Pharmacognosy Magazine [Phcog Mag]
Vol 5, Issue 20 (Suppl.), Oct-Dec, 2009 Page 407-411
(An Official Publication of Pharmacognosy Network Worldwide)
Received: 28 Sep, 2009
Modified: 14 Oct, 2009
Accepted: 03 Nov, 2009

PHCOG MAG.: Research Article

Triterpenoids and Steroids from *Holarrhena pubescens* Seeds

Bhattachartya Sanjib[1]*, Tarafdar Saswati[2], Saha Chandra Nath[3]

[1] *Bengal School of Technology (A College of Pharmacy), Delhi Road, Sugandha, Hooghly 712102, West Bengal, India.*
[2] *Gupta College of Technological Sciences, Ashram More, G. T. Road, Asansol 713301, West Bengal, India.*
[3] *Research Associate, Mepro Pharmaceuticals Pvt. Ltd., Q Road, G.I.D.C., Surendranagar 363035, Gujrat, India.*

*** Author for Correspondence:** *E-mail: sakkwai@yahoo.com*

ABSTRACT

In present study, three known pentacyclic triterpenoids namely lupeol, betulinaldehyde, and betulinic acid and a steroidal compound stigmasterol were isolated from the seeds of *Holarrhena pubescens* (Buch. Ham.) (Apocynaceae); commonly known as *Kurchi* in commerce. Their structures were elucidated on the basis of spectroscopic evidences and comparison with the authentic samples.

Keywords: *Holarrhena pubescens,* triterpenoids, seeds, betulinic acid, lupeol, stigmasterol.

INTRODUCTION

Holarrhena pubescens (Buch. Ham.) Wall. ex G. Don. (Synonym: *H. antidysenterica* L. Wall.) (Apocynaceae), commonly called *Kurchi* in Bengali and Hindi and also commercially; *Kutaja* is Sanskrit and Conessi or Bitter oleander in English, is a large shrub to small deciduous tree with white flowers and found throughout the dry forests of Indian subcontinent. The different parts of the plant were used since antiquity in the indigenous systems of medicine. The stem bark of this plant has been used traditionally for the treatment of dysentery. Its stem bark possesses amoebicidal property and used traditionally as anti-dysenteric, expectorant, tonic, febrifuge, digestive, anthelmintic, antiperiodic, digestive, astringent, in treatment of amoebic dysentery, arthritis, asthma, diarrhea, malaria, boils, cough and cold. The seeds are traditionally used as anti-dysenteric, antidiarrhoeal, antibilious, antipyretic, anthelmintic, stomachic and carminative; for promoting conception, also for toning up vaginal tissues after delivery (1–7). However, the antidysenteric action of seeds is lower than that of its stem bark. Around 30 steroidal alkaloids have been isolated from this plant, mostly from the stem bark. These include kurchinine, kurchinine, kurchinidine, holarrifine, holadiene, regholarrhenines, pubescine, norholadiene, pubescimine, kurchilidine, kurchamide, kurcholessine, kurchessine, conessine, conessimine and isoconessimine (5, 8–10). Stem bark of the plant, has been extensively investigated chemically and pharmacologically mainly for their antimicrobial and antidiarrhoeal properties (10–20). The seeds were also reported to possess steroidal-type alkaloids (21, 22). An enzyme, serine protease was also isolated from kurchi seeds (23). Previous workers reported antidiabetic, antihyperlipidemic, antibacterial and antidiarrhoeal effects of kurchi seed and its alkaloids (21–24). However, reports on the chemical constituents of kurchi seed are comparatively scanty. Present study was therefore aimed to isolate and report the non-alkaloidal constituents viz. triterpenoid and steroidal compounds present in the seeds of *H. pubescens* from India.

MATERIALS AND METHODS

Plant material

The ripe seeds of *H. antidysenterica* were collected from Keonjhar, Orissa, India, during November 2007. The specimen was identified by Dr. M. S. Mondal, at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India, and a voucher specimen (ST/01/08) was deposited at Gupta College of Technological Sciences, Asansol, West Bengal 713301, India, for reference. Just after collection the seeds were washed thoroughly with tap water and shade dried at room temperature (24–26 °C) and then ground mechanically into a coarse powder.

General experimental techniques

Melting point was determined on an XT-4 micro melting point apparatus. Optical rotation was measured on a Perkin-Elmer 241 polarimeter. IR spectrum was recorded with a Perkin-Elmer 683 FT-IR spectrometer. NMR (^{1}H, ^{13}C) spectra were recorded on a Bruker AV300 Supercon NMR System with chemical shifts being represented in parts per million (ppm, δ values) and tetramethylsilane (TMS) as an internal standard. EI-MS and HR-FAB-MS were recorded on a Autospec-Ultima ETOF MS spectrometer at an ionization energy of 70 eV. Column chromatography was performed on silica gel (200–300 mesh, Sisco Research Lab. Pvt. Ltd., Mumbai, India). Fractions were monitored by analytical thin layer chromatography (TLC) and the spots were visualized by spraying the developed TLC plates with Libermann-Burchard and anisaldehyde-H_2SO_4 reagent followed by heating at 100 °C for 5–10 mins. The TLC employed pre-coated silica gel plates (aluminium sheets 20×20 cm, Silica gel 60 F_{254} of Merck K GaA, Germany). All solvents and reagents used were of analytical grade obtained from Merck.

Extraction and isolation

The air-dried and powdered plant material (277.06 g) was macerated with methanol at room temperature (26–28 °C) for 72 h. The extract was filtered and evaporated to dryness *in vacuo* using a rotary evaporator at 40 °C to provide the crude methanol extract (28.13 g). The crude extract was fractionated into *n*-hexane (6.97 g), dichloromethane (16.84 g) and finally the aqueous fraction (2.21 g).

The *n*-hexane soluble fraction was subjected to silica gel column chromatography, eluted with *n*-hexane: ethyl acetate (gradient, 1 : 0 → 0 : 1) to yield total 32 fractions, monitored by TLC. Compounds 1 (167 mg), 2 (132 mg) and 3 (59 mg) were obtained by evaporating the fractions eluted with 10, 15 and 50 % ethyl acetate in *n*-hexane, respectively to dryness *in vacuo* at 40 °C . Compounds 1 and 3 were colourless crystals whereas compound 2 was amorphous powder.

The dichloromethane soluble fraction was subjected to silica gel column chromatography, eluted with *n*-hexane: dichloromethane: methanol = 2: 5: 1 (isocratic) to provide total 21 fractions, monitored by TLC. Fractions 13 to 18 were combined together and evaporated *in vacuo* at 40 °C to afford compound 4 (97 mg) as white amorphous mass which was further crystallized in methanol to afford a white crystalline powder.

The isolated compounds were characterized by physical and extensive spectral analyses, the structure of compounds were established by comparison of these data with previously reported literature data and by co-chromatography with the authentic samples.

Compound 1: Betulinaldehyde: Colourless crystals; ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 9.67 (1H, s, CHO), 4.74 (1H, br. s, Ha - 29), 4.62 (1H, br. s, H_b -29), 3.18 (1H, dd, *J*= 11.0, 4.8 Hz, H-3), 2.85 (1H, m, H-19), 1.69 (3H, s, Me -30), 0.97 (3H, s, Me -26), 0.95 (3H, s, Me -23), 0.91 (3H, s, Me -27), 0.81 (3H, s, Me -25), 0.74 (3H, s, Me -24).

Compound 2: Stigmasterol: Amorphous powder; ^{1}H NMR data were found in accordance with previously reported data.

Compound 3: Lupeol: Colorless crystals;^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 4.74 (1H, br. s, H_a-29), 4.61 (1H_b, br. s, H-29), 3.19 (1H, dd, *J* = 11.2, 4.8 Hz, H-3), 1.69 (3H, s, Me-30), 0.98 (3H, s, Me -26), 0.97 (3H, s, Me -23), 0.94 (3H, s, Me -27), 0.82 (3H, s, Me -25), 0.76 (3H, s, Me -24).

Compound 4: Betulinic acid : White crystalline powder; ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 4.73 (1H, br. s, Ha -29), 4.60 (1H, br. s, Hb -29), 3.18 (1H, dd, *J* = 11.2, 4.9 Hz, H-3), 2.98 (1H, m, H-19), 1.68 (3H, s, Me -30), 0.97 (3H, s, Me -26), 0.96 (3H, s, Me -23), 0.93 (3H, s, Me -27), 0.81 (3H, s, Me -25), 0.74 (3H, s, Me -24).

RESULTS AND DISCUSSION

Column chromatographic separation and purification of the *n*-hexane and dichloromethane soluble fractions of methanol extract from *H. pubescens* seeds afforded three known pentacyclic triterpenoids including betulinic acid, betulinaldehyde and lupeol; and one steroidal compound stigmasterol. The structure of isolated compounds (Fig. 1 and 2) was determined by means of physical and spectral analyses (FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectrometry) and by comparing the experimental data with corresponding literature values and also by co-chromatography (analytical TLC) with the authentic samples. The HR-FAB-MS spectra ascertained the

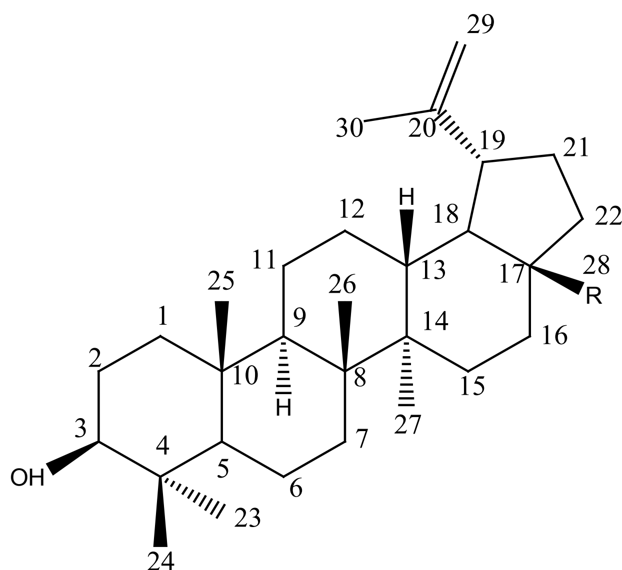

Figure 1: Structures of lupeol (R = CH_3), betulinaldehyde (R = CHO) and betulinic acid (R = COOH)

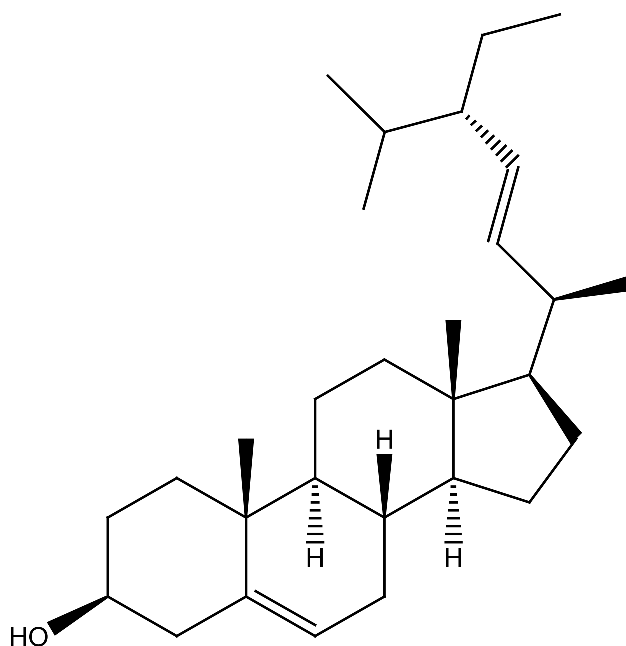

Figure 2: Structure of stigm asterol

molecular formula of the compounds. The recorded ^{13}C-NMR spectra demonstrated excellent concordance with corresponding literature data, especially for betulinic acid and stigmasterol. The typical important ^{1}H-NMR spectral features of isolated compounds are being discussed here.

In the ^{1}H-NMR spectrum of compound 1, the presence of a lupene skeleton having an angular aldehyde group was evident. The spectrum displayed signals attributable to exomethylene protons at δ 4.62 and 4.74 (1H, each, br. s) which together with an allylic methyl at δ 1.69 demonstrated an isopropenyl moiety. The 1H NMR spectrum also showed singlets at δ 0.74, 0.81, 0.91, 0.95 and 0.97 (3H, each) suggestive of the presence of five methyl groups in this compound. These were attributed to H3-25, H3-27, H3-26, H3-23 and H3-24 (Me-10, Me-14, Me-8, Me-14 and Me-4), respectively. The double doublet (*J* =11.0, 4.8 Hz) centered at δ 3.17 could be assigned to the oxymethyline proton at C-3. The large coupling of this proton (H-3) with the vicinyl methylene protons suggested a β orientation of the hydroxyl group at C-3.

In addition, the spectrum also showed a multiplet at δ 2.85 for the methine proton at C-19. On the basis of the aforesaid spectral features, compound 1 was identified as betulinaldehyde, the identity of which was established by comparison of these data with those reported for betulinaldehyde and by co-TLC with an authentic sample as well (25).

The structure of compound 2 was evidently determined by comparison of spectral data with corresponding literature data and co-TLC with an authentic sample as stigmasterol, a common phytosterol (26).

The ^{1}H-NMR spectrum of compound 3 showed one double doublet of one proton intensity at δ 3.19 (J =11.2, 4.8 Hz) typical for H-3 of a triterpene type carbon skeleton. The spectrum displayed two singlets at δ 4.74 and δ 4.61 (1H each) assignable to protons at C-29. A multiplet of one proton intensity at δ 2.36 was assigned to H-19. The spectrum also displayed six singlets at δ 0.76, 0.82, 0.94, 0.97, 0.98, and 1.69 (3H each) assignable to protons of methyl groups at C-4 (H3-23, H3-24), C-10 (H3-25), C-8 (H3-26), C-14 (H3 -27), and C-20 (H3-30), respectively. By comparing the spectral data of compound 3 with those of previously reported values as well as by co-TLC with an authentic sample established its identity as lupeol (27, 28).

The ^{1}H-NMR spectrum of compound 4 revealed the presence of a lupene type carbon skeleton. It displayed signals attributable to an exomethylene group at δ 4.60 and 4.73 (1H, each, br.s) which together with an allylic methyl at δ 1.68 which indicated an isopropenyl function. The double doublet δ 3.18 with couplings of 11.2 and 4.9 Hz centered at could be assigned to H-3. The large coupling of this proton (H-3) with the vicinyl methylene protons suggested a β (beta) orientation of the hydroxyl group at C-3. In addition, the spectrum also showed a multiplet at δ 2.98 for the methine proton at C-19 and five methyl group resonances at 0.74, 0.81, 0.93, 0.96 and 0.97. On the basis of the above spectral features, compound 4 was identified as betulinic acid. The identity of compound 4 as betulinic acid was confirmed by comparison the spectral data with corresponding literature values as well as by co-TLC with an authentic sample (29–31).

Betulinic acid 3β-hydroxy-lup-20(29)-en-28-oic acid, is a widely distributed pentacyclic lupane-type triterpenoid in the plant kingdom along with its derivatives viz. betulin, betulinaldehyde, dihydrobetulinic acid, amide derivatives and side chain modified analogues etc. Betulinic acid, originally isolated from birch bark, *Betula alba* has been reported to exhibit a variety of biological effects including antiretroviral and anti-cancer activity. There are several methods reported for isolation of betulinic acid and its derivatives from different plants, as it often becomes difficult to isolate them when these are present in combination with other similar triterpenoids (32, 33). Lupeol is a common pentacyclic triterpenoid occurring in free form and as saponin glycosides especially. It has anti-cancer and other biological effects (27). Phytosterols are common plant constituents found widely in higher plants. Sitosterol is most abundantly occurring phytosterol. Stigmasterol is also a common one. It has mainly hypoglycemic and hypercholesterolemic property, also used as a precursor in the synthesis of steroidal drugs (26, 34).

To our best knowledge, present study is the first report of the isolation of pentacyclic triterpenoids viz. lupeol, betulinic acid and betulinaldehyde; and stigmasterol (a phytosterol) from the seeds of *H. pubescens*. Isolation of other possible non-alkaloidal and alkaloidal compounds from *H. pubescens* seeds and biological evaluations of isolated compounds are presently underway.

ACKNOWLEDGEMENTS

The authors are thankful to the Director, Chembiotek Research International, Kolkata, India; and the Director, Indian Institute of Chemical Biology (IICB), Kolkata, India for instrumental facilities. The second author expresses her sincere thanks to the authority of Gupta College of Technological Sciences, Asansol 713301, West Bengal, India for necessary facilities.

REFERENCES

1. Duke J.A., Bogenschuz-Godwin M.J., duCellier J., Duke P.K. *Handbook of Medicinal Plants*, 2nd Ed., (CRC Press, Boca-Raton, 2002) 219.
2. Nadkarni A.K., Chopra R.N. *Indian Materia Medica*. Vol. **I**. (Popular Prakashan, Bombay, 1976) 927.
3. *Ibid*. Vol. II. 636.
4. Chopra R.N., Nayar S.L., Chopra I.C. *Glossary of Indian Medicinal Plants*, (Council of Scientific and Industrial Research, New Delhi, 1956) 187.
5. Khare C.P. *Indian Medicinal Plants: An Illustrated Dictionary*, (Springer, Berlin, Heidelberg, 2007) 312–313.
6. Khan I.A., Khanum I.A. *Medicinal and Aromatic Plants of India*, (Ukkaz Publications, Hyderabad, 2005) 201–202.
7. Prajapati N.D., Purohit S.S., Sharma A.K., Kumar T. *A Handbook of Medicinal Plants: A Complete Source Book*, (Agrobios (India), Jodhpur, 2004) 273.
8. Handa S.S., Kaul M.K. *Supplement to Cultivation and Utilization of Medicinal Plants*, (Regional Research Laboratory, Jammu-Tawi, 2006) 566.
9. Henry T.A. *The Plant Alkaloids*, (J. & Churchill Ltd., London, 1949) 496.
10. Rahman A.U. and Choudhary M.I. Chemistry and Biology of Steroidal Alkaloids. In: Cordell G.A. Ed. *The Alkaloids, Chemistry and Biology*, (Academic Press, San Diego, California, 1998) 63.
11. Siddiqui S. and Shamsuddin B.A. Isolation and structure of holarrifine, a new alkaloid from the bark of *Holarrhena antidysenterica* Linn. *Pak J Sci Ind Res*. **32**: 1–3 (1989).
12. Siddiqui B.S., Usmani S.B., Begum S. and Siddiqui S. (1993). Steroidal alkaloids and an androstane derivative from the bark of *Holarrhena pubescens*. *Phytochemistry*. **33**: 925–928 (1993).
13. Siddiqui B.S., Usmania S.B., Alib S.T., Beguma S. and Rizwani G.H. Further constituents from the bark of Holarrhena pubescens *Phytochemistry*. **58**: 1199–1204 (2001).

14. Kumar N., Singh B., Bhandari P., Gupta A.P. and Kaul V.K. Steroidal alkaloids from *Holarrhena antidysenterica* (L.) Wall. *Chem Pharm Bull.* **55**: 912–914 (2007).
15. Raman M.S., Anwar M.N. and Chowdhury A.Z.M.S. Antibacterial activity of Secondary metabolites from *Holarrhena antidysenterica* stem bark. *Bangladesh J Microbiol.* **16**: 101–105 (1999).
16. Raman M.S., Sultana N. and Anwar M.N. *In Vitro* antimicrobial Activity of Holarrifine 24-ol Isolated from the Stem Bark of *Holarrhena antidysenterica*. *Int J Agri Biol.* **6**: 698–700 (2004).
17. Ballal M., Srujan D., Bhat K.K., Shirwaikar A. and Shivananda P.G. Antibacterial activity of *Holarrhena antidysenterica* [kurchi] against the enteric Pathogens. *Indian J Pharmacol.* **33**: 392–393 (2001).
18. Rani P. and Khullar N. Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant *Salmonella typhi*. *Phytother Res.* **18**: 670–673 (2004).
19. Chakraborty A. and Brantner A.H. Antibacterial steroid alkaloids from the stem bark of *Holarrhena pubescens*. A. *J Ethnopharmacol.* **68**: 339–344 (1999).
20. Kavitha D. and Niranjali S. Inhibition of enteropathogenic *Escherichia coli* adhesion on host epithelial cells by *Holarrhena antidysenterica* (L.) Wall. *Phytother Res.* **9**: 1229–36 (2009).
21. Kumar A. and Ali M. A new steroidal alkaloid from the seeds of *Holarrhena antidysenterica Fitoterapia.* **71**: 101–104 (2000).
22. Kavitha D., Shilpa P.N., Devaraj S. and Niranjali S. Antibacterial and antidiarrhoeal effects of alkaloids of *Holarrhena antidysenterica* Wall. *Indian J Exp Biol.* **42**: 589–594 (2004).
23. Khan H., Subhan M., Mehmood S., Durrani M.F., Abbas S. and Khan S. Purification and Characterization of Serine Protease from Seeds of *Holarrhena antidysenterica. Biotechnology.* **7**: 94–99 (2008).
24. Ali K.M., Chatterjee K., De D., Bera T.K. and Ghosh D. Efficacy of aqueous extract of seed of *Holarrhena antidysenterica* for the management of diabetes in experimental model rat: A correlative study with antihyperlipidemic activity. *Int J Appl Res Nat Prod.* **2**(3): 13–21 (2009).
25. Zhong S., Waterman P.G. and Jeffreys J.A.D. Naphthoquinones and triterpenes from African *Diospyros* species. *Phytochemistry.* **23**: 1067–72 (1984).
26. Panda S., Jafri M., Kar A. and Meheta B.K. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma. Fitoterapia.* **80**: 123–126 (2009).
27. Aratanechemuge Y., Hibasami H., Sanpin K., Katsuzaki H., Kunio I.K. and Komiya T. Induction of apoptosis by lupeol isolated from mokumen (*Gossampinus malabarica* L. Merr) in human promyelotic leukemia HL-60 cells. *Oncol Rep.* **11**: 289–92 (2004).
28. Bhattacharyya J. and Barros C.B. Triterpenoids of *Cnidosculus urens. Phytochemistry.* **25**: 274–276 (1985).
29. Ikuta A. and Itokawa H. Triterpenoids of *Paeonia japonica* callus tissue. *Phytochemistry.* **27**: 2813–15 (1988).
30. Chatterjee P., Pezzuto J.M. and Kouzi S.A. Glucosidation of betulinic acid by *Cunninghamella* species. *J. Nat Prod.* **62**: 761–763 (1999).
31. Patra A., Chaudhury S.K. and Panda S.K. Betulin-3-caffeate from *Quercus suber.* ^{13}C-NMR spectra of some Lupenes. *J Nat Prod.*; **51**: 217–220 (1988).
32. Yogeeswari P. and Sriram D. Betulinic acid and its derivatives: A Review on their biological properties. *Curr Med Chem.* **12**: 657–666 (2005).
33. Nyasse B., Nono J.J., Nganso Y., Ngantchou I., Schneider B. *Uapaca* genus (Euphorbiaceae), a good source of betulinic acid. *Fitoterapia.* **80**: 32–34 (2009).
34. Evans W.C. *Trease and Evans Pharmacognosy.* 15th Ed. (W.B. Saunders, Edinburgh, 2002) 294.