Isolation of potential liver x receptor alpha agonist and antioxidant compounds from *Hypericum microcalycinum* Boiss. & Helldr.

Seçil Sarıkaya Aydın¹, Vahap Murat Kutluay¹, Toshiaki Makino², Makoto Inoue³, Ümmühan Şebnem Harput⁴, İclal Saraçoğlu¹

¹Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, Turkey
²Nagoya City University, Graduate School of Pharmaceutical Sciences, Nagoya, Japan
³Aichi Gakuin University, School of Pharmacy, Laboratory of Medicinal Resources, Nagoya, Japan
⁴Freelance Scientist, Previous address: Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, Turkey

ORCID IDs of the authors: S.S.A. 0000-0003-4692-9117; V.M.K. 0000-0003-4135-3497; T.M. 0000-0002-2524-8745; M.I. 0000-0003-0116-320X; Ü.S.H. 0000-0002-2641-3263; İ.S. 0000-0003-0555-6262

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ABSTRACT

**Background and Aims:** *Hypericum microcalycinum* Boiss. & Helldr. is used for inflammatory diseases in Anatolia. The involvement of liver X receptors (LXRs) and free radicals in inflammatory diseases, activation of LXRs, and high radical contents in cancerous tissue and organs prompted us to determine the radical scavenging and LXRα agonist activity of aqueous fraction of methanol extract and fractions of *H. microcalycinum* along with the isolation studies from active fractions.

**Methods:** Isolation studies were carried out on chromatographic techniques. DPPH, NO, and SO radical scavenging activity methods were used for the determination of antioxidant activity, and a LXRE reporter gene assay was used for the determination of LXRα agonist activity.

**Results:** While the extract showed weak LXRα agonist activity, phenolic compounds- rich fractions showed moderate activity. DPPH radical scavenging capacities of the extract and some fractions seemed to be very high as well as some isolated compounds. Bioactivity- guided studies resulted in the isolation of catechin (1), epicatechin (2), apigenin-8-C-(2-O-acetyl)-glucopyranoside (3), quercetin-3-O-β-glucopyranoside (4), quercetin-3-O-β-arabinopyranoside (5), kaempferol-3-O-β-arabinopyranoside (6), luteolin-8-C-β-glucopyranoside (orientin) (7).

**Conclusion:** According to our results, compounds 1, 2, 4, and 5 may be responsible for the anti-inflammatory effects of *H. microcalycinum* as a function of LXRα agonist and free radical scavenging effects.

**Keywords:** *Hypericum microcalycinum*, phenolic compounds, free radical scavenging, LXRα agonist activity

INTRODUCTION

Natural products or therapeutic agents derived from natural sources have an important role in human health. In a continuation of our studies to find new bioactive compounds from herbal sources, we have focused on the *Hypericum* species, which are well known for their antidepressant, anti-inflammatory, antiproliferative and antimicrobial activities (Boga et al., 2016; Fobofou et al., 2015). In Anatolia, *Hypericum* species are used as an antispasmodic, sedative, and anthelmintic internally; antiseptic and for wound healing externally (Baytop, 1984). The constituents of the genus have been previously investigated, and several types...
of compounds were determined mainly naphthodianthrones, flavonoids, acylchlorogluconol derivatives, tannins, xanthones, and essential oils (Eroglu, Aksu, & Mat, 2008; Zorzetto et al., 2015). As part of our continuing research for bioactive metabolites from herbal medicines, we carried out chemical and biological investigations on the aerial parts of *H. microcalycinum* Boiss. & Helder since detailed biological activity studies on this plant are lacking in the literature. *H. microcalycinum* has different synonyms which are *H. elongatum* C. A. Mey. var. *microcalycinum* (Boiss. & Helder) A. Ramos (Nunez, 1985) and *H. hyssopifolium* Chaix. subsp. *elongatum* (Ledeby) Woron var. *microcalycinum* (Boiss.& Helder) (Robson, 1980).

The role of free radicals and reactive oxygen in the species is becoming increasingly important in the pathogenesis of diabetes, arteriosclerosis, cardiovascular diseases, cancer, and several neurodegenerative disorders (Aktas, Genc, Gozcelioglu, Konuklugil, & Harput, 2013). Nuclear receptors are one of the major targets for the development of new potential agents in diseases like inflammation, rheumatoid arthritis, obesity, diabetes, and cancer (Vedin, Gustafsson, & Steffensen, 2013). Since nuclear receptor signaling has an important function during the burying of dead cells and suppression of inflammation, nuclear receptors such as glucocorticoid receptors, PPAR (peroxisome proliferator activated receptors), and liver X receptors (LXR), are important therapeutic targets in inflammatory diseases (Szendy, Garabuzzi, Joos, Tsay, & Sarang, 2014).

Liver X receptors were first identified in the mid-1990s (Fessler, 2018). The LXRs, LXRα (NR1H3), and LXRβ (NR1H2) form a heterodimer with the retinoid x receptor (RXR), and its activity can be regulated by ligands for either LXR or RXR (Willy, 1995; Wang, Nakashima, Hirai, & Inoue, 2019). While LXRα and LXRβ have similar DNA and ligand binding domains in humans, their distribution differs in tissues. LXRα is found predominantly in liver, intestine, kidney, spleen, macrophages, and adipose tissues, whereas LXRβ is found more ubiquitously (Viennois et al., 2012). Previous studies have shown that both isoforms of LXR play a role in the inhibition of some inflammatory genes such as iNOS, COX2, IL6, the chemokines monocyte chemoattractant protein-1 (MCP-1) and MCP-3, and matrix metalloproteinase-9 (MMP9) (Joseph, Castrillo, Laffitte, Mangelsdorf, & Tontonoz, 2003).

It has been shown that LXRα has also a role in preventing oxidative stress and can affect oxidative stress response by regulating the expression of antioxidant genes (Gong et al., 2009). Nuclear receptors are one of the major targets for the development of new potential agents in diseases like inflammation, rheumatoid arthritis, obesity, diabetes, and cancer (Vedin et al., 2013).

Natural products and nature-derived compounds attract more attention day by day in the treatment of human and animals’ diseases. The members of the *Hypericum* genus have been used in the treatment of various diseases worldwide and have been traded in the global marketplace. In this study, we focus on *H. microcalycinum*, which has not been studied in detail, to determine the potential antioxidant and anti-inflammatory activities. We aimed to identify the anti-inflammatory potential of the extract and to determine the bioactive compounds. For this purpose, the LXR response element (LXRE) reporter gene assay (to determine LXRα agonist activity) and DPPH, NO, and SO radical scavenging activities (to determine antioxidant activity) for the aqueous fraction of methanol extract and different polyamide column fractions of *H. microcalycinum* were tested. As *Hypericum* species have been shown to be involved in inflammatory mechanisms, we hypothesized that it could be a function of the plant as a nuclear receptor activator and/or the extract’s antioxidant activity.

**MATERIALS AND METHODS**

**General**

Fractionation and isolation studies were carried out on chromatographic techniques such as silica gel (Kieselgel 60, 60–230 mesh, Merck, Darmstadt, Germany), polyamide (Sigma Aldrich, St. Louis, MI, USA). In medium pressure liquid chromatography (MPLC) LiChroprep C18 (40–63 μm, Merck) was used as an adsorbent. The system was equipped with a Büchi column (3.5 x 45 cm). 5-15 bar pressure and 5 ml/min flow rates were used. Silica gel 60 F254 plates (Merck) were used during isolation studies and CHCl3–MeOH–H2O (61:32:7, 70:30:3, 80:20:2) was used as the solvent system.

NMR spectra were recorded on an Agilent Varian VN5500 spectrometer (Agilent, Santa Clara, CA, USA). Positive-mode ESI-TOFMS was obtained on a JEOL JMS-T100LP AccuTOF LC-plus 4G spectrometer (JEOL, Tokyo, Japan) using a sample dissolved in MeOH.

3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), DPPH, nitro blue tetrazolium (NBT), sodium nitroprusside, Folín–Ciocalteu reagent, gallic acid, ascorbic acid (AA) were obtained from Sigma–Aldrich. 3,3‘-dibutyl-4-hydroxyanisole (BHA) was purchased from Nacalai Tesque (Kyoto, Japan). Sulfanilamide and naphthylethylenediamine dihydrochloride were obtained from Merck.

HEK293 (Human embryonic kidney) cell line was obtained from Riken Bioresource Center Cell Bank (Japan). Minimum Essential Medium Earle’s salts (MEM’s Earle), trypsin were obtained from Medium Earle’s salts (MEM’s Earle), trypsin were obtained from MERCK KGaA (Darmstadt, Germany). In medium pressure liquid chromatography (MPLC) LiChroprep C18 (40–63 μm, Merck) was used as the solvent system.

**Plant material**

The aerial parts of *H. microcalycinum* were collected from Ağrı-Doğubeyazıt in July 2011 and identified by Prof. Dr. Hayri Duman (Gazi University, Faculty of Science, Department of Botany, Ankara, Turkey). A voucher specimen has been deposited in the Herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 11006).

**Extraction and isolation**

The air-dried aerial parts of the plant (90.8127 g) were extracted with MeOH at 40°C for 12 h (4 x 400 mL). A rotary equipped with a water bath and a 2 L flask was used for the extraction. The MeOH solutions were evaporated under vacuum to yield MeOH extract and it was dissolved in water and partitioned with petroleum ether to remove chlorophyll and other lipophilic compounds.
The aqueous fraction was lyophilized to yield 12.85 g dry weight. The aqueous fraction was applied to polyamide column chromatography to get four sub-fractions using increasing concentrations of methanol (0–25–50–75–100%). The aqueous fraction of methanol extract and polyamide column fractions were tested for radical-scavenging and LXRα agonist activities. Further isolation studies were continued on the active and phenolic compound rich fraction (Fraction D; 795 mg). Fr. D was applied to MPLC using 0–100% methanol as solvent system. Five sub-fractions were obtained, and the fraction of 27% methanol was applied to silica gel column chromatography using different concentrations of CHCl3: CH3OH as a mobile phase to give compound 1 (20.3 mg) and compound 2 (70.8 mg) in pure form. MPLC fraction of 45% methanol was applied to preparative TLC to get compound 3 (3.4 mg) in pure form. MPLC fraction of 40% methanol was applied to preparative TLC to get compound 4. The compound was dissolved in DMSO. A total of 100 μL of alkaline DMSO (1 mL DMSO containing 5 mM NaOH in 0.1 mL water) was added to reaction mixture containing nitro blue tetrazolium (1 mg/mL solution in DMSO) and the sample or standard compounds was dissolved in DMSO. A total of 100 μL of alkaline DMSO (1 mL DMSO containing 5 mM NaOH in 0.1 mL water) was added to yield a final volume of 140 μL. The absorbance was measured at 560 nm by using a microplate reader (Kunchandy & Rao, 1990; Jensen, Gottfredsen, Harput, & Saracoglu, 2010). DPPH (1 mM) solution was added to the MeOH solution of the extract, fractions, or compounds at various concentrations. The reaction mixture was shaken vigorously, and the absorbance of the remaining DPPH was measured at 517 nm after 30 min. All the analyses were done in triplicate. Radical scavenging activity was expressed as the inhibition percentage.

DPPH radical scavenging activity

The DPPH radical scavenging effect was assessed by the discoloration of methanol solution of DPPH spectrophotocically; AA and BHA were used as positive controls (Hatano et al., 1989; Jensen, Gottfredsen, Harput, & Saracoglu, 2010). DPPH (1 mM) solution was added to the MeOH solution of the extract, fractions, or compounds at various concentrations. The reaction mixture was shaken vigorously, and the absorbance of the remaining DPPH was measured at 517 nm after 30 min. All the analyses were done in triplicate. Radical scavenging activity was expressed as the inhibition percentage.

SO radical scavenging activity by alkaline DMSO method

The method of Kunchandy and Rao was used to detect SO radical–scavenging activity of the samples, with slight modification. In brief, a SO radical was generated in a nonenzymatic system. The reaction mixture containing nitro blue tetrazolium (1 mg/mL solution in DMSO) and the sample or standard compounds was dissolved in DMSO. A total of 100 μL of alkaline DMSO (1 mL DMSO containing 5 mM NaOH in 0.1 mL water) was added to yield a final volume of 140 μL. The absorbance was measured at 560 nm by using a microplate reader (Kunchandy & Rao, 1990; Srinivasan, Chandrasekar, Nanjan, & Suresh, 2007).

NO radical scavenging activity

To determine NO radical scavenging activity of the samples, a serial diluted sample was added to a 96-well flat-bottomed plate. 10 mM sodium nitroprusside, dissolved in phosphate-buffered saline, was added to each well and the plate was incubated under light at room temperature for 150 minutes. Finally, an equal volume of the Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride, 2.5% H3PO4) was added to each well to measure the nitrite content. After chromophore was formed at room temperature in 10 minutes, absorbance at 577 nm was measured on a microplate reader (Tsai, Tsai, Yu, & Ho, 2007).

LXRα agonist activity

The LXRE reporter gene assay was used to determine LXRα agonist activity. HEK293 cells were cultured in MEM (Minimum Essential Medium) supplemented with 10% FBS, 1% nonessential amino acids, 50 U/mL penicillin, and 50 μg/mL streptomycin at 37 °C in a humidified atmosphere of 5% CO2 in the air.

The calcium phosphate co- precipitation method was used for transfection. For LXR luciferase reporter assay, pBApo-CMV-hLXR-α (30 ng), pGL4.1-DR4-Luc (120 ng), pCMX-β-gal expression vector (30 ng), and carrier DNA pUC18 were used to yield a total of 600 ng of DNA per well. After 6 hours, the cells were treated with test samples for 36 hours of incubation. The luciferase and β-galactosidase activities were analyzed from cell lysates using a luminescence reader and a spectrophotometer, respectively. The results were given in fold induction values relative to vehicle-treated cells after normalization of luciferase activity by β-galactosidase (Kotani, Tanabe, Mizukami, Makishima, & Inoue, 2010; Nakashima, Murakami, Tanabe, & Inoue, 2014).

RESULTS AND DISCUSSION

The methanol extract of H. microcalycinum aerial part was suspended in water and partitioned with petroleum ether. The aqueous fraction was used for the phytochemical and biological analysis. The aqueous fraction was subjected to polyamide column chromatography to afford four main fractions.

To evaluate LXRα agonist activity, the aqueous fraction of H. microcalycinum extract and its main polyamide column fractions were tested at a concentration of 100 μg/mL. In this study, an LXRα agonist, T0901317, was used as a positive control at the concentrations of 1, 10 and 100 nM. The results were given as fold induction values normalized by β-galactosidase. While the extract showed weak LXRα agonist activity, Fr. D, the phenolic compound-rich fraction, showed moderate activity with a fold value of 1.37 (Figure 1, Table 1).

Table 1. LXRα agonist activities of the Hypericum microcalycinum extract and fractions* Effects of Hypericum microcalycinum (aerial parts) and fractions on LXRE reporter gene transcription at 100 μg/mL concentration were given as fold values.

| Samples        | Fold Value |
|----------------|------------|
| Aqueous extract| 0.96±0.02  |
| Fr. A          | 1.15±0.06  |
| Fr. B          | 1.6±0.06   |
| Fr. C          | 2.22±0.07  |
| Fr. D          | 1.37±0.08  |
| T0901317 1 nM  | 1.22±0.11  |
| T0901317 10 nM | 2.91±0.13  |
| T0901317 100 nM| 5.49±0.34  |
| Control        | 1±0.03     |

*Three independent test results were considered, averages and standard error means were given in the table.
The aqueous *Hypericum microcalycinum* extract and fractions of polyamide column were tested against 2,2-diphenyl-1-picylhydrazyl (DPPH), NO, and SO radicals for antioxidant activity. Results were evaluated with the known antioxidants ascorbic acid (AA), butyl-4-hydroxyanizole (BHA), and quercetin. Tested fractions showed radical scavenging activity depending on their phenolic contents. Fr. A is the non-phenolic fraction of the polyamide column that showed the lowest radical scavenging activity. Fr. D, the phenolic-rich fraction, was found to possess high radical scavenging activity against the tested radicals. Its radical scavenging activity was comparable to that of known antioxidants AA, BHA, and quercetin. Radical scavenging activity (inhibition %) of Fr. D at a concentration of 200 µg/mL against DPPH, NO and SO radicals were found as 90.5, 59.9, and 65.5 respectively, whereas radical scavenging activity (inhibition %) of Fr. A at a concentration of 200 µg/mL against DPPH, NO and SO radicals were found as 0.8, 24.4, and 42.1 respectively.

Repeated chromatographies of the flavonoid fraction (Fr. D), which was eluted with 75–100% methanol, resulted in the isolation of seven compounds. The spectroscopic data (1D and 2D-NMR, and ESITOFMS) of the isolated compounds were compared to the data of the compounds that were given in the references and their structures were identified as follows: Catechin (1), epicatechin (2), Apigenin-8-C-(2-O-acetyl)-glucopyranoside (3), Quercetin-3-O-glucopyranoside (4), Quercetin-3-O-arabinopyranoside (5), Luteolin-8-C-glucopyranoside (orientin) (6) and (7) Apigenin-8-C-(2-O-acetyl)-β-glucopyranoside (orientin) (Figure 2). All data is provided in the Supplementary document. Catechin and epicatechin were obtained as a mixture (ratio 1:3). Apigenin-8-C-(2-O-acetyl)-β-glucopyranoside was isolated for the first time from a *Hypericum* species in this study.

**Figure 1.** Effects of *Hypericum microcalycinum* (aerial parts) on LXRE reporter gene transcription at 100 µg/mL concentration. Three independent test results were considered, averages and standard error means were given in the figure.

**Figure 2.** Isolated compounds from *Hypericum microcalycinum* Boiss.&Heldr.

Catechin (1): 'H NMR (500 MHz, CD3OD): 6.87 (1H, d, j = 1.9, H-2'), 6.80 (1H, dd, j = 8.0 Hz, H-5'), 6.76 (1H, dd, j=8.2/1.3, H-6'), 5.96 (1H, d, j = 2.31 Hz, H-8), 5.89 (1H, d, j = 2.31 Hz, H-6), 4.59 (1H, d, j = 7.5 Hz, H-2), 4.00 (1H, ddd, H-3), 2.54-2.90 (2H, dd, H-4), 13C NMR (125 MHz, CD3OD): 157.86 (C-7), 157.59 (C-9), 156.93 (C-5), 146.26 (C-3'), 146.24 (C-4'), 132.30 (C-1''), 120.04 (C-6'), 116.08 (C-5'), 115.27 (C-2'), 100.82 (C-10), 96.29 (C-8), 95.50 (C-6'), 82.87 (C-2'), 68.83 (C-3'), 65.5 respectively.

Apigenin-8-C-(2-O-acetyl) glucopyranoside (3): 'H NMR (500 MHz, CD3OD): 8.07 (1H, d, j = 8.8 Hz, H-2'), 6.99 (1H, d, j = 8.8 Hz, H-3'), 6.64 (1H, s, H-3), 6.25 (1H, s, H-6), 5.57 (1H, dd j = 4.5/10.2 Hz, H-2''), 5.11 (1H, d, j = 10.2 Hz, H-1''), 4.52 (1H, d, j = 2.1/12.1, H-6''a), 3.86 (1H, dd, j = 5.7/12.1, H-6''b), 3.74 (1H, d, H-3''), 3.74 (1H, d, H-4''), 3.54 (1H, m, H-5''), 1.70 (3H, s, CH3); 13C NMR (125 MHz, CD3OD): 184.15 (C-4), 171.91 (C=O), 166.71 (C-2), 163.90 (C-7), 162.99 (C-5), 158.58 (C-9), 130.13 (C-2'/6'), 123.66 (C-1'), 116.97 (C-3'/5'), 105.75 (C-10), 103.66 (C-3), 103.60 (C-8), 98.97 (C-6), 83.14 (C-5'), 77.74 (C-3'), 74.08 (C-2'), 73.00 (C-1''), 72.24 (C-4'), 62.93 (C-6''), 20.52 (CH3).
Quercetin-3-O-glucopyranoside (4): 1H NMR (500 MHz, CD3OD): 7.75 (1H, d, j = 2.1 Hz, H-2”), 7.61 (1H, dd, j = 2.1/8.4 Hz, H-6”), 6.88 (1H, d, j = 8.4 Hz, H-5”), 6.26 (1H, d, j = 1.8 Hz, H-8”), 6.11 (1H, d, j = 1.8 Hz, H-6”), 5.15 (1H, d, j = 7.8 Hz, H-1”), 3.74 (1H, dd, j = 2.3/11.9 Hz, H-6”-a), 3.62 (1H, dd, j = 5.2/11.9 Hz, H-6”-b), 3.52 (1H, dd, j = 7.8/9.0 Hz, H-2”), 3.45 (1H, m, H-3”), 3.39 (1H, m, H-4”), 3.25 (1H, m, H-5”), 13C NMR (125 MHz, CD3OD): 178.60 (C-4”), 170.30 (C-7”), 162.59 (C-5”), 158.89 (C-2”), 158.13 (C-9”), 150.45 (C-4”), 146.06 (C-3”), 135.39 (C-3”), 123.07 (C-6”), 122.96 (C-1”), 117.30 (C-2”), 116.05 (C-5”), 105.09 (C-1”), 103.65 (C-10”), 102.20 (C-6”), 96.33 (C-8”), 78.33 (C-5”), 78.20 (C-3”), 75.02 (C-2”), 71.14 (C-4”), 62.53 (C-6”).

Quercetin-3-O-arabinopyranoside (5): 1H NMR (500 MHz, CD3OD): 7.78 (1H, d, j = 2.1 Hz, H-2”), 7.61 (1H, dd, j = 2.1/8.5 Hz, H-6”), 6.91 (1H, d, j = 8.5 Hz, H-5”), 6.44 (1H, d, j = 2.1 Hz, H-8”), 6.24 (1H, d, j = 2.1 Hz, H-6”), 5.20 (1H, d, j = 6.5 Hz, H-1”), 3.94 (1H, dd, j = 6.5/8.4 Hz, H-2”-a), 3.89 (1H, m, H-5”-a), 3.88 (1H, m, H-4”), 3.68 (1H, dd, j = 3.2/8.4 Hz, H-3”), 3.48 (1H, d, j = 10.4, H-5”-b): 13C NMR (125 MHz, CD3OD): 179.49 (C-4”), 164.07 (C-7”), 163.07 (C-5”), 158.71 (C-9”), 158.44 (C-2”), 149.96 (C-4’”), 154.99 (C-3’”), 135.65 (C-1”), 123.03 (C-6”), 122.89 (C-1”), 117.45 (C-2”), 116.17 (C-5”), 105.65 (C-10”), 104.62 (C-1”), 99.87 (C-6”), 94.69 (C-8”), 74.12 (C-3”), 72.87 (C-2”), 69.10 (C-4”), 66.94 (C-5”).

Kaempferol-3-O-arabinopyranoside (6): 1H NMR (500 MHz, CD3OD): 6.15 (1H, d, j = 2.0 Hz, H-6”), 6.02 (1H, d, j = 2.0 Hz, H-8”), 8.05 (1H, d, j = 8.8 Hz, H-2’-H-6”), 6.85 (1H, d, j = 8.7 Hz, H-3’-H-5”), 4.95 (1H, d, j = 6.5, H-1’”), 3.92 (1H, dd, j = 8.4/6.5, H-2’”), 3.65 (1H, dd, j = 3.4/8.3, H-3’”), 3.81 (1H, m, H-4’”), 3.46 (1H, dd, j = 12.5/10, H-5”-b), 3.83 (1H, d, j = 12, H-5”-a); 13C NMR (125 MHz, CD3OD): 158.5 (C-2’-j), 135.5 (C-3’-j), 170.29 (C-4’), 159.31 (C-5’), 97.40 (C-6’), 162.36 (C-7’), 103.62 (C-8’), 154.38 (C-9’), 102.50 (C-10’), 122.1 (C-1’-i), 131.97 (C-2’), 116.89 (C-3’), 163.2 (C-4’), 116.89 (C-5’), 131.97 (C-6’), 105.40 (C-1’-i), 99.87 (C-6’), 94.69 (C-8’), 74.12 (C-3’), 72.87 (C-2’), 69.10 (C-4’), 66.94 (C-5”).

Luteolin-8-C-glucopyranoside (Orientin) (7): 1H NMR (500 MHz, CD3OD): 7.56 (1H, brs, H-2”), 7.51 (1H, dd, j = 8.2, H-6”), 6.92 (1H, dd, j = 8.2, H-5”), 6.51 (1H, s, H-3”), 6.23 (1H, s, H-6”), 5.05 (1H, d, j = 9.9, H-1’”) 4.15 (1H, t, H-2’”), 3.97 (1H, d, j = 10, H-6”-a), 3.86* (1H, m, H-4’”), 3.60 (1H, m, H-3’”), 3.53 (1H, m, H-5”); 13C NMR (125 MHz, CD3OD): 183.68 (C-8”), 163.68 (C-7”), 166.30 (C-2’), 164.29 (C-5”), 158.03 (C-9”), 151.72 (C-4’), 147.34 (C-3’), 123.72 (C-1’), 120.73 (C-6’), 116.80 (C-5”), 114.53 (C-2”), 104.48 (C-3”), 104.00 (C-10”), 103.62 (C-8”), 97.40 (C-6”), 82.83 (C-5”), 80.48 (C-3’), 75.5 (C-1’”), 72.90 (C-2”-a), 72.50 (C-4”), 63.10 (C-6’). As seen in Table 2, five of the isolated compounds were tested for their radical scavenging activity because of their adequate amount for testing. While four of them showed strong scavenging activity, compounds 1 and 2, which were a 1:3 mixture of catechin-epicatechin, exhibited the strongest free radical scavenging activity. Its activity was found to be more potent or very close to that of AA, BHA, and quercetin in the case of nitric oxide and superoxide radicals.

Catechin and epicatechin have shown high antioxidant activity due to their high number of hydroxyl groups (Prochazkova, Bousova, & Wilhelanova, 2011). In previous studies have reported that the o-dihydroxy group in the B ring of flavonoids and the functional hydroxyl groups in the 3- and 5- positions also mediated antioxidant activity. Glycosylation at position 3 also decreases the radical scavenging (Prochazkova et al., 2011). The results of our study supported these data. According to the present research, it was found that compounds 1, 2, 4, and 5 show higher antioxidant activity. Comparison results of compounds 4 and 5 with quercetin support that glycosylation decreases the antioxidant effect. The results of DPPH radical scavenging assay in this study were compared to results obtained from previous literature. The IC50 values of compounds 1 and 2 (catechin-epicatechin mixture), compound 4 (quercetin-3-O-glucopyranoside), compound 5 (quercetin-3-O-arabinopyranoside), and the positive control (quercetin) were found as 23.1, 27.9, and 7.7 μg/mL, respectively (Razavi, Zahri, Zarrini, Nazemiyeh, & Mohammadi, 2009; Sarian et al., 2017; Zhang et al., 2005).

Previous studies on the structure-activity relationship studies of flavonoids suggested that hydroxyl group at position 3 could be important to activate LXRα. For example, while quercetin acts as an agonist, luteolin shows antagonist activity for LXRα (Fouache et al., 2019). The studies also revealed that not only aglycons, but also flavonoid glycosides could be important to activate LXRα. For example, while quercetin-3-O-glucopyranoside also showed potent activity for LXRα with an EC50 value of 1.8 μM. Quercetin-3-O-gluc-
uronide was also shown to activate LXRα (Ohara et al., 2013). These findings suggest that compounds 4, 5, and 6 might be responsible for the LXRα agonist activity of the extract.

CONCLUSION

Hypericum species has widespread use in traditional and contemporary medicine for its antidepressant, anti-inflammatory, antiproliferative and antimicrobial activities. According to our results, isolated compounds, particularly compounds 1, 2, 4 and 5 from active fractions may be responsible for the anti-inflammatory effects of *H. microcalycinum* as a function of LXRα agonist and free radical scavenging effects.

Also, this is the first study for the isolation of apigenin-8-C-(2-O-acetyl)-β-glucopyranoside, 3 from a *Hypericum* species.

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