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Analytical Methods

Assessment of antimicrobial and antiprotozoal activity of the olive oil macerate samples of *Hypericum perforatum* and their LC–DAD–MS analyses

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ABSTRACT

Twenty-one samples of traditionally-prepared (home-made) and ready-made (commercial) St. John's Wort olive oil macerates were profiled for their *in vitro* antimicrobial and antiprotozoal activity. Their cytotoxic potential was evaluated on MRC-5 fibroblasts. In the antiprotozoal assays, ten of the oils inhibited *Trypanosoma brucei rhodesiense* (IC_{50} 15.9–64.5 μg/mL), while only one oil exerted antimicrobial activity towards *Staphylococcus aureus* (IC_{50} = 88.7 μg/mL). LC–DAD–MS data revealed the presence of pseudohypericin (0.135–3.280 μg/g) and hypericin (0.277–6.634 μg/g) in all the oils, whereas chlorogenic acid (1.063 μg/g) was detected only in one oil sample. Hyperforin was detected in four (0.977–2.399 μg/g) and adhyperforin in six samples (0.005–3.165 μg/g). Hypericin and pseudohypericin were common in the active oils, whereas hyperforin, adhyperforin, and chlorogenic acid were absent in these samples. Our results indicated that if the correct plant material is used, the infused oils from *Hypericum perforatum* may contain active components.

1. Introduction

Hypericum perforatum L. (Hypericaceae), commonly known as "St. John's wort", is one of the best-studied medicinal plants throughout the world and its chemical constituents are well-characterised. The phytopharmaceuticals based on standardized extracts obtained from the flowering tops of this plant have been approved against mild to moderate depression (Bilia, Gallori, & Vincieri, 2002; Di Carlo, Borrelli, Ernst, & Izzo, 2001; Francis, 2005). The bioactive compound classes found in *H. perforatum* comprise the naphthodianthrone derivatives (hypericin and pseudohypericin), acylated phloroglucinol derivatives (hyperforin and adhyperforin), and flavonoids such as quercetin, quercitrin, hyperoside, rutin, kaempferol, biapigenin, and amentoflavone. Among its constituents, hyperforin is probably the best-known constituent, which has been reported to exert antidepressant, antibiotic, and antitumor activities (Beerhues, 2006). Adhyperforin has also been stated to contribute to the antidepressant effect of the plant (Lensen, Hansen, & Nielsen, 2001). *H. perforatum* has been recorded for its use in several other disorders, such as wounds, burns, cuts, haemorrhoids, gastric spasms, insomnia, and muscular pain (Gürhan & Ezer, 2004; Jaric et al., 2007). The traditional way of preparing the oil macerate of St. John's wort is to soak the fresh flowering tops of the plant into the olive oil for about 15 days to one month, resulting in a dark red oil macerate. In Turkish folk medicine, the oil is used externally on wounds and drunk for stomach disorders (Gürhan & Ezer, 2004). A clinical study has indicated that the oily extract of *H. perforatum* enhances healing of surgical wounds from caesarean section by augmentation in epithelial reconstruction (Lavagna et al., 2001). The *in vivo* wound healing activity of traditionally prepared Turkish *H. perforatum* extract in olive oil has recently been confirmed by excision and incision models in mice (Süntar et al., 2010).

Several antimicrobial activity studies have been published on extracts of different *Hypericum* species (Dall'Agnol et al., 2003; Rabanal, Arias, Prado, Hernández-Pérez, & Sánchez-Mateo, 2002; Radulovic et al., 2007) of which some species have been reported to be used as antiseptic in Turkish folk medicine (Erdogrul, Azirak, & Tosyali, 2004). The antimicrobial properties might be involved in its wound healing activity as it is essential to keep the wound free of infection and complications. Some *Hypericum* species such as

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Hypericum lanceolatum (syn. *Hypericum revolutum* subsp. *revolutum*), *Hypericum angustifolium*, *Hypericum madagascariense* and *Hypericum revolutum* in traditional Cameroon medicine (Zofou et al., 2011) as well as *Hypericum erectum* in South Korea (Moon, 2010) were reported to be used against protozoal diseases such as malaria. Taking the above information on the traditional use of *Hypericum* species into account, the current study specifically aimed to investigate the *in vitro* antimicrobial and antiprotozoal activity of twenty-one home-made and ready-made Turkish St. John's wort oil macerates against a panel of microorganisms by establishing their IC_{50} values. Potential cytotoxicity of the oils was tested on human lung fibroblast (MRC-5) cell line. In addition, naphthodiantrone (hypericin and pseudohypericin), acylated phloroglucinol (hyperforin and adhyperforin), flavonoid (quercetin and biapigenin), and (chlorogenic acid) contents of the oil samples were analyzed and quantified by LC–DAD–MS.

2. Materials and methods

2.1. Oil macerates

The places and dates purchased for home-made and commercial types of St. John's Wort olive oil macerates are listed in Table 1.

Table 1
Purchase dates, sites and of the producer companies for the St. John's wort oils.

Oil No.	Dates purchased	Places purchased	Producers of the oils
1	November, 2006	Konya province	Home-made
2	April, 2006	Konya province	Home-made
3	April, 2007	Adana province	Home-made
4	April, 2007	Adana province	Home-made
5	April, 2007	Adana province	Home-made
6	April, 2007	Adana province	Home-made
7	August, 2006	Aydin province	Home-made
8	May, 2005	Manisa province	Home-made
9	May, 2005	Aydın province	Home-made
10	April, 2007	Ankara province	Home-made
11	April, 2007	Ankara province	Akhtar & Akhtar St. John's Wort oil
12	August, 2006	Aydin province	Bosphorus Flora St. John's Wort oil
13	November, 2006	Konya province	Kardelen St. John's Wort oil
14	November, 2006	Konya province	Karden St. John's Wort oil
15	September, 2006	Konya province	Talya St. John's Wort oil
16	September, 2006	Konya province	Sepe St. John's Wort oil
17	September, 2006	Konya province	Mecitefendi St. John's Wort oil
18	April, 2007	Adana province	Defne/Doga St. John's Wort oil
19	April, 2007	Adana province	Nurs Lokman Hekim St. John's Wort oil
20	March, 2007	Hatay province	Ege Lokman St. John's Wort oil
21	April, 2007	Aksaray province	Kirinti St. John's Wort oil

2.2. Chemicals

Rutin trihydrate (SR04-072-D), hyperoside (SR04-093-A), quercitrin (Bu04-015-A) and hyperforin (SY04-047-A) were kindly provided by Dr. Willmar Schwabe Pharmaceuticals (Germany). Quercetin hydrate (34120) was purchased from Serva Chemical Co. (NY, USA), while hypericin (H9252), pseudohypericin (H9416), I3,II8-biapigenin (73962), adhyperforin (APH-20012), and chlorogenic acid (C-3878) were purchased from Sigma–Aldrich Co. (Taufkirchen, Germany).

Chromatographic grade-double distilled water, HPLC grade acetonitrile (Merck-1.00030) and analytical grade formic acid 98% (Merck-263) were used in LC–DAD–MS analyses.

2.3. Preparation of the oil samples for LC–DAD–MS analysis

The samples of the oil macerates were prepared for LC–DAD–MS analysis as described by Isaachi et al. (2007) with some minor modifications: Accurately weighed oil samples (around 2 g) were extracted with 10 mL of methanol; after vortexing for 2 min, the methanol solutions were completed up to 10 mL in volumetric flask and filtered through a cartridge type sample filtration unit prior to LC analysis.

2.4. Chromatographic conditions for LC–DAD–MS analysis

Analyses were performed using an Agilent Technologies 1200 series HPLC, including a binary pump, vacuum degasser, autosampler, diode array detector and coupled to a single quadrupole mass spectrometer (MS) equipped with multimode ionisation interface. Chromatographic separations were achieved at room temperature using Eclipse XDB-C18 column (15 cm × 4.6 mm, 5 μm). The mobile phase consisted of acetonitrile (solution A) and 40 mM formic acid in water (solution B). All solvents were filtered through a 0.45 m Milipore filter prior to use and degassed in an ultrasonic bath. A linear gradient program was applied with a slight modification of (Brolis et al.'s method, 1998) using a flow rate of 1.0 mL/min and an injection volume of 10 μL. Quantification was measured at 270 nm using photo-diode array detector (DAD). The chromatographic run time was 60 min and the column void volume was 1.60 min. Retention time values for chlorogenic acid, rutin, hyperoside, quercitrin, quercetin, 13,II8-biapigenin, pseudohypericin, hypericin, hyperforin, and adhyperforin were 9.31, 17.45, 18.01, 18.38, 21.07, 29.28, 34.77, 37.70, 39.05, 51.82, and 54.37 min, respectively.

All calculations concerning the quantitative analyses were performed with external standardization method by measurement of peak areas. The LC–MS instrumentation used a quadrupole MS system operating in selective ion mode (SIM) mode, which precludes the ability to detect simultaneously and identify non-target analytes. The API-ES process was used for mass spectral measurements. The positive-ion mass spectrum of chlorogenic acid, rutin, hyperoside, quercitrin, quercetin, 13,II8-biapigenin, pseudohypericin, hypericin, hyperforin, and adhyperforin were recorded in the total-ion monitoring mode using a series of fragmentor potentials to establish their fragmentation patterns. The mass spectrum (MS) consisted of the protonated molecular ion $[M+H]^+$ at *m/z* 355 for chlorogenic acid, *m/z* 303 for quercetin, *m/z* 611 for rutin, *m/z* 465 for hyperoside, *m/z* 449 for quercitrin, *m/z* 539 for 13, II8-biapigenin, *m/z* 537 for hyperforin, *m/z* 551 for adhyperforin, *m/z* 521 for pseudohypericin, and *m/z* 505 for hypericin. The fragmentor was set at 20 V for all compounds to observe the pseudomolecular ion. Spray chamber parameters were as follows: 5.0 L/minute drying gas, 325 °C drying gas temperature, 200 °C vaporizer temperature, 60 psig. nebulizer pressure and 2000 V capillary voltage. The

constituents were identified by LC–MS analysis and by comparing the retention time of the peaks in the oil samples with those of the authentic reference samples. The purity of the peaks was controlled by DAD. The UV spectra of the peaks were compared with those of the authentic samples.

Quantification of the constituents was performed using rutin as external standard and consideration of each constituent and relative response factor (RRF) with respect to rutin, using the following equation: $\text{Content\%} \equiv A_{\text{Sample}} \times 100/\text{RF}_{\text{std}} \times \text{Conc}_{\text{sample}} \times \text{RRF}$, in which A_{Sample} is the peak of the considered constituent in the test solution (area count), RF_{std} the mean response factor of rutin in the reference solutions [response factor = area × 100/($\text{Conc}_{(\text{mg/ml})}$ purity], C_{sample} the concentration of the test solution (μg/mL) and RRF the response factor of the considered constituent relative to rutin.

2.5. Determination of in vitro antiprotozoal and antimicrobial activity

A general overview of the different models and endpoint parameters was previously published (Cos, Vlietinck, Vanden Berghe, & Maes, 2006). A brief description of the assays is given below. All tests were performed in duplicate.

2.5.1. Antifungal assays

Candida albicans, Trichophyton rubrum and *Aspergillus fumigatus* were cultured in RPMI-1640 medium supplemented with Mops buffer and glucose at 37 °C (yeasts) or 27 °C (moulds). Assays were performed in 96-well microtiter plates, each well containing 10 μl of the oil macerate dilutions together with 190 μL of fungal inoculum (5×10^3 CFU/mL). After 24 h (yeasts) or 7 days (moulds) incubation, fungal viability was assessed fluorimetrically after addition of 10 μL resazurin per well (λex 550 nm, λem 590 nm). The results are expressed as % reduction in fungal growth/viability compared to control wells and an IC_{50} (50% inhibitory concentration) is determined. Miconazole was the reference for *C. albicans*, whereas terbinafine was used as the reference for *T. rubrum* and *A. fumigatus.*

2.5.2. Antibacterial assays

Staphylococcus aureus and *Escherichia coli* were cultured in MHB (Mueller Hinton Broth) at 37 °C. Assays are performed in 96-well microtiter plates, each well containing 10 μL of the oil macerate dilutions together with 190 μL of inoculum (5×10^3 CFU/mL). After 17 h incubation, bacterial viability is assessed fluorimetrically after addition of resazurin, as described above. Doxycyline (*S. aureus*) and ampicillin (*E. coli*) were used as the reference compounds.

2.5.3. Antiprotozoal assays

Leishmania infantum MHOM/MA(BE)/67 is maintained in the golden hamster and spleen amastigotes are collected for infection. Primary peritoneal mouse macrophages (PMM) were used as host cells and the assays were performed in 96-well microtiter plates, each well containing 10 μL of the oil macerate dilutions together with 190 μl of macrophage-parasite inoculum (3.10^5 cells + 3.10^6 parasites/well in RPMI-1640 + 5% FCSi). After 5 days of incubation, total amastigote burdens were microscopically assessed after Giemsa staining. Miltefosine was employed as the reference drug.

Trypanosoma rhodesiense (STIB-900 strain) is maintained in HMI-9 medium supplemented with 10% FCSi. The assays were performed in 96-well microtiter plates, each well containing 10 μL of the compound dilutions together with 190 μL of the parasite suspension (7×10^4 parasites/mL). After 3 days of incubation, parasite growth is assessed fluorimetrically after addition of resazurin. Suramin was included as the reference drug.

Plasmodium falciparum (K1 strain) is maintained in RPMI-1640 medium supplemented with 0.37 mM hypoxanthine, 25 mM Hepes, 25 mM $NaHC0_3$ and 10% O^+ human serum together with 2% washed human O^+ erythrocytes. Assays were conducted under 4% CO_2, 3% O_2 and 93% N_2 admosphere in 96-well microtiter plates, each well containing 10 μL of the oil macerate dilutions together with 190 μL of the malaria parasite inoculum (1% parasitaemia, 2% haematocrit). After 72 h incubation, plates were frozen and stored at −20 °C. After thawing, 20 μL of each well was transferred into another plate together with 100 μL Malstat™ reagent and 20 μL of a 1:1 mixture of PES (phenazine ethosulfaat, 0.1 mg/mL) and NBT (nitro blue tetrazolium, 2 mg/mL). Change in colour is measured spectrophotometrically at 655 nm. Chloroquine was employed as the reference drug.

Trypanosoma cruzi (Tulahuen LacZ, nifurtimox-sensitive) is maintained on $MRC\text{-}5_{SV2}$ (human lung fibroblast) cells in MEM medium, supplemented with 200 mM L-glutamine, 16.5 mM $NaHCO_3$ and 5% FCSi at 37 °C under an atmosphere of 5% CO_2. The assays were performed in 96-well microtiter plates, each well containing 10 μL of the oil macerate dilutions together with 190 μL of MRC-5 cell/parasite inoculum (2×10^4 cells/mL + 2×10^5 parasites/mL). After 7 days of incubation, parasite burdens were assessed after adding the substrate CPRG (chlorophenolred β-D-galactopyranoside): 50 μL/well of a stock solution containing 15.2 mg CPRG + 250 μL Nonidet in 100 mL PBS. The change in colour was measured at 540 nm after 4 h incubation at 37 °C. Nifurtimox was used as the reference drug.

2.6. Cytotoxicity

$MRC\text{-}5_{SV2}$ cells were cultured in Earl's MEM + 5% FCSi. The assays were performed in 96-well microtiter plates, each well containing 10 μL of the oil macerate dilutions together with 190 μL of MRC-5 2×10^4 cells/mL. After 3 days of incubation, cell viability was assessed fluorimetrically after addition of resazurin. Tamoxifen was included as the reference.

3. Results

3.1. Antiprotozoal and antimicrobial activity and cytotoxicity of the oil macerates (Table 2)

Nine of the oil macerate samples (No. 1, 2, 6, 8, 12, 14, 15, 16 and 23) exerted a mild inhibitory activity against *T. rhodesiense* with IC_{50} values varying between 19.7 and 90.0 μg/mL. One oil (No. 12) showed marginal activity against *S. aureus* (IC_{50} of 88.7 μg/mL). The rest of the oils exhibited no activity (IC_{50} > 128 - μg/mL) against the tested protozoa, bacteria, and fungi. None of the macerates were cytotoxic against MRC-5 cell line at the highest test concentration (IC_{50} > 128 μg/mL).

3.2. LC–DAD–MS analysis of the oil macerates (Table 3)

All twenty-one oil macerates were analyzed for their naphthodianthrone (hypericin and pseudohypericin), acylated phloroglucinol (hyperforin and adhyperforin), flavonoid (quercetin and biapigenin) and chlorogenic acid content by LC–DAD–MS. Chlorogenic acid was detected only in one sample (No. 17) amounting for 1.03 μg/g. Among the flavonoids, quercetin was found in six oil samples (No. 1, 4, 8, 9, 17 and 22) in concentrations ranging between 0.25 ± 0.03 and 4.18 ± 0.03 μg/g, whereas biapigenin was present in eight samples (No. 1, 2, 4, 8, 9, 12, 17 and 20) between 0.10 ± 0.06–0.40 ± 0.04 μg/g. Pseudohypericin (0.14 ± 0.05–3.28 ± 0.16 μg/g) and hypericin (0.28 ± 0.01–6.63 ± 0.06 μg/g) were detected in all of the oils analyzed. Hyperforin (0.977 ± 0.01–2.399 ± 0.02 μg/g) was present in four oil samples (No. 4, 7, 9, and 18), while adhyperforin (0.01 ± 0.01–3.17 ± 0.01 μg/g) was detected in six oil samples (No. 3, 4, 6, 7, 9, and 18). Notably, querce-

Table 2
In vitro antiprotozoal and antimicrobial activity of the *H. perforatum* oil macerates.

Oil No.	IC_{50} (μg/mL) (mean of two replicates)									
	T. cruzi	*T. b. rhodesiense*	*L. infantum*	*P. falciparum* (K1)	*S. aureus*	*E. coli*	*C. albicans*	*T. rubrum*	*A. fumigatus*	MRC-5
1	>128	**19.7**	>128	>128	>128	>128	>128	>128	>128	>128
2	>128	**22.9**	>128	>128	>128	>128	>128	>128	>128	>128
3	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
4	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
5	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
6	>128	**15.9**	>128	>128	>128	>128	>128	>128	>128	>128
7	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
8	>128	**19.9**	>128	>128	>128	>128	>128	>128	>128	>128
9	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
10	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
11	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
12	>128	**62.6**	>128	>128	**88.7**	>128	>128	>128	>128	>128
13	>128	**38.1**	>128	>128	>128	>128	>128	>128	>128	>128
14	>128	**90.0**	>128	>128	>128	>128	>128	>128	>128	>128
15	>128	**64.4**	>128	>128	>128	>128	>128	>128	>128	>128
16	>128	**64.4**	>128	>128	>128	>128	>128	>128	>128	>128
17	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
18	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
19	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
20	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
21	>128	**64.4**	>128	>128	>128	>128	>128	>128	>128	>128
Ref.	**3.0**[a]	**0.013.0**[b]	**3.6**[c]	**0.34**[d]	**0.36**[e]	**5.81**[f]	**3.55**[g]	**0.05**[h]	**0.73**[h]	**10.0**[i]

Reference compounds (IC_{50} in μM). Bold numbers mean notable activity.
[a] Benznidazol.
[b] Suramin.
[c] Miltefosine.
[d] Chloroquine.
[e] Doxycycline.
[f] Ampicillin.
[g] Miconazole.
[h] Terbinafine.
[i] Tamoxifen.

tin, biapigenin, hyperforin, and adhyperforin were observed to be more abundant in home-made type of St. John's Wort oils.

4. Discussion

The infused oil obtained from *H. perforatum* has been reputed for its wound-healing effect in traditional medicine and quite a number of studies are available on the phytochemical constituents of the oil. Considering naphthodiantrone, phloroglucinol, and flavonoid composition of St. John's Wort oils, Maisenbacher and Kovar (1992) analyzed *Oleum Hyperici* according to the method described in the German pharmacopoeia (DAB 6) using TLC and HPLC techniques after solid-phase extraction and revealed the presence of hyperforin and its analogues as well as some flavonoids (quercetin, 13,II8-biapigenin) and xanthones. They also attributed the red colour and fluorescence of the oil to breakdown products of hypericin, most likely pseudohypericin. This is consistent with our present data since hypericin and pseudohypericin were detected in all samples. In a study by Isaachi et al. (2007), different preparation techniques were applied to St. John's Wort oils in order to obtain the highest concentration and stability of the phloroglucinol derivatives and five oils obtained by modifications of various parameters such as duration and temperature were extracted with 10 mL of chloroform:methanol (4:6) prior to HPLC-MS analysis. We applied the same extraction method as Isaachi et al. (2007) to analyze our samples by LC–DAD–MS. However, we observed a better separation when the oils were extracted only with 10 mL of methanol instead of chloroform:methanol mixture (4:6, 10 mL). Besides, Isaachi et al. (2007) analyzed only 13, II8-biapigenin, hyperforin, adhyperforin, and furohyperforin contents in the oils, whereas we analyzed chlorogenic acid, quercetin, hypericin, and pseudohypericin in Turkish oil samples in addition to 13, II8-biapigenin, hyperforin, and adhyperforin. As presented in Table 3, pseudohypericin and hypericin were detected in all samples, while hyperforin and adhyperforin were relatively rare. Interestingly, these two compounds were more abundant in the home-made samples which may underline the importance of using the traditional preparation method properly to obtain bioactive components in a higher concentration.

Despite of several studies proclaiming antiprotozoal activity of *Hypericum* species (Decosterd, Hoffmann, Kyburz, Bray, & Hostettmann, 1991; Moon, 2010; Zofou et al., 2011), we have not encountered any antiprotozoal report related to the lipophilic extract or oils of this genus. Hence, lipophilic components found in oily macerates and oils could be responsible for the moderate antiprotozoal activity of the oil samples No. 1, 2, 6, 8, 12, 14, 15, and 16. The non-polar components such as medium- and long-chain fatty acids (Krugliak et al., 1995; Wang & Johnson, 1992) and β-sitosterol (Nweze, Anene, & Asuzu, 2011) have been reported to contribute to antiprotozoal activity. Biapigenin, the biflavonoid derivative, was shown to possess activity against *Trypanosoma brucei rhodesiense* (Weniger, Vonthron-Sénécheau, Kaiser, Brun, & Anton, 2006). Since it seems to be a common compound in most of the active oils in our study, biapigenin may be speculated to be responsible for the observed antiprotozoal effect of the oils. On the other hand, hyperforin was previously reported to possess a remarkable antiplasmodial activity (Verotta, Appendino, Bombardelli, & Brun, 2007), whereas it was found in only four of our oil samples. Consequently, lack of antiplasmodial activity could be correlated partly with absence of hyperforin in most of the oil samples investigated herein. On the other hand, absence of hyperforin

Table 3
Quantities (μg/g ± SEM) of some flavonoids, naphthodiantones, floroglucinols, and chlorogenic acid found in the St. John's Wort oil samples.

Oil No.	Chlorogenic acid	Quercetin	Biapigenin	Pseudohypericin	Hypericin	Hyperforin	Adhyperforin
1	–[a]	0.623 ± 0.01	0.276 ± 0.01	1.110 ± 0.02	0.472 ± 0.08	–	–
2	–	–	0.359 ± 0.02	0.348 ± 0.06	1.478 ± 0.02	–	–
3	–	–	–	0.918 ± 0.01	0.775 ± 0.04	–	3.165 ± 0.01
4	–	1.257 ± 0.01	0.402 ± 0.04	0.845 ± 0.09	0.891 ± 0.01	0.977 ± 0.01	1.186 ± 0.02
5	–	–	–	0.886 ± 0.01	0.277 ± 0.01	–	–
6	–	–	–	0.135 ± 0.05	0.860 ± 0.06	–	0.005 ± 0.01
7	–	–	–	2.612 ± 0.02	1.596 ± 0.01	1.510 ± 0.02	1.082 ± 0.14
8	–	1.239 ± 0.01	0.157 ± 0.01	0.583 ± 0.09	6.634 ± 0.06	–	–
9	–	0.246 ± 0.03	0.169 ± 0.02	0.403 ± 0.02	1.343 ± 0.01	2.399 ± 0.02	2.238 ± 0.06
10	–	–	–	0.446 ± 0.04	0.727 ± 0.02	–	–
11	–	–	–	0.270 ± 0.04	0.746 ± 0.01	–	–
12	–	–	0.140 ± 0.02	0.421 ± 0.03	0.832 ± 0.09	–	–
13	–	–	–	0.520 ± 0.04	2.634 ± 0.30	–	–
14	–	–	–	0.326 ± 0.11	1.240 ± 0.18	–	–
15	–	–	–	0.315 ± 0.01	0.972 ± 0.02	–	–
16	–	–	–	0.494 ± 0.07	2.063 ± 0.08	–	–
17	1.063 ± 0.000009	4.177 ± 0.03	0.268 ± 0.09	3.280 ± 0.16	1.972 ± 0.05	–	–
18	–	–	–	1.174 ± 0.06	1.069 ± 0.06	0.677 ± 0.04	0.000881 ± 0.07
19	–	–	0.096 ± 0.06	0.665 ± 0.05	0.378 ± 0.39	–	–
20	–	0.579 ± 0.05	–	0.745 ± 0.11	2.440 ± 0.03	–	–
21	–	–	–	0.403 ± 0.06	1.199 ± 0.12	–	–

[a] Not found.

in most of the oils samples can be explained by the verity that since hyperforin is known to be an unstable compound, it might have been degraded in the infused oil samples as they are subjected to sunlight during the preparation.

A high number of antimicrobial studies have been performed on *Hypericum* species along with their individual constituents. Jayasuriya, Clarck, and McChesney (1991) suggested several filicinic acid derivatives to be antimicrobial principles of *H. drummondii* leaves isolated through bioactivity-guided fractionation of the hexane extract. On the other hand, the olive oil macerates of *H. scabrum* were found to be completely ineffective in antimicrobial activity (Erdogrul et al., 2004). The antimicrobial activity of *H. perforatum* has been investigated against a wide spectrum of microorganisms (Saddiqe, Naeem, & Maimoona, 2010) and most of those studies identified only hyperforin and hypericin as the major antimicrobial components. Relevantly, Reichling, Weseler, and Saller (2001) had already suggested hyperforin as the active principle of *H. perforatum*. However, hyperforin and adhyperforin were shown to be absent in our active oil samples. In fact, since the hypericin-rich oil sample (No. 8) was not active in the antimicrobial assays, it could be speculated whether hypericin and hyperforin alone may not be responsible for the antimicrobial activity of this plant.

Lack of the antimicrobial activity and absence of some active components specific to *H. perforatum* in most of the oil samples such as hyperforin could make also think one about a possibility that wrong plant material such as another species of *Hypericum* with similar appearance to that of *H. perforatum* could have been used in preparation of the oil macerates instead of *H. perforatum*. This is not a far prospect as over 80 species of the genus *Hypericum* grow naturally in throughout Turkey.

5. Conclusion

In this study, we have identified and quantified naphthodiantrones (hypericin and pseudohypericin), acylated phloroglucinols (hyperforin and adhyperforin), flavonoid derivatives (quercetin and biapigenin), and chlorogenic acid by LC–DAD–MS in twenty-one samples of St. John's Wort oils prepared traditionally (homemade) and commercially produced by different companies in Turkey. All of the oils were found to contain bioactive constituents, such as hypericin, pseudohypericin, hyperforin, adhyperforin, and flavonoids in varying amounts. Some of the oils displayed antitrypanosomal activity, but no significant antimicrobial activity was observed. In conclusion, our results underline that the traditional way of preparing infused oil from *H. perforatum* using olive oil is more capable of getting bioactive constituents in the oil in case of the fact that if a correctly-identified sample of *H. perforatum* has been used.

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