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Phytochemical Investigation of *Plumbago capensis* Thunb.

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Abstract

Capensisone, isoshinanolone, diomuscinone , α-amyrin acetate, plumbagin, α-amyrin , β-sitosterol and β-sitosterol-3β-D- glucoside have been isolated from the methanol extract of the roots of *Plumbago capensis***.** Capensisone, a novel quinone is the first report from the roots of *Plumbago capensis*. The structure of these compounds has been established on the basis of FT- IR, ^{1}H NMR, and ^{13} C NMR spectral data.

Key-Words: *Plumbago capensis,* Capensisone, Isoshinanolone, Diomuscinone

Introduction

Plumbago capensis Thunb. Syn. *P. auriculata* (Plumbaginaceae) is a small sub-scandent shrub with oblong spathulate leaves and pale blue flowers, indigenous to South Africa and grown in gardens in India as an ornamental plant. In Tamil it is known as neela kodiveli, Krishna chithraka in Sanskrit and blue colured lead wort in English. A decoction of the plant is taken as a remedy for black water fever. The powdered root is taken as a snuff to relieve headache; it caused warts to disappear when smeared over them[1-2]. Anti-inflammatory activity of hydro alcoholic extract of the root of *P. capensis* showed a maximum of 46.88 % inhibitory action on carrageenan induced paw edema at the dose of 250 mg/kg and inhibited the leukocyte migration in a dose dependent manner[3]. Crude antifungal protein 20 KD from the young leaves was isolated and its toxic property was tested against the pathogen, *Macrophomina phaeolina*[4]. Flowers of the plant reported to contain azalein and capensinidin, capensinidin-3-rhamnoside[5]. Leaves contain apigenin, luteolin and their glycosides[6]. Preliminary chemical examination on the roots of karunkoduveri has been reported[7]. As there were no reports on the chemicals present in the roots, the present paper deals with the phytochemical investigation of the methanolic extract of the roots of *P. capensis.*

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Material and Methods

Collection of plant materials

Roots of *Plumbago capensis* were procured from local market at Nagercoil, Tamil Nadu. The drug was identified by Dr. P.Brindha, botanist at Captain Srinivasa Murti Drug Research Institute for Ayurveda and Siddha (CCRAS), Arumbakkam Chennai-106. A voucher specimen *P. capensis* (00583), have been deposited in the herbarium of this institute.

Solvent Extraction

2 kg of the air dried and coarsely powdered roots of the plant were extracted with methanol in an aspirator bottle by cold percolation method (72 h).The solvent was removed by distillation on a water bath, the last traces being removed *in vacuo* . All chemicals and solvents used were of analytical grade. All melting points are uncorrected and taken on a heating block apparatus. FT- IR – spectra were taken on Perkin Elmer grating spectrophotometer in KBr disc. The ^{1}H NMR and ^{13}C NMR spectra were taken on Bruker instrument at 400 and 100 MHz, respectively. The solvents used were $CDCl_3$, DMSO- d_6, CD_3OD with TMS as the internal standard[8-9].

Column Chromatography

Acme's silica gel (100-200 mesh or finer than 200 mesh) was used for column chromatography. The methanol extract was chromatographed over silica gel (1:22) and eluted with solvents of increasing polarity in the order n-hexane, benzene, chloroform, ethyl acetate and methanol, respectively. The fractions were monitored by TLC over silica gel G plates (20 x 5 cm).

Acme's silica gel G with gypsum as binder was used. Visualisation was carried out either by exposure to iodine vapour or by spraying with 1: 1 H_2SO_4. Earlier fractions yielded α-amyrin acetate, plumbagin, α-amyrin, β-sitosterol and β-sitosterol-3- β-D-glycopyranoside .

Compound 1 (Diomuscinone)

Earlier fractions of the column eluted with n-hexane, and mixtures of n-hexane and benzene yielded α-amyrin acetate, plumbagin, α-amyrin, β-sitosterol. Elution of the column with benzene gave a compound which answered for phenol and quinone. It is purified by rechromatography over silica gel and is obtained as yellow crystals from n-hexane - ether (mp 85ºC, Literature mp 85 -85.5ºC) yield, 30 mg. It gave a single spot (R_f = 0.6) on TLC over silica gel with toluene: ethyl acetate (4:1) as the developing system. On exposure to ammonia vapours the yellow spot changed to orange showing it to be a quinone. It also gave positive ferric reaction for phenol.

IR ν max (KBr) cm^{-1}: 3423 (chelated hydroxyl), 2924, 2854, 1624 (chelated carbonyl), 1640 (unchelated carbonyl), 1614, 1456, 703 (aromatic system). ^{1} H NMR (δ, $CDCl_3$, 400 MHz) : 1.18(3H, s, - CH_3), 2.69, 3.33 (1H, each, d, J = 16.8 Hz, H-3), 3.43, 3.95(1H each, d, J = 11.2 Hz -C**H**$_2$ OH), 7.29(1H, m, overlapping with solvent signal, H-6), 7.5 (1H, m, H-7), 7.6 (1H m, H-8), 11.9 (1 H, br s, C-5 OH). ^{13}C NMR (δ, $CDCl_3$, 100 MHz) : 202.9(C-1), 50.5(C-2), 46.6(C-3), 201.0(C-4),161.2(C-5), 118.8(C-6), 137.0(C-7), 124.1(C-8), 134.0(C-9), 117.4 (C-10), (67.8 -CH_2OH), 21.5 (-CH_3).

Compound 2 (Capensisone)

Chloroform extract is column chromatographed over silica gel. Elution of the column with chloroform gave a pale yellow amorphous solid. It answered for phenol but not for quinone. It gave a single spot (R_f = 0.4) on TLC over silica gel with toluene: ethyl acetate 4:1 as the developing system. The spot appeared fluorescent yellow in UV 366 nm and turned blue on spraying with vanillin - sulphuric acid reagent and heating the plate at 105°C till the colour of the spots appeared.

IR ν_{max} (KBr) cm^{-1} : 3436 (chelated hydroxyl), 2920, 2850, 1645 (chelated carbonyl), 1455, 1257, 1027, 704. ^{1}H NMR (δ ppm,$CDCl_3$, 400 MHz) : 1.29(3H, s, - CH_3), 4.78(d, J = 2.8 Hz, H-1), 2.47 (1H, m, H-2), 2.61, 2.86(1H, each, m, H-3 eq, and H-3 ax), 6.96(1H, m, H-6 and H-8), 7.51(1H, m, H-7), 12.45(1H, br s, C-5 OH).

Compound (3) Isoshinanolone

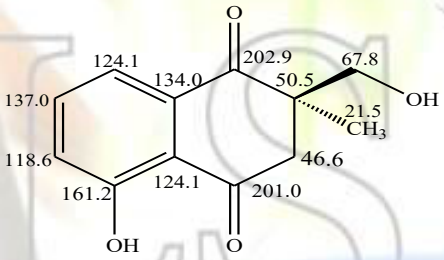
Elution of the column with ethyl acetate gave colourless crystalline solid (mp 256ºC, decomp., literature. mp 255ºC) on crystallisation from methanol. It answered positive ferric reaction for phenol but not for quinone. It gave a single spot (R_f = 0.52) on TLC over silica gel with toluene: ethyl acetate (2:3) as the developing system.

^{1}H NMR (δ, DMSO, 400 MHz) : 4.65(1H, d, J = 2.3 Hz H-1), 2.32 – 2.38(1H,m, H-2), 0.99(3H, d, J = 6.8 Hz, - CH_3), 2.61(1H, dd, J = 17 and 4.5 Hz, H-3 eq.), 2.70(1H, dd, J = 17 and 9.3 Hz, H-3 ax.), 6.88(1H, br d, J = 8.3 Hz H-6), 7.00(1H, br d, J = 7.5 Hz, H-8), 7.55(1H, m, H-7), 5.38(1H, br s, C-1 OH), 12.38(1H, br s, C-5 OH).

Results and Conclusion

Systematic fractionation of the methanolic extract of *P. capensis* resulted in the isolation and identification of α-amyrin acetate, plumbagin, α-amyrin, β-sitosterol along with diomuscinone (1), capensisone (2) and isoshinanolone (3) and β-sitosterol-3- β-D-glycopyranoside. Compounds 1, 2 and 3 are related to plumbagin, the naphthaquinone isolated from all species of the genus *Plumbago*. All the known compounds were identified by comparison with authentic sample (by comparison with mp, mmp, super imposable IR and co-TLC).

Compound 1 (Diomuscinone)



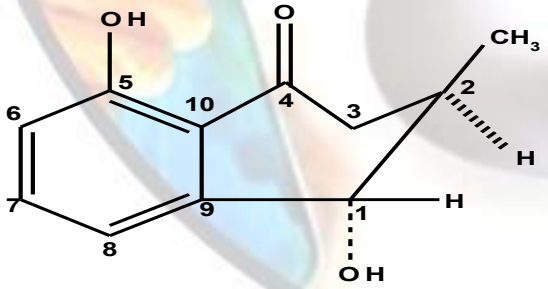
(1)

The ^{1}H NMR and ^{13}C NMR spectra of the compound confirmed its structure as diomuscinone. The fact that 2(3) double bond in plumbagin (2-methyl -5- hydroxy 1,4 – naphthaquinone) is reduced and C-2 is attached to –CH_2OH group instead of a methyl group which was evident from both^{1}H and ^{13}C NMR spectral data. In the ^{1}H NMR, the methyl proton attached to C-2 appeared as singlet at δ 1.18. H-3 methylene protons each appeared as doublet at δ 2.69 and δ 3.33 as one proton doublets with J = 16.8 Hz. The large coupling constant is due to the geminal coupling of the protons in the ring system. Hydroxy methylene protons at C-2 appeared at δ 3.43 and 3.95 each as a one proton doublet with J=11.2 Hz. The presence of CH_2OH group was also evident in the ^{13}C NMR spectrum as the hydroxy methylene carbon appearing at δ 67.8. The three aromatic protons in ring A as in plumbagin appeared in the region δ 7.29 – 7.6. The chelated hydroxyl proton attached to carbon C-5 was seen as a broad singlet at δ 11.9. ^{13} C NMR spectrum showed the presence of 12 carbons. There were one quartet, two triplets, three

doublets and six singlets. Of the six singlets, the two quinone carbonyls appeared at δ 202.9 (C-1) and 201.0 (C-4). The carbon C-2 attached to hydroxy methylene and methyl group appeared at δ 50.5. C-5 carrying phenolic hydroxyl was observed at δ 161.2. The two C-9 and C-10 carbon atoms at the ring junction appeared at δ 134.0 and 117.4, respectively. The three aromatic carbons C.6, C-7 and C-8 appeared as doublets at δ 118.8, 134.0 and 124.1 respectively. The two triplets at

Compound 2 (Capensisone)

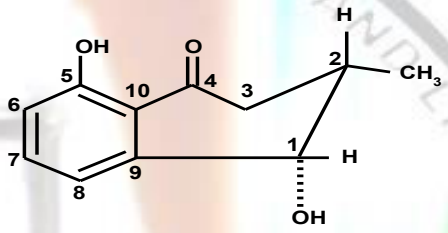
The IR spectrum showed chelated hydroxyl 3436, and chelated quinone carbonyl 1645 cm-[1]. The [1]H NMR of the compound was very similar to that of isoshinanolone. It showed the presence of three aromatic protons of ring A along with the chelated phenolic hydroxyl at C-5. The two H-3 protons adjacent to the carbonyl also appeared at δ 2.61(H-3 eq.) and 2.86 (H-3 ax.) similar to isoshinanolone. H-1 appeared at δ 4.78 as doublet coupling to H-2 with J = 2.8 Hz showing equatorial – equatorial coupling. This can be explained by inverting the configuration of isoshinanolone at C-2, the methyl group becoming axial. The chemical shifts of both the methyl and H-2 have changed with respect to isoshinanolone. The methyl group has moved downfield to δ 1.29 from δ 0.99. Similarly, H-2 moved downfield to δ 2.47 from δ 2.35. The configuration at C-1 must be the same as isoshinanolone since the chemical shift value for H-1 has not much changed. For axial H-2 as in epi-isoshinanolone[10] the chemical shift value is upfield at δ 4.30. Hence, capensisone must be (**2**) being the C-2 epimer of isoshinanolone. This is a new natural product reported. The other isomer with diaxial protons at C-1 and C-2 reported was epi-isoshinanolone reported by Bhattacharaya *et al* (1986) together with isoshinanolone from *Plumbago zeylanica*.



(2)

Compound (3) isoshinanolone

δ 67.8 and 46.6 corresponded to the hydroxy methylene carbon and C-3. The methyl quartet appeared at δ 21.5. Based on the above data, compound **1** was identified as diomuscinone. The compound has been previously reported from the methanolic extract of the leaves and roots of *Dionaea muscipula*[11]. This is the first report of the compound from the genus *Plumbago*.



(7)

Compound **3** eluted with ethyl acetate answered for phenol but not for quinone.The compound was identified as isoshinanolone in which the unchelated carbonyl of plumbagin is reduced to alcohol while ring A of plumbagin is intact.The hydroxy methine proton at C-1 which is equatorial appeared at δ 4.65 as one proton doublet of J = 2.3 Hz due to coupling with the axial proton at C-2. H-2 appeared as a multiplet at δ 2.32 – 2.38 Hz. The equatorial methyl at C-2 appeared as a three proton doublet (J = 6.8 Hz) at δ 0.99. H-3 eq and H- 3 ax appeared each as one proton double doublets at δ 2.61 and 2.70 respectively. H-6 and H-8 appeared at δ 6.88 and 7.0 as broad doublets (J = 8.3 and 7.5 Hz) due to ortho coupling with H-7. H-7 appeared as one proton multiplet at δ 7.55. The presence of chelated phenolic hydroxyl at C-5 was confirmed by the broad peak at δ 12.38 integrating for one proton. The alcoholic proton at C-1 appeared as a broad peak at δ 5.38. The above data corresponded to those reported for isoshinanolone. Isoshinanolone has been reported from *Diospyros maritime*[12]and later from *Plumbago zeylanica*[13].

Phytochemical examination of the methanol extract of *P.capensis* roots has yielded α-amyrin acetate, plumbagin, α-amyrin, β-sitosterol, diomuscinone, capensisone, isoshinanolone and β-sitosterol-3β–glucopyranoside. Isoshinanolone and diomuscinone are reported for the first time from the genus *Plumbago*. Capensisone is a new natural product characterized from *P. capensis*.

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