Phytochemical and Pharmacological Investigation of *Hibiscus rosasinensis* Linn.

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

Major efforts have been directed during the last three decades to diagnose and treat patients with hypertension. This certainly contributes significantly to the decreased incidence in cardiovascular diseases observed in developed countries, but much remains to be done, since high blood pressure still represents one of the leading causes of morbidity and mortality world wide[1]. 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 treatment[2]. 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 sometimes these side effects lead to severe complications. Hence present trend is diverting towards the screening of traditional herbal medicines to treat this fatal disease.

Hibiscus rosasinensis Linn. (Family Malvaceae), is widely distributed throughout the world. As a traditional medicine, the fresh juice of flower of wild variety is used to treat gonorrhaea, the powdered root is used to treat menorrhagia, and the infusion of the petals is used as refrigerant drink in fevers[3]. The alcoholic extract of flowers of *H. sabdariffa* inhibited angiotensin-I converting enzymes[4]. The alcoholic extract of flowers of *H. rosasinesis* has been proved to posseses anticonvulsant property[5]. Powdered leaves of *H. rosasinesis* showed lowering of blood pressure[6].

Inspite of its use in cardiovascular ailments, the hypotensive activity of the flowers of *H. rosasinesis* has not been explored. Prompted by these findings, in the present study various extracts of flowers of *H. rosasinesis* have been prepared and evaluated for hypotensive activity. In an effort to isolate the active constituent(s) responsible for this activity, five new compounds were isolated which were not reported in literature[7-12] and their chemical structures were characterized by spectral data (IR, NMR and Mass).

MATERIALS AND METHODS

Plant material:

Flowers including buds of *Hibiscus rosasinensis* 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 was kept in our laboratory for future reference. The flowers were powdered.

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 dueteriated chloroform

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solvent using tetramethylsilane as internal standard. The FAB mass spectra were scanned on a Jeol SX-102/DA-6000 Mass spectrometer. m-Nitrobenzylalcohol (NBA) was used as the matrix for recording mass spectra. The m/z values of only intense peaks have been mentioned.

Preparation of the extract:

The flower powder was extracted with petroleum ether (60-80°), the solvent was removed under vacuum and a crude solid mass was obtained. The marc was then re-extracted with chloroform and the solvent was removed under vacuum and a crude solid mass was obtained. The marc, after extraction with chloroform, was successively extracted with ethanol and water mixture (7:3) to get a solid crude mass. These dried crude extracts (petroleum ether, $CHCl_3$, EtOH-H_2O (7:3)) were stored in a desiccator and used for further experiment after suspending in sodiumcarboxymethylcellulose (1% w/w) solution. 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 sugars.

Column chromatography of hydroalcoholic extract:

Hydroalcoholic extract obtained from 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 and 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 and their structures were shown in fig. 1.

***n*-Docosane (SM-1):**

Elution of the column with benzene yielded yellow semisolid compound SM-1. It was recrystallised from chloroform-methanol (1:1) mixture, R_f: 0.75 (benzene:chloroform, 9:1); IR (KBr) (cm^{-1}): 2956, 2853,1463 (CH); NMR ($CDCl_3$) (δ ppm): 1.58 (2H, m, CH_2), 1.52 (2H, brs, CH_2), 1.47 (2H, brs, CH_2), 1.37 (2H, brs, CH_2), 1.18 (28H, brs, 14×CH_2), 1.01 (4H, s, 2×CH_2), 0.80 (3H, J=6.5 Hz, Me-1), 0.78 (3H, J=6.1 Hz, Me-22); $^+$ve ion FAB-MS (m/e) (relative intensity): 310 $[M]^+$ ($C_{22}H_{46}$) (8,7), 295 (15.6), 281 (32.1), 267 (12.3) 253 (8.7) 239 (13.5), 225 (11.6), 211 (12.5), 197 (12.7), 183 (12.9), 169 (21.3), 155 (23.7), 141 (26.9), 127 (27.1), 113 (41.8).

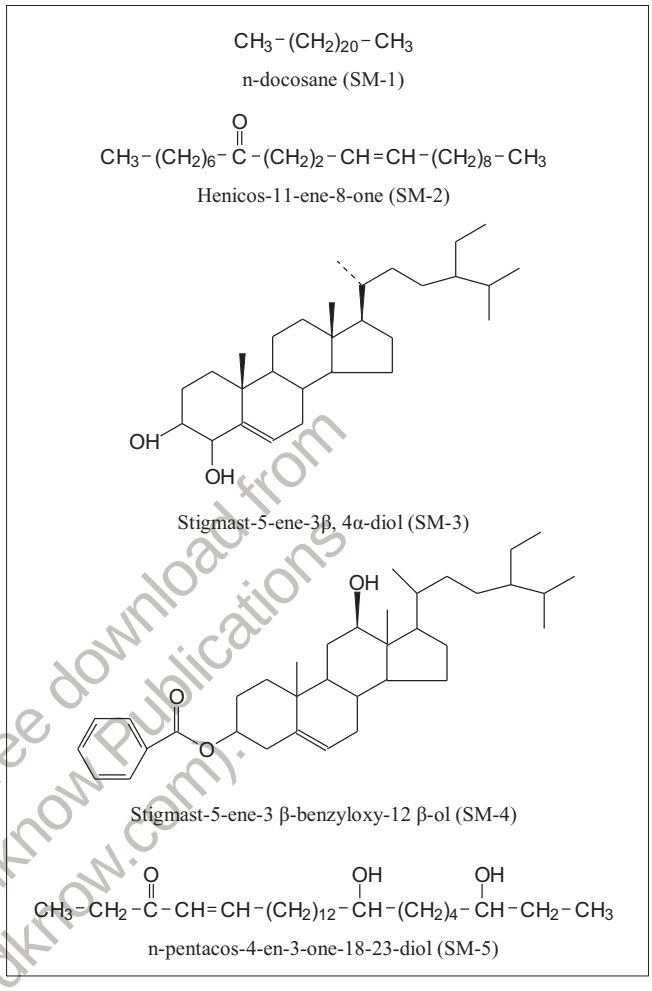

Fig. 1 : Structures of the isolated chemical constituents

Henicos-11-ene-8-one (SM-2):

Elution of the column with benzene furnished white solid compound SM-2. It was recrystallised from chloroform-methanol (1:1) mixture, R_f: 0.60 (benzene:chloroform, 9:1); mp: 34-36°; IR (KBr) (cm^{-1}): 1710 (C=O), 1600 (C=C); NMR ($CDCl_3$) (δ ppm): 5.29 (1H, brs, H-11) 5.27 (1H, brs, H-12), 2.28 (2H, brs, H_2-7),1.95 (2H, brs, H_2-9), 1.56 (4H, brs, H_2-10, H_2-13), 1.18 (24H, br, 12´CH_2), 0.81 (6H, br, Me-1, Me-21); $^+$ve ion FAB-MS (m/e) (relative intensity): 308$[M]^+$ ($C_{21}H_{40}O$) (22.8), 265 (11.2), 251 (12.3) 181 (16.3), 155 (100), 127 (21.9).

Stigmast-5-ene-3β, 4α-diol (SM-3):

Further elution of the column with benzene yielded yellow semisolid compound SM-3. It was recrystallised from chloroform-methanol (1:1) mixture, R_f: 0.40 (benzene:chloroform: methanol, 1:1:0.1); IR (KBr) (cm^{-1}): 3490 (OH), 1645 (C=C), 1120 (C-O); NMR ($CDCl_3$) (δ ppm): Table 1; $^+$Ve ion FAB-MS (m/e) (relative intensity): 430$[M]^+$ ($C_{29}H_{50}O_2$) (21.6), 412 (51.8), 397 (100), 382 (17.5), 342 (2.5), 290 (5.9), 289 (7.8), 276 (7.5), 275 (7.5), 271 (18.2),

TABLE 1: NMR SPECTRAL DATA OF COMPOUND SM-3

Position	NMR		Position	NMR	
	Alpha	Beta		Alpha	Beta
1	1.34 m	2.27 m	17	1.43 m	-
2	1.86 m	1.82 m	18	.67 brs	-
3	3.52 br ($W_{1/2}$) 16.5)	-	19	1.01brs	-
4	-	4.09 d (6.6)	20	-	2.27 m
6	5.36 brs	-	21	0.93 d (6.0)	-
7	1.61 m	1.66 m	22	1.54 m	1.06 m
8	-	1.12 m	23	1.26 m	1.80 m
9	1.50 m	-	24	1.18 m	-
11	2.02 m	1.48 m	25	1.53 m	-
12	1.08 m	1.06 m	26	0.84 d (6.9)	-
14	1.14 m	-	27	0.80 d (6.3)	-
15	1.08 m	1.48 m	28	1.14 m	1.25 brs
16	1.61 m	1.50 m	29	0.82 d (6.3)	-

Coupling constant values (in Hertz) are provided in parenthesis

256 (23.8), 250 (3.9), 241 (13.7), 214 (24.7), 208 (11.3), 201 (20.1), 199 (23.8), 194 (15.6), 190 (17.6), 180 (18.3), 176 (11.3), 175 (21.3), 172 (26.5), 165 (28.1), 162 (14.8), 158 (76.1), 157 (46.5), 154 (38.7), 149 (23.6), 147 (52.1), 144 (73.6), 140 (36.4), 136 (35.8), 135 (29.3), 134 (57.8), 129 (52.6), 122 (45.5), 120 (47.3), 118 (63.7),109 (66.8), 107 (86.5), 104 (96.9).

Stigmast-5-ene-3β-benzyloxy-12β-ol (SM-4):

Elution of the column with benzene-chloroform (3:1) yielded yellow semisolid compound SM-4. It was recrystallised from chloroform-methanol (1:1) mixture, R_f: 0.25 (benzene:chloroform:methanol,1:1:0:1); IR (KBr) (cm^{-1}): 3406 (OH),1725 (OCO), 1461 (C=C), NMR ($CDCl_3$) (δ ppm): Table 2; $^+$ve ion FAB-MS (m/e) (Relative intensity): 534 $[M]^+$ ($C_{36}H_{54}O_3$) (1.1), 429 (19.6), 413 (42.3), 397 (43.1),

TABLE 2: NMR SPECTRAL DATA OF COMPOUND SM-4

Position	NMR		Position	NMR	
	Alpha	Beta		Alpha	Beta
1	1.48 m	2.27 m	20	-	2.27 m
2	1.86 m	2.01 m	21	0.97 d (6.5)	-
3	4.30 brm ($W_{1/2}$) 16.5)	-	22	1.25 brs	1.25
4	2.01 m	2.20 m	23	1.28 brs	1.25 brs
6	5.34 brs	-	24	1.28 brs	-
7	1.63 m	1.82 m	25	1.54 m	-
8	-	1.14 m	26	0.84 d (6.96)	-
9	1.55 m	-	27	0.80 d (6.6)	-
11	2.04 m	1.57 m	28	1.25 brs	1.25 brs
12	4.09 dd (5.9,8.37)	-	29	0.82 d (6.6)	-
14	1.14 m	-	1'	-	-
15	1.63 m	1.48 m	2'	7.73 dd (3.3,8.4)	-
16	1.63 m	1.57 m	3'	7.55 m	-
17	1.48 m	-	4'	7.29 m	-
18	0.67 brs	-	5'	7.55 m	-
19	1.00	-	6'	7.73 dd (3.3,8.4)	-

Coupling constant values (in Hertz) are provided in parenthesis

288 (11.3), 272 (8.3), 254 (13.3), 252 (8.1), 239 (9.9), 222 (15.7), 212 (8.6), 207 (10.9), 197 (8.9), 149 (100), 137 (26.3), 123 (21.5), 111 (17.3).

***n*-Pentacos-4-en-3-one-18, 23-diol (SM-5):**

Elution of the column with benzene-chloroform (1:1) furnished yellow shiny flakes of compound SM-5. It was recrystallised from chloroform-methanol (1:1) mixture, R_f: 0.50 (benzene:chloroform,1:1); mp 90-92°; IR (KBr) (cm^{-1}): 3500 (OH), 1710 (C=O), 1632 (C=C), 759 (C-C), 723 (C-C); NMR ($CDCl_3$) (δ ppm): 5.36 (2H, brs, H-4, H-5), 4.28 (1H, brm, H-18), 4.11(1H, m, H-23), 2.77 (2H, m, H_2-2), 2.34 (2H, m, H_2-6), 2.01 (2H, m, H_2-17), 1.82 (2H, m, H_2-19), 1.62 (2H, m, H_2-22), 1.30 (6H, brs 3×CH_2), 1.25 (20H, brs, 10×CH_2), 0.87 (3H, t, J=6.5 Hz, Me-1), 0.83 (3H, t, J=6.0 Hz, Me-25); $^+$Ve ion FAB-Ms (m/e) (relative intensity): 396$[M]^+$ ($C_{25}H_{48}O_3$) (16.3), 339 (31.9), 337 (21.8), 313 (13.8), 281 (17.5), 265 (15.7), 253 (13.1), 239 (18.1), 217 (21.6), 203 (26.1), 189 (22.6), 175 (27.1), 159 (31.8), 145 (46.3), 115 (17.1).

Hypotensive activity:

Various extracts, *viz.* petroleum ether, chloroform and ethanol-water (7:3) extracts of the flowers of *H. rosasinensis* were tested for hypotensive activity in normotensive rats, non-invasively, by tail and cuff method using LE 5001 Pressure Meter[13]. Student-t-test was performed for all the activities to ascertain the significance of the exhibited activities. The test compounds and the standard drugs were administered in the form of a suspension (1% sodium CMC as vehicle) in oral route at dose levels 50, 100, 200 mg/kg animal body weight. Each group consisted of five animals. The animals were maintained in a colony cages at 25±2°, 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. The Institutional Animal Ethics Committee of Jamia Hamdard, Hamdard university, New Delhi has approved the experimental protocol (173/CPCSEA, P. NO. 48).

After 3 h 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 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 was within the normal range, start switch was pressed and the recorder records the blood pressure as systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial

blood pressure (MABP), the same displayed on the monitor. The Blood pressure was read from the precaliberated monitor. 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 BP after administration of the extracts is shown in Table 3. The same procedure was adopted for determining reduction in BP for isolated constituents and the results are depicted in Table 4.

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 Wistar rats[13]. The results of hypotensive activity studies of crude extracts (Table 3) indicate that the hydroalcoholic extract exhibited prominent activity when compared to the standard minoxidil followed by chloroform extract. Hence ethanol-water (7:3) extract was column chromatographed with the aim to isolate pure constituents responsible for hypotensive activity. The following compounds were isolated and structurally characterized on the basis of spectral data.

The compound SM-1 was obtained as yellow semisolid after eluting the column with benzene. It was found to possess molecular formula $C_{22}H_{46}$ (m/z 310, $[M]^+$) on the basis of FAB mass spectrometry. It did not decolourise bromine water and react to any alkylating or oxidizing reagent, suggesting saturated compound devoid of any functional group. Its IR spectrum was devoid of any band in the functional group region. The NMR spectrum of SM-1 displayed two triplets at δ 0.78 (J=6.1 Hz) and 0.80 (J=6.5 Hz), each integrating for three protons, assigned to C-1 and C-22 terminal methyl protons, respectively. Six broad singlet at δ 1.01 (4H), 1.18 (28H), 1.37 (2H), 1.47 (2H), 1.52 (2H) and 1.58 (2H) were associated with the methylene protons.

Compound SM-1 exhibited the molecular ion peak at m/z 310, corresponding to molecular formula of a saturated hydrocarbon, C_nH_{2n+2}. The ion fragments at m/z 281, 267, 253, 239, 225, 211, 197, 183, 169, 155, 141, 127 and 113 were observed due to loss of CH_2 units. On the basis of this foregoing account, the structure of SM-1 has been elucidated as n-docosane.

The compound SM-2 was obtained as white solid from benzene as eluant. It decolourised bromine solution and formed 2,4-DNP derivative indicating the presence of vinylic bond and carbonyl group, respectively. Its IR spectrum showed absorption bands at 1710 and 1600 cm^{-1} indicating the presence of aliphatic ketone and double bond, respectively. The NMR spectrum of SM-2 displayed two one-proton broad signals at δ 5.29 and 5.27 assigned to H-11 and H-12 ethylenic proton, respectively. Two broad signals at δ 2.28 and δ 1.95 integrating two protons each, were attributed to C-7 and C-9 methylene protons adjacent to carbonyl function at C-3 position. A four-proton broad signal at δ 1.56 was ascribed to C-10 and C-13 methylene protons adjacent to vinylic protons.

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

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

TABLE 4: HYPOTENSIVE ACTIVITY OF ISOLATED CONSTITUENTS OF FLOWERS OF *HIBISCUS ROSASINENSIS*

Constituents	25 mg/kg			50 mg/kg		
	SBP	DBP	MABP	SBP	DBP	MABP
SM-1	1.4 ±0.3	1.3±0.5	1.3±0.5	2.0±0.2	1.5±0.2	1.6±0.2
SM-2	4.8±0.4	2.1±0.5	3.0±0.5	6.2±0.3	3.5±0.5	4.3±0.3
SM-3	6.2±0.5	7.2±0.7	6.4±0.8	7.9±0.5	8.3±0.7	8.2±0.5
SM-4	9.4±0.6	3.3±1.5	6.3±1.2	12.4±0.5	9.2±0.7	10.2±0.6
SM-5	1.0±0.5	0.8±0.6	0.7±0.6	7.2±0.7	5.6±0.8	8.6±0.8
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 percent reduction of blood pressure (mean) ± SEM (n = 5). Significance levels *P*<0.01, SBP - Systolic blood pressure, DBP - Diastolic blood pressure, MABP - Mean arterial blood pressure

A twenty-four proton broad signal at δ 1.18 was accounted to remaining methylene protons. The C-1 and C-21 terminal methyl protons resonated as a broad signal at δ 0.81.

Compound SM-2 showed the molecular ion peak at m/z 308 $[M]^+$ corresponding to the molecular formula of an aliphatic unsaturated ketone $C_{21}H_{40}O$. The important ion fragments appeared at m/z 155, due to the presence of fragment, $[CH_3(CH_2)_6CO(CH_2)_2]^+$ suggesting the existence of the carbonyl group at C-8. The spectrum also showed the peak at m/z 256 $[M\text{-}C_3H_7]^+$, 251 $[M\text{-}C_4H_9]^+$, and 127 for $[CH_3(CH_2)_6CO]^+$ fragment ion. Based on these evidences, the structure of the compound SM-2 has been formulated as henicos-11-en-8-one.

The compound SM-3 was isolated as a pale yellow semisolid and had the molecular formula $C_{29}H_{50}O_2$ as confirmed on the basis of FAB mass spectrometry (m/z 430,$[M]^+$). It gave positive Libermann-Burchard test, indicating the presence of steroid nucleus. IR spectrum of SM-3 exhibited a band at 3490 cm^{-1} (OH), 1120 cm^{-1} (C-O, alcoholic) and 1640 cm^{-1} (C=C) which indicated the presence of hydroxyl group and ethylenic bond, respectively. Its NMR spectrum showed signals of three protons each at δ 0.67 (brs), 0.80 (d, J=6.3 Hz), 0.82 (d, J=6.3 Hz), 0.84 (d, J=6.9 Hz), 0.93 (d, J=6.0 Hz), and 1.01 (brs) indicating the presence of six methyl groups at C-18, C-27, C-29, C-26, C-21 and C-19, respectively. A broad multiplet at δ 3.52 was due to carbinolic protons at C-3 position. The half-width of multiplet was found to be 16.50 Hz, indicating the α-orientation of the carbinolic proton. A one-proton doublet at δ 4.09 (J= 6.6 Hz) was attributed to the β-proton at C-4 position. A one-proton broad multiplet at δ 5.36 was accounted for H-6 vinylic proton.

The compound SM-3 showed molecular ion peak at m/z 430 corresponding to the molecular formula $C_{29}H_{50}O_2$. The mass spectrum showed a sharp peak at m/z 412 $[M\text{-}H2O]^+$ and m/z 289 $[M\text{-}C_{10}H_{21}]^+$. The later peak indicated the number of carbon atoms in the side chain. The mass spectrum also clearly proved the absence of double bond in the side chain and the ring A and D. the presence of this double bond at $\Delta^{5(6)}$ was confirmed on the basis of sharp peak observed at m/z 342, 180, 154 and 140 in mass fragmentation pattern. On the basis of the above spectral data, the structure of compound SM-3 was assigned as stigmast-5-ene-3β, 4α-diol.

The compound SM-4 was isolated as a pale yellow semisolid. It gave positive Libermann-Burchard test, suggesting the presence of steroid nucleus. Its IR spectrum exhibited absorption bands at 3406 cm^{-1} (OH), 1725 cm^{-1} (OCO), 1461 cm^{-1} (ethylenic double bond) suggesting the presence of alcoholic hydroxyl, ester group and double bond, respectively in the structure. The other bands present at 2957, 2853, 1378, 1220, 1073 and 980 cm^{-1} also provided supportive evidence for the presence of above functional and aromatic groups in the structure. The NMR spectrum of SM-4 showed the peaks at δ 0.67 (brs), 0.80 (δ, J=6.6 Hz), 0.82 (δ, J=6.6 Hz), 0.84 (δ, J=6.6 Hz), 0.97 (δ, J=6.5 Hz), and 1.00 (brs) indicating the presence of six methyl groups present at C-18, C-27, C-29, C-26, C-21 and C-19 position in the steroid nucleus. A broad multiplet at δ 4.30 that may be due to carbinolic proton at C-3 position. The half-width of multiplet was observed at 16.5 Hz, indicating the α-orientation of carbinolic proton. A one-proton broad signal at δ 5.34 was accounted to vinylic H-6. The peaks at δ 7.29, 7.5 and 7.73 (dd, J= 3.3, 8.4 Hz) indicated the presence of aromatic protons.

The mass spectrum of compound SM-4 showed molecular ion peak at m/z 534 $[M]^+$, corresponding to the molecular formula $C_{36}H_{54}O_3$. The mass spectrum showed peak at m/z 429 $[M\text{-}C_6H_5CO]^+$, 413 $[M\text{-}C_6H_5COO]^+$, 288 $[M\text{-}C_6H_5CO$ and side chain$]^+$, 272 $[M\text{-}C_6H_5COO$ and $C_{10}H_{21}]^+$ and 254 $[M\text{-}C_6H_5COO$, $C_{10}H_{21}$, $H_2O]^+$. These peaks suggested the presence of benzoyloxy group at C-3 position. The peak at m/z 254 and 207 (due to C/D ring fission) suggested the presence of hydroxyl group at C-12 position. The peaks at m/z 272 and 254 also suggested the number of carbon atoms present in the side chain. The presence of band at $\Delta^{5(6)}$ is confirmed on the basis of peaks observed at m/z 207, 17, 137 and 123 in mass fragmentation pattern. The mass spectrum also clearly proved the absence of double bond in the side chain and ring A and D. On the basis of above spectral and chemical evidences, the structure of compound SM-4 was assigned as stigmast-5-ene-3β-benzyloxy-12β-ol.

The compound SM-5 was obtained as yellow shiny flakes after eluting the column with benzene: chloroform (1:1). It decolourized bromine solution and formed 2,4-DNP derivative, supporting the presence of a vinylic double bond and a carbonyl group. The IR spectrum of compound SM-5 displayed characteristic absorption bands at 3500, 1710 and 1632 cm^{-1} suggesting the presence of hydroxyl, keto and double bond, respectively. Absorption bands present at 759 and 723 cm^{-1} provided supportive evidences for the presence of aliphatic chain in the

molecule. The NMR spectrum of compound SM-5 exhibited two triplets at δ 0.83 (J=6.0 Hz) and 0.87 (J= 6.5 HZ) assigned to terminal methyl groups at C-1 and C-25 positions, respectively. The signals at δ 1.25, 1.30, 1.62, 1.82, 2.08, 2.34, and 2.77 were associated to CH_2 group present in different magnetic environment. The quartet observed at δ 2.34 and 2.77 are due to CH_2 group adjacent to ethylenic double bond and keto group at C-2 and C-6 position, respectively. A two-proton broad signal at δ 5.36 was due to H-4 and H-5 ethylenic protons, as suggested in IR spectrum. Two one-proton multiplets at δ 4.11 and 4.28 were associated with C-18 and C-23 hydroxymethine protons.

The mass spectrum showed molecular ion peak at m/z 396 $[M]^+$, corresponding to the molecular formula $C_{25}H_{48}O_3$. The ion peak at m/z 339 $[M\text{-}COC_2H_5]^+$ suggested the presence of keto group at C-3 position. The ion fragment at m/z 313 $[M\text{-}CH{=}CHCOC_2H_5]^+$ supported the presence of double bond at $\Delta^{4(5)}$ position. The ion peaks at m/z 337 and 145 indicated the presence of two hydroxyl groups at C-18 and C-23 position, respectively. On the basis of above spectral data, the structure assigned to the compound SM-5 is n-pentacos-4-en-3-one-18, 23-diol.

Thus five new phyto constituents *viz.* n-Docosane (SM-1), henicos-11-ene-8-one (SM-2), stigmast-5-ene-3β,4α-diol (SM-3), stigmast-5-ene-3β-benzyloxy-12β-ol (SM-4) and n-entacos-4-en-3-one-18,23-diol (SM-5) were isolated first time from the flowers of *H. rosasinensis*. These isolated novel constituents, when subjected to the hypotensive activity (Table 4), the compound stigmast-5-ene-3β-benzyloxy-12β-ol (SM-4) showed highest activity. In contrast, the hypotensive activity shown by the ethanol-water (7:3) extract is more when compared to the isolated constituent SM-4. These findings suggested that there must be synergistically acting constitutent(s) present in the 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 show the expected hypotensive activity.

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