Bioactive Constituents of *Pulicaria jaubertii*: A promising Antihypertensive Activity

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**ABSTRACT**

**Objective:** Phytochemical investigation and evaluation of antihypertensive activity of the alcoholic extract of *Pulicaria jaubertii* aerial parts. **Material and Methods:** The chloroform and ethyl acetate fractions were chromatographed on silica gel and sephadex LH 20 to afford six compounds. Their structures were elucidated on the bases of extensive NMR (1H, 13C, DEPT, COSY, HSQC, HMBC, NOESY) and MS analysis. The antihypertensive activity of the alcoholic extract was evaluated against LNAME–induced hypertensive rats. **Results:** One new monoterpane glucoside; (2R,4S)-p-menth-1-ene-2,7-dimethyl-2,6,8,10-tetrahydro-3H,7H-dibenzo[b,d]pyran-10-carboxylic acid-1-O-β-D-glucopyranoside [1] along with five known compounds; thymoquinol 2-O-β-D-glucopyranoside (zataroside-B) [2], quercetin 7, 3’-di-O-methyl ether (quercetin) [3], quercetin 7,7,3,3’-tetra-O-methyl ether (hamnazin) [4], quercetin 3’-O-methyl ether (isorhamnetin) [5] and stigmasterol 3-O-β-D-glucopyranoside [6] were isolated. The alcoholic extract significantly reduced the elevated SBP and partially restored the diminished nitric oxide metabolites. **Conclusion:** Compound [1] is a new and compounds [2-6] are reported for first time from *Pulicaria jaubertii*. The possible antihypertensive activity of the alcoholic extract may be due to its flavonoids and phenolic contents. **Key words:** *Pulicaria jaubertii*, Monoterpane glucoside, Flavonoids, Antihypertensive activity, Nitric oxide. **Address for Correspondence:** Usama Shaheen (Ph.D), Department of Pharmacognosy, Faculty of Pharmacy, Umm Al-Qura University, Makkah, 21965, SAUDI ARABIA. Phone no: +966597019100 E-mail: usamayosef2003@yahoo.com DOI: 10.5530/pj.2016.1.18

**INTRODUCTION**

Hypertension is a primary risk factor for stroke, heart disease and renal failure and remains one of the most critical issues for human health. Both lifestyle and hereditary factors are known to be related to the development of hypertension, including eating habits. Attempts by the low-income group, particularly the rural dwellers in the developing countries, to control hypertension and its attendant complications in the face of the scarce socioeconomic resources, have led more people opting for herbal remedy.¹ The renewed interest in the search for new drugs from natural sources, especially from plant sources, has gained global attention during the last two decades. Many foods as well as herbal remedies are claimed by folk medicine to be effective in the prevention and/or treatment of this serious disease. The aim of the present study was identify the main active constituents of *Pulicaria jaubertii* and to evaluate the antihypertensive effects of its extract in rats made hypertensive by reducing their body ability to produce the potent vasodilator radical NO. *Pulicaria jaubertii* Gamal-Eldin is a fragrant perennial herb belonging to the Asteraceae family and widely distributed in Arab peninsula especially south of Saudi Arabia and Yemen, it is known as Alkhaw‘ah and used traditionally as diuretic and to relieve fever of urogenetic diseases.²,³ Although this plant reported to many uses and biological activities, there is only one phytochemical study concerning the isolation of its active constituents.⁴ Several sesquiterpenes, diterpenes, triterpenes and flavonoids were previously isolated from different *Pulicaria* species.⁵-⁷ Its essential oil composition was also investigated.⁸-¹⁰ This paper describe the isolation and structure elucidation of a new monoterpane glucoside (1) and five known compounds (2-6) from the aerial parts of *Pulicaria jaubertii*. The alcoholic extract was standardized to its total phenol and flavonoid contents. The possible antihypertensive activity of the alcoholic extract was also evaluated.

**MATERIAL AND METHODS**

UV spectra were determined with a Shimadzu UV-1650PC spectrophotometer; IR spectra were carried out on a Nicolet 205 FT IR spectrometer connected to a Hewlett-Packard Color Pro. Plotter. The 1H-and 13C-NMR measurements were obtained with Bruker Avance spectrometers operating at 600, 500 and 400 MHz (for 1H) and 150, 125 and 100 MHz (for 13C) in DMSO-d₆ solution, and chemical shifts were expressed in δ (ppm) with reference to TMS, and coupling constant (J) in Hertz. 13C multiplicities were determined by the DEPT pulse sequence (135°). HMBC, HSQC, COSY and NEOXY NMR experiments were carried out using Bruker AV-600, 500 and 400 spectrometers. ESIMS (positive ion acquisition mode) was carried out on a XEVO TQD triple quadruple instrument (Waters Corporation, Milford, MA 01757, USA) mass spectrometer. EIMS was carried on Scan EIMSTIC, VG-ZAB-HF, X-mass (158.64, 800.00) mass spectrometer (VG Analytical, Inc.). Si gel (Si gel 60, Merck), was used for open column chromatography. Solid phase extraction was performed on SPE-C₁₅ cartridges (A Strata column, Phenomenex, USA). TLC was carried out on precoated silica gel 60 F254 (Merck) plates. Developed chromatograms were visualized by spraying with 1% vanillin-H₂SO₄ followed by heating at 100 for 5 min.

**Plant material**

The aerial parts of *Pulicaria jaubertii* were collected in August 2012 from Yemen, Aljar region of Hajja. The plant was authenticated by Dr. Abd El-Rahman S. Aladbee, Professor of Plant Taxonomy, Faculty of Science, Sana’a university, Yemen. A voucher specimen [P-01] has been deposited in the Department of Pharmacognosy, College of Pharmacy, Al-Azhar University, Cairo, Egypt.
Extraction and isolation

The air-dried *Pulicaria jaubertii* aerial parts powdered (1.0 kg) were subjected to exhaustive extraction with ethanol (5 L x 2). The combined ethanolic extracts were concentrated under vacuum to dryness. The concentrated ethanolic extract (40 g) was suspended in distilled water (300 ml) and partitioned successively with *n*-hexane, chloroform, ethyl acetate and *n*-butanol to give 16 gm, 3.4 g, 5.5 g, and 4.2 g, respectively. The chloroform extract (3.4 g) was applied to a silica gel column eluted with *n*-hexane: chloroform 50:50 to 100% chloroform to give five fractions of A (450 mg), B (50 mg), C (430 mg), D (733 mg) and E (590 mg). The B fraction (50 mg) was chromatographed on a Sephadex LH-20 column eluted with MeOH to give compound 3 (4 mg). Fraction C (430 mg) was applied to a Sephadex LH-20 column eluted with MeOH to give two sub fractions of C-1 (120 mg) and C-2 (65 mg). The sub fraction C-2 was further repeatedly chromatographed on silica gel columns eluted with pet. ether: ethyl acetate 85:15 to 80:20, followed by solid phase extraction (RP-C<sub>18</sub>) using 10:90-60:40 acetonitrile: water system to obtain compounds 4 (10 mg) and 5 (10 mg). The ethyl acetate extract (5.5 g) was subjected to a silica gel column eluted with *n*-hexane: ethyl acetate 50:50 to 100% ethyl acetate to obtain five fractions of A (800 g), B (350 mg), C (330 mg), D (290 mg) and E (13 mg). Fraction E (1.3 g) was purified on Si gel CC eluted with pet. ether: ethyl acetate 20:80 to give sub fractions E-1 (225 mg) and E-2 (400 mg). On addition of methanol to sub fraction E-2, a white precipitate was observed. Three washes of the precipitate with methanol following by decanting the supernatant enabled the purification of compound 6 (15 mg). The supernatant was applied to a Sephadex LH-20 column eluted with MeOH to give three fractions of S1 (20 mg), S2 (160 mg) and S3 (70 mg). Fractions S2 and S3 were subjected separately to solid phase extraction (C<sub>18</sub> column) eluted with 100% water to 80% water in acetonitrile to afford compounds 2 (60 mg) and 1 (25 mg), respectively.

**Estimation of total flavonoid content**

The total concentration of flavonoid was determined spectrophotometry using AlCl<sub>3</sub> method. A standard calibration curve was done using quercetin (0-50 μg/mL). All analyses were repeated three times.

**Estimation of total phenolic content**

The total phenol content of *Pulicaria jaubertii* extract was determined using the reagent of Folin-Ciocalteu reagent using Agilent-Cary 60 spectrophotometer. Gallic acid was used to make a standard calibration curve for determining total phenolic content in mg of gallic acid equivalents (GAE)/g of extract. All assays were repeated three times.

**Evaluation of potential antihypertensive activity**

Adult male albino Sprague-Dawley rats weighing 150-200g were used in our experiments. They were purchased from the animal facility of the pharmacology department, College of Pharmacy, King Abdul-Aziz University, Jeddah, KSA. Animals were housed in cages kept under constant environmental and nutritional conditions throughout the period of investigation. They were allowed a free access to water and diet consisting of standard pellet chow. The study was carried out according to The European Communities Council Directive of 1986 (86/609/EEC) and approved by the Ethical Committee for Animal Experimentation at the Faculty of Pharmacy, Umm Al Qura University, Makkah, KSA. L-NAMe was obtained from Sigma-Aldrich Company, USA. It was freshly prepared in normal saline in a concentration adjusted so that the volume administered is 1ml/100 g animal weight. The standardized lyophilized hydroalcoholic extract of *Pulicaria jaubertii* aerial parts was prepared as discussed under the methods, and was freshly reconstituted prior to use in normal saline so that the volume administered is 1ml/100 g animal weight. Hypertension was induced in rats by once daily intraperitoneal (i.p) injection of L-NAMe for 10 days in a dose of 75 mg/kg. A non invasive blood pressure (NIBP) technique utilizing tail cuff and volume pressure recording (VPR) sensor was followed to record the systolic blood pressure (SBP) of conscious animals in our experiment. This was carried out using a computerized NIBP monitor (CODA Monitor, Kent Scientific, Kentucky, USA). During the procedure, core body temperature of all the animals was maintained constant by keeping their body surface temperature around 32 ± 0.5°C via a UV warming pad, as monitored by a non contact IR thermometer (TW2 Thermometer, ThermoWorks, USA).

**Estimation of blood nitric oxide total metabolites**

Nitric oxide (NO) metabolites (nitrates and nitrites) were assayed in serum following the colorimetric assay method of Griess after enzymatic conversion of nitrates to nitrites by nitrate reductase using nitrate/nitrite colorimetric assay kit (Sigma-Aldrich, MI, USA). Determination of total nitrate and nitrite in serum samples is an indicator for NO radical formation.

**Experimental design and treatment protocol**

Twenty rats were randomly allocated into 3 groups, consisting each of 6-7 rats treated as follows: Group 1 (normotensive control group): Animals in this group were i.p. injected with normal saline for 10 days, then continued for further week together with oral gavage of the saline to rule out the possible effect of manipulation during the process of feeding by the gastric tube. Group 2 (hypertensive control group): Animals in this group were rendered hypertensive as described above. Hypertensive animal were maintained on L-NAMe for a further week, during which they were orally given normal saline. Group 3 (hypertensive treated group): Here, animals were maintained on L-NAMe for a further week together with daily gavage of the plant extract in a dose of 150 mg/Kg. At the end of the experimental period (17 days), all animals were subjected to NIBP measurement as described above. After NIBP measurement, blood samples were collected from the retro-orbital plexus under light ether anesthesia and serum was prepared for nitric oxide metabolites estimation as described above.

**Statistical Analysis**

All values in this study are expressed as mean±standard error of the mean (M±SEM). Data were analyzed by one-way analysis of variance (ANOVA). When variation among groups was found significant, Tukey-Kramer multiple comparison tests were carried out to compare between groups. Differences were considered significant when p value was <0.05.

**RESULTS AND DISCUSSION**

The dried aerial ethanol extract of *Pulicaria jaubertii* was suspended in water and fractionated with *n*-hexane, chloroform, ethyl acetate and *n*-butanol. The chloroform and ethyl acetate fractions were subjected to subsequent purification using several chromatographic techniques (repeated silica gel columns, solid phase extraction “C<sub>18</sub> columns” and sephadex LH-20 columns); one new and five known compounds were isolated (Figure 1). Their structures were elucidated by comparison of its spectroscopic data with reported data in the literature as thymoquinol 2-0-β-D-glucopyranoside (zataroside-B) [2]<sup>13,14</sup>, quercetin 7, 3′-di-O-methyl ether [3] (rhamnazin) <sup>15,16</sup>, quercetin 7-O-methyl ether (rhamnetin) <sup>4</sup>, quercetin 3′-O-methyl ether <sup>5</sup> and stigmasteryl 3-O-β-D-glucopyranoside <sup>6</sup>.

(2R,4S)-p-menth-1-ene-2-0-β-D-glucopyranoside [1]: Colorless crystals; IR <sup>ν<sub>max</sub></sup> (KBr): 3350, 1080, 1640 cm<sup>-1</sup>; ESIMS <sup>m/z</sup> 339 [M+Na]<sup>+</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>,400 MHz) and <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 100.0 MHz) see (Table 1).
Figure 3: Effect of one week’s treatment with Pulicaria jaubertii extract (150 mg/Kg p.o. once daily) on serum NO metabolites of male albino L-NAME induced hypertensive rats. Values are represented as mean±SEM (n=6-7 per group).

*Significantly different from normotensive control value at p<0.05.

*Significantly different from hypertensive control value at p<0.05.
Thyromoquinol 2-O-β-D-glucopyranoside (zaratoside-B) [2]: Colorless crystals; IR $\nu_{\text{max}}$(KBr): 3440 cm$^{-1}$; ESI-MS $m/z$ 351 [M+Na]$^+$; 1H NMR (DMSO-$d_6$, 400 MHz) aglycon $\delta=8.96$ (1H, brs, H-1), 8.68 (1H, brs, H-3), 6.51 (1H, brs, H-6), 3.08 (1H, t, $J=6.8$ Hz, H-8), 2.09 (3H, s, H-7), 1.11 (6H, d, $J=6.4$ Hz, H$_2$-9), sugar $\delta=4.53$ (1H, d, $J=7.2$ Hz, H-1′), 3.68 (1H, d, $J=11.6$ Hz, H-6′a), 3.48 (1H, dd, $J=11.6$, 5.2 Hz, H-6′b), 3.24 (1H, m, H-5′), 3.22 (1H, m, H-2′), 3.20 (1H, t, $J=8.8$ Hz, H-3′), 3.17 (1H, t, $J=8.0$ Hz, H-4′); 13C NMR (DMSO-$d_6$, 100.0 MHz) aglycon $\delta=149.35$ (s, C-5), 149.20 (s, C-4), 132.49 (s, C-6), 125.29 (s, C-1), 117.0 (d, C-6), 114.98 (d, C-3′), 26.64 (d, C-8), 23.09 (q, C-9), 23.0 (q, C-10), 16.18 (q, C-7), sugar $\delta=103.25$ (d, C-1′), 77.42 (d, C-5′), 77.23 (d, C-2′), 73.91 (d, C-2′), 70.46 (d, C-4′), 61.36 (t, C-6′). The spectroscopic data (1H and 13C NMR) were comparable with published value.

Assignments were confirmed by HSQC and HMBC experiments.

Quercetin 7, 3′-di-O-methyl ether (rhamnazin) [3]: Pale yellow powder, UV $\lambda_{\text{max}}$(MeOH): 256, 357 nm; IR $\nu_{\text{max}}$(KBr): 3400, 1655, 1610, 1585 cm$^{-1}$; ESI-MS $m/z$ 316 [M]$^+$; 1H NMR (DMSO-$d_6$, 500 MHz) $\delta=12.15$ (1H, brs, OH-5), 7.79 (1H, brs, H-2′), 7.75 (1H, d, $J=8.2$ Hz, H-6′), 6.95 (1H, d, $J=8.4$ Hz, H-5′), 6.78 (1H, brs, H-8), 6.35 (1H, brs, H-6), 3.87 (7-OCH$_3$); 13C NMR (DMSO-$d_6$, 125 MHz) $\delta=175.42$ (C-4′), 164.37 (C-7′), 159.73 (C-5′), 155.52 (C-9′), 148.36 (C-4′), 146.85 (C-2′), 146.51 (C-3′), 135.55 (C-5′), 130.21 (C-1′), 114.96 (C-5′), 111.19 (C-2′), 103.42 (C-10), 96.91 (C-6′), 91.50 (C-8), 55.46 (7-OCH$_3$), 55.27 (3′-OCH$_3$). The spectroscopic data (1H and 13C NMR) were comparable with published value. Assignments were confirmed by HSQC and HMBC experiments.

Quercetin 7-O-methyl ether (rhamnetin) [4]: Pale yellow powder, UV $\lambda_{\text{max}}$(MeOH): 254, 355 nm; IR $\nu_{\text{max}}$(KBr): 3450, 1646, 1610, 1590 cm$^{-1}$; ESI-MS $m/z$ 316 [M]$^+$; 1H NMR (DMSO-$d_6$, 600 MHz) $\delta=12.47$ (1H, brs, 5-OH), 9.65 (1H, brs, 4′-OH), 9.47 (1H, brs, 3-OH), 9.30 (1H, brs, 3′-OH), 7.71 (1H, d, $J=2.4$ Hz, H-2′), 7.56 (1H, dd, $J=8.4$, 2.4 Hz, H-6′), 6.88 (1H, d, $J=8.4$ Hz, H-5′), 6.69 (1H, d, $J=2.4$ Hz, H-6′), 6.33 (1H, d, $J=1.8$ Hz, H-6), 3.85 (OCH$_3$); 13C NMR (DMSO-$d_6$, 150 MHz) $\delta=175.92$ (C-4′), 164.86 (C-7′), 160.32 (C-5′), 156.12 (C-9′), 147.81 (C-4′), 147.24 (C-2′), 145.05 (C-3′), 135.78 (C-3′), 121.93 (C-1′), 120.0 (C-6′), 115.53 (C-5′), 115.19 (C-2′), 103.96 (C-10), 97.42 (C-6), 91.86 (C-8), 55.98 (OCH$_3$). The spectroscopic data (1H and 13C NMR) were comparable with published values. Assignments were confirmed by HSQC and HMBC experiments.

Stigmasteryl 3-O-β-D-glucopyranoside [6]: White amorphous powder; IR $\nu_{\text{max}}$(KBr): 3432, 1635 cm$^{-1}$; ESI-MS $m/z$ 575 [M+H]$^+$; 1H NMR (DMSO-$d_6$, 500 MHz) aglycon $\delta=5.33$ (m, H-6), 5.15 (dd, $J=15.0$, 8.5 Hz, H-22), 5.02 (dd, $J=15.0$, 8.5 Hz, H-23), 3.63 (m, H-3), 0.99 (d, $J=6.5$ Hz, Me-21), 0.96 (s, Me-19), 0.81 (d, $J=6.5$ Hz, Me-26), 0.79 (t, $J=6.0$ Hz, Me-29), 0.79 (d, $J=6.5$ Hz, Me-27), 0.65 (t, Me-18), sugar $\delta=4.22$ (d, $J=7.6$ Hz, H-1′), 3.65 (d, $J=11.0$ Hz, H-6′a), 3.41 (m, H-6′b), 3.10 (m, $J=8.7$ Hz, H-3′), 0.09 (m, H-5′), 0.30 (s, $J=8.8$ Hz, H-4′), 2.91 (t, $J=8.1$ Hz, H-2′), 1H NMR (DMSO-$d_6$, 125.0 MHz) aglycon $\delta=140.94$ (C-5′), 138.51 (C-22), 129.31 (C-23), 121.70 (C-6′), 79.62 (C-3′), 56.65 (C-14), 55.83 (C-17), 51.06 (C-24), 50.08 (C-9), 42.33 (C-13), 40.45 (C-20), 38.78 (C-4′), 38.77 (C-12), 37.30 (C-1′), 36.70 (C-10), 31.90 (C-7), 31.85 (C-8), 31.80 (C-25), 27.94 (C-2′), 28.97 (C-16), 25.90 (C-28), 24.35 (C-21), 21.58 (C-21), 21.41 (C-26), 21.07 (C-11), 20.19 (C-27), 19.32 (C-19), 12.60 (C-18), 12.32 (C-29), sugar $\delta=101.25$ (C-1′), 77.42 (C-3′), 77.21 (C-5′), 73.94 (C-2′), 70.59 (C-4′), 61.57 (C-6′). The spectroscopic data (1H and 13C NMR) were comparable with published values. Assignments were confirmed by HSQC and HMBC experiments.
The new compound 1 was obtained as colorless crystals. Its ESIMS showed a molecular ion peak at m/z 339 [M+Na]+ corresponding to the molecular formula C_{14}H_{25}O_{6}. The IR spectrum showed strong absorption bands at 3350 and 1640 cm\(^{-1}\) due to the hydroxyl functions and olefinic double bond, respectively. \(^1^C\) NMR spectrum (Table 1) and DEPT experiment of 1 displayed the presence of one \(\beta\)-D-glucopyranosyl unit from the signals at \(\delta\) 104.95 (C-1), 74.29 (C-2), 77.16 (C-3), 70.64 (C-4), 76.86 (C-5) and 61.65 (C-6) together with 10 carbon signals for the aglycon as follows: 3 methyl groups (\(\delta\) 19.51, 19.63 and 20.09), 2 methylene groups (\(\delta\) 28.66 and 35.89), 4 methines (\(\delta\) 32.17, 37.91, 79.74 and 124.26) and one quaternary carbon (\(\delta\) 136.39). The carbon signal at \(\delta\) 79.74 indicated the attachment of one hydroxyl group to this carbon.\(^{23}\) On the other hand, \(^1^H\) NMR spectrum of 1 revealed the presence of one methyl singlet (\(\delta\) 1.69) and two methyl doublets (\(\delta\) 0.88 and 0.83) assignable for Me-7, 9 and 10, respectively.\(^{24}\) The broad singlets at \(\delta\) 4.0 and 5.38 were assigned for H-2 and H-6, respectively. Moreover, the one proton triplet signal at \(\delta\) 1.42 in the \(^1^H\) NMR spectrum of 1 was interpreted for H-8 while the four protons multiplet at \(\delta\) 1.3-2.18 were assigned for two methylene groups (H-3 and 5). The \(^1^H\) NMR spectrum of 1 showed also resonances for the sugar moiety and by the HSQC and \(^1^H\)-\(^1^H\)-COSY analyses, and their coupling patterns indicated the sugar moiety to be glucose. The \(\beta\)-configuration of the glucopyranosyl unit was deduced from the coupling constant (7.7 Hz) of the anomeric proton doublet at \(\delta\) 4.24 in the \(^1^H\) NMR spectrum of 1.\(^{12}\) The glucose moiety was determined to be in the D-pyranose form from its \(^1^C\) NMR data. The attachment of the \(\beta\)-glucosyl moiety to C-2 of the aglycon was based on the downfield shift of this carbon (\(\delta\) 79.74) in addition to the long-range correlation observed in HMBC spectrum between H-2 (\(\delta\) 4.0) and the anomeric carbon (\(\delta\) 104.95) and between the anomeric proton (\(\delta\) 4.24) and C-2 (\(\delta\) 79.74) of the aglycon. In HMBC spectrum, long-range correlations observed between the methyl protons at \(\delta\) 1.69 (H-7) and C-1 (136.39), C-2 (79.74) and C-6 (124.26) and between the olefinic proton at \(\delta\) 5.38 (H-6) and C-7 (\(\delta\) 20.09) proved the attachment of the hydroxyl group to C-2 of the aglycon together with the location of the double bond between C-1 and C-6. The relative stereochemistry of 1 was confirmed by NOESY (Table 1). Consequently the structure of compound 1 was assigned as (2R, 4S)-p-menth-1-ene-2-O-\(\beta\)-D-glucopyranoside.

**Total flavonoids and total phenolic contents**

In order to standardized the alcoholic extract of *Pulicaria jaubertii*, the total phenol content was analyzed using F-C analytical method, and total flavonoid content using UV-vis colorimetric method. The total phenol content was appeared to be 104.5858 mg gallic acid equivalent/g dry extract and the total flavonoid content was appeared to be 78.5858 mg quercetin equivalent/g dry extract.

**Antihypertensive activity**

In the present investigation, values of SBP of control normotensive animals fell all within normal values recorded for rats in previous studies, giving a mean of 122 ± 1.73 mmHg. After 17 days of daily injection of L-NAME, this value was significantly elevated to reach 184.34 ± 3.06 mm Hg. After one week treatment with the extract, hypertensive animals showed a significant reduction of SBP down to 138.4 ± 4.14 mm Hg, representing 25% reduction from control hypertensive value (Figure 2). As shown in (Figure 3), NO-deficient hypertensive rats showed a sharp fall in serum NO metabolites down to 1.735 ± 0.512 μmol/L as compared to normotensive animals (20.25 ± 0.755 μmol/L). Treatment with the plant extract for one week preserved the radical level and elevated its metabolites to 15.056 ± 1.919 μmol/L, a value which significantly higher than control hypertensive group.

L-NAME, the non selective nitric oxide synthase inhibitor, reduces NO synthesis in the endothelium and induces changes in vascular compliance to vasoactive mediators. Its continued administration causes persistent elevation of blood pressure by various mechanisms, including lack of tonic vasodilatation, activation of the sympathetic nervous system, activation of the renin angiotensin system, as well as interference with calcium channels and arachidonic acid derivatives.\(^{26-28}\) L-NAME induced hypertension has been also associated with the generation of free oxygen species and that this can be overcome by the administration of free radical scavengers.\(^{28}\) It is thus clear, that agents that can decrease blood pressure in L-NAME induced hypertension, like the investigated extract, could do so in more than one way.

The candidate constituents of the extract that could have antihypertensive properties are probably the flavonoid components which have been previously reported to possess antihypertensive properties. Indeed, blood pressure-lowering effect of (-)-epicatechin the most abundant flavanol present in cocoa seeds in L-NAME-treated rats was found to be associated with restored nitric oxide levels, reduced superoxide anion production and elevated nitric oxide synthase (NOS) activity, favoring an increase in NO bioavailability.\(^{30,31}\)

In the present study, the total nitrite concentration in the serum of the animals was determined as a measure for NO concentration and availability. Hypertensive animals showed a marked reduction in serum nitrite concentration, while treated animals showed partially restored level of the radical. The diminished NO bioavailability observed in the L-NAME group is probably a result of NOS inhibition and/or increased NO oxidation to peroxynitrite secondary to an increased production of superoxide anion.\(^{32}\) Consequently, the restoration of NO concentrations observed following the extract administration can include an increase in eNOS activity or expression as well as a direct superoxide anion scavenging potential of the flavonoid content of the extract.

Other constituents in the extract may modulate its activity, or may have other antihypertensive properties by their own. As is often the case with herbal extracts, it is sometimes difficult to attribute an overall activity to a single component, but rather to the extract as a whole. Further studies are being carried out to determine the possible involvement of the rennin-angiotensin aldosterone system, and to test the effect of the extract on vascular reactivity in an attempt to gain more insight in its mechanism of action.

**CONCLUSION**

The present study has identified the isolation and characterization of a new monoterpene glucoside along with one known monoterpene glucoside, three known flavonol and one known sterol glucoside from aerial parts of *Pulicaria jaubertii*. All compounds are reported for first time in this plant. The standardized *Pulicaria jaubertii* extract significantly reduced SBP in L-NAME–induced hypertensive rats. Further investigations using the isolated fractions of the extract are now being carried out to elucidate the active principle(s) that owe this effect.

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**CONFLICT OF INTEREST**

The authors declare that, no conflict of interest.
ABBREVIATION USED

L-NAME: Nω-Nitro-L-arginine methyl ester hydrochloride; NO: Nitric Oxide; NOS: Nitric Oxide Synthase.

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