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NEW ALKALOIDS FROM *Casimiroa edulis* FRUITS AND THEIR PHARMACOLOGICAL ACTIVITY

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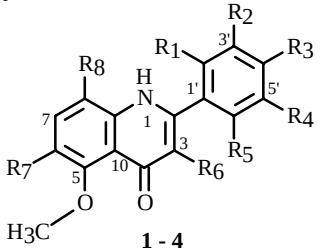
The extract of Casimiroa edulis *was investigated for antihypertensive activity. The ethanol and total alkaloids (in chloroform) extracts were found to have antihypertensive properties at doses of 500 and 200 mg/kg, respectively. Four quinolinone alkaloids were isolated and identified as: 2-(2′-hydroxy-4′-methoxyphenyl)-5,8-dimethoxy-3-propyl-1*H*-quinolin-4-one (**1**), 5,8-dimethoxy-2-(3′-methoxyphenyl)-3-propyl-1*H*-quinolin-4-one (**2**), 5,8-dimethoxy-2- (3′,4′-dimethoxyphenyl)-3-propyl-1*H*-quinolin-4-one (**3**), and 5,6-dimethoxy-2-(2′,5′,6′-trimethoxyphenyl)-1*H*-quinolin-4-one (**4**). Interestingly, compounds **1**, **2**, and **3** were found to be new alkaloids. The four isolated alkaloids showed antihypertensive activity at doses of 50, 100, 200, and 300 mg/kg, respectively.*

Key words: quinolinone alkaloids, antihypertensive, *Casimiroa edulis*.

Natural products are vastly becoming the most important field of research all over the world in the attempt to discover cheaper drugs with lower side effects [1]. There are many plants that possess several biological activities and are currently used in folk medicine. *Casimiroa edulis* Llave et Lex (Rutacae) is one of such plants, which is known for its sedativelike effect as a sleep inducer [2, 3]. The tree is cultivated in Egypt for its edible fruits. Earlier, extensive studies have been carried out on the isolation and identification of the chemical constituents of the bark, seeds, and root of *Casimiroa edulis* [4–6]. The isolated compounds include casimiroedine and dimethylhistamine [5], *N*α,*N*α-dimethylhistamine [6], zapoterin, imidazole [7], and 2-quinolinone, and 4-quinolones [8]. The aqueous extract of *Casimiroa edulis* leaves has antiinflammatory and diuretic activities [8]. However, the alcoholic extract of the leaves has anticonvulsant, antimutagenic, and sedative activities. The compounds isolated from leaves include isoimpinellin, casimiroin, skimmianine, 1-methyl-2-phenyl-4-quinol, edulein, and scopoletin methyl ether [9, 10]. The fruits of *C. edulis* have never been subjected to any investigation. So, the ripened fruits of *C. edulis* are studied for determination of the pharmacological activity and isolation of its active constituents.



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	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
1:	OH	H	OCH_3	H	H	$(CH_2)_2CH_3$	H	OCH_3
2:	H	OCH_3	H	H	H	$(CH_2)_2CH_3$	H	OCH_3
3:	H	OCH_3	OCH_3	H	H	$(CH_2)_2CH_3$	H	OCH_3
4:	H	OCH_3	H	OCH_3	OCH_3	H	OCH_3	H

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TABLE 1. Antihypertensive Effect of Extracts and Isolated Compounds of *Casimiroa edulis* Fruits

Treament.	Body weight, mg/kg	Duration/min			
		5	10	30	60
Control		164.0±17.1	164.0±17.1	164.0±17.1	164.0±17.1
α-Methyldopa	25	154.0±13.42	141.0±15.97*	141.0±15.97*	141.0±15.97*
Total alcohol extract	500	140.0±13.56*	135.0±12.44*	135.0±12.44*	135.0±12.44*
Total alkaloids	200	120.1±11.10**	120.0±13.54**	115.0±13.54**	115±13.54**
1	50	100.0±8.30**	90.0±9.31**	85.0±9.31**	80.0±10.31**
	100	90.2±9.90***	80.2±8.23***	75.2±6.23***	70.2±7.23***
	200	80.4±7.49***	70.0±7.19***	65.0±6.19***	60.0±5.19***
	300	60.0±6.29***	55.3±5.24***	50.3±5.24***	50.3±5.24***
2	50	120.0±8.22**	115.0±11.32**	110.0±10.32**	105.0±9.32**
	100	110.1±13.1**	105.0±10.90***	95.0±9.90***	85.0±8.90***
	200	95.2±9.90***	85.1±9.31***	75.1±9.31***	70.1±9.31***
	300	85.0±9.23***	80.1±9.90***	70.1±8.90***	65.1±7.90***
3	50	120.0±10.32**	110.0±9.32	100.0±10.32**	95.0±8.32***
	100	107.1±8.21**	95.1±8.61***	95.1±8.61***	95.1±7.61***
	200	100.0±7.19***	90.3±7.19***	90.3±8.19***	90.3±9.19***
	300	90.0±7.92***	80.0±9.94***	80.0±9.94***	80.0±8.94***
4	50	125.0±9.32**	125.0±10.32**	125.0±10.32**	125.0±9.32**
	100	120.0±8.43**	120.0±9.21**	120.0±9.21**	120.0±8.21**
	200	115.1±7.67**	115.2±8.76**	115.2±9.76**	115.2±7.76**
	300	110.0±9.12***	110.5±7.13**	110.5±8.13***	110.5±9.13**

*Significant at <0.05; **significant <0.01; ***significant <0.001.

The antihypertensive activity of *C. edulis* fruits was determined using male dogs, intravenously anesthetized with pentobarbital sodium (30 mg/kg b.wt.). The alcoholic and total alkaloidal extracts of *C.edulis* ripened fruits showed a significant antihypertensive activity. The alkaloidal extract of *C. edulis* showed the most significant antihypertensive activity (125.0±13.54). Further fractionation of this alkaloidal fraction by several chromatographic methods yielded four compounds three of which were found to be new alkaloid compounds: 2-(2′-hydroxy-4′-methoxyphenyl)-5,8-dimethoxy-3-propyl-1*H*-quinolin-4-one (**1**), 5,8-dimethoxy-2-(3′-methoxyphenyl)-3-propyl-1*H*-quinolin-4-one (**2**), 5,8-dimethoxy-2-(3′,4′-dimethoxyphenyl)-3-propyl-1*H*-quinolin-4-one (**3**), and 5,6-dimethoxy-2-(2′,5′,6′-trimethoxyphenyl)-1*H*-quinolin-4-one (**4**). All the isolated compounds showed yellow fluorescence in UV light and gave an orange color on TLC when sprayed with Dragendorff's reagent and precipitated with Mayer's reagent. They gave a positive test for the presence of nitrogen [11]. The results of the current study revealed that the four isolated alkaloids have the 4-quinolinone structure [12]. This suggestion was supported by the UV/viz λ_{max} 255–265 and 310–338 nm peaks in IR (KBr, ν_{max}, cm^{-1}) at 1690 (C=O), 300–3100, 675, 870 (C-H aromatic), 3300–3500 (N-H), and 1590 (aryl NH). The ^{13}C NMR spectra of the isolated alkaloids possess the signal of the carbonyl group (at 178–180 ppm), methoxy groups at 55.9–62, and the signal of the aliphatic CH_2-CH_2-CH_3 at 13.95–24.63, and the four compounds were found to have related ^1H NMR spectra. However, the number of protons and the deshielded methoxy groups suggested that all compounds contain the phenylquinolinone structure. The signals of the compounds **1**, **2**, and **3** in the ^1H NMR indicated the same moiety except the differences in the number and position of methoxy groups. Some of them showed a sharp singlet in the range of 6–7 ppm, which is likely to be the signal of the proton in position 8 (from HMBC and HMQC), and a signal in the range 0.92–2.56 ppm for the CH_2-CH_2-CH_3 which from HMQC can be attached to position 3. On the other hand, compound **4** showed a slight difference due to the absence of such signals in the range 0.92–2.56 and the appearance of a signal at 6.7 ppm which indicates a proton in position 3 (from its correlation in HMBC and HMQC). All the previous evidences were supported by the mass fragmentation pattern.

Compound 1. On the basis of chromatographic properties and UV and IR spectral data, compound **1** was expected to be a benzoquinoline alkaloid. This concept was supported by the EI/MS spectrum, which exhibited a molecular ion peak at *m/z* 369 and fragment peaks at 357 and 329 corresponding to the molecular formula of $C_{21}H_{23}NO_5$, the ^1H NMR spectrum of **1** showed a 2′,4′-disubstituted β-ring due to the δ and J values of the protons H-3′, H-5′, and H-6′, which show a singlet at 7.49

and a doublet at 7.29 and 7.26. Also, the presence of a propyl group at the 3-position was deduced from its characteristic signals at δ 0.96–2.45 ppm and absence of the H-3 singlet resonance, the first signal at δ 2.45 ppm was for the CH_2 (it appeared as a multiplet) attached to C-3 (from HMBC and HMQC), and the middle CH_2 was at 1.59 while the CH_3 was at 0.96. Three singlet resonances each integrated to three protons were observed at δ 3.98, 3.97, and 3.93, indicating the presence of three methoxy groups. The ^{13}C NMR spectrum showed 21 carbon resonances among which the most downfield signal was that at 178.56 for the carbonyl resonance (C-4). In the aliphatic region, three methoxy groups were identified on the basis of the characteristic three carbon resonances at δ 61.9, 56.27, and 56.77. Also, three carbon resonances at δ 0.96, 1.59, and 2.45 were assigned to CH_2-CH_2-CH_3 (propyl group), respectively, at the 3-position. On the other hand, there were two other signals, each one of which was a doublet appearing at 7.97 and 7.86 for C-6 and C-7, and their positions were established from HMQC. Thus, compound **1** was identified as 2-(2′-hydroxy-4′-methoxyphenyl)-5,8-dimethoxy-3-propyl-1*H*-quinolin-4-one.

Compound 2. The proton ^1H NMR spectrum of this compound indicated mono substitution in ring C. This was postulated from the complex absorption pattern in the aromatic region, a multiplet at δ 7.14 ppm integrated for one proton of H-4′, and another multiplet at δ 7.34–7.61 ppm integrated for three protons at C-2′, C-5′, and C-6′. The mass spectrum fragment at *m/z* 132 confirmed the presence of one methoxy group in C ring. The presence of a broad signal at 0.96–2.45 indicated the type of aliphatic proton ($CH_2CH_2CH_3$) attached to position 3. Other signals were found to be similar to compound **1** except the absence of the OH group at C-2′. From the previously mentioned data and by comparison with data of 3′-methoxyphenol [7], compound **2** was identified as 5,8-dimethoxy-2-(3′-methoxyphenyl)-3-propylquinolin-4 (1*H*)-one.

Compound 3. ^1H NMR and ^{13}C NMR spectra of this compound showed the same signal pattern of compound **2** (ring A and B) and the presence of four deshielded methoxy groups. The fragment ion peak at *m/z* 162 confirmed the presence of two methoxy groups in ring C. The 2′, 3′-dimethoxy substitution in ring C was indicated in the ^1H NMR spectrum by the presence of a doublet at δ 7.2 ppm (J_m = 1.8 Hz) for H-2′, a quartet at 7.25 (1H, dd, J = 8.5, 2.5 Hz, H-6′), and a doublet at δ 7.30 ppm (J_m = 1.8 Hz, J_0 = 7.6 Hz) for H-5′. The attachment postions were obtained from both HMBC and HMQC. From the above data, this compound was identified as 5, 8-dimethoxy-2-(2′, 3′-dimethoxyphenyl)-3-propylquinolin-4(1*H*)-one.

Compound 4. The ^1H NMR of this compound indicated 5,6-dimethoxy substitution of ring B by the presence of two doublets with an ortho-coupling constant of 9.2 Hz at δ 7.37–7.49 and 7.47–7.56 ppm (H-7 and H-8; cf 5, 6-dimethoxyflavones), as the resonance of H-3 in the corresponding 2′-methoxyflavones is shifted downfield, the expected substitution pattern in 2′,6′-dimethoxyquinolone (this was verified by comparison with the H-3 shift in the zapotin flavonoid isolated from the seed of this plant). The presence of the signal from five methoxy groups in addition to a sharp singlet at δ 6.2 may be due to the proton in position 3. Mass spectrum showed an increase of 30 mass unit to that recorded for compound **3**, the fragment of *m/z* 192. Thus this compound can be identified as 5, 6-dimethoxy-2-(2′,5′,6′-trimethoxy-phenyl)-1*H*-quinolin-4-one. This compound has been isolated before from the leaf of this plant [12].

Finally, all substitution positions in ^1H NMR and ^{13}C NMR (DEPT, 145 & 90) resonances were achieved by the correlation cross peaks in the 2D NMR experiments (HMBC and HMQC).

Antihypertensive Activity. The value of LD_{50} was over 3000 mg/kg body weight, for fruit extract, which means that the plant is safe for human use. The total alcoholic and alkaloidal extracts of the ripened fruits and the four isolated compounds possess an antihypertensive effect when injected intravenously into pentobarbital anaesthetized dog (Table 1) in doses of 50, 100, 200, and 300 mg/kg body weight; the highest antihypertensive activity was obtained after 10 min and persisted for more than one hour and proportionally correlated with the dose (dose dependent). The heart rate was increased, but the difference was not large, and the respiration was shallow and slow, which means that all the tested extracts have very promising antihypertensive activity with minimal effect on heart rate and respiration. The results were compared with the antihypertensive drug α-methyldopa.

EXPERIMENTAL

Plant. The edible fruits of *C. edulis* were obtained from Dakahlia governorate, Nile delta, Egypt in August 2001. The identification of this plant was verified by Prof. N. El-Hadidi, Professor of Taxonomy, Botany Dept., Faculty of Science, Cairo University. A voucher specimen of the titled plant was kept in the Herbarium of Desert Research Center.

Apparatus. UV spectra were measured on a Shimadzu 1201 spectrophotometer. IR spectra were run in KBr disc using a Perkin–Elmer 783 spectrophotometer. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected;

El/MS (scan ES/ 3.72 e9). ^{1}H and ^{13}C NMR spectra, using external electronic referencing through the deuterium resonance frequency of the solvent, were determined at 600.17 or 150.91 MHz, respectively, with a JEOL ECA 600 spectrometer fitted with an auto 5mm X/H probe. Carbon atom types were established in the ^{13}C NMR spectrum by employing a combination of broad- and proton-decoupled and distortionless enhancement by polarization transfer (DEPT) experiments with 64 K data points over a spectrum width of 17, 605.6 Hz. [$^{1}J_{C-H}$] and [$^{2}J_{C-H}$], and [$^{3}J_{C-H}$]. ^{1}H-^{13}C correlations were established using HMQC and HMBC pulse sequences, respectively. TLC was carried out on precoated Kieselgel 60 (0.25 mm, Merck), and spots were visualized by spraying with Dragendorff's reagent.

Extraction and Isolation. Two kilograms of fleshy ripened fruits of *C. edulis* were exhaustively extracted by blending with 95% EtOH. Evaporation of the combined organic solvents gave brown syrup residues weighing approximately 80 g, which were acidified with HCl (3%) and filtered. The filtrate was extracted with chloroform to remove undesirable matters. The acidic aqueous layer was adjusted to alkaline pH with ammonia and the liberated alkaloid bases were extracted by chloroform till exhaustion. The chloroform extract was filtered over Na_2SO_4 and dried under reduced pressure at a temperature not exceeding 35°C to yield 13 g (total alkaloids); this residue was then subjected to column chromatography on a column packed with silica gel (360 g) and eluted with $CHCl_3$–MeOH 99:1. A hundred fractions were collected (70 mL each) and reduced into three main groups according to the R_f and color of the spots after spraying with Dragendorff's reagent, and concentrated to yield 500, 400, and 1000 mg. Each fraction group was separately reapplied on silica gel columns (30, 20, and 40 g) and eluted with $CHCl_3$ with a gradually increasing amount of methanol, from which four compounds were isolated in semipurified condition, therefore they were purified by preparative TLC and repurified by applying on a column packed with Sephadex LH 20 and eluted with methanol–water 1:1. The crystallization procedure was performed from chloroform to get compounds **1–4**; the yield was 100, 150, 120, and 150 mg, respectively.

2-(2′-Hydroxy-4-methoxyphenyl)-5,8-dimethoxy-3-propyl-1*H*-quinolin-4 one (1). This was obtained as a white colorless crystal ($CHCl_3$): mp 115–116°C; UV (EtOH, λ_{max}, ε): 280 (14294), 300 (8140), 320 (6590) nm; IR (KBr, ν_{max}, cm^{-1}): 3452, 2955, 1655, 1591; ^{1}H NMR($CDCl_3$, 600 MHz, δ, ppm, J/Hz): 7.97 (d, J = 9.2, H-7), 6.81 (d, J = 9.2, H-6), 7.49 (s, H-2′), 7.29 (d, J = 9.2, H-5′), 7.26 (d, J = 9.2, H-6′), 3.92 (s, OCH_3), 3.85 (s, $2OCH_3$), 0.96 (m, CH_2 prop. attached to Ar.), 1.58 (m, CH_2 prop. middle), 2.45 (m, CH_3). ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm): 178.3 (C-4), 163.9 (C-2), 162.1 (C-3′), 152.32 (C-5), 133.4 (C-1′), 131.12 (C-4′), 121.34 (C-7), 120.14 (C-6′), 119.21 (C-2′), 116.81 (C-9), 117.31 (C-10) 114.94 (C-8), 113.31 (C-3), 108.14 (C-5′), 61.90 (OCH_3), 57.13 (OCH_3), 56.11 (OCH_3), 24.63 (CH_2-Ar), 21.92 (CH_2 middle), and 13.94 (CH_3); EIMS *m/z* 371 (M^+ +2) (76), 370 (M^+ +1) (43), 356 (25), 337 (30), 326 (15), 323 (17), 307 (9), 165 (10), 137 (15), 132 (6).

5,8-Dimethoxy-2-(3′-methoxyphenyl)-3-propyl-1*H*-quinolin-4-one (2). This was obtained as a white colorless crystal ($CHCl_3$): mp 130–131°C; UV (EtOH, λ_{max}, nm, ε): 267 (22965), 304 (12218), 330 (9580); IR (KBr, ν_{max}, cm^{-1}): 3450, 2955, 1655, 1591; ^{1}H NMR ($CDCl_3$, 600 MHz, δ, ppm, J/Hz): 7.95 (d, J = 9.2, H-7), 7.81 (d, J = 9.2, H-6), 7.31 (dd, J =7.6, 1.8, H-6′), 7.25 (d, J = 7.6, H-5′), 7.15 (d, J = 1.8, H-2′), 3.83 (s, OCH_3), 3.79 (s, OCH_3), 3.71 (s, $2OCH_3$), 0.96 (m, CH_2 prop.attached to Ar.), 1.58 (m, CH_2 prop. middle.), 2.45 (m, CH_3); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm): 179.10 (C-4), 164.21 (C-2), 155.62 (C-4′), 152.92 (C-3′), 152.32 (C-5), 130.41 (C-1′), 131.12 (C-4′), 126.14 (C-6′), 122.4 (C-5′), 120.94 (C-7), 119.25 (C-2′), 117.31 (C-10), 116.81 (C-9), 115.14 (C-8), 113.41 (C-3), 61.10 (OCH_3), 59.73 (OCH_3), 56.11 (OCH_3), 24.63 (CH_2-Ar), 21.92 (CH_2 middle), and 13.94 (CH_3); EIMS *m/z* 355 (M^+ +2)(100), 356 (M^+ +1) (70), 372 (56), 357 (30), 353 (35), 329 (17), 296 (19), 281 (10), 156 (15), 114 (6), 137 (23).

5,8-Dimethoxy-2-(2′-methoxyphenyl)-3-propyl-1*H*-quinolin-4-one (3). This was obtained as a white colorless crystal ($CHCl_3$): mp 120–121°C; UV (EtOH, λ_{max}, nm, ε): 285 (14451), 310 (8145), 325 (6595); IR (KBr, ν_{max}, cm^{-1}): 3450, 3630, 2950, 1655, 1590; ^{1}H NMR ($CDCl_3$, 600 MHz, δ, ppm, J/Hz): 7.95 (d, J = 9.2, H-7), 6.88 (d, J = 9.2, H-6), 7.56 (d, J = 1.8, H-2′), 7.49 (dd, J = 7.6, 1.8, H-6′), 7.39 (d, J = 8, H-5′), 3.98 (s, OCH_3), 3.97 (s, OCH_3), 3.93 (OCH_3), 0.96 (m, CH_2 prop. attached to Ar.), 1.58 (m, CH_2 prop. middle.), 2.45 (m, CH_3); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm): 178.56 (C-4), 158.79 (C-2), 151.781 (C-4′), 149.77 (C-5), 147.79 (C-1′), 147.12 (C-7), 145.29 (C-6), 120.14 (C-6′), 119.61 (C-2′), 117.83 (C-10), 116.51 (C-9), 114.94 (C-8), 113.31 (C-3), 108.14 (C-5′), 61.90 (OCH_3), 57.27 (OCH_3), 56.77 (OCH_3), 24.63 (CH_2-Ar), 21.92 (CH_2 middle), and 13.94 (CH_3); EIMS *m/z* 385 (M^+ +2) (10), 384 (M^+ +1) (23), 329 (25), 328 (60), 313 (65), 299 (35), 239 (9), 149 (17), 137 (15).

5,6-Dimethoxy-2-(2′,5′,6′-trimethoxyphenyl)-1*H*-quinolin-4-one (4). This was obtained as a white colorless crystal ($CHCl_3$): mp 156–157°C; UV (EtOH, λ_{max}, nm, ε): 250 (11165), 259 (11156), 328 (5480); IR (KBr, ν_{max}, cm^{-1}): 3450, 3000, 1655, 1591; ^{1}H NMR ($CDCl_3$, 600 MHz, δ, ppm, J/Hz): 7.57 (d, J = 9.2, H-8), 7.35 (d, J = 9.2, H-7), 7.13 (d, J = 8.5, H-4′), 6.75 (d, J = 8.5, H-3′), 6.27 (s, H-3), 3.99 (s, OCH_3), 3.89 (s, OCH_3), 3.87 (s, OCH_3), 3.85 (s, OCH_3), 3.74 (s, OCH_3); ^{13}C NMR

($CDCl_3$, 600 MHz, δ, ppm): 180.16 (C-4), 162.3 (C-2), 153.6 (C-6′), 152.62 (C-2′), 150.91 (C-5), 148.89 (C-6), 147.85 (C-5′), 121.41 (C-7), 116.81 (C-9), 119.23 (C-1′), 118.12 (C-10), 116.75 (C-4′), 116.11 (C-3′), 115.95 (C-8), 108.21 (C-3), 61.90 (OCH_3), 57.83 (OCH_3), 57.56 (OCH_3); EIMS *m/z* 373 (M^+ +2) (85), 372 (M^+ +1) (100), 357 (30), 353 (35), 343 (17), 341 (19), 193 (10), 165 (15), 1149 (6), 137 (23).

Pharmacological Activity. The plant extract was prepared by extraction of fleshy ripened fruits with 95% ethanol by blending as before, and then the ethanol was evaporated under reduced pressure at a temperature not greater than 35°C. Each of the four compounds and the total fruits extract (alcohol and alkaloids) were prepared separately in aqueous solution. The LD_{50} of the investigated extracts was determined using a statistical analysis [13], from which the therapeutic index was also calculated. The effect of the tested extracts and isolated compounds on the arterial blood pressure of dogs was studied as follows [14]: 35 adult dogs of both sexes weighing 14–24 kg were used. Animals were divided into seven groups (for each extract and each compound) and anesthetized intravenously with 30 mg/kg body weight of pentobarbital sodium. They were artificially ventilated using a Narco biosystem apparatus 20571. The abdominal aorta and posterior vena cava were canulated through the femoral vessels for measuring blood pressure (BP) and intravenous (IV) injection of the extracts. The blood pressure was monitored by a pressure transducer and was recorded together with lead II electrocardiography on a Narco Biosystem Physiograph recorder. A thermodilution canula was introduced into the jugular vein to the pulmonary artery under fluoroscopic examination. The ethanolic and alkaloidal extracts, in addition to the four isolated compounds, were separately administered IV, at a dose of 50, 100, 200, 300, and 500 mg/kg body weight. The extracts and compounds were dissolved in 0.9% sodium chloride. Also α-methyldopa was used for comparison in a dose of 25 mg/kg body weight.

Statistical Analysis. All data are expressed as the mean ±SD. The statistical significance was determined by the "one-way ANOVA" test using a computerized statistical package. The data were considered to be significant if the probability had a value of 0.05 or less.

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