthodianthrones and phloroglucinols ESI +/- Scan mode m/z 120-650	

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			hypericin						
9.	*H. perforatum* *H. canariense*	naphthodianthrones	hypericin pseudohypericin protohypericin hyperforin	Measurement of *Hypericum*-specific metabolites	C18, 30 X 2, 1.8	A-0.05 M ammonium acetate in water B-acetonitrile	3.3	ESI – QQQ MRM	[90]
10.	*H. andinum* *H. brevistylum* *H. silenoides* *H. laricifolium* (aerial parts)	flavonoids naphtodianthrones dimeric acylphloroglucinols	chlorogenic acid rutin hyperoside guaijaverin quercetin phloroglucinols uliginosin isouliginosin isohyperbrasilol uliginosin B hyperbrasilol B No naphtodiantrones detected	Phytochemical profiles and antidepressant-like activity-four Peruvian *Hypericum* species	C18, 100 X 2.1, 1.9	A-water B-acetonitrile and methanol 8:2	15	ESI + QTOF detection	[26]
11.	*H. perforatum* *H. maculatum*	phenolic acids flavonoids	quinic acid derivative	Comparison of chemical composition of *H. perforatum*	C18, 150 X 2.1, 5	A-0.1 % formic acid in water	42	ESI - ion trap	[27,97]

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
	(air dried leaves and flowers)	naphthodianthrones	caffeic acid glucoside vanillic acid glucoside neochlorogenic acid chlorogenic acid catechin epicatechin myricetin glucoside, hyperoside isoquercitrin rutin quercetin pentoside, quercitrin kaempferol glucoside kaempferol rutinoside	and *H. maculatum*		B-acetonitrile		Scan mode m/z 50-1000	

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			quercetin hyperforin adhyperforin, protopseudohypericin pseudohypericin hypericin						
12.	*H. perforatum* roots, non-flower shoots, flower shoots	phenolic acids flavan-3-ols flavonol glycosides flavonoid aglycones anthocyanins naphthodianthrones acyl-phloroglucinols xanthones	phenolic acids quinic acid chlorogenic acid 3-p-coumaroylquinic acid 3-feruloylquinic acid flavan-3-ols catechin B-type procyanidin dimer procyanidin trimer B-type	Phytochemical composition of *H. perforatum* from Northern Republic of Macedonia.	C18, 150 × 4.6, 5 μm	A-water–formic acid (99:1) B-methanol	90	ESI + ESI - ion trap Full scan	[18]

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			procyanidin dimer (Epi)catechin B-type procyanidin dimer flavonol glycosides quercetin 3-O-galactoside) quercetin 3-O-rutinoside guaijaverin quercetin 3-O-arabinoside kaempferol 3-O-glucoside quercetin 3-O-rhamnoside) kaempferol 3-O-rutinoside flavonoid						

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			aglycones quercetin I3–II8 Biapigenin amentoflavone anthocyanins cyanidin 3-O-glycoside cyanidin 3-O-rhamnoside naphthodianthrones pseudohypericin padiaxanthone brasilixanthone dimethylmangiferin xanthone derivative 3,6-dihydroxy-1,5,						

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			7-trimethoxy-xanthone cadensin C mangostin 5-O-Methyl-2-deprenylrheediaxanthone B cadensin G garcinone C cadensin C isomer						
13.	*H. perforatum* (aerial parts)	phenolic acids flavonoids naphthodianthrones	caffeoylquinic acid p-coumaroylquinic acid chlorogenic acid derivative catechin quercetin 3-O-galactoside quercetin rutinoside quercetin	Optimisation of polyphenol extraction from *H. perforatum* using aqueous glycerol	C18, 150 X 2, 4	A-2.5 % acetic acid B-methanol	60	ESI + Single quadrupole	[57]

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			rhamnoside derivative hypericin						
14.	*H. cardonae* *H. myricariifolium* *H. laricifolium* *H. humboldtianum* *H. garciae* *H. carinosum* *H. cuatrecasii* (aerial parts)	Flavonols Flavan-3-ols Stilbenoids Coumarins Cinnamic Acids Flavones	caffeic acid 3.5-dihydroxybenzoic acid gallic acid vanillic acid 2.6-di-OH-benzoic acid p-OH-benzoic acid cinnamic acid kampferol quercetin-3-glucoronide quercetin-3-rabinoside quercetin quercetin-3-glucoside kampferol-3-gluco	Phenolic profile, chemical relationship and antifungal activity of Andean *Hypericum* species	HSS T3, 150 × 2.1, 1.8	A-0.1% formic acid in water B-0.1% formic acid in acetonitrile	15	ESI + ESI – Triple quadrupole MRM	[40]

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			ronide kampferol −3-glucoside iso-rhamnetin syringetin-3-glucoside iso-rhamnetine-3-glucoside myricetin cathechin epicathechin gallocathechin epicgallocathechin procyanidin B1 procyanidin B2 c-piceid wsculin neochlorogenic acid cryptochlorogenic acid						

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			chlorogenic acid coniferyl alchol phlorizin luteolin naringenin luteolin 7-O-glucosid naringenin-7-glucoside						
15.	*H. perforatum* *H. calycinum* *H. confertum* (flowering aerial parts)	multiple classes	coumarin hesperidin p-coumaric acid o-coumaric acid gallic Acid 6-caffeic Acid vanilic Acid salicylic Acid quinic Acid 4-OH-benzoic acid ferulic acid chlorogenic acid	Anti-aging potential and anti-tyrosinase activity of three Hypericum species with focus on phytochemical composition by LC–MS/MS	C18, 100 × 2.1, 2	A-10mM ammonium formate and 0.1% formic acid in water B-acetonitrile	50	ESI + ESI – Triple quadrupole MRM 2 transitions /compound	[14]

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			rosmarinic acid protocatechuic acid cinnamic Acid sinapinic Acid fumaric Acid vanilin pyrocatechol malic acid syringic acid hesperetin naringenin rutin quercetin quercitrin apigenin chrysin liquiritigenin isoquercitrin apigetrin rhoifolin						

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			nicotiflorin fisetin luteolin myricetin kaempferol						
16.	*H. perforatum* (aerial parts)	flavonols flavonoids phenolic acids	(+)-catechin (−)-epicatechin chlorogenic acid hyperoside I3, II8-biapigenin rutin.	Improvement of antioxidant activity and polyphenol content of *H. perforatum*	C18, 150, 4.6, 3	A-0.5% formic acid in water B-acetonitrile.	35	ESI – Scan mode Single quadrupol e	[38]
17.	*H. reflexum*, *H. canariense* *H. grandifolium* (top flowering aerial parts)	naphthodianthron es	hypericin	Phytochemical analysis and in vitro biological activity of three *Hypericum* species from the Canary Islands	C18, 250 X 4.6, 5	A-water B-acetonitrile/metha nol (90:10 v/v)	15 C18 (250 × 4.6 mm, 5 μ)	ESI - Ion Trap SIM/TIC/E IC	[19]
18.	*H. perforatum* (whole plant)	phenolic acids flavonols epicatechin procyanidins naphtodianthrone	phenolic acids: gallic acid protocatechuic acid protocatechuic	Oxidative stress induced by lanthanum and cadmium produces modifications in metabolic profile of *H. perforatum*	C18, 50 X 3, 2.7	A-methanol B-0.2% (v/v) acetic acid in water	8	ESI – for procyandi ns MRM	[96]

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
		s	aldehyde p-OH-benzoic acid p-OH-benzoic aldehyde vanillic acid vanillin syringic acid salicylic acid chlorogenic acid caffeic acid ferulic acid p-coumaric acid flavonols: astragalin hyperoside quercetin quercitrin rutin epicatechin						

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			naphtodianthrones: hypericin hyperforin						
19.	*H. perforatum ssp. veronense* *H. perfoliatum* *H. empetrifolium ssp. empetrifolium* *H. tri-quetrifolium* (aerial parts)	multiples caffeoylquinic acids flavonoids flavonoid glycosides biflavones xanthones naphthodianthrones phloroglucinols or phloroglucinol derivatives	63 compounds	Metabolomic fingerprinting and genetic discrimination of four Hypericum taxa from Greece	C18, 100 × 2.1, 1.7	A-0.1 % aqueous formic acid B-acetonitrile	25 minutes	ESI – ESI + Full scan m/z 85-1200 QTOF	[48]
20.	*H.perforatum* *H. rumeliacum* *H. triquetrifolium* (extracts)	phenolic acids flavonols biflavones, naphthodianthron	naphthodianthrones: protohypericin protopseudohyper	Compund identification and correlation with necrotic cell-death activity in human leukemic cells	RP, 100 × 2.1, 3	A-0.1 % acetic acid in water B- methanol	24 minutes	ESI - Orbitrap	[68]

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
		es phloroglucinols,	icin hypericin pseudohypericin phloroglucinols: hyperforin hyperforin hyperjovinol B flavonoids: flavonoid-O-glycosides quercetin 3-O-pentoside skyrin-2-O-glucopyranoside rutin miquelianin hyperoside isoquercitrin astilbin						

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			quercitrin aglycon quercetin biflavones: biapigenin amentoflavone chlorogenic acids: caffeic, ferulic and p-coumaric acid quinic acid chlorogenic acid 3-O-feruloylquinic acid						
21.	*H. perforatum*	flavonols flavonoids	rutin hyperoside, quercitrin and quercetin adducts with ABTS+	ABTS+ scavenging potency of selected flavonols from *H. perforatum*	C18, 100 X 4.6, 5	A-0.1% formic acid in water B-methanol	21.0 min	ESI + ESI – QQQ MRM	[95]
22.	*H. aegypticum*	naphthodianthron	naphtodianthrone	Phytochemical profiles,	C18, 250 X 4.6, 5 μm	polyphenols:	50	ESI +	[30]

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (µm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
	H. androsaemum *H. calycinum* *H. hircinum* *H. hirsutum* *H. montanum* *H. patulum* *H. perfoliatum* *H. perforatum* *H. pubescens* *H. tetrapterum* (flowering tops)	es acylphloroglucinol s cinnamic acids flavonoids biflavones	s: protopseudohyper icin pseudohypericin protohypericin hypericin acylphloroglucinol s: hyperforin adhyperforin cinnamic acids 3-O-caffeoylquinic acid p-cumaroylquinic acid 5-O-caffeoylquinic acid p-Coumaric acid	phototoxic and antioxidant properties of eleven *Hypericum* species – a comparative study		A- B- 2.5% formic acid in acetonitrile naphtodiantrones and phloroglucinols: B-acetonitrile A-20 mM ammonium acetate in water		orbitrap Full scan m/z 120-1500	

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
			flavonoids: catechin quercetin-3-O-rutinoside myricetin-3-O-rhamnoside quercetin-3-O-galactoside quercetin-3-O-glucoside quercetin-3-O-arabinoside quercetin-3-O-rhamnoside quercetin biflavones: biapigenin amentoflavone						
23.	*H. hengshanense*	phloroglucinol	Multiple – 52	UPLC-QTof-MS chemical	C18, 100 X 2.1, 1.7	A-10mM aqueous	16	ESI -	[42]

LC-MS and LC-MS/MS Techniques									
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length X diameter (mm X mm), particle dimension (μm)**	**Mobile Phase**	**Analysis time (min.)**	**Detection**	**Ref.**
	H. petiolulatum subsp. yunnanense *H. seniawinii* *H. daliense* *H. himalaicum* *H. wightianum* *H. japonicum* *H. perforatum* (aerial parts and roots)	derivatives flavonoids chromones glycerophospholipids	compounds	profiling and characterization of antiproliferative and anti-inflammatory compounds from seven *Hypericum* species in PR China		ammonium acetate buffer, adjusted to pH=5.0 with formic B-acetonitrile:-methanol (9:1)		ESI + Q-TOF Full scan m/z 100-1500 MS^{e+}	

Table S3. GC, GC-MS and GC-MS/MS Techniques for the analysis of Hypericum Species.

GC, GC-MS and GC-MS/MS techniques										
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length (m), type**	**Vector Gas**	**Analysis Time (min)**	**Detection**	**Identification/ Quantification**	**Ref.**
1.	*H. perforatum, H. perfoliatum, H. tomentosum H. ericoides* (aerial parts, top of 2/3)	fatty acids-total and specific tocopherols	caprylic acid capric acid lauric acid myristic acid palmitic acid palmitoleic acid stearic acid oleic acid linoleic acid a-linolenic acid arachidic acid behenic acid saturated fatty acids (SFA) unsaturated fatty acids (UFA) SFA/UFA	Fatty acid composition and tocopherol content in four Tunisian *Hypericum* species	RT-2560, 100, capillary	N_2	97	FID	comparison of the retention time with those of reference standards	[66]
2.	*H. perforatum* (the top of 2/3 plants)	essential oils fatty acids	168 compounds	Altitudinal impacts on chemical content and	CP-5MS, 30, film coated capillary column	He	120	MS-EI	fragmentation patterns Kovats index	[47]

GC, GC-MS and GC-MS/MS techniques										
No.	Plant	Class of Compounds	Compounds Specific	Application	Chromatographic column Chemistry, length (m), type	Vector Gas	Analysis Time (min)	Detection	Identification/ Quantification	Ref.
				composition of H. perforatum					Spectral library Area normalization	
3.	*H. perforatum* (dried leaves)	monosaccharides polysaccahrides	mono-saccharides L-arabinose D-galactose rhamnose mannose, glucose xylose	Characterization of *H. perforatum* polysaccharides with antioxidant and antimicrobial activities alditol acetates	HP-5MS, 30, film coated capillary column	He	47	MS	-	[21]
4.	*H. calycinum* (cell cultures)	different benzoic acids derivatives	benzoic acid derivatives transcinnamic acid trimethylsilyl (TMS) derivatization of the acidic protons present	Involvement of Cytosolic aromatic aldehyde dehydrogenase for xanthone biosynthesis starting from benzoic acid derivatives	ZB-5MS, 30, film coated capillary column	He	18	MS	identification of transcinnamic acid based on stanrd and MS spectra	[54]
5.	*H. perforatum* (shoot cultures)	terpenes (monoterpenes and	35 compounds	Biological confirming that nano-perlite and	HP-5MS-dimethylpolysiloxan, 30, fused silica capillary	He	65	MS-EI	-	[55]

GC, GC-MS and GC-MS/MS techniques										
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length (m), type**	**Vector Gas**	**Analysis Time (min)**	**Detection**	**Identification/ Quantification**	**Ref.**
		sesquiterpenes) fatty acids hydrocarbons		MnO2/perlite to H. perforatum have positive effects						
6.	*H. perforatum* (aerial parts)	essential oils fatty acids long chain alcohols	72 compounds	Solvent-free microwave extraction influence VS conventional hydrodistillation over types and oil content	Elite 5MS non-polar, 30, film coated capillary column	He	110	MS-EI	GC linear retention indices (RI) reference to a homologous series C5–C32 n-alkanes comparison of the mass spectral with those stored in the MS database percentage composition- summation of peak areas of the total oil.	[67]
7.	*H. perforatum*	fatty acids		Characterization of	ZB-WAX, 30, film	He	80	FID	identification	[63]

GC, GC-MS and GC-MS/MS techniques										
No.	Plant	Class of Compounds	Compounds Specific	Application	Chromatographic column Chemistry, length (m), type	Vector Gas	Analysis Time (min)	Detection	Identification/ Quantification	Ref.
	(the top 6 cm comprising the flowers)	(methyl esters)		*H. perforatum* macerates prepared with different fatty oils phytochemical profile of oil extracts comprising fatty acids and terpenes	coated capillary column on-column derivatization with trimethylsulfonium hydroxide (TMSH)			MS-EI	relative to standards, mass spectra in NIST data-base, determination of average chain length	
8.	*H. asperulum* *H. scabrum* *H. vermiculare* (aerial part of plants)	essential oils	78 compounds	Essential oil composition of three Iranian *Hypericum* species collected from different habitat conditions	GC-FID VF-5MS, 30, film coated capillary column GC-MS HP-5, 30, film coated capillary column	He	75	FID MS (m/z 40-400)	mixture of n-alkanes (C8–C40) arithmetic retention index (AI) of peaks. MSD ChemStation NIST Mass Spectral peak assignment	[75]
9.	*H. lydium* *H. orientale* *H. confertum*	essential oils hydrocarbons aldhehydes	80 compounds	*Hypericum spp.* volatile profiling and the potential	HP-WAX, 30, film coated capillary	GC-FID N_2	GC-FID 42	FID MS-ion trap	reference standards linear retention indices relative to	[73]

GC, GC-MS and GC-MS/MS techniques										
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length (m), type**	**Vector Gas**	**Analysis Time (min)**	**Detection**	**Identification/ Quantification**	**Ref.**
	(aerial parts)	hydrocarbon monoterpenes oxygenated mononoterpenes hydrocarbon sesquiterpenes oxygenated sesquiterpenes		significance in the quality control of new valuable raw material	columns HP-5, 30, film coated capillary column GC-MS DB-5, 30, film coated capillary columns	GC-MS He	GC-MS 60		n-hydrocarbons library mass spectra NIST 98 and ADAMS	
10.	*H. perforatum* *H. annulatul* *H. calycinum* *H. hirsutum* *H. hookerianum* *H. humifusum* *H. maculatum* *H. olympicum* *H. pseudohenryi* (aerial part-flowering phase)	secondary metabolites naphthodianthrones flavonoids phenolic acids	hyperforin pseudohypericin hypericin rutin hyperoside amentoflavone (-)-epicatechin (+)-catechin chlorogenic acid neochlorogenic acid quercetin	Phytochemical profile of *Hypericum* plants, Identification of potential marker distinguishing *H. perforatum* from other *Hypericum* species.	HP-5, 30, film coated capillary column	He	18	FID	reference standards	[69]

GC, GC-MS and GC-MS/MS techniques										
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length (m), type**	**Vector Gas**	**Analysis Time (min)**	**Detection**	**Identification/ Quantification**	**Ref.**
			quercitrin isoquercetin							
11.	*H. perforatum* (aerial parts)		Structural elucidation two novel β-diketones, 2,6,9-trimethyl-8-decene-3,5-dione and 3,7,10-trimethyl-9-undecene-4,6-dione	Structural elucidation identification of two novel prenylated β-diketones, structurally related to (ad)hyperforin.	DB-5MS (5% phenylmethylsiloxane), 30, film coated capillary column	He	79	MS-EI (m/z 35-850)	spectra based elucidation	[35]
12.	*H. reflexum* *H. canariense* *H.grandifolium* (top flowering aerial parts)	essential oils alkanes monoterpene hydrocarbons oxygenated monoterpenes sesquiterpenes hydrocarbons oxygenated sesquiterpenes	160 compounds	Phytochemical analysis and in vitro biological activity of three Hypericum species from the Canary Islands	GC-FID HP-5 (5% phenylmethylpolysiloxane, 30. film coated capillary column GC-MS HP-5 MS (5% phenylmethylpolysiloxane, 30, film coated	He	GC-FID 65 GC-MS 65	FID MS-EI	identifcation by co-injection with authentic standards FID peak-area internal normalization without using correction factors.	[19]

GC, GC-MS and GC-MS/MS techniques										
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length (m), type**	**Vector Gas**	**Analysis Time (min)**	**Detection**	**Identification/ Quantification**	**Ref.**
					capillary column				MS data consisting the computer matching with the WILEY275, NIST 08 ADAMS, FFNSC 2 and	
13.	*H. perforatum ssp. veronense* *H.perfoliatum* *H. empetrifolium* ssp. *empetrifolium, H. triquetrifolium* (aerial parts)	volatile metabolites in essential oils	113 compunds	Metabolomic fingerprinting and genetic discrimination of four Hypericum taxa from Greece	HP-5 MS, 30, film coated capillary column	He	81	MS	retention indices series of alkane standards (C8–C40) library-NIST/EPA/NIH Mass Spectral Library, Version 2.0d; NIST, Gaithersburg, MD	[48]
14.	*H. rochelii* *H. umbellatum* (fresh aerial parts)	monoterpene hydrocarbon oxygenated monoterpenes sesquiterpene hydrocarbon	*H. rochelii*-79 compounds *H. umbellatum*-126 compounds	The chemical composition and antimicrobial activity studies on the essential oils of H. rochelii	HP-5MS (5% phenylmethylsiloxane), 30, film coated capillary column	He	54	FID MS-EI (m/z 35-500)	oil constituents-linear retention indices (relative to n-alkanes)	[74]

GC, GC-MS and GC-MS/MS techniques										
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length (m), type**	**Vector Gas**	**Analysis Time (min)**	**Detection**	**Identification/ Quantification**	**Ref.**
		oxygenated sesquiterpenes diterpenoids triterpenoids non-terpenoids		and H. umbellatum					mass spectra comparison with those of authentic standards, Wiley 6, NIST02, MassFinder 2.3 homemade MS library with the spectra corresponding to pure	
15.	*H. perforatum*, *H. hookerianum* *H. bellum* *H. pseudohenryi* (aerial parts)	multiple compounds	*H. perforatum*-56 compounds *H. hookerianum*-71 compounds *H. bellum*-43 compunds	Chemical characterization, neuroprotective, antimicrobial and enzyme inhibitory activities of Hypericum volatile oils	SLB-5 MS, 10, fused-silica capillary column	He	46.5	MS-EI (m/z 30-500)	retention index relative to a series of n-alkanes (C7-C30). mass spectrum library with reference to Wiley 275 and NIST 2017 databases	[17]

GC, GC-MS and GC-MS/MS techniques										
No.	**Plant**	**Class of Compounds**	**Compounds Specific**	**Application**	**Chromatographic column Chemistry, length (m), type**	**Vector Gas**	**Analysis Time (min)**	**Detection**	**Identification/ Quantification**	**Ref.**
			H. pseudohenryi,-57 compunds							
16.	*H. perforatum* (aerial parts flowering stage)	monoterpenes hydrocarbons oxygenated monoterpens sesquiterpene hydrocarbons oxygenated sesquiterpens	46 compounds	Chemical characterization of the essential oil compositions from Iranian populations of H. perforatum	GC-FID BP5, 30, film coated capillary column GC-MS HP-5MS, 30, film coated capillary column	GC-FID He GC-MS He	GC-FID 75 GC-MS 20.6	FID MS-EI (m/z 50-550)	authentic standards chromatographic linear retention indices, determined relative to a series of n-alkanes (C8 C30) MS data matching with the WILEY275, NIST 05, ADAMS, and a home-made library	[76]