**EXPLORING THE ANTIDIABETIC AND ANTIOXIDANT POTENTIAL OF NEPETA ADENOPHYTA HEDGE IN HEPG2 CELLS AND ITS PHYTOCHEMICAL STUDIES**

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**ABSTRACT**

The current study is based on the phytochemical isolation and biological activity evaluation of *Nepeta adenophyta* Hedge. This plant species is traditionally used for abdominal pain, kidney pain, stomach problems, urine problems, and diarrhea and is examined to control bleeding. Five compounds were isolated using column chromatography over normal silica gel from the ethanolic crude extract of *Nepeta adenophyta* and their structures were characterized with the help of spectroscopic techniques. In addition, the crude extract was evaluated for its antioxidant and antidiabetic potential in a human hepatoma cell line HepG2 cells and the crude extract showed good activities. The phytochemical isolation studies and the biological activities are conducted for the first time on the study sample.

**Keywords:** Cherchoomoro, Isolation, Ethanolic crude, Glucose consumption, Oxidative stress

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1. **INTRODUCTION**

*Nepeta adenophyta* Hedge (Lamiaceae) is a medicinal plant endemic to Astore valley (Gilgit, Gilgit-Baltistan, Pakistan). *N. adenophyta* (Figure 1) is locally called Cherchoomoro and it is reported for abdominal pain, kidney pain, stomach problems, urine problems and diarrhoea and examined to control bleeding. Many local people use this plant for stomach disorders and to treat general weakness of their cattle [1]. In a recent study, *N. adenophyta* has shown potent analgesic and anti-inflammatory effects [2].

*Figure 1: Nepeta adenophyta* Hedge (image credit: Rubab Ilyas).

*N. adenophyta* has been studied for preliminary phytochemicals that reported positive results for the presence of polyphenols, flavonoids, and reducing sugars [3]. The GC-MS
analysis of the crude has shown the presence of phenolics, nepetalactones, flavonoids etc. [2]. 

*Nepeta adenophyta* Hedge is a herbal remedy commonly used in Astore valley, Gilgit-Baltistan [1]. In literature, phytochemical study of *Nepeta* species has shown terpenoids (mono, di, tri), glycosides, flavonoids, iridoïd, particularly [4, 5]. Particularly terpenoids have been isolated from *Nepeta* species such as di, and triterpenoids were isolated from *N. obtusicrena* and showed anti-Alzheimer activity [6]. As from *N. teydea* many diterpenes, triterpenes have been isolated [7]. *N. cataria* is also reported to have diterpenes and triterpenes [8]. Some diterpenes are reported from *N. sorgerae* [9]. Triterpenes are also majorly found in *Nepeta* species like in *N. hindostana* [10]. Pimarane diterpenoid has been mainly isolated from many species of family Lamiaceae [11]. Derivatives of most pimarane diterpenoid have shown promising biological properties i.e., antitumor, anti-inflammatory, antimicrobial [12]. Recently, *Nepeta* species have been reported to treat depression [13], hyperuricemia [14], acute hypoxia [15] etc.

*N. adenophyta* has not been thoroughly investigated for phytochemical isolation. The present study aims at the isolation of phytoconstituents from the ethanolic crude extract of *N. adenophyta* (NAE). In addition, NAE has also been evaluated for its antidiabetic and antioxidant properties in a human hepatoma cell line (HepG2 cells). The phytochemical studies, the antidiabetic and antioxidant potential in HepG2 cells are investigated for the first time.

### 2. MATERIAL AND METHODS

#### 2.1 Solvents and reagents

The solvents of commercial grade *n*-hexane, ethyl acetate, dichloromethane, methanol and ethanol were purchased from Musaji Adam & Sons (Abbottabad, Pakistan). 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from Solarbio Science and Technology Co., Ltd. (Beijing, China). MilliQ water (Millipore, Bedford, MA, USA) was used to prepare all buffers and solutions. Foetal bovine serum (FBS), dulbecco’s modified Eagle medium (DMEM), phosphate-buffered saline (PBS), streptomycin, and penicillin were purchased from Jinan Kang-Mai Science & Technology Co., Ltd. (Jinan, China). A glucose test kit was used to instruct the glucose content (Rong-Sheng Biotech Co., Ltd., Shanghai, China). Reactive Oxygen Species Assay Kit was purchased by Beyotime Biotechnology (Beijing, China).

#### 2.2 Structure Determination

The masses of isolated compounds were determined by ESI-MS (positive ion mode and negative ion mode), carried out on Agilent 6520 Q-TOF (Agilent, Santa Clara, CA, USA). The NMR spectra were recorded on a Bruker AV-400 spectrometer (Bruker BioSpin, Rheinstetten, Germany) with DMSO, or CDCl as NMR solvent, TMS was used as an internal standard. The chemical shift (δ) values were determined in parts per million (ppm), and the coupling constant (J) was measured in Hz.

#### 2.3 Chromatography

Silica gel 60 F plates (Merck) were used to check the purity of semi pure and pure fractions. For column chromatography, silica gel 60 (Merck) mesh size 60-230 was used. For the identification of UV active compounds, TLCs were checked by UV lamp (254 & 365 nm). TLCs were visualized by spraying ceric sulphate spray and heated for analyzing spray active compounds. For ceric sulphate spray, 0.1 g ceric sulphate and 1 g trichloroacetic acid were
dissolved in 4 mL distilled water. The solution was boiled and concentrated H$_2$SO$_4$ was added drop wise till turbidity disappearance.

2.4 Plant Material

The plant material of *N. adenophyta* Hedge was collected from Astore, Gilgit-Baltistan. The plant was identified by Dr. Ali Noor, and the voucher number (180-KUH) was submitted to the Department of Biological Sciences, Karakoram International University, Gilgit. The whole plant material were collected and dried under the shade.

2.5 Extraction of *Nepeta adenophyta*

The air-dried *N. adenophyta* whole plant material (5.5 kg) was soaked and extracted thrice (3 × 24 h) under room temperature in absolute ethanol, keeping the ratio of solid and liquid (1:15). The extractives were combined and evaporated to dryness by rotary evaporator yielding the ethanol residue (NAE; 480 g).

2.6 Isolation & Characterization

The crude extract NAE (460 g) was subjected to normal column chromatography with increasing polarity order (n-hexane, DCM, ethyl acetate, methanol), and as a result 48 fractions (N1-N48) were obtained. The fractions N6-N9 (A, 40% DCM:n-hexane), and N10-18 (B, 70% DCM:n-hexane to 10% EtOAc:DCM) were combined separately, dried and rechromatographed separately over normal silica gel using (n-hexane, ethyl acetate, methanol) solvent system. From part A, Total 66 sub fractions (NA1-NA66) were obtained. The sub fractions NA23-NA24 (10% EtOAc:n-hexane), NA30-NA36 (15% EtOAc:n-hexane) were separately combined which yielded compounds 1, and 2, respectively. While the sub fractions NA41-NA56 (1:1 EtOAc:n-hexane to 100%EtOAc) were combined and furthermore rechromatographed that yielded compound 3 (100% EtOAc). The part B was rechromatographed over silica gel, and total 24 fractions were obtained. The fraction 9-11 (10-20% EtOAc:n-hexane) yielded compound 4, and the fraction 18 (100% EtOAc) was further rechromatographed. Total 54 fractions were obtained and fraction 33 (1:1%EtOAc:n-hexane to 80%EtOAc:n-hexane) yielded compound 5.

2.7 Antidiabetic Activity

The antidiabetic activity was performed following the reported protocol [16]. HepG2 (a human hepatoma cell line) cells were analyzed for glucose consumption assay. In brief, 96-well cell culture plates were filled with a 100 μL of the HepG2 cell suspension (5 × 10$^4$ /mL). In culturing the cell suspension, 37 °C temperature was provided along with 5% CO$_2$. The used medium was discarded after culturing the plates for 24 h and washed the cultured plates two or three times using PBS (phosphate-buffered saline) solution. Afterwards, the insulin-containing solution with the serum-free DMEM (Dulbecco’s modified Eagle medium) medium was added to the cells. For 36 h, the supernatant was cultured and then aspirated. The cells were poured with serum-free drug containing, or drug-free DMEM added. In this experiment, three groups were classified: component treatment group (25-100 μg/mL), blank control group, and insulin group (10-6 mmol/L). According to the glucose test kit (Beijing Applygen Technologies Inc., China), the glucose content was detected after 24 h of culture at 505 nm. The given formula was used to calculate the glucose consumption rate.

$$\Delta GC = (\text{glucose conc. of blank wells} - \text{glucose conc. of cell-inoculated}).$$

2.8 Antioxidant Activity

Hydrogen peroxide-induced oxidative stress was determined in HepG2 cells to check
the sample’s antioxidant capability. The reported protocol [17] with slight modification was followed in the current experiment. Test sample was prepared by adding stock solution with DMSO. HepG2 cells were grown in 96-well plates at 5 × 10⁴ cells/well and cultured for 24 h at 37 °C with 5% CO₂. Then different samples were added to the plates and were left for 24 h. Last, HepG2 cells were incubated with hydrogen peroxide solutions (200 μM) for 6 h. Then 1 mg/mL MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) solution was prepared and added to each well about 50 μL of MTT solution and incubated the samples containing MTT solution further for 4 h at 37 °C. Then the supernatant was removed and 500 μL DMSO (dimethyl sulfoxide) was added to lyse or split the cells on a gyratory shaker. The absorbance of each sample was recorded at 490 nm by using the microplate reader. The results were expressed as the mean of cell survival normalized to control (without treatment). However, the minor modification that was employed in the present study is illustrated here. In the reported protocol [17], the authors used 24 multi-well plate, 100 μM hydrogen peroxide, 100 μM of DCFH-DA (2,7-dichlorodi-hydrofluorescein diacetate), and finally analyzed the samples at 485 and 535 nm. While in the present experiment, the minor modification was adopted using 96 multi-well plates, using 200 μM hydrogen peroxide, 1 mg/mL MTT solution, and finally analyzing the samples at 490 nm.

3. RESULTS AND DISCUSSION

3.1 Structure Characterization

Five compounds (1-5) were isolated in pure form from N. adenophyta by repeated column chromatography over normal silica gel. The structures of 1-5 were elucidated by various spectral techniques. Moreover, the spectral data were compared and found in agreement with the literature. Compound 1: The ESI-MS (positive ion mode) showed molecular ion [M+H]+ at m/z 581.2538 corresponding to the molecular formula C₃₅H₄₂O₇ (calcd. 581.4928). In proton NMR, the signals for methyl protons were observed at different chemical shift values. A double bond proton appeared at δH 5.40, which corresponded to position 6. A signal at δH 3.70 ppm attributed to H-3. Likewise, a signal with δH 5.06 appeared for highly deshielded proton attached between two electronegative atoms at 3’ position. The 13C NMR showed 39 signals including 8 methyl, 13 methylene, 14 methine and 4 quaternary carbon atoms, as determined by 2D NMR. The structure was elucidated for compound 1 as 3α-[β-sitosteryl-3β-oxo]-dihydronepetalactone. The experimental data was found in correlation with previous literature [18]. 1H NMR (400 MHz, in CDCl₃): δH 5.40 (1H, d, J = 4.7 Hz, H-6), 5.06 (1H, d, J = 8.6, H-3’), 3.70 (1H, m, H-3), 1.25 (3H, d, J = 6.6 Hz, Me-8’), 1.06 (3H, d, J = 7.1 Hz, Me-9’), 1.06 (3H, s), 0.91 (3H, d, J = 7.5 Hz), 0.87 (3H, d, J = 6.7 Hz), 0.85 (3H, d, J = 6.9 Hz), 0.83 (3H, t, J = 6.9 Hz), 0.64 (3H, s). 13C NMR (100 MHz, in CDCl₃): δC 173.4 (C-1’, C=O), 139.7 (C-1, CH₂), 120.7 (C-2, CH₂), 103.9 (C-3’), 77.8 (C-3, CH), 55.7 (C-4, CH₂), 55.0 (C-5), 49.1 (C-6, CH), 49.0 (C-7α’, CH), 48.6 (C-7, CH₂), 41.3 (C-8, CH), 39.3 (C-9, CH), 39.1 (C-4α’, CH), 38.7 (C-5’), 38.1 (C-10), 37.1 (C-11, CH₂), 36.2 (C-7’, CH), 36.1 (C-12, CH₂), 35.5 (C-13), 35.1 (C-6’), 32.9 (C-14, CH), 32.4 (C-15, CH₂), 32.2 (C-16, CH₂), 30.6 (C-17, CH), 29.2 (C-18, CH₃), 29.1 (C-19, CH₃), 26.1 (C-20, CH), 24.3 (C-21), 22.5 (C-22, CH₂), 21.1 (C-23, CH₂), 19.8 (C-9’, CH₃), 19.6 (C-24, CH), 19.6 (C-25, CH), 19.4 (C-26, CH₂), 19.1 (C-27, CH₃), 15.4 (C-8’, CH₃), 10.9 (C-28, CH₃), 10.8 (C-29, CH₃).
**Compound 2**: The ESI-MS (positive ion mode) showed molecular ion [M+H]+ at m/z 731.6175 corresponding to the molecular formula C_{40}H_{32}O_{6} (calcd. 731.6184). The aromatic protons resonating at δ_H 7.70 and 7.53 ppm attributed to H-3/6 and H-4/5, respectively. The 13C NMR revealed 2 methyl, fifteen methylene, 2 aromatic sp^2 carbon atoms, 2 methine and two quaternary carbon atoms for half of the compound, as determined by 2D NMR spectra. All these promising signals helped to elucidate the structure of the compound 2, identified as bis[7-ethyl-2-(2-hydroxyethyl)pentadecyl] phthalate. The experimental data was found in correlation with previous literature [19]. 1H NMR (400 MHz, CDCl3): δ_H 7.70 (2H, m, H-3, H-6), 7.53 (m, H-4, H-5), 4.15-4.28 (4H, m, H-1′, H-1′′), 3.59-3.67 (4H, m, H-17′, H-17′′), 1.47-1.53 (4H, m, H-2′, H-2′′), 1.66, 1.81 (2H, m, H-16′, H-16′′), 1.38-1.41 (2H, m, H-7′, H-7′′), 1.30-1.34 (4H, m, H-4′, H-4′′), 1.20-1.22 (24H, m, H-3′, H-5′, H-6′, H-7′, H-9′, H-14′, H-18′, H-18′′, H-3′′, H-5′′, H-6′′, H-9′′, H-14′′). 0.89 (6H, m, H-19′, H-19′′), 0.87 (6H, m, H-15′, H-15′′). 13C NMR (100 MHz, CDCl3): δ_C 167.9 (2CO, C-1, -8), 132.5 (2C, C-2, -7), 130.8 (2CH, C-4, -5), 128.8 (2CH, C-3, -6), 68.1 (2CH2, C-1′, -1′′), 63.3 (2CH2, C-17′, -17′′), 38.7 (2CH2, C-2′, -2′′), 32.8 (2CH2, C-7′, -7′′), 31.9 (2CH2, C-8′, -8′′). 29.6 (2CH2, C-6′, -6′′), 29.5 (12CH2, C-9′ to -14′, -9′′ to -14′′), 28.9 (2CH2, C-5′, -5′′), 25.7 (2CH2, C-18′, -18′′), 23.7 (2CH2, C-3′, -3′′), 22.9 (2CH2, C-4′, -4′′), 22.7 (2CH2, C-16′, -16′′), 14.1 (2CH3, C-15′, -15′′), 10.9 (2CH3, C-19′, -19′′).

**Compound 3**: The ESI-MS (negative ion mode) gave the quasi molecular ion peak at m/z 303.2260 [M-H]- corresponding to C_{20}H_{36}O_{10} (calcd. 303.2318). The ESI-MS (positive ion mode) showed molecular ion [M-H+O-H]+ signal at m/z 287.1674. The 1H NMR of the compound represented doublet of doublet for double bond protons resonating at δ_H 5.90 (dd, J = 17.6, 10.9 Hz). A multiplet of a single proton appeared at δ_H 5.29 showed a double bond at carbon 7. A proton attached to electronegative atom at position 12 appeared at chemical shift of δ_C 3.56 (dd, J = 11.5, 4.4 Hz) connected with δ_C 7.39 at position 12, one proton appeared at δ_H 3.48 and δ_C 3.48 ppm. Likewise, four quaternary carbons were observed at C-4, C-8, C-10 and C-13 with δ_C of 32.4, 135.3, 39.0 and 42.0 ppm, respectively. However, all the spectral information was in agreement with reported values [20] and the structure of the pimarane diterpenoid was deduced as (2R,4aS,4bS,8aS)-2-ethenyl-1,2,3,4,4a,4b,5,6,7,8a,9-dodecahydro-2,4b,8,8-tetramethyl-3,5-phenanthrenediol (3) with the molecular formula C_{20}H_{32}O_{2}. 1H-NMR (DMSO-d6, 400 MHz): δ_H 5.90 (1H, dd, J = 17.6, 10.9 Hz, H-15), 5.29 (1H, m, H-7), 4.92 (2H, m, H-16), 3.56 (1H, dd, J = 11.5, 4.4 Hz, H-12), 3.48 (1H, m, H-11), 2.54 (1H, m, H-9), 1.97 (2H, m, H-14), 1.85 (2H, m, H-6), 1.61 (2H, m, H-3), 1.57 (2H, m, H-11), 1.44 (2H, m, H-2), 1.36 (1H, m, H-5), 0.89 (3H, s, H-18), 0.84 (3H, s, H-19), 0.78 (3H, s, H-20), 0.75 (3H, s, H-17). 13C-NMR (DMSO-d6, 100 MHz): δ_C 148.5 (CH, C-15), 135.3 (C-8), 120.5 (CH, C-7), 111.3 (CH2, C-16), 73.9 (CH, C-12). 69.4 (CH, C-1), 45.2 (CH2, C-14), 43.8 (CH, C-5), 42.0 (C-13), 41.3 (CH, C-9), 39.0 (C-10), 34.1 (CH2, C-3), 33.8 (CH3, C-19), 32.5 (C-4), 28.5 (CH2, C-11), 25.9 (CH2, C-2), 23.4 (CH3, C-6), 23.0 (CH3, C-18), 15.4 (CH3, C-17), 15.2 (CH3, C-20).

**Compound 4**: The ESI-MS (positive ion mode) showed m/z at 309.2790 [M+H]+ showing molecular formula C_{20}H_{32}O_{2} (calcd. 309.2788). The 1H NMR data represented singlet for methyl protons resonating at δ_H 0.95, 0.86, 0.85 and 1.69 ppm which represent protons at H-18, H-19, H-20, and H-16 respectively. The chemical shift values for protons at δ_H 2.15 (2H, t, J = 8.1) and δ_H 5.41 (1H, t, J = 8.1) confirmed the protons at positions 12 and 14, respectively. The peak at δ_H 4.18 (2H, d, J = 7.9 Hz) was assigned to proton at...
position 15. The experimental data was found in correlation with previous literature [21] and the structure was confirmed as labd-13(E)-ene,8α,15-diol (4). 1H NMR (400 MHz, CDCl₃): δH 5.41 (1H, t, J = 8.1, H-14), 4.18 (2H, m, H-13), 2.15 (2H, m, H-12), 1.69 (3H, s, H-16), 1.12 (3H, s, H-17), 0.95 (3H, s, H-18), 0.86 (3H, s, H-19), 0.85 (3H, s, H-20). The remaining protons were observed as multiplet signals. 13C NMR (400 MHz, CDCl₃): δC 140.9 (CH₂, C-1), 123.1 (CH₂, C-2), 74.3 (CH₂, C-3), 61.1 (C, C-4), 59.2 (CH, C-5), 56.1 (CH₂, C-6), 44.5 (CH₂, C-7), 43.8 (C, C-8), 42.8 (CH, C-9), 39.7 (C, C-10), 39.2 (CH₂, C-11), 33.3 (CH₂, C-12), 32.2 (C, C-13), 23.9 (CH, C-14), 23.5 (CH₂, C-15), 21.5 (CH₃, C-16), 20.5 (CH₃, C-17), 18.4 (CH₃, C-18), 16.4 (CH₃, C-19), 15.4 (CH₃, C-20).

**Compound 5**: The ESI-MS showed molecular ion peak [M+H]+ at m/z 315.08642. The 1H NMR showed two signals at δH 6.81 and δH 6.86 ppm for hydrogens at C-3 for deshielded methine proton and for aromatic hydrogen at C-8, respectively. The signal at δC 182.6 was assigned to carbonyl carbon at C-4. The chemical shifts at δC 132.3 and δC 159.0 ppm were assigned to C-6 and C-7, respectively. So all these promising signals helped for elucidation of structure and the compound 5 was named as cirsimaritin with molecular formula C₁₇H₁₄O₆ (calcd. 315.0863).

**Table 1.** Antidiabetic activity in HepG2 cells of crude extract of Nepeta adenophyta Hedge

<table>
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<th>Sample code &amp; concentration</th>
<th>Glucose uptake (mmol/L)</th>
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<tr>
<td></td>
<td>25 (μg/mL)</td>
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<tr>
<td>NAE</td>
<td>3.908078</td>
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<td>4.086351</td>
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*Control (6.210515, 6.159660, 6.454621); Model (3.514492, 4.079756, 3.805199); Metformin (6.051500, 5.966000, 6.546210)*
Figure 3. Glucose consumption assay on HepG2 cells

The test samples were taken in concentrations of 25, 50, and 100 μg/mL. Each sample was examined three times. Alongside the results for the control group, the model group and metformin are also provided in Table 1. The glucose uptake in HepG2 cells increased with increasing concentrations of sample, i.e., 25, 50, and 100 μg/mL as shown in Fig. 3. The ethanol extract was found active. Significant activity (p<0.05) was found at all tested concentrations.

Results of Antioxidant Activity

The antioxidant potential of the test sample was determined in HepG2 cells. For almost six hours, the cells were treated with 200 μM hydrogen peroxide, and the results are shown in Table 2, which also shows the results for the control group, the model group and quercetin.

Table 2. Antioxidant activity in HepG2 cells of crude extract of Nepeta adenophyta Hedge

<table>
<thead>
<tr>
<th>Sample code &amp; concentration</th>
<th>Cell viability (% of control)</th>
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<td></td>
<td>25 (μg/mL)</td>
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<tr>
<td>NAE</td>
<td>0.1288</td>
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<td></td>
<td>0.1301</td>
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<td>0.1301</td>
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<td>0.1411</td>
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* Control (0.520, 0.530, 0.520); Model (0.2904, 0.2754, 0.1985); Quercetin (0.4810, 0.4456, 0.4710)

Figure 4. The cell viability of components on HepG2 cells

The crude extract of the study plant was used for antioxidant potential which were checked in hydrogen peroxide induced HepG2 cells. As presented in Table 2, the test sample was analyzed in three different concentrations, i.e., 25, 50, and 100 μg/mL. Each sample was analyzed in triplicate.

As shown in Fig. 4, the sample was tested by taking three different concentrations i.e., 25, 50, and 100 μg/mL. The cell viability was found lesser than 50%, and the standard antioxidant quercetin was used. The increase in concentration showed good results.

The mint family (Lamiaceae) is a source for healing and managing various metabolic disorders, included diabetes [4]. *N. cataria* is the most studied species of the genus *Nepeta*, it is traditionally used for fever, pneumonia, etc., and it has previously shown many biological properties like antioxidant and most importantly against diabetes [23, 24]. Likewise, *N. racemosa* showed antidiabetic and antioxidant activities.
which are due to the presence of phenolic and flavonoid content [25]. N. hindostana, N. gracilliflora and N. cataria have previously shown high potential for antioxidant, anti-inflammatory and anti-diabetic properties [26]. Similarly, recently, N. baytopii, Turkey’s endemic specie, has been reported for its antioxidant and anti-diabetic potential [27] and N. deflersiana has exhibited high content of antibacterial and anti-diabetic properties [28]. Herein, Nepeta adenophyta Hedge showed antioxidant and anti-diabetic potential, which favors its traditional uses and could be an important source in search of medicine for such diseases in future.

CONCLUSIONS

The Himalayan Nepeta adenophyta Hedge was studied for the isolation, and five compounds were isolated by using chromatographic techniques. The compounds namely 3α-[β-sitosteryl-3β-oxy]-dihydronepetalactone (1), bis [7-ethyl-2-[2-hydroxyethyl] pentadecyl] phthalate (2), a pimarane diterpenoid (3), labd-13(E)-ene,8α,15-diol (4), and cirsimaritin (5) were isolated in pure form using column chromatography over normal silica gel. The plant crude material (NAE) showed good results in antioxidant and anti-diabetic activities in HepG2 (a human hepatoma cell line) cells. The current study has unlocked new ways for the phytochemical and bioactivity evaluation of this medicinal plant. The compounds like nepetalactones, diterpenes and flavones are present in the plant sample, and in future studies, some other related compounds of these classes might be isolated.

DECLARATIONS

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Authors’ contributions:
Rubab Ilyas: Investigation. M. Ali: Investigation. I. Ali: Conceptualization; Formal analysis; Funding acquisition; Methodology; Project administration; Resources; Supervision; Roles/ Writing - original draft. Baraa Siyo: Formal analysis; Writing - review & editing. Wenliang Xu: Investigation. Amjad Hussain: Formal analysis; Writing - review & editing. Wang Wei: Formal analysis. Long Chen: Investigation. Zamarrud: Formal analysis; Resources; Writing - review & editing. Xiao Wang: Project administration; Supervision; Writing - review & editing.

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