Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2013, 5(5):63-67
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Isolation and characterization of steroids and a triterpenoid from *Phyllanthus lawii*

Chandrashekar K. Subraya[1]*, Satyanarayana D.[2] and Yamini[1]

[1]Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, Karnataka, India
[2]Department of Pharmaceutical Chemistry, Vikas Institute of Pharmaceutical Sciences, Nidigatla Road, Rajahmundry, Andra Pradesh, India

---

ABSTRACT

Phyllanthus lawii Grah. is a glabrous shrub usually growing along the bank of rivers towards the Konkan Ghats.Treatment of P. lawii together with powdered sugar candy is taken orally for 7 days in stomach and other cancers barring brain and lung cancer. Extensive literature survey revealed that no systematic study on phytochemical investigation has been carried out so far. Hence an effort has been made to isolate and characterize the phytoconstituents from the whole plant of Phyllanthus lawii. Two steroids β-Sitosterol and Stigmasterol and a triterpenoidα- amyrin acetate has been isolated from aerial part of P.lawii and their structures were established by spectral analysis and direct comparison with authentic samples. This is a first report of occurrence of these compounds from P.lawii.

Key words: *Phyllanthus lawii*, triterpenoid, steroids

INTRODUCTION

Some species of Phyllanthus L. (Euphorbiaceae) are reported to be bitter, astringent, stomachic, diuretic, febrifuge, and antiseptic and have been used as hepatoprotective agents in Ayurvedic medicine[1,2]. The chemical and pharmacological properties and clinical studies of some Phyllanthus species have been summarized in a review article [3,4] . Of the Phyllanthus market samples, about 76% contains *P. amarus*Schum. &Thonn.and the remaining percentage is shared by several other species [5]. *Phyllanthus lawii*Grah. is a glabrous shrub usually growing along the bank of rivers towards the Konkan Ghats. It is also found in Nilatwar near Kaladghi, bank of the Gatpraba River, Bihar, Central Provinces and Western Peninsula[6] . Treatment of *P. lawii* together with powdered sugar candy is taken orally for 7 days in stomach and other cancers barring brain and lung cancer [7]. The aerial parts of *P. lawii* was tested for hepatoprotective activity against CCl4 in rats and it has shown significant activity, lowering the serum enzymes like SGOT and SGPT in rats intoxicated with CCl_4[8]. Diuretic activity in rats and hypothermic effect in mice of *P. lawii* has been reported earlier [9]. The present study is to investigate the phytoconstituents from the aerial partof *P. lawii*.

MATERIALS AND METHODS

All the melting points were recorded in a Toshniwal melting point apparatus and were uncorrected. UV spectra were recorded on uv-vis Spectrophotometer (Shimadzu uv – 160 A). IR spectra of the compounds were recorded using the KBr pellet method on a Perkin – Elmer model 700 IR spectrophotometer. 1HNMR spectra of the compound were taken on varian EM- 360 (270MHz) NMR spectrometer using $CDCl_3$ as solvent. ^{13}CNMR was recorded on Brukur instrument with DMSO – d6 as the solvent at 75.5 MHz. Mass spectra were recorded on a MAT 312 spectrophotometer and FAB – MS (positive) data on JEOL SX 102/DA–600. TLC was carried out using Si – gel (Merck). Column chromatography was carried out on Si gel (Merck, 70-230 mesh) and neutral alumina (S.D. fine chemicals Pvt.Ltd., Bombay). All the chemicals and reagents used were obtained in high purity either from S.D.fine chemicals Pvt. Ltd; Bombay, India or E.Merck Pvt. Ltd., Bombay, India.

Plant Material

The aerial parts (including leaves, flowers, stem and branches) of *P. lawii* were collected from Udupi, Karnataka, India during Oct – December 2002 and its botanical identity was confirmed by Dr. GopalkrishnaBhat, Department of Botany, Poornaprajna College, Udupi, Karnataka.

Extraction and Isolation

The shade dried powdered aerial parts (5 kg) were soaked in ethanol (95%) and kept aside for two days. After two days, the ethanol layer was decanted off. The process was repeated four times. The solvent from the total extract was distilled off and the concentrate was evaporated on a water bath to a syrupy consistency and then evaporated to dryness (750 gm).

The extract (750 g) was suspended in distilled water (1,500 ml) and then extracted with petroleum ether (60 – 80°C, 8 X 500 ml). The yield obtained was 65g. The petroleum ether extract (49 g) was saponified with 20% ethanolic KOH (300ml) for 2h. The contents of the flask were then evaporated to remove all traces of EtOH, the lost volume being replaced by water from time to time. The unsaponifiable portion was then extracted with ether (5 X 300ml). All the ethereal fractions were combined, washed with distilled water (40ml), dried over anhydrous sodium sulphate and the solvent was evaporated to afford a yellow residue (10 g).

The residue (10 g) was dissolved in $CHCl_3$ (20 ml) and adsorbed onto neutral alumina (20 g). After evaporation of the solvent it was loaded onto a neutral alumina column (150 g) prepared in petroleum ether (60–80°C). The column was eluted first with petroleum ether (60-80°C), petroleum ether (60-80°C): benzene graded mixtures (95:5, 90:10, 80:20 and 50:50), then with benzene followed by graded mixtures of benzene: chloroform (95:5, 90:10, 80:20 and 50:50), chloroform and finally chloroform : methanol (95:5,90:10, 80:20 and 50:50). The elution was monitored by TLC (Silica gel G; visualization: vanillin-sulphuric acid reagent heated at 110°C). Each time 5 ml were collected and identical eluates (TLC monitored) were combined and concentrated to 5 ml and kept in a refrigerator.

Elutions carried out with petroleum ether (60-80°C): benzene graded mixture 90:10 resulted in a mixture of three compounds. After removing the solvent, a residue 250 mg was resulted. This residue was subjected to preparative TLC in the solvent system viz: petroleum ether: benzene (90:10). The three compounds were isolated as pure compounds by recrystallisation with petroleum ether. These were designated as compound I (45 mg) and compound II (48 mg) and compound III (45mg).

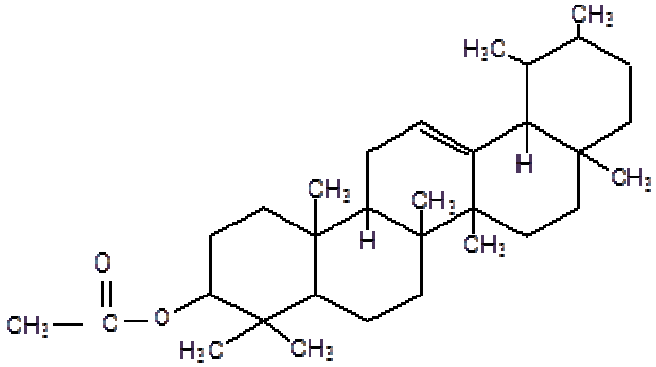
The concentration of other eluates gave only brown resinous masses which were not processed further.

RESULTS AND DISCUSSION

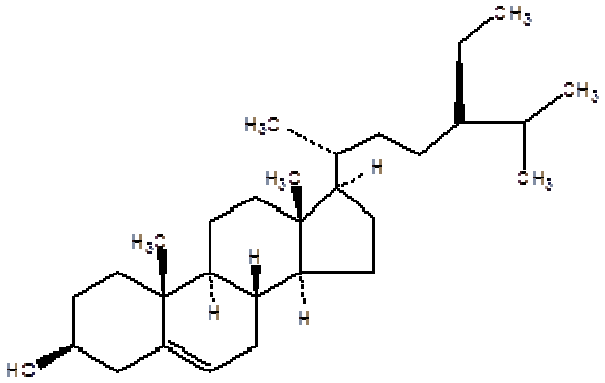
COMPOUNDI: (α -amyrin acetate). It wascrystallized from chloroform as white crystals, m.p. 226-227°C. IR (KBr cm^{-1})γ_{max}3417 (br, OH), 2942 and 2800 (C-H str. in CH_3 and CH_2), 1628 (C=C str.), 1461(C-H deformation in CH_3), 1377 (C-H deformation in gem dimethyl), 1056 (C-O str. of secondary alcohol). ^{1}HNMR ($CDCl_3$) δ 0.87 to δ 1.1 (m,18H, 6xCH_3), δ 1.1 to δ 1.20 (m,18 H, 9 x CH_2), δ 1.8 to δ 2.3 (8 H , methine protons), δ 3.5 (br, 1 H, CHOH), δ 5.36 (br,1 H, vinylic proton), δ 5.00 and δ 5.16 (2H, br, olefinic protons). ^{13}CNMR ($CDCl_3$) δ145 (C-5), δ138 (C-22), δ128 (C-23), δ121 (C-6), δ78 (C-3), δ56 (C-17), δ50 (C-20), δ51 (C-13), δ40 (C-4), δ38 (C-12), δ37 (C-15), δ33 (C-1),δ35 (C-8), δ35.1(C-10), δ32 (C-18), δ32.5 (C-7), δ29.12 (C-25), δ30.25 (C-24), δ30 (C-14),

δ28.24 (C-27), δ28 (C-29), δ31.6 (C-26), δ19.8 (C-19), δ18.7 (C-21), δ18.24 (C-18), δ26 (C-2), δ18.8 (C-28). Mass spectra (EI-MS) 412 (M+, $C_{29}H_{48}O$, 76 %), 394 (M+ - H_2O, 2%), 273 (M+- side chain, 8%), 255 (M+ - (side chain + H_2O 25 %).It gave positive response (pink colour) to Liebermann – Burchard's test for triterpenoids. An ester linkage was discernible from the characteristic IR adsorption at 1735.8cm^{-1}. The acetate nature of the ester was indicated by the characteristic acetyl protons singlet at δ 2.069. Further more mass fragment m/z 408 is suggestive of the loss of acetic acid (60) from the molecular ion (M^+) 468. The peaks at 250 and 218 (Base peak) m/z were due to Retro-Dials -Alder fragmentation [10] with the usual hydrogen transfer, characteristic of the left and right half arising from triterpene having δ^{12}-oleanane /ursane structure. The peak at 190 (250-60) m/z was due to loss of acetate moiety from the left half providing conclusive proof for the attachment of the acetate grouping at C-3.

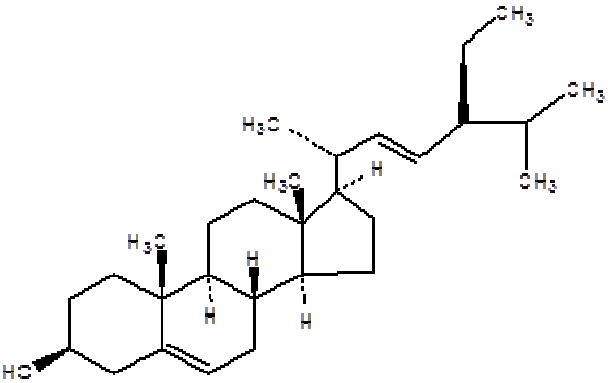
The fact that it is the acetate of α amyrin was established by saponification leading to the isolation of the triterpene alcohol, melting point of which (184°C) was in good agreement with that reported for α-amyrin (lit 186 °C)[11]



COMPOUND II: β(–Sitosterol)It wascrystallized from chloroform as white crystals, m.p.138 – 140 °C. IR (KBr cm^{-1}) γ_{max}3417 (br, OH), 2942, 2800(C-H str. in CH_3 and CH_2), 1628 (C=C str.), 1461 (C-H deformation in CH_3), 1377 (C-H deformation in gem dimethyl), 1056 (C-O str. of secondary alcohol). ^{1}HNMR ($CDCl_3$)δ 0.87 to 1.1(18 H,6 X CH_3), δ1.020 to 1.1 (22 H,11XCH_2), δ 1.8 to 2.3 (m, 8H, methine protons), δ 3.5 (m, 1H,OH), δ5.4 (m, 1H, Vinylic proton).^{13}CNMR ($CDCl_3$) δ145 (C-5), δ121 (C-6) δ56 (C-17), δ50 (C-13), δ45 (C-13), δ40 (C-4), δ36 (C-22), δ37 (C-5), δ33(C-1), δ35 (C-8), δ34 (C-23), δ35.1 (C-10), δ32 (C-16), δ33 (C-7), δ29.12 (C-25), δ30.25 (C-24), δ30 (C-14), δ28.24 (C-27), δ28 (C-29), δ31.6 (C-26), δ78.7 (C-28).Mass spectra (EI-MS) 414 (M^+, $C_{29}H_{50}O$,54%), 397 (18%), 329(12%), 303(10%), 288(4%), 273 (10%), 255 (M^+ - Side chain +H_2O, 6%), 231 (10%), 199(20%), 161 (30%), 147 (34%), 133 (24%), 105 (50%), 91 (76%), 71 (44%) 57 (100%).It gave positive response (pink colour) to Liebermann – Burchard's test for steroid.The yellow colour obtained with tetranitromethane confirmed unsaturation in the molecule. Its acetate melted at 126-128°C (lit 127-128°C) [12]. ^{13}CNMR spectral data matched exactly with that of β–Sitosterol [13]. The most downfield signals at δ145 was accommodated for sp^2 (olefinic) carbon at C-5 and the next downfield signal at δ121 to C-6 carbon. The oxygenated carbon at C-3 gave a downfield signal at δ 78 ppm. The next downfield at δ56 was accommodated for C-17. Other carbon atoms of the steroidal skeleton except that in the side chain appeared in the range δ45-δ30 ppm. The angular methyl groups and the side chain methyl carbons gave signal in the region δ19.8 – δ 18.24 ppm. Its identity as β–sitosterol was further confirmed by IR, ^{1}HNMR spectral characters and by co-chromatography with an authentic sample (Sigma Chemical Co., USA).



COMPOUND III:(Stigmasterol) It wascrystallized from chloroform as white crystals, m.p.167-170°C.IR (KBr cm-1) γmax3351(br, OH), 2919 and 2800 (C-H str. in CH_3 and CH_2), 1630 and 1627 (C=C str.), 1457 (C-H deformation in gem dimethyl), 1057 (C-O str. of secondary alcohol), 625 (rocking vibration of CH_2).1HNMR ($CDCl_3$) δ 0.87 to δ 1.1 (m,18H, 6xCH_3), δ 1.1 to δ 1.20 (m,18 H, 9 x CH_2), δ 1.8 to δ 2.3 (8 H , methine protons), δ 3.5 (br, 1 H, CHOH), δ 5.36 (br,1 H, vinylic proton), δ 5.00 and δ 5.16 (2H, br, olefinic protons). ^{13}CNMR ($CDCl_3$)δ145 (C-5), δ138 (C-22), δ128 (C-23), δ121 (C-6), δ78 (C-3), δ56 (C-17), δ50 (C-20), δ51 (C-13), δ40 (C-4),δ38 (C-12), δ37 (C-15), δ33 (C-1),δ35 (C-8), δ35.1 (C-10), δ32 (C-18), δ32.5 (C-7), δ29.12 (C-25), δ30.25 (C-24), δ30 (C-14), δ28.24 (C-27), δ28 (C-29), δ31.6 (C-26), δ19.8 (C-19), δ18.7 (C-21), δ18.24 (C-18), δ26 (C-2), δ18.8 (C-28).Mass spectra (EI-MS)412 (M^+, $C_{29}H_{48}O$, 76 %), 394 (M^+ - H_2O, 2%), 273 (M^+- side chain, 8%), 255 (M^+ - (side chain + H_2O 25 %). It showed positive response to Liebermann Burchardt's test and Salkowski's test. It formed an acetate m.p. 143 –145°C (lit 144°C) [14]. ^{13}CNMR spectral data matched exactly with that of Stigmasterol [15, 16, 17]. The most downfield signals at δ145 was accommodated for sp^2 (olefinic) carbon at C-5 and the next downfield signal at δ138 ppm and 128 ppm to C-22 and C-23. The downfield signal at δ 121 is to C-6. The oxygenated carbon at C-3 gave a downfield signal at δ78 ppm. The next downfield signal at δ56 ppm was accommodated for C-17. Other carbon atoms of the steroidal skeleton except that in the side chain appeared in the range δ 45- δ 30 ppm. The angular methyl groups and the side chain methyl carbons gave signals in the region δ 19.8 – δ 18.24 ppm. Its identity as stigmasterol was further confirmed by IR, ^{1}HNMR spectral characters and by co–chromatography with an authentic sample (Sigma Chemical Co., USA).



CONCLUSION

The constituents isolated and characterized from aerial part of *P.lawii* in the present study can be categorized under the sterols andtriterpene. The constituents isolated from aerial parts of *P.lawii*consisted of twosterolsnamelyβ–Sitosterol and stigmasterol and a triterpenoids namelyα-amyrinacetate. These compounds were isolated for first time from this plant.

Acknowledgements
Authors are thankful to Nitte University for providing necessary facility to carry out this research work.

REFERENCES

[1]. K. Sayyada.,R.Vartika.,K.R. Ajay.,M. Shanta., *J. Ethnopharmacol*.,**2006**, 104, 79-86.
[2]. K.S.Chandrashekar., S. Santanu., K.S. Prasanna.,*Pharmaceutical Crops*, **2011**, 2, 8-10
[3]. B.C. João.,R.S. Adair., C.F. Valdir., A.Y. Rosendo., *Med. Res. Rev*., **1998,** 18, 225-258.
[4]. K.S. Chandrashekar.,A.B. Joshi..,D.Satyanarayana., E.V.S. Subrahmanyam., *Natural Product Sciences*, **2004**; 10(3): 101–103.
[5]. R. Srirama., U.Santilkumar., N.Sreejayan., G. Ravikanth., B.R.Gurumurthy., M.B.Shivanna., M.Sanjappa., K.N.Ganeshaiah.,R. Uma Shaanker., *J. Ethnopharmacol*., **2010**, 130, 208-215.
[6]. T. Cooke.,*The Flora of the Presidency of Bombay*, **1906**, Vol III, 82.
[7]. A.R. Prust.i,K.K. Behera., *Ethnobot. Leaflet*, **2007**, 11, 148-163.
[8]. K.S. Chandrashekar., K.S. Prasanna., *Intl. J. Pharm. Biol. Arch*., **2010,** 1, 187-188.
[9]. B.N. Dhawan., G.K. Patnik., R.P. Rastogi., K.K. Singh., J.S. Tandonn., *Ind. J. Exp. Biol*., **1977**, 15, 208-219.
[10]. H. Budzikzewic., J.M. Wilson.,C.J. Djerassi.,*AmChemSoc***1963;** 85: 3688.
[11]. The Merck Index of Chemicals, *Drugs and Biologicals*, 12th ed., Merck and Co (NJ), Merck research laboratories; **1966**; 659.
[12]. The Merck Index of Chemicals, *Drugs and Biologicals*, 12th ed., Merck and Co (NJ), Merck research laboratories; **1966**; 8698.
[13]. .J.B. Harborneand T.J. Mabry.*The flavonoids: Advances in research*, Chapmann and Hall, New York, **1982,** 41.
[14]. The Merck Index of Chemicals, *Drugs and Biologicals*, 12th ed., Merck and Co (NJ), Merck research laboratories; **1966**; 8976.
[15]. J.L.C. Wright., A.G. Mclnnes., J.G. Shimizu Smith, J.A.Walter, D.Idler, W.Khalil,*Can J Chem***1978,** 56: 1898.
[16]. A. B. Joshi, P. M. Surlikar, M. Bhobe, *Der PharmaChemica*, **2013**, 5(4):112-115
[17]. K.S. Chandrashekar, S. Santanu,K.Prasanna. *Der PharmaChemica*, **2010**, 2(5):434-437