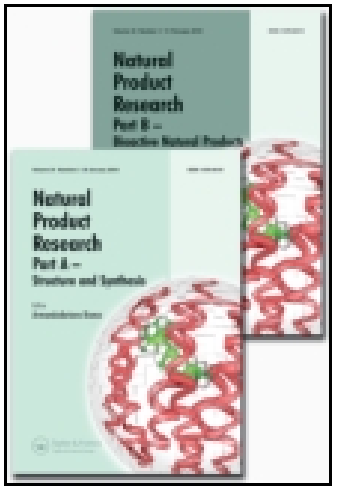

Natural Product Research: Formerly Natural Product Letters
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

Pregnane glycoside from Hemidesmus indicus as a potential anti-oxidant and anti-dyslipidemic agent

Arun Sethi [a] , Akriti Bhatia [a] , Sanjay Srivastava [b] , Geetika Bhatia [c] , M.M. Khan [c] , A.K. Khanna [c] & J.K. Saxena [c]

[a] Department of Chemistry , University of Lucknow , Lucknow, India

[b] Department of Applied Sciences , Institute of Engineering & Technology , Lucknow, India

[c] Division of Biochemistry , Central Drug Research Institute , Lucknow, India

Published online: 18 Feb 2010.

To cite this article: Arun Sethi , Akriti Bhatia , Sanjay Srivastava , Geetika Bhatia , M.M. Khan , A.K. Khanna & J.K. Saxena (2010) Pregnane glycoside from Hemidesmus indicus as a potential anti-oxidant and anti-dyslipidemic agent, Natural Product Research: Formerly Natural Product Letters, 24:15, 1371-1378, DOI: 10.1080/14786410802265084

To link to this article: http://dx.doi.org/10.1080/14786410802265084

Natural Product Research
Vol. 24, No. 15, 20 September 2010, 1371–1378

Taylor & Francis
Taylor & Francis Group

Pregnane glycoside from *Hemidesmus indicus* as a potential anti-oxidant and anti-dyslipidemic agent

Arun Sethi[a]*, Akriti Bhatia[a], Sanjay Srivastava[b], Geetika Bhatia[c], M.M. Khan[c], A.K. Khanna[c] and J.K. Saxena[c]

[a]*Department of Chemistry, University of Lucknow, Lucknow, India;* [b]*Department of Applied Sciences, Institute of Engineering & Technology, Lucknow, India;* [c]*Division of Biochemistry, Central Drug Research Institute, Lucknow, India*

(*Received 11 April 2008; final version received 22 August 2008*)

A new pregnane glycoside hindicusine (**1**) was isolated from the $CHCl_3$–EtOH (3 : 2) extract of *Hemidesmus indicus*, whose structure was established on the basis of spectroscopic studies. The glycoside (**1**) and its acetylated derivative (**5**) were evaluated for their anti-oxidant and anti-dyslipidemic activities.

Keywords: Asclepiadaceae; *Hemidesmus indicus*; pregnane glycoside; hindicusine; anti-oxidant; anti-dyslipidemic

1. Introduction

The side effects and associated toxicities of modern drugs have made oriental plant-based medicinal systems the focus of pharmaceutical research. Plants belonging to the family Asclepiadaceae are used in oriental medicine and are a rich source of biologically active cardiac (Kaneda et al., 1992) and pregnane glycosides (Deepak, Srivastav, & Khare, 1997). Plant pregnanes and their glycosides are known to possess anti-tumour and anti-cancer activities (Deepak et al., 1997; Pan, Chang, Wei, & Wu, 2003). Recently, pregnane glycosides isolated from plants have shown anti-proliferative activity (Leo et al., 2005; Plaza et al., 2005) on J774, A1, HEK-293 and WEHI-164 cell lines. *Hemidesmus indicus* (Asclepiadaceae) is widely used in Ayurveda and Unani medicine. Recent studies have shown that *H. indicus* possesses protective effects against diethyl nitrosoamine-induced hepatocarcinogenesis (Iddamaldeniya, Wickramasinghe, Thabrew, Ranatunge, & Thammitiyagodage, 2003) and reno protective effects in gentamicin-induced renal toxicity (Kotnis, Patel, Menon, & Sane, 2004). Further, it has been shown to possess antioxidant (Ravishankara, Shrivastava, Padh, & Rajani, 2002) and anti-ulcerogenic properties (Anoop & Jegadeesan, 2003), anti-nociceptive activity (Verma, Joharapurkar, Chatpalliwar, & Asnani, 2005), anti-inflammatory and anti-pyretic activities (Lakshman, Shivaprasad, Jaiprakash, & Mohan, 2006).

In our continuing studies (Oberai, M. Khare, & A. Khare, 1985; Prakash, Sethi, Deepak, A. Khare, & M. Khare, 1991; Chandra, Deepak, & Khare, 1994; Deepak,

*Corresponding author. Email: alkaarunsethi@rediffmail.com

ISSN 1478–6419 print/ISSN 1029–2349 online

DOI: 10.1080/14786410802265084
http://www.informaworld.com

Srivastav, & Khare, 1995, 1997a; Sigler, Saksena, Deepak, & Khare, 2000; Sethi, S.S. Srivastava, & S. Srivastava, 2006) on pregnane glycosides from *H. indicus* as biologically active components, the structure of hindicusine (**1**) isolated from the $CHCl_3$–EtOH (3 : 2) extract of the plant is herein reported.

2. Result and discussion

Hindicusine (**1**), m.p. 105–109°C, $[\alpha]_D$ −39° ($c = 0.55$, $CHCl_3$), $C_{34}H_{48}O_7$, ESI MS m/z 607$[M+K]^+$, responded positively to Liebermann–Burchardt (Prakash et al., 1991), xanthydrol (Prakash et al., 1991) and Keller–Killiani (Prakash et al., 1991) tests, indicating it to be a steroidal glycoside of 2,6-dideoxy hexose. The molecular formula was also confirmed by ^{13}C NMR and DEPT spectroscopic analysis, suggesting the presence of a pregnane glycoside. The presence of one anomeric proton and carbon at δ 4.14 and 99.6 in its ^1H and ^{13}C NMR spectra, respectively, suggested it to be a monoglycoside. In the ^1H NMR spectrum of (**1**) the presence of methylene group signals in the region δ 2.35–2.41 (1H) and 1.76–1.84 (1H) for respective equatorial and axial protons and the three proton doublet at δ 1.38 ($J = 7.0$ Hz) for the secondary methyl function further confirmed the presence of a 2,6-dideoxy sugar. The presence of five aromatic protons at δ 7.65(2H), 7.61(1H), 7.48(2H) in the ^1H NMR spectrum along with the carbon signals at δ 132.30, 129.22, 131.30, 133.72 and a carbonyl group signal at δ 167.5 in the ^{13}C NMR suggested the presence of a benzoyl function in the molecule.

Mild acid hydrolysis (0.05 N H_2SO_4 in dioxane) (Chandra et al., 1994) of (**1**) afforded the genin (**2**) and chromatographically pure sugar (**4**) (Figure 1). The genin (**2**) on methanolysis by the Zemplen method (Chandra et al., 1994) afforded a crystalline product (**3**) which was identified as calogenin (Prakash et al., 1991; Sethi, Deepak, M. Khare, & A. Khare, 1988) by comparison with the authentic sample (m.p., TLC, $[\alpha]_D$). The sugar was identified as D-digitoxose by direct comparison with the authentic sample (TLC, PC, $[\alpha]_D$) (Prakash et al., 1991) and by preparing its known acid phenyl hydrazide derivative (Prakash et al., 1991).

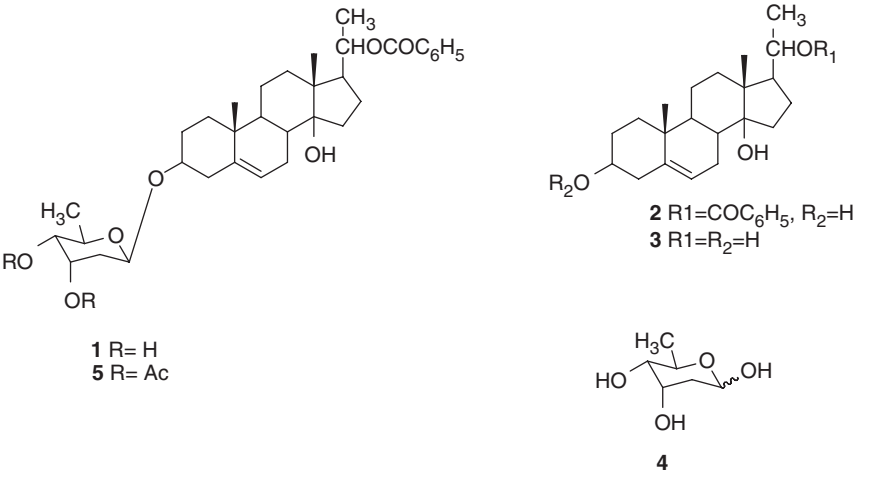

Figure 1. Structures of compounds **1**–**5**.

In the NMR spectrum (^{13}C, DEPT 135, DEPT 90) of (**1**), chemical shifts of all signals due to the steroid nucleus were almost identical to those of calogenin (Srivastav, Deepak, & Khare, 1994), except that of C-20, which was shifted downfield, indicating that the C-20 hydroxyl function carried the benzoyl substituent. The presence of the benzoyl function at C-20 hydroxyl was further supported by the mass fragments at m/z 441 and 277 due to $[M–Sugar–H_2O + K]^+$, and $[M–Sugar–H_2O–CH_3CHOCOC_6H_5–CH_3 + K]^+$, respectively. The loss of the benzoylated C-17 side chain in the mass spectrum also confirmed that the D-digitoxose is linked to the only available free secondary C-3 hydroxyl of the aglycon. The fragment at m/z 369 (retro Diels Alder rearrangement at C-2 and C-3 of sugar, followed by loss of benzoic acid and one angular methyl group) further confirmed the proposed structure.

The ^1H NMR spectrum of (**1**) showed the anomeric proton as a double doublet at δ 4.14 ($J = 9.0$ and 2.0 Hz). The large coupling constant of the double doublet showed the presence of D-digitoxose in $^4C_1$(D) conformation linked through β-glycosidic linkage. The acetylation of (**1**) yielded a di-*O*-acetyl hindicusine (**5**), confirming the presence of only two free acetylable hydroxyl groups in (**1**). The structure of (**1**) was thus defined as 20-*O*-benzoyl calogenin-3-*O*-β-D-digitoxopyranoside.

2.1. *Effect of pregnane glycosides on hyperlipidemia*

Administration of Triton WR-1339 in rats induced marked hyperlipidemia, as evidenced by increase in the plasma level of Tc (3.92 fold), Pl (3.59 fold) and Tg (3.74 fold) as compared to the control (Table 1). Treatment of hyperlipidemic rats with pregnane glycosides at the dose of 100 mg kg^{-1} p.o. reversed the plasma levels of the lipids with varying extents (Table 1). These data compared with the standard drug gemfibrozil at the dose of 100 mg kg^{-1} showed a decrease in plasma levels of Tc, Pl and Tg by 34, 35 and 37%, respectively. The order of lipid lowering activity by these pregnane glycosides in the above model was **5** > **1** (Table 1).

2.2. *Effects of 1 and 5 on oxygen free radical generation* in vitro

The scavenging potential of pregnane glycosides at 200 μg mL^{-1} against the formation of O^{-2} and $OH^{\cdot}$ in a non-enzymic system was also studied (Table 2). Compound **5** showed greater anti-oxidant activity in the above tests as compared to **1** (Table 2).

3. Experimental

3.1. *General experimental procedures*

The melting points were recorded on an electrically heated melting point apparatus and were uncorrected. Optical rotations were recorded on an ORIBA, SEPA-300 digital polarimeter. ^1H and ^{13}C NMR spectra were recorded on a Bruker Advance DRX-300 MHz spectrometer or DPX 200 FT spectrometers using TMS as an internal reference. ESI MS were recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer. Chemical analysis was carried out on a Carlo-Erba-1108 instrument. Solvents used were of laboratory grade, purified and dried according to standard procedures. Column chromatography was performed with silica gel (60–120 mesh). Paper chromatography was conducted on Whatman No. 1 paper.

Table 1. Lipid lowering activity of pregnane glycosides in triton-treated hyperlipidemic rats.

Treatment	Total cholesterol (Tc)	Phospholilid (Pl)	Triglyceride (Tg)
Control	84.62 ± 6.27	75.34 ± 6.77	80.22 ± 5.62
Triton treated	332.22 ± 20.88 (+3.92F)***	270.70 ± 18.77 (+3.59F)***	300.22 ± 23.33 (+3.74F)***
Triton + **1**	280.14 ± 24.41 (−16)*	230.33 ± 18.84 (−15)*	238.44 ± 19.92 (−20) **
Triton + **5**	252.16 ± 17.16 (−24)**	220.86 ± 14.50 (−18)**	244.99 ± 20.14 (−18)**
Triton + Gemfibrozil ($100\,mg\,Kg^{-1}$) standard drug	220.00 ± 15.55 (−34)***	175.81 ± 12.80 (−35)***	190.11 ± 12.44 (−37)***

Notes: Unit: $mg\,dL^{-1}$. Each value is the mean ± SD of 2 rats. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Hyperlipidemic group was compared with control, hyperlipidemic + pregnane glycoside treated with hyperlipidemic.

Table 2. Anti-oxidant activity of pregnane glycosides *in vitro*.

Treatment	Concentration	Formation of superoxide anions[a]	Formation of hydroxyl radicals[b]
1	None	70.80 ± 5.21	8.62 ± 0.07
	200	68.75 ± 6.00 (−3) NS	8.50 ± 0.05 (−1.3) NS
5	None	90.27 ± 7.24	3.80 ± 0.02
	200	63.88 ± 5.11 (−29)***	2.83 ± 0.01 (−26)***
Alloperinol ($20\,\mu g\,mL^{-1}$)	None	26.55 ± 0.57	
	20	$2.60 \pm (0.41)(90)$***	
Mannitol ($100\,\mu g\,mL^{-1}$)	None		32.62 ± 1.31
	100		17.12 ± 2.68 (−48)***

Notes: [a]Nmol formazone formed per minute; [b]nmole MDA h^{-1}. Each value is the mean ± SD of four separate observations. $*P<0.05$, $**P<0.001$. NS = non-significant as compared to the systems without drug treatment.

3.2. *Plant material, extraction and isolation*

Stems of mature *H. indicus* were collected from Dehradun forest, India. The method of extraction was same as reported earlier (Prakash et al., 1991; Sethi et al., 2006). Fractionation of the crude extract of *H. indicus* yielded $CHCl_3$–EtOH (3 : 2) extract (1.2 g). Repeated column chromatography of $CHCl_3$–EtOH (3 : 2) extract over silica gel (60–120 mesh) using different polarities of $CHCl_3$–MeOH as eluent afforded different fractions. The purified compound hindicusine (**1**) (82 mg) was obtained by using $CHCl_3$: MeOH (98 : 3) as eluent.

3.3. *Structure and identification*

3.3.1. *Hindicusine (**1**)*

Compound **1** crystallised from MeOH as colourless needles, m.p. 105–109°C, $[\alpha]_D - 39°$ ($c = 0.55$, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.65 (2H, d, $J = 7.5$ Hz, H-2′, 6′) 7.61 (1H, t, $J = 7.5$ Hz, H-4′), 7.48 (2H, t, $J = 7.5$ Hz, H-3′, 5′), 5.35 (1H, m, H-6), 5.03–4.92 (1H, m, H-20), 4.52–4.47 (1H, m, H-3), 4.14 (1H, dd, $J = 9.0$ & 2.0 Hz, H-1″), 3.91–3.88 (1H, m, H-5″), 3.72–3.69 (1H, m, H-3″), 3.20–3.18 (1H, m, H-4″), 2.41–2.35 (1H, m, H-2″eq), 1.84–1.76 (1H, m, H-2″ ax), 1.38 (3H, d, $J = 7.0$ Hz, 6″ CH_3), 1.26 (3H, d, $J = 7.0$ Hz, 21 CH_3), 1.18 (3H, s, 18 CH_3), 0.84 (3H, s, 19 CH_3). ESI MS m/z 607 $[M + K]^+$(10), 455 [607-C_6H_5COOH–$2CH_3$](20), 441 [607-Sugar–H_2O] (100), 369[$C_{32}H_{42}O_5$–C_6H_5COOH–CH_3](25), 292 [369-CH_3–H_2O–CH_2CHOH] (10), 277 [441-$CH_3CHOCOC_6H_5$–CH_3](15), 263 [455-Sugar–CH_2CHOH](10). Elemental analysis C 71.80, H 8.51 Calcd for $C_{34}H_{48}O_7$, C 71.72, H 8.42. (^{13}C NMR, Table 3).

3.3.2. *Di-O-acetylhindicusine (**5**)*

Compound **1** (12 mg) on acetylation with Ac_2O (0.15 mL) in pyridine (1.1 mL) at room temperature and the usual workup yielded **5** as an amorphous residue (12 mg), $[\alpha]_D - 12°$ ($c = 0.10$, $CHCl_3$) ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (2H, d, $J = 7.5$ Hz, H-2′, 6′) 7.67 (1H, t, $J = 7.5$ Hz, H-4′), 7.51(2H, t, $J = 7.5$ Hz, H-3′, 5′), 5.39 (1H, m, H-6), 5.06–4.95

Table 3. ^{13}C NMR spectral data of **1** in $CDCl_3$.

	1		**1**		**1**
1	36.20 t	13	50.40 s	3″	69.20 d
2	29.32 t	14	86.20 d	4″	74.80 d
3	78.17 d	15	30.75 t	5″	69.90 d
4	39.12 t	16	28.20 t	6″	18.20 q
5	139.00 s	17	54.90 d	Benzoyl at C-20	
6	120.20 d	18	14.40 q	COO	167.50 s
7	29.70 t	19	17.30 q	1′	132.30 s
8	33.30 d	20	74.30 d	2′/6′	129.22 d
9	55.60 d	21	19.20 q	3′/5′	131.30 d
10	37.50 s	Digitoxose		4′	133.72 d
11	23.30 t	1″	99.60 d		
12	36.10 t	2″	39.80 t		

Notes: Multiplicity was determined by DEPT experiments (s = quaternary, d = methine, t = methylene, q = methyl).

(1H, m, H-20), 4.21 (dd, $J = 9.0$ and 2.0 Hz, H-1″), 2.08(3H, s, $OCOCH_3$), 2.05(3H, s, $OCOCH_3$), 1.41(3H, d, $J = 7.0$ Hz, 6″ CH_3) 1.28 (3H, d, $J = 7.0$ Hz, 21 CH_3), 0.97 (3H, s, 18-CH_3), 0.83 (3H, s, 19-CH_3). ESI MS m/z 675 $[M + Na]^+$(10), 627(675-2CH_3–H_2O) (20), 406 (675-2CH_3COOH–$CH_3CHOCOC_6H_5$) (100).

3.3.3. *Mild hydrolysis of hindicusine (**1**)*

To a solution of **1** (15 mg) in 80% 1,4-dioxane (1.5 mL) was added 0.05 N H_2SO_4 (1.5 mL) and the mixture was warmed at 50°C for 30 min. The usual work up (Oberai et al., 1985; Sigler et al., 2000) followed by column chromatography afforded genin (**2**) and syrupy sugar **4** (3 mg) $[\alpha]_D + 40°$(c 0.10, MeOH). The specific rotation, TLC and PC comparison with the authentic sample showed it to be identical to D-digitoxose.

3.3.4. *Hydrolysis of **2** by the Zemplen method*

To a solution of **2** (2 mg) in absolute MeOH (0.5 mL) was added NaOMe (0.05 mL) and the mixture was kept at room temperature. After 15 min it was neutralised with IR 120$[H]^+$ resin and filtered. Methanol was removed under reduced pressure, yielding **3** (1.1 mg) m.p. 200–203°C, $[\alpha]_D$ −50° (c 0.12, MeOH).

3.3.5. *Animals*

Rats (Charles Foster strain, male, adult, body weight 100–125 g) were kept in a room with controlled temperature (25–26°C), humidity (60–80%) and 12/12 h light/dark cycle (light on from 8.00 am to 8.00 pm) under hygienic conditions. Animals, which were acclimatised for one week before starting the experiment, had free access to the normal diet and water.

3.3.6. *Lipid lowering activity*

The procedure adopted was the same as reported earlier (Sethi et al., 2007). Rats were divided into five groups: control, triton induced, triton plus **1**, **5** and gemfibrozil

(100mg/Kg) treated groups, containing two rats in each group. Two pregnane glycosides and gemfibrozil were macerated with gum acacia, suspended in water and fed simultaneously with triton at a dose of 100 mg kg^{-1} p.o. to the animals.

3.3.7. *Anti-oxidant activity (generation of free radicals)*

The procedure adopted was the same as reported earlier (Sethi et al., 2007). Superoxide anions (O^{-2}) were generated enzymatically by xanthine (160 mM), xanthine oxidase (0.04 units) and nitroblue tetrazolium (320 μM) in the absence or presence of compounds **1** and **5** (200 μg mL^{-1}) in 100 mM phosphate buffer (pH 8.2). In another set of experiments, the effect of compounds **1** and **5** (200 μg mL^{-1}) on the generation of hydroxyl radical (OH) was also studied by non-enzymic reactants.

Acknowledgement

The authors are grateful to the RSIC Division of the Central Drug Research Institute for the analytical data.

References

Anoop, A., & Jegadeesan, M. (2003). Biochemical studies on the anti-ulcerogenic potential of *Hemidesmus indicus* R.Br. var. *Indicus*. *Journal of Ethnopharmacology, 84*, 149–156.

Chandra, R., Deepak, D., & Khare, A. (1994). Pregnane glycosides from *Hemidesmus indicus*. *Phytochemistry, 35*, 1545–1548.

Deepak, D., Srivastav, S., & Khare, A. (1995). Indicusin – A pregnane diester triglycoside from *Hemidesmus indicus* R.Br. *Natural Product Letters, 6*, 81–86.

Deepak, D., Srivastav, S., & Khare, A. (1997). Pregnane glycosides. *Progress in the Chemistry of Organic Natural Products, 71*, 169–325.

Iddamaldeniya, S.S., Wickramasinghe, N., Thabrew, I., Ranatunge, N., & Thammitiyagodage, M.G. (2003). Protection against diethylnitrosoamine-induced hepatocarcinogenesis by an indigenous medicine comprised of *Nigella sativa, Hemidesmus indicus* and *Smilax glabra*: a preliminary study. *Journal of Carcinogenesis, 2*, 6.

Kaneda, N., Chai, H., Pezzuto, J.M., Kinghorn, A.D., Farnsworth, R., Tuchinda, P., et al. (1992). Cytotoxic activity of carenolides from *Beaumontia brevituba* stems. *Planta Medica, 58*, 429–431.

Kotnis, M.S., Patel, P., Menon, S.N., & Sane, R.T. (2004). Renoprotective effect of *Hemidesmus indicus*, a herbal drug used in gentamicin-induced renal toxicity. *Nephrology, 9*, 142–152.

Lakshman, K., Shivaprasad, H.N., Jaiprakash, B., & Mohan, S. (2006). Anti-inflammatory and anti-pyretic activities of *Hemidesmus indicus* root extract. *African Journal of Traditional Complementary and Alternative Medicine, 3*, 90–94.

Leo, N.D., Tommasi N, N.D., Sanogo, R., Autore, G., Marzocco, S., Pizza, C., et al. (2005). New pregnane glycosides from *Caralluma dalzielii*. *Steriods, 70*, 573–585.

Oberai, K., Khare, M.P., & Khare, A. (1985). A pregnane ester diglycoside from *Hemidesmus indicus*. *Phytochemistry, 24*, 2395–2397.

Pan, W., Chang, F., Wei, L., & Wu, Y. (2003). New flavans, spirostanol and a pregnane genin from *Tupistra chinensis* and their cytotoxicity. *Journal of Natural Products, 66*, 161–168.

Plaza, A., Perrone, A., Balestrieri, M.L., Felice, F., Balestrieri, C., Hamed, A.J., et al. (2005). New unusual pregnane glycosides with antiproliferative activity from *Solenostemma argel*. *Steriods, 70*, 594–603.

Prakash, K., Sethi, A., Deepak, D., Khare, A., & Khare, M.P. (1991). Two pregnane glycosides from *Hemidesmus indicus*. *Phytochemistry, 30*, 297–299.

Ravishankara, M.N., Shrivastava, N., Padh, H., & Rajani, M. (2002). Evaluation of antioxidant properties of root bark of *Hemidesmus indicus* R.Br. (Anantmul). *Phytomedicine, 9*, 153–160.

Sethi, A., Maurya, A., Tewari, V., Srivastava, S., Faridi, S., Bhatia, G., et al. (2007). Expeditious and convenient synthesis of pregnanes and its glycosides as potential anti-dyslipidemic and anti-oxidant agents. *Bioorganic & Medicinal Chemistry, 15*, 4520–4527.

Sethi, A., Deepak, D., Khare, M.P., & Khare, A. (1988). A novel pregnane glycoside from *Periploca calophylla*. *Journal of Natural Product, 51*, 787–790.

Sethi, A., Srivastava, S.S., & Srivastava, S. (2006). Pregnane glycoside from *Hemidesmus indicus* R.Br. *Indian Journal of Hetrocyclic Chemistry, 16*, 191–192.

Sigler, P., Saksena, R., Deepak, D., & Khare, A. (2000). C21 Steroidal glycosides from *Hemidesmus indicus*. *Phytochemistry, 54*, 983–987.

Srivastav, S., Deepak, D., & Khare, A. (1994). Three novel pregnane glycosides from *Leptadenia reticulata* Wright and Arn. *Tetrahedron, 50*(3), 789–798.

Verma, P.R., Joharapurkar, A.A., Chatpalliwar, V.A., & Asnani, A.J. (2005). Antinociceptive activity of alcoholic extract of *Hemidesmus indicus* R.Br. in mice. *Journal of Ethnopharmacology, 102*, 298–301.