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ORIGINAL ARTICLE

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GC/MS Analysis of *Hypericum perforatum* L. (Hypericaceae) Species

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Hypericum perforatum L. aerial parts have been extracted with tetrahydrofuran solvent and their chemical composition has been investigated by Gas Chromatography-Mass Spectrometry (GC/MS) analysis. GC/MS chromatogram of the 4 tetrahydrofuran studied *H. perforatum* genotypes (HP) revealed that the main constituents for HP1 were: 9-Octadecenamide (Z)-(Oleic acid) (65.551%), Hexadecenamide (20.681%) and Dodecenamide (5.595%). Whereas, for HP2, they were 9-Octadecenamide (Z)- (Oleic acid) (63.117%), Hexadecenamide (19.107%) and Dodecenamide (5.585%). As for HP3, they were 9-Octadecenamide (Z)-(Oleic acid) (63.496%), Hexadecenamide (18.891%) and Dodecenamide (5.961%). Whereas, they were 9-Octadecenamide (Z)-(Oleic acid) (62.048%), Hexadecenamide (19.325%) and Dodecenamide (5.914%) for HP4. Thereby, isolation of these constituents and investigation of their biological activity is requested.

Key words: Hypericum perforatum L., phytochemical analysis, gas chromatography-mass spectrometry (GC/MS), chemical constituents

Hypericum perforatum L. species belongs to *Hypericum* genus and Hypericaceae family which included approximately 500 species of flowering plants (Schepetkin *et al.*, 2020). Wild *H. perforatum* L. is one of 21 *Hypericum* species existent in Syria (Mouterde, 1970). *H. perforatum* L. is commonly known as St. John's wort and it is a perennial herb native to relatively dry temperature zones of Europe and North America (Çırak *et al.*, 2010). Its richness in different secondary metabolites including essential oils, amino acids, tannins, flavonoids, xanthones, naphthodianthrones, phloroglucinols, procyanidins, phenylpropanes and other water-soluble components (Çırak *et al.*, 2010) make them as one of the most commonly-investigated medicinal plants of the last two decades. It displayed different pharmacological properties *e.g*. as anticholinesterase and antioxidant properties (Božin *et al.*, 2013); antidepressant, antibacterial, antifungal, antiviral, relaxing smooth muscle contraction, inhibiting protein kinase C, potentiating wound healing and photodynamic effects (Ivetic *et al.*, 2011). It became one of the most commercially plants used due to its medicinal value beside its traditional applications in folk medicine and its importance as an ornamental plant (Saleh, 2019). Thereby, many researches have been done to investigate its extracts and essential oils chemical composition using different analytical methods; *e.g*. Gas chromatography-mass spectrometry (GC/MS) (Çakir *et al.*, 1997; Seger *et al.*, 2004; Chatzopoulou *et al.*, 2006; Çırak *et al.*, 2010; Helmja *et al.*, 2011; Chauhan *et al.*, 2011; Pirbalouti *et al.*, 2014; Đorđević, 2015; Parchin and Ebadollahi, 2016; Saleh, 2019; Schepetkin *et al.*, 2020); high-performance layer chromatography (HPLC) (Nuevas-Paz *et al.*, 2005; Božin *et al.*, 2013); high-performance layer chromatography (HPLC) with a diode-array detector (DAD)- mass spectrometry (MS) - (MS) coupling (HPLC–DAD–MS–MS) HPLC– (Silva *et al.*, 2005); liquid chromatography (LC)-with a diode-array detector (DAD)-mass spectrometry (MS) - (MS) coupling (LC-DAD-MS/MS) (Rusalepp *et al.*, 2017) and fourier transform raman spectroscopy (FT-Raman) (Saleh, 2020).

In the current study, tetrahydrofuran extract of wild *Hypericum perforatum* L. aerial parts was collected from four *H. perforatum* genotypes grown in Lattakia-Syria and their chemical composition has been investigated by GC/MS analysis.

MATERIALS AND METHODS

Plant Material

Wild *Hypericum perforatum* L. (HP) aerial parts (10 plants/sample) were collected from four genotypes in Lattakia-Syria. Sampling has been carried out during blooming stage, from four collection sites differ in their altitude (80-680 m) and annual rainfall (750-1250 mm) (Table 1). Samples were shade dried for two weeks, milled to fine powder by special electric mill and stored separately in glass bowls until extracts preparation.

Extract preparation

The fine powder for each sample was extracted with tetrahydrofuran solvent as flowing: 1 g of fine powder was extracted with 10 mL tetrahydrofuran overnight, filtrated with filter papers (Whatman no.1). Then, all extracts were kept in tightly fitting stopper bottles and stored at 4 °C. The final obtained extracts were then analyzed using GC/MS analysis.

GC/MS assay

To investigate chemical components in tetrahydrofuran *H. perforatum* L. aerial parts extract, GC Chromatec-Crystal 5000 system, supported with Chromatec Crystal Mass Spectrometry Detector (Chromatec, Russia) has been employed. GC/MS analysis has been carried out according to the following conditions: The range scan was 42-850 MU, the column [BP-5-MS (30 m × 0.25 mm × 0.25 µm)], carrier gas (0.695 ml/min flow of Helium gas). Oven temperature was programmed initially at 35 °C for 1 min, then an increase by 10°C /1 min till 220 °C, then increase to 230 °C by 1°C /1 min followed by 10 °C /1 min increasing till 255 °C (hold for 5 min). Injector temperature was 275 °C and detector temperature was 280 °C and ionization energy was 70 ev. Each extract component was identified by comparing retention time values of gas chromatography on polar columns and by comparing mass spectrum and NIST library databases.

RESULTS AND DISCUSSION

GC/MS analysis of the 4 tetrahydrofuran studied *H. perforatum* genotypes extracts has been performed. GC/MS chromatogram revealed 20, 18, 19 and 20 chemical constituents were identified in the 4 tetrahydrofuran studied *H. perforatum* genotypes HP1 (Table 2), HP2 (Table 3), HP3 (Table 4) and HP4 (Table 5) extracts. Of which eleven constituents commonly occurred in the 4 tetrahydrofuran studied *H. perforatum* genotypes extracts (Figure 1). Whereas, the remaining constituents were presented in scare amounts.

GC/MS chromatogram in the 4 tetrahydrofuran studied *H. perforatum* genotypes revealed that the main constituents for HP1 were: 9-Octadecenamide (Z)- (Oleic acid) (65.551%), Hexadecenamide (20.681%), Dodecenamide (5.595%), 13-Docosenamide, (Z) (2.114%) and Pentadecanal (1.25%). Whereas, for HP2, they were 9-Octadecenamide (Z)- (Oleic acid) (63.117%), Hexadecenamide (19.107%), Dodecenamide (5.585%), 13-Docosenamide, (Z) (2.514%) and Pentadecanal (1.419%). As for HP3, they were 9-Octadecenamide (Z)- (Oleic acid) (63.496%), Hexadecenamide (18.891%), Dodecenamide (5.961%), 13-Docosenamide, (Z) (2.481%) and Pentadecanal (1.041%). Whereas, they were 9-Octadecenamide (Z)- (Oleic acid) (62.048%), Hexadecenamide (19.325%), Dodecenamide (5.914%), 13-Docosenamide, (Z) (2.739%) and Pentadecanal (1.046%) for HP4.

In the current study, Glycerol 1-palmitate and 9-Octadecenamide (Z)- (Oleic acid) presented in the 4 tetrahydrofuran *H. perforatum* extracts and Phytol in tetrahydrofuran *H. perforatum* HP2 and HP4 extracts were supported by Seger *et al.* (2004). Moreover, n-Hexadecenoic acid as a common constituent occurred in the 4 tetrahydrofuran *H. perforatum* extracts in the current study, was reported in the same species and supported by Saleh (2019). Indeed, among chemical constituents, Hexadecenoic acid, Octadecanoic acid and 9-Octadecenamide (Z)- presented in the 4 tetrahydrofuran *H. perforatum* extracts in the current study, were reported in ethanolic *Psorospermum febrifugum* extracts belonging to the same family (Asogwa *et al.*, 2019).

Some studies deal with *H. perforatum* extracts phytochemical analysis. In this regards, Seger *et al.* (2004) reported 9 alkanes (C21–C32), four primary (C24, C26, C28 and C30) and one secondary alkanol (C28), one aldehyde (C32), alkanoic acids (C14–C32), linoleic acid, oleic acid, methyl linoleate, glyceryl-1-palmitate, as well as 42 wax esters (C29–C48). Moreover, one sesquiterpene alcohol, nerolidol, two diterpenes, neophytadiene and phytol, two pentacyclic derivatives, -amyrin and lupeol and six triterpenes, squalene, the sterols -sitosterol, -stigmasterol, and nervisterol were observed in *H. perforatum* supercritical fluid extracts (carbon dioxide without modifiers extract) using GC/MS. Whereas, Nuevas-Paz *et al.* (2005) reported protopseudohypericin, pseudohypericin, protohypericin and hypericin in methanolic *H. perforatum* extract using HPLC analysis. Moreover, Silva *et al.* (2005) reported rutinacetyl and Kaempferol 3-rutinoside were identified for the first time in total ethanolic *H. perforatum* extracts using HPLC–DAD–MS–MS analysis. Moreover, Božin *et al.* (2013) reported ethanolic *H. perforatum* extract using HPLC analysis. They reported that total phenolics ranged between 14.35-16.72%, whereas, total flavonoids ranged between 1.33-2.48%. Indeed, performance phenolic composition of ethanolic *H. perforatum* extract has been done and these phenolic constituents including phenolic acids [Chlorogenic (0.37-0.65%) and Caffeic (nd-0.07%)], Flavonoids [Rutin (0.33-0.66%) and Quercitrin (0.09-0.19%)], Phloroglucinols [Hyperforin (0.76-1.71%)] and Naphtodianthrones [Hypericin (0.56-1.11%)]. Whereas, Rusalepp *et al.* (2017) reported phytochemical composition of methanolic *H. perforatum* L. aerial parts using LC-DAD-MS/MS analysis. Phytochemical analysis revealed that total flavonols ranged between 3.15 - 4.72%, chlorogenic acids 0.79 - 1.27% , total phenolics ranged between 4.62 - 6.93%, total hypericins ranged between 0.26 - 0.62% and total hyperforins ranged between 2.41 - 11.91%; of which hyperoside ranged between 1.70 - 2.76% and hyperforin ranged between 2.15 - 10.60%.

Other studies however focused on *H. perforatum* essential oils phytochemical analysis. In this regards, Çakir *et al.* (1997) previously reported that α-pinene (61.7%), 3-carene (7.5%), β-caryophyllene

(5.5%), myrcene (3.6%) and cadalene (3.2%) were mainly identified in aerial parts *H. perforatum* L. essential oils using GC/MS analysis. Whereas, Chatzopoulou *et al.* (2006) reported wild and cultivated *H. perforatum* essential oils composition using GC/MS analysis. They reported 69 identified compounds of which Germacrene D was the main compound presented in its oils from wild (22.8%) and cultivated (16.9%) types, 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%). Indeed, 14 compounds were presented in wild type and not in cultivated one. Whereas, Çırak *et al.* (2010) reported *H. perforatum* essential oils using GC/FID and GC/MS analyses. They reported that 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 the main compounds occurred in *H. perforatum* essential oils. 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. Moreover, Helmja *et al.* (2011) reported 34 compounds of which Germacrene D (13.7%), Spathulenol (2.9%) and Caryophyllene oxide (2.5%) were mainly presented in *H. perforatum* essential oils using GC/MS. Indeed, Chauhan *et al.* (2011) reported 40 constituents in cultivated aerial parts *H. perforatum* essential oils using GC/MS analysis, of which germacrene D (22.1%), b-caryophyllene (11.3%), a-pinene (8.6%), a-cadinol (4.4%), b-pinene (3.8%), 2-methyl-octane (3.7%), terpinen-4-ol (3.3%), caryophyllene oxide (3.3%), a-muurolol (2.9%) and spathulenol (2.8%) were mainly presented. Whereas, Pirbalouti *et al.* (2014) reported flowers *H. perforatum* essential oils composition using GC/MS analysis. They reported that α-pinene (12.52-49.96%), β-pinene (6.34-9.70%), (E)-β-ocimene (4.44-12.54%), β-caryophyllene (1.19-5.67%), and germacrene-D (2.34-6.92%) were presented as major constituents in its essential oils. Whereas, Đorđević (2015) reported 134 compounds in *H. perforatum* L. essential oils using GC/MS analysis, of which germacrene D (18.6%), (E)-caryophyllene (11.2%), 2-methyloctane (9.5%), α-pinene (6.5%), bicyclogermacrene (5.0%) and (E)-β-ocimene (4.6%) were mainly presented in its essential oils. Indeed, Parchin and Ebadollahi (2016) reported 14 chemical constituents in aerial parts *H. perforatum* essential oils using GC/MS analysis. They reported that Decane (59.58%), Dodecene (12.93%), ethylcyclohexane (6.84%), 5-methylnonane (4.71%), 3-methylnonane (4.32%) and tetradecane (3.82%) were mainly presented. Furthermore, Saleh (2019) reported 52 chemical constituents in aerial parts *H. perforatum* essential oils using GC/MS. Of which, ß -Selinenol (18.13%), Elemol (12.77%), ß-Elemene (10.73%), γ-Eudesmol (6.62%), n-Hexadecenoic acid (6.46%), ß-Selinene (5.98%), Valencene (4.59%), 1S,Cis-Calamenene (3.82%), Aromadendren epoxide-(I) (3.16%) and Germacrene D (2.88%) were mainly identified.

Recently, Schepetkin *et al.* (2020) reported 30 compounds were detected in flowers *H. perforatum* essential oils using GC/MS analysis, of which 3-methoxy-2,3-dimethylcyclobutene (9.8%), cis-p-menth-3-en-1,2-diol (9.1%), terpinen-4-ol (7.4%), α-terpineol (6.1%), trans-ascaridol glycol (4.6%), 4-hydroxy-4-methyl-cyclohex-2-enone (3.4%), limonen-4-ol (3.2%), p-cymen-8-ol (2.9%), myrtenol (2.7%), and α-pinene (2.2%) were mainly presented; whereas the sesquiterpenes were found in trace amounts. While, leaves *H. perforatum* essential oils inversely comprised sesquiterpenes (63.2%) of which germacrene D (25.7%) and β-caryophyllene (9.5%) were mainly presented. Indeed, they also contained oxygenated monoterpenes like terpinen-4-ol (2.6%). Whereas, Saleh (2020) reported 7, 5, 6 and 6 peaks for the same HP1, HP2, HP3 and HP4 *H. perforatum* genotypes, respectively using FT-Raman analysis. Of which three peaks were common for the four studied *H. perforatum* genotypes: peak of 1250 cm^{-1} assigned to C–O stretch-Carboxylic acids group, peak of 1600 cm^{-1} assigned to C=C stretch aromatic-Aromatics group and peak of 2850 cm^{-1} assigned to C–H stretch-Alkanes group.

Overall, the four tetrahydrofuran *H. perforatum* extracts showed some differences in their chemical constituents. These observed differences could be

attributed to the geographical distribution where the studied samples were collected. Where, samples were collected from four collection sites differ in their altitude (80-680 m) and annual rainfall (750-1250 mm). This observation was in coherent of Tangpao *et al.* (2018) and Saleh (2019, 2020) findings who reported that the geographical distribution is a main factor affect plants phytochemical composition.

Table 1: Description of the 4 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: GC/MS analysis of the tetrahydrofuran studied *H. perforatum* genotype1 (HP1) extract.

Peak No	RT (min)	Name of Compound	Peak area (%)
1	17.7	Hexadecenoic acid	0.042
2	19.7	Tetradecanal	0.446
3	19.9	Neophytadiene	0.128
4	21.4	n-Hexadecenoic acid	0.598
5	22.3	Pentadecanal	1.25
6	23.2	Palmitoleonitrile	0.268
7	23.7	Hexadecenenitrile	0.22
8	23.8	Furanone	0.147
9	24.7	Octadecanoic acid	0.39
10	25.1	Hexadecenamide	20.681
11	25.6	1-Hexacosanol	0.008
12	27.3	Clorophene -α-Amyrin	0.182
13	29.5	9-Octadecenamide (Z)-	65.551
14	30.1	Dodecenamide	5.595
15	32.3	Erucic acid	0.658
16	32.6	Glycerol 1-palmitate	0.477
17	33.6	Deoxyspergualin	0.079
18	33.8	13-Docosenamide, (Z)	2.114
19	33.9	Palmitoleamide	0.808
20	34.3	Octadecenamide	0.357

Table 3: GC/MS analysis of the tetrahydrofuran studied *H. perforatum* genotype2 (HP2) extract.

Peak No	RT (min)	Name of Compound	Peak area (%)
1	17.8	1-Octanol,3,7-dimethyl-	0.117
2	19.7	Tetradecanal	0.449
3	19.9	Neophytadiene	0.237
4	21.4	n-Hexadecenoic acid	2.279
5	22.3	Pentadecanal	1.419
6	23.2	Palmitoleonitrile	0.25
7	23.6	Hexadecenenitrile	0.228
8	23.8	Phytol	0.219
9	24.7	Pentadecanoic acid	0.76
10	25.1	Hexadecenamide	19.107
11	29.6	9-Octadecenamide (Z)-	63.117
12	30.2	Dodecenamide	5.585
13	32.3	Erucic acid	0.772
14	32.6	Glycerol 1-palmitate	0.319
15	33.6	Tricosane, 2-methyl-	1.448
16	33.8	13-Docosenamide, (Z)	2.514
17	33.9	Palmitoleamide	0.903
18	34.3	Octadecenamide	0.511

Table 4: GC/MS analysis of the tetrahydrofuran studied *H. perforatum* genotype3 (HP3) extract.

Peak No	RT (min)	Name of Compound	Peak area (%)
1	17.6	CycloDodecene	0.102
2	19.7	Tetradecanal	0.405
3	19.9	Neophytadiene	0.125
4	21.4	n-Hexadecenoic acid	1.997
5	22.3	Pentadecanal	1.163
6	23.2	Palmitoleonitrile	0.144
7	23.6	Octadecanenitrile	0.163
8	23.8	Tricosane, 2-methyl-	0.071
9	24.7	Octadecanoic acid	0.54
10	25.1	Hexadecenamide	18.891
11	27.3	Oxazolidine	0.115
12	29.6	9-Octadecenamide (Z)-	63.496
13	30.1	Dodecenamide	5.961
14	32.3	Butanoic acid, tridec-2-ynyl ester	0.631
15	32.6	Glycerol 1-palmitate	0.639
16	33.6	Hexadecenoic	1.499
17	33.8	13-Docosenamide, (Z)	2.481
18	33.9	Palmitoleamide	1.041
19	34.3	Octadecenamide	0.536

Table 5: GC/MS analysis of the tetrahydrofuran studied *H. perforatum* genotype4 (HP4) extract.

Peak No	RT (min)	Name of Compound	Peak area (%)
1	17.8	1-Undecanol	0.293
2	19.7	Tetradecanal	0.31
3	19.9	Neophytadiene	0.208
4	21.4	n-Hexadecenoic acid	2.418
5	22.3	Pentadecanal	0.958
6	23.2	Palmitoleonitrile	0.168
7	23.6	Hexadecenenitrile	0.198
8	23.8	Phytol	0.632
9	24.7	9-Octadecenoic acid (Z)-, oxiranymethyl ester	0.766
10	25.1	Hexadecenamide	19.325
11	25.6	2,6,9,12,16-Pentamethylheptadeca-2,6,11,15-tetraaene-9- carboxylic acid	0.265
12	27.3	Oixren-5(1aH)-one,2,7,9,10-tetrakis (acetyloxy) decahydro-	0.144
13	29.6	9-Octadecenamide (Z)-	62.048
14	30.2	Dodecenamide	5.914
15	32.3	Erucic acid	0.661
16	32.6	Glycerol 1-palmitate	0.393
17	33.6	Nonadecane	0.878
18	33.8	13-Docosenamide, (Z)	2.739
19	33.9	Palmitoleamide	1.046
20	34.3	Octadecenamide	0.635

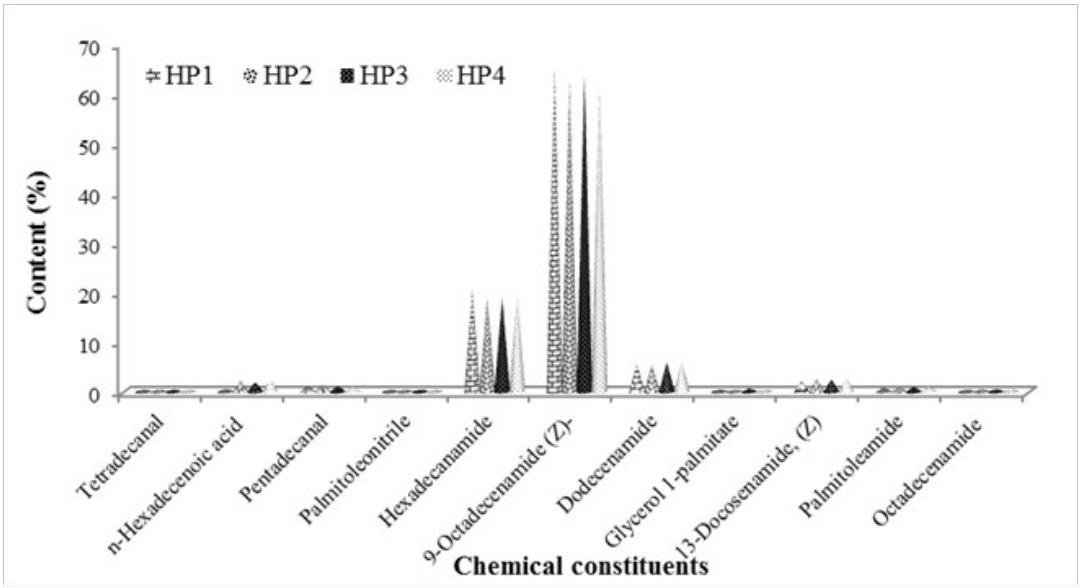

Figure 1. Common constituents occurred in the 4 tetrahydrofuran studied H. perforatum genotypes extracts.

CONCLUSION

Chemical composition of the 4 tetrahydrofuran studied *H. perforatum* HP1, HP2, HP3 and HP4 genotypes extracts has been assessed using GC/MS analysis. GC/MS chromatogram revealed eleven constituents commonly occurred in the 4 tetrahydrofuran studied *H. perforatum* genotypes extracts. In this regards, 9-Octadecenamide (Z)- (Oleic acid),

Hexadecenamide and Dodecenamide were mainly occurred in the 4 tetrahydrofuran studied *H. perforatum* extracts. GC/MS chromatogram showed some differences in chemical composition of the studied *H. perforatum* genotypes extracts, could be attributed to the geographical distribution. This study highlighted GC/MS chemical composition of *H. perforatum* genotypes for the first time in Syria.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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