
Isolation and Hypotensive Activity of New Phytoconstituents from Chloroform Extract of *Hibiscus rosasinensis* Linn Flowers

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Three extracts of *Hibiscus rosasinensis* Linn have been prepared and hypotensive activity was evaluated. Hydroalcoholic extract was found to exhibit prominent activity when compared to the reference standard minoxidil followed by chloroform extract. In an attempt to isolate the active constituents responsible for this activity, five new phytoconstituents were isolated and their structures were elucidated from spectral evidence (IR, NMR and Mass spectra). Hypotensive activity of these compounds was also studied.

Diseases of the arterial tree cause more premature deaths than all other diseases such as cancer and infections combined. Among the major risk factors for arterial diseases, high blood pressure has been identified as the most powerful one¹. Lowering blood pressure in hypertensive patients requires not only a broad choice of effective and well tolerated medications, but also skills to motivate them to comply with life long treatment². In recent years many synthetic drugs are in clinical practice to treat diverse cardiac problems. Among these, hypertension is the major one. Along with the specific action of the drugs on heart, they produce different side effects depending on their mechanism of action. Hence recent studies, diverting towards the screening of traditional herbal medicines to treat this fatal disease.

Hibiscus rosasinensis Linn (Family Malvaceae), a plant with flowers widely distributed throughout the world. As a traditional medicine, the infusion of the petals used as refrigerant drink in fevers³. The alcoholic extract of flowers of *H. sabdariffa* inhibited angiotensin-I converting enzyme⁴. The alcoholic extract of flowers of *H. rosasinensis* has been proved to possess anticonvulsant property⁵. Powdered Leaves of *H. rosasinensis* showed reduction of blood

pressure⁶. In spite of its use in cardiovascular ailments, the hypotensive activity of the flowers of *H. rosasinensis* had not been explored. In view of this and continuing phytochemical investigations on the constituents of *H. rosasinensis*, in the present study various extracts of flowers of *H. rosasinensis* have been prepared and their hypotensive activity was studied.

MATERIALS AND METHODS

Plant Material:

Flowers including buds of *Hibiscus rosasinensis* Linn were collected from Japanese Garden, Rohini, New Delhi, during August 2002. It was authenticated in the department of botany, Jamia Hamdard, New Delhi. A voucher specimen has been kept in our laboratory for future reference.

Melting points were recorded on a Perfit apparatus and are uncorrected. Infrared spectra were recorded on a Jasco-410 Spectrometer by using potassium bromide pellet and nujol mull for solid and semisolid compounds, respectively. NMR spectra were recorded on a Jeol JNM FX-100 FTNMR Spectrometer in deuteriated chloroform solvent using tetramethylsilane as internal standard. The FAB mass spectra were scanned on a Jeol SX-102/DA-6000 Mass Spectrometer. m-Nitrobenzyl alcohol (NBA) was used as the matrix for recording mass spectra. The m/z values of only intense peaks have been mentioned.

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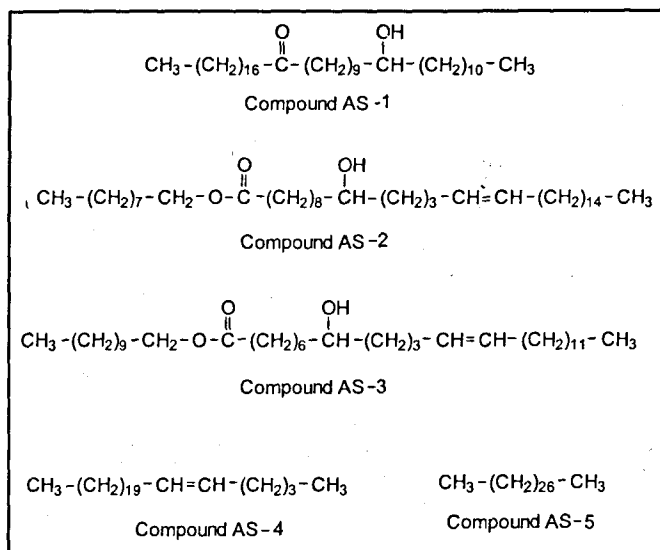


Fig. 1: Structures of the Isolated chemical constituents

Preparation of the extract:

The powdered flower was extracted with petroleum ether (60-80°), the solvent was removed under vacuum and a crude solid mass was obtained. The flowers extracted with petroleum ether were then re-extracted with chloroform and the solvent was removed under vacuum and a crude solid mass was obtained. The same flowers after extraction with chloroform, successively extracted with ethanol water (7:3) mixture to get solid crude mass. This dried crude extracts (petroleum ether, CHCl_3 , $\text{EtOH-H}_2\text{O}$ (7:3)) were stored in a desiccator and used for further experiment after suspending in sodium carboxymethylcellulose (1% w/w).

The chemical constituents of the extract were identified by preliminary qualitative analysis and confirmed by thin layer chromatography (TLC) for the presence of steroids, flavonoids, tannins and reducing sugars⁷.

Column chromatography of chloroform extract:

The chloroform extract of the flowers of *H. rosasinensis* was adsorbed on silica gel (60-120 mesh) for column chromatography. The slurry was air-dried to remove any adsorbed moisture on surface. It was then loaded on the top of the column of silica gel packed with petroleum ether.

The polarity of the column solvent was gradually increased with the disappearance/appearance of the existing/new spot, visualized on TLC. Various compounds isolated from the extract are listed below along with their spectral data.

n-nonatriacontan-12-ol-22-one (AS-1):

Elution of the column with benzene-chloroform (1:1) yielded yellow solid compound AS-1. It was recrystallized from chloroform-methanol (1:1); R_f 0.25 (chloroform); m.p. 60-62°; IR (KBr cm^{-1}): 3420 (OH) and 1710 (C=O); NMR (CDCl_3) (δ ppm): 3.03 (1H, m, $w_{1/2}$, 5.2 Hz, H-12 β), 2.61 (2H, brs, $J=5.3$ Hz, CH_2 -21), 2.25 (2H, m, $J=5.6$ Hz, CH_2 -23), 1.53 (2H, brs, $J=5.7$ Hz, CH_2), 1.25 (64 H, brs, $J=6.0$ Hz, 32 x CH_2), 0.87 (3H, t, $J=6.1$ Hz, Me-39), 0.83 (3H, t, $J=6.2$ Hz, Me-1); *Ve ion FAB-MS (m/e) (Relative intensity): 578 [M]⁺ ($\text{C}_{39}\text{H}_{78}\text{O}_2$) (23.6), 423 (12.3), 393 (16.1), 339 (43.1), 311 (21.6), 267 (9.8), 239 (22.6), 185 (13.9), 155 (24.3). Anal. Calcd. for $\text{C}_{39}\text{H}_{78}\text{O}_2$: C. 80.89, H.13.57; Found: C. 80.76, H. 13.63.

TABLE 1: HYPOTENSIVE ACTIVITY OF VARIOUS EXTRACTS OF FLOWERS OF *HIBISCUS ROSASINENSIS*

Extract	50 mg/kg			100 mg/kg			200 mg/kg		
	SBP	DBP	MABP	SBP	DBP	MABP	SBP	DBP	MABP
Petroleum ether	4.3	6.1	5.8	11.3	12.4	12.6	13.9	15.4	14.6
	± 0.7	± 0.6	± 0.6	± 0.8	± 1.1	± 1.1	± 1.5	± 1.0	± 1.1
Chloroform	17.1	7.9	10.7	25.3	20.3	21.9	26.7	23.0	25.0
	± 0.8	± 0.9	± 8.2	± 0.8	± 1.1	± 1.1	± 1.5	± 1.0	± 1.1
Ethanol-water (7:3)	8.7	7.2	7.5	20.6	16.7	17.9	26.9	22.9	24.5
	± 0.7	± 0.8	± 0.8	± 0.7	± 1.0	± 0.9	± 1.2	± 1.3	± 0.8
Minoxidil	6.35	8.24	6.60	46.0	32.0	43.39	58.7	57.7	58.4
	± 0.8	± 1.0	± 1.0	± 0.8	± 1.4	± 1.5	± 1.2	± 0.6	± 0.3

Each value represents the mean ± SEM (n=5). Significance levels $P < 0.01$. SBP-Systolic blood pressure, DBP- Diastolic blood pressure, MABP Mean arterial blood pressure

***n*-nonyl-*n*-triacont-14-en-10-ol-1-oate (AS-2):**

Elution of the column with benzene yielded yellow solid compound AS-2. It was recrystallised from chloroform-methanol (1:1); R_f 0.25 (chloroform); m.p. 72-74°; IR (KBr cm^{-1}): 3446 (OH), 1739 (COO) and 1559 (C=C); NMR (CDCl_3) (δ ppm): 5.26 (2H, m, $J=7.8$ Hz, H-14, H-15), 4.03 (2H, brs, $J=8.0$ Hz, H_2 -1'), 2.27 (1H, d, $J=9.15$ Hz, H-2a), 2.23 (1H, d, $J=8.32$ Hz, H-2b), 1.97 (2H, brs, $J=8.12$ Hz, H_2 -13), 1.59 (6H, brs, 3 CH_2), 1.25 (56H, brs, 28 CH_2), 0.91 (3H t, $J=6.1$ Hz, Me-9'), 0.83 (3H, t, $J=6.2$ Hz, Me-30); *ve ion FAB-MS (m/e) (Relative intensity): 592 [M]⁺ ($\text{C}_{39}\text{H}_{76}\text{O}_3$) (3.5), 355 (18.30), 313 (23.6), 283 (12.3), 279 (11.8) 237 (8.1), 171 (17.3), 143 (30.7), 127 (23.1). *Anal.* Calcd. for $\text{C}_{39}\text{H}_{76}\text{O}_3$: C. 78.98, H. 12.91; Found: C. 78.92, H. 12.95;

***n*-undecyl-*n*-pentacos-8-ol-12-en-1-oate (AS-3):**

Elution of the column with benzene-chloroform (1:1) yielded yellow solid compound AS-3, recrystallized from chloroform:methanol mixture (3:1); R_f 0.3 (benzene:methanol, 9:1); m.p. 46-48°; IR (KBr cm^{-1}): 3446 (OH), 1739 (OCO) and 1640 (C=C); NMR (CDCl_3) (δ ppm): 5.23 (2H, m, $J=4.7$ Hz, H-12, H-13), 4.08 (2H, brs, $J=4.8$ Hz, H_2 -1'), 3.61 (1H, m, $w_{1/2}$ 4.95 Hz, H-8 β), 2.23 (1H-d, $J=7.20$ Hz, H-2a), 2.06 (1H, d, $J=7.20$ Hz, H-2b), 1.96 (2H, brs, $J=6.8$ Hz, H_2 -1), 1.62 (4H, brs, H_2 -14, H_2 -2'), 1.25 (50H, brs, 25 CH_2), 0.89 (3H, t, $J=6.5$ Hz, Me-25), 0.85 (3H, t, $J=6.2$ Hz, Me-11'); *Ve ion FAB-MS (m/e) (Relative intensity): 550 [M]⁺ ($\text{C}_{36}\text{H}_{70}\text{O}_3$) (11.3), 395 (9.8), 381 (7.3), 355 (11.3), 351 (6.5), 313 (53.6), 283 (11.6), 267 (11.7), 237 (5.6), 99 (13.3), 195 (12.6), 171 (18.6), 169 (17.5), 155 (22.5). *Anal.* Calcd for $\text{C}_{36}\text{H}_{70}\text{O}_3$: C. 78.55, H. 12.73; Found: C. 78.67, H. 12.76.

***n*-hexacos-5-ene (AS-4):**

Elution of the column with chloroform-alcohol (49:1) yielded yellow solid compound AS-4. It was recrystallised from petroleum ether-chloroform (2:1); R_f 0.8 (benzene:methanol, 9:1); m.p. 36-38°; IR (KBr cm^{-1}): 2922 and 1653 (CH); NMR (CDCl_3) (δ ppm): 5.21 (2H, m, $J=5.8$ Hz, H-5, H-6), 2.17 (2H, m, $J=5.9$ Hz, H_2 -7), 1.67 (2H, m, $J=6.0$ Hz, H_2 -4), 1.25 (40H, brs, 20 CH_2), 1.89 (3H, t, $J=6.0$ Hz, Me-26), 1.85 (3H, t, $J=6.1$ Hz, Me-1); *ve ion FAB-MS (m/e) (Relative intensity): 364 [M]⁺ ($\text{C}_{26}\text{H}_{52}$) (7.8), 307 (16.9), 281 (33.7). *Anal.* Calcd. for $\text{C}_{26}\text{H}_{52}$: C. 85.63, H. 14.37. Found: C. 85.51, H. 14.31.

***n*-octacosane (AS-5):**

Elution of the with chloroform-alcohol (49:1) yielded cream solid powder of compound AS-5. It was recrystallised

from petroleum ether-chloroform (2:1); R_f 0.4 (benzene:methanol, 9:1); m.p. 94-96°; IR (KBr cm^{-1}): 2923 and 2853 (CH); NMR (CDCl_3) (δ ppm): 0.85 (3H, t, $J=6.2$ Hz, Me-1,) 0.89 (3H, t, $J=6.5$ Hz, Me-28,) 1.03 (48H, brs, $J=6.7$ Hz, 24 CH_2) and 1.25 (4H, m, 2 CH_2); *ve ion FAB-MS (m/e) (Relative intensity): 394 [M]⁺ ($\text{C}_{28}\text{H}_{58}$) (21.1). *Anal.* Calcd for $\text{C}_{28}\text{H}_{58}$: C. 85.19, H. 14.80; Found: C. 85.23, H. 14.85.

Hypotensive activity:

The Institutional Animal Ethics committee of Jamia Hamdard, Hamdard University approved the protocol used for experiments. The crude extracts and isolated compounds were evaluated for hypotensive activity. Student-t-test was performed for exhibited activities to ascertain the significance. Adult Wistar rats of either sex, weighing 200-250 g, were used for the screening of extracts for hypotensive activity. The animals were maintained in colony cages at $25 \pm 2^\circ$, relative humidity of 45-55 %, maintained under 12 h light and dark cycle and were fed with standard animal feed. All the animals were acclimatized for a week before use. Various extracts, viz. petroleum ether, chloroform and ethanol-water (7:3) extract of the flowers of *H. rosasinensis* Linn were tested for hypotensive activity in normotensive rats, non-invasively, by tail and cuff method using LE 5001 Pressure Meter[®]. Suspension of all the three extracts was prepared by using 1% sodium carboxymethylcellulose and was administered orally at dose levels 50, 100 and 200 mg/kg animal body weight to different groups of rats, each containing five. Control group received an equivalent quantity of sodium carboxymethyl cellulose suspension. Blood pressure was measured in stepwise manner as follows. After 3 hours of administration of the extract, animal was shifted to the restrainer, which restricts the movement of animal. The tail was cleaned with moist cotton to remove the dirty matter and cotton to remove the dirty material and talcum powder was sprayed on tail to make its surface smooth. A tail-cuff and pulse transducer was fixed around the tail. Initially animal shows particular pulse level. When this pulse rate is within the normal range, Pushing the start button initiates recording of systolic BP, diastolic BP and mean arterial BP displayed on monitor. The Pressure can be easily read from the precalibrated monitor. Once all the values are displayed, the recorder is switched off and for next measurement same procedure is followed once sufficient pulse level is attained. Percent reduction in blood pressure was calculated in comparison to minoxidil, which was used as standard, at the same dose levels. Results were analyzed by using student's t-test. Percent reduction in blood pressure after administration of

TABLE 2: HYPOTENSIVE ACTIVITY OF ISOLATED CONSTITUENTS OF FLOWERS OF *HIBISCUS ROSASINENSIS*

Constituents	25 mg/kg body weight			50 mg/kg body weight		
	SBP	DBP	MABP	SBP	DBP	MABP
AS-1	1.5±0.3	2.2±0.4	2.1±0.4	4.9±0.4	4.9±0.5	5.2±0.4
AS-2	1.9±0.6	1.1±0.7	1.7±0.6	5.2±0.5	5.5±1.0	5.6±0.8
AS-3	6.8±0.3	7.6±0.8	7.3±1.0	8.9±0.4	9.5±0.6	10.2±0.4
AS-4	3.0±0.3	3.0±0.4	3.0±0.3	5.5±0.3	5.3±0.6	5.7±0.5
AS-5	2.1±0.2	2.2±1.1	2.2±0.9	4.1±0.8	4.4±1.1	4.4±0.9
Minoxidil	14.1±0.7	14.5±1.1	14.7±0.9	29.4±0.8	32.4±1.1	31.4±0.9

Each value represents the mean±SEM (n=5). Significance levels $P < 0.01$. SBP-Systolic blood pressure, DBP- Diastolic blood pressure, MABP-Mean arterial blood pressure

the extracts is shown in Table 1. The same procedure has been adopted for determining percent reduction in blood pressure for isolated constituents and the results are depicted in Table 2.

RESULTS AND DISCUSSION

The flowers of *H. rosasinesis* including buds were collected, processed and extracted with various solvents and the crude extracts were subjected to the hypotensive by tail-cuff method using wister rats⁸. The results of hypotensive activity studies of crude extracts (Table 1) indicate that the hydroalcoholic extracts exhibited prominent activity when compared to the standard minoxidil followed by chloroform extract. Hence chloroform extract was column chromatographed with the aim to isolate pure constituents responsible for hypotensive activity. In an effort to isolate the active constituent(s) responsible for this activity, five new phytoconstituents were isolated from chloroform extract (which are not reported in literature⁹⁻¹⁴) and their structures were characterized by spectral data (IR, NMR and Mass spectra). These isolated constituents were evaluated for hypotensive activity.

The Compound AS-1 was obtained as a colourless mass from benzene:chloroform (1:1) eluants. Its mass spectrum suggested its molecular formula as $C_{39}H_{78}O_2$ (m/z 578, $[M]^+$). It formed 2,4-DNP derivative, indicating the presence of carbonyl group. Its IR spectrum showed characteristic absorption bands at 3420 and 1710 cm^{-1} suggesting the presence of alcoholic hydroxyl and carbonyl functional group. The NMR spectrum of AS-1 displayed two three-proton triplets at δ 0.83 ($J=6.2$ Hz) and 0.87 ($J=6.1$ Hz), assigned to terminal primary methyl protons at C-1 and C-39 position. Two multiplets at δ 1.25 and 1.53, each

corresponding to sixty-four and two protons were attributed to thirty-two and one methylene groups, respectively. Two two-proton multiplets at δ 2.25 and 2.61 were ascribed to methylene protons at C-23 and C-21 position adjacent to carbonyl functional group. A one-proton multiplet at δ 3.03 was accounted to H-12 carbinol proton. Its half-width of 5.2 Hz suggested its β -orientation. The FAB mass spectrum of AS-1 exhibited the molecular ion peak at m/z 578, corresponding to molecular formula $C_{39}H_{78}O_2$. The important ion peaks of diagnostic importance appeared at m/z 239, 329 [C22-C23 fission]⁺, 267, 311 [C21-C22 fission]⁺, 393, 185 [C12-C13 fission]⁺, and 155, 423 [C11-C12 fission]⁺, supporting the existence of the hydroxyl group at C-12 and keto group at C-22. On the basis of above spectral data, compound AS-1 has been identified as *n-nonatriacontan-12-ol-22-one*.

The Compound AS-2 was obtained as a colourless solid on elution of the column with benzene-chloroform (1:1). Its FAB mass spectrometry suggested its molecular formula $C_{39}H_{76}O_3$ (m/z 592, $[M]^+$). It decolourized bromine solution and responded 2,4-DNP test suggesting the presence of vinylic double bond and carbonyl functional group in the molecule.

Its IR spectrum showed characteristic absorption bands at 3446 (OH), 1739 (COO) and 1559 (C=C) cm^{-1} . The band at 1259 cm^{-1} confirms that carbonyl function is present in the form of ester group. The NMR spectrum of AS-2 displayed two three-proton triplets at δ 0.83 ($J=6.2$ Hz) and 0.91 ($J=6.1$ Hz) assigned to terminal methyl protons at C-30 and C-9' position. Three broad signals at δ 1.25 (56H), 1.59 (6H), and 1.97 (2H) were associated with thirty-two methylene protons. Two one-proton doublets at δ 2.23

($J=9.15$ Hz) and 2.27 ($J=9.13$ Hz) were associated to non-equivalent proton attached to position C-2 adjacent to the ester group. A two-proton signal at δ 4.03 was attributed to oxygenated methylene H-1'. A two-proton signal at δ 5.26 was attributed to vinylic H-14 and H-15. In the mass spectrum, the important ion fragments appearing at m/z 127 [C1-O fission]⁺, 143 [O-CO fission]⁺ and 171 (C1-C2 fission)⁺ suggested esterification of nonyl alcohol with a triacontanoic acid. The ion peaks at m/z 283 [C9-C10 fission]⁺ and 313, 279 [C10-C11]⁺ supported the location of the hydroxyl group at C-10. The location of the vinylic bond at Δ^{14} was inferred from the intensified ions arose at m/z 355, 237 [C13-C14 fission]⁺ and 381 [C15-C16 fission]⁺. On the basis of above evidences, compound AS-2 was characterized as *n*-nonyl-*n*-triacont-14-en-10-ol-1-oate.

It was isolated as a colorless solid from benzene : chloroform (1:1) eluants. Its FAB mass spectrometry suggested its molecular formula $C_{36}H_{70}O_3$ (m/z 550 [M]⁺). It decolourized bromine solution suggesting unsaturated nature of the compound. Its IR spectrum exhibited absorption bands at 3446 and 1739 cm^{-1} characteristic for alcoholic hydroxyl group and acyclic ester group, respectively. The presence of a band at 1640 cm^{-1} indicated the presence of double bond. The NMR spectrum of AS-3 showed two three-proton triplets at δ 0.85 ($J=6.2$ Hz) and 0.89 ($J=6.5$ Hz) assigned to terminal methyl protons at C-11' and C-25 positions. A broad signal at δ 1.25 was corresponded to fifty methylene protons. Two signals at δ 1.62 (2H) and 1.96 (2H) were accounted to H_2 -11 and H_2 -14 adjacent to vinylic bond. Two one-proton doublets at δ 2.06 ($J=7.2$ Hz) and 2.03 ($J=7.20$ Hz) were ascribed to methylene H-2 adjacent to the ester group. Two carbinol signals at δ 3.61 and 4.08 were attributed to oxygenated H-1' methylene protons. Its half width 4.95 Hz indicated its β -orientation. A de shielded signal at δ 5.23 was assigned to vinylic H-12 and H-13. The important ion peaks at m/z 155, 395 [C1-O fission]⁺, 171 [O-C1 fission]⁺ and 199, 351 [C1-C2 fission]⁺ indicated the esterification of an undecyl alcohol with a C-25 acid. The existence of the hydroxyl group at C-8 was inferred from the intensified ion peaks generated at m/z 283, 267 [C7-C8 fission]⁺ and 313, 237 [C8-C9 fission]⁺. The ion peaks at m/z 355, 195 [C11-C12 fission]⁺ and 381, 169 [C13-C14 fission]⁺ supported the location of the vinylic linkage at Δ^{12} . The data led to establish the structure of AS-3 as *n*-undecyl-*n*-pentacos-8-ol-12-en-1-oate.

It was obtained as a colourless mass from chloroform : methanol (49:1) eluants. Its molecular formula was found to be $C_{26}H_{52}$ (m/z 364, [M]⁺) with the help of FAB mass

spectroscopy. It decolourized bromine solution indicating presence of double bond in the molecule. Its IR spectrum showed the absorption bands at 2922 and 1653 cm^{-1} indicating the presence of CH stretching and double bond. The NMR spectrum of AS-4 displayed two three-proton triplets at δ 0.85 ($J= 6.1$ Hz) and 0.84 ($J=6.0$ Hz) assigned to terminal methyl groups at C-1 and C-26. A forty proton broad signal at δ 1.25 was associated with twenty methylene groups. Two multiplets at δ 1.67 and 2.17 were accounted to methylene groups at C-4 and C-7 position adjacent to vinylic carbon at C-5 and C-6. FAB mass spectrum showed the presence of molecular ion peak at m/z 364 [M]⁺ (7.8) corresponding to the molecular formula $C_{26}H_{52}$. The intensified ion peaks at m/z 307 and 281 supported the location of the vinylic linkage at $\Delta 5$. On the basis of above evidences, the structure of compound AS-4 has been characterized as *n*-hexacos-5-ene.

It was obtained as colourless amorphous powder on elution the column with chloroform:methanol (49:1). Its FAB mass spectrometry suggested its molecular formula $C_{28}H_{58}$ (m/z 394, [M]⁺); The IR spectrum of AS-5 was devoid of any absorption band in the functional group region; The NMR spectrum displayed two three-proton up field triplets at δ 0.85 ($J= 6.2$ Hz) and 0.89 ($J=6.5$ Hz) assigned to terminal methyl groups at C-1 and C-28 positions. A broad signal at δ 1.03 was corresponding to 48 protons was accounted to 24 methylene protons, a multiplet present at δ 1.25 was associated to two methylene protons; These spectral data suggested that compound AS-5 is an acyclic hydrocarbon corresponding to general formula C_nH_{2n+2} , and the structure was characterized as *n*-octacosane.

These isolated constituents, when subjected to the hypotensive activity (Table 2), all the compounds shown the moderate hypotensive activity. These findings suggested that there must be synergistically acting constituent(s) present in crude extract, responsible for its hypotensive activity and these compounds are only effective in combination with each other, and not alone. Hence further more pharmacological investigations are required to find out proper combination of two or more constituents, which may shows the expected hypotensive activity.

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