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Phytochemical Constituents of Root Bark of *Alstonia scholaris*. R. Br

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Two triterpenoids α-amyrin acetate and lupeol, and a steroid β-sitosterol have been isolated from the root bark of *Alstonia scholaris* and their structures were established by spectral analysis.

Key Words: *Alstonia scholaris*, Triterpenoids, Steroids, Apocyanaceae.

INTRODUCTION

Alstonia scholaris R. Br (Apocyanaceae) commonly known as "Devils tree" or "Dita bark", is a widely distributed in South and Southeast Asia. The bark of this tree is used in traditional medicines throughout the region to treat dysentery and Malaria[1]. *Alstonia scholaris* is known to be rich of alkaloids and there is interest among the scientists to use this for therapeutic purposes, *i.e.* alkaloids stand as a class of major importance of development of new drugs because alkaloids posses a great variety of chemical structures and have been identified as responsible for pharmacological properties of medicinal importance[2]. Almost all the parts of plant (bark, flowers, root, leaves) are found to contain active principles. The species of *Alstonia scholaris* is used[3] in commercial formulation Ayush 64. The new indole alkaloids were isolated from the bark of *Alstonia scholaris* collected in Timor Indonesia. The structures of all compounds were elucidated by spectroscopic method[4]. Various authors have described in their publications, some of indole alkaloids picrinine[5], scholarine[6], scholaricine[7], new indole alkaloids like alstonamine and strickine[8] and other several alkaloids from the leaves of *Alstonia scholaris* in Taiwan, Thailand, Indonesia and Philippines[9].

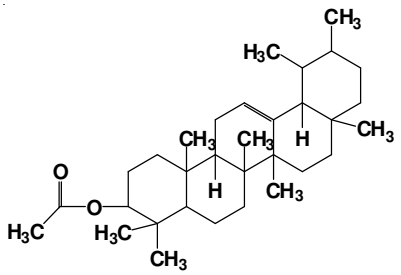
In this paper, the isolation and characterization of major triterpenes and sterol from the root bark of *Alstonia scholaris* is reported.

EXPERIMENTAL

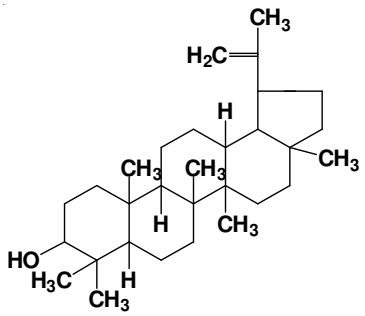
Alstonia scholaris (Apocynaceae) was collected from the medicinal garden of SDM College of Ayurveda, Udupi and authenticated by Dr. T. Sridhar Bairy by comparison with the standard specimens deposited at the Department of Drava Guna, SDM College of Ayurveda, Udupi. Voucher specimen is kept at the NGSM Institute of Pharmaceutical Science, Deralakatte, Mangalore, India.

Extraction and isolation: The air-dried, powdered root bark of *Alstonia scholaris* was extracted (3 kg) with petroleum ether by cold percolation (8-10 draining were necessary) and this extract was concentrated to remove the solvent. The bright yellowish coloured residue (52 g) was dissolved in 500 mL of ethyl alcohol, boiled for 15 min on water bath, cooled in a refrigerator for 0.5 h. Precipitation of wax took place, it was then filtered and the filtrate was concentrated. The concentrated (30 g) was dissolved in $CHCl_3$ (20 mL) and chromatographed through a column of Brockmann alumina (diam. 4 cm × length 32 cm). The column being successively eluted with petroleum ether and benzene. Total 500 fractions of each 10 mL were collected. Fractions of 186-265 eluent gave 2 spots on TLC. These were combined and concentrated to 10 mL when it yielded as white solid. It was separated by preparative TLC using silica gel (petroleum ether:benzene; 8:2) into compound **1** and **2**. Pure compound **1** (69 mg) was obtained through recrystaliaztion from petroleum ether. Pure compound **2** (54 mg) was obtained through recrystillization from petroleum ether. Fraction 270-292 gave **1** spot on TLC. These were combined and concentrated (compound **3**). The compound **3** (72 mg) was obtained through recrystilazation.

Compound 1 (α-amyrin acetate): Compound **1**, (69 mg), crystallized from the mixture of ethanol and ether as a colourless solid; m.p. 244-246 ºC shown positive Liberman's Burchards test for triterpenoids. IR (KBr, γ_{max}, cm^{-1}): 2944, 2871 (C-H *str.* in CH_3 and CH_2), 1715 (ester, C=O *str.*), 1642 (C=C *str.*), 1463 (C-H deformation in CH_3) 1380, 1361 (C-H gem dimethyl *str.*), 1245 (C-O *str.* in acetate), 1096 (C-O *str.* in secondary alcohol); EIMS m/z (rel. int., %) m/z 468 [M^+] ($C_{32}H_{52}O_2$); FABMAS (+ve mode) m/z 453 (12), 425 (52), 409 (48), 218 (100), 453 (12), 189 (25), 135 (54), 109 (59), 95 (70); ^{13}C NMR ($CDCl_3$): δ 38.1 (C-1), 27.0 (C-2), 77.3 (C-3), 38.5 (C-4), 55.3 (C-5), 18.2 (C-6), 32.7 (C-7), 39.6 (C-8), 47.7 (C-9), 37.1 (C-10), 23.4 (C-11), 121.2 (C-12), 145.2 (C-13), 41.5 (C-14), 26.2 (C-15), 27.5 (C-16), 32.5 (C-17), 47.3 (C-18), 46.8 (C-19), 29.9 (C-20), 34.8 (C-21), 34.8 (C-21), 37.1 (C-22), 28.3 (C-23), 14.7 (C-24), 15.7 (C-25), 16.9 (C-26), 25.9 (C-27), 28.7 (C-28), 33.2 (C-29), 23.7 (C-30), 170.7 (C=O, s), 21.1 (CH_3-C=O). It was found to be identical with α-amyrin acetate on comparison with authentic sample (mixed. m.p., CO-TLC and Superimposible IR).

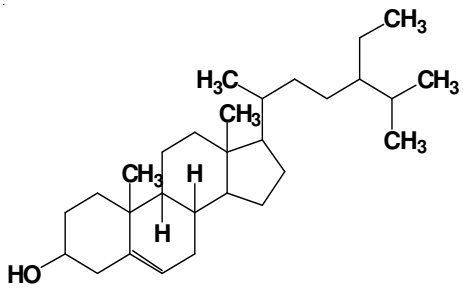


Compound 2 (Lupeol): Compound **2** (53 mg), crystallized from the mixture of ethanol and ether as a colourless needles; m.p. 215-217 ºC shown positive Liberman's Burchards test for triterpenoids. IR (KBr, γ_{max}, cm^{-1}): 3316 (OH *str.*), 2944 (C-H *str.* in CH_3 and CH_2), 1638 (C=CH_2), 1189 (C=C *str.*), 1042 (C-O *str.* in secondary alcohol); EIMS m/z (rel. int., %) 426 [M^+] ($C_{30}H_{50}O$) (55), 411 (25), 393 (7), 315 (20), 218 (68), 207 (79), 189 (88), 175 (38), 161 (43), 147 (42), 135 (67), 121 (71), 109 (100), 81 (92), 69 (76); ^{13}C NMR ($CDCl_3$): δ 79.0 (C-1), 41.1 (C-2), 45.3 (C-3), 32.0 (C-4), 47.5 (C-5), 36.1 (C-6), 52.3 (C-7), 41.6 (C-8), 55.3 (C-9), 43.0 (C-10), 40.0 (C-11), 26.2 (C-12), 27. 3 (C-13), 33.3 (C-14), 17.4 (C-15), 35.1 (C-16), 24.1 (C-17), 30.4 (C-18), 28.7 (C-19), 43.5 (C-20), 49.9 (C-21), 20.0 (C-23), 19.3 (C-24), 21.5 (C-25), 14.6 (C-26), 18.4 (C-27), 18.0 (C-28), 145.2 (C-29), 109.3 (C-30), (>C-OH), 21.3 (C-31). It was found to be identical with lupeol on comparison with authentic sample (mixed. m.p., CO-TLC and Super-imposible IR).



Compound 3 (β-sitosterol): Compound **3** (64 mg), crystallized from the mixture of ethanol and ether as a colourless crystals; m.p. 137-139 ºC shown positive Liberman's Burchards test and Salkowski test for sterols. IR (KBr, γ_{max}, cm^{-1}): 3374 (OH *str.*), 2944, 2871 (C-H stretching in CH_3 and CH_2), 1641 (C=C *str.*), 1381 (C-H deformation in CH_3), 1095 (C=O *str.*), 996 (C-H deformation out of plane); EIMS m/z (rel. int., %) m/z 414 [M^+] ($C_{29}H_{50}O_2$); FABMAS (+ve mode) m/z 396 (85), 381 (38), 365 (5), 329 (30), 309 (9), 289 (14), 273 (15), 241 (5), 231 (30), 213(83), 199 (20), 173 (22), 159 (40), 145 (37), 133 (93), 107 (90), 95 (100); ^{13}C NMR ($CDCl_3$):

δ 31.0 (C-1), 30.4 (C-2), 37.2(C-3), 46.9 (C-4), 139.6 (C-5), 121.0 (C-6), 31.3 (C-7), 31.8 (C-8), 50.5 (C-9), 36.4 (C-10), 21.3 (C-11), 40.0 (C-12), 42.2 (C-13), 55.3 (C-14), 23.7 (C-15), 28.7 (C-16), 55.3 (C-17), 13.9 (C-18), 19.3 (C-19), 35.6 (C-20), 18.4 (C-21), 34.8 (C-22), 30.0 (C-23), 41.5 (C-24), 33.8 (C-25), 21.5 (C- 26), 22.5 (C-27), 106.0 (C-28), 28.1 (C-29). It was found to be identical with ß-sitosterol on comparison with authentic sample (mixed. m. p., CO-TLC and Superimposible IR).



RESULTS AND DISCUSSION

Chromatographic resolution of petroleum ether extract of root bark of *Alstonia scholaris* furnished compound **1**, **2** and **3** which were characterized as α-amyrin acetate, lupeol and β-sitosterol by detailed spectral analysis *i.e.*, IR, ^{13}C NMR, FABMAS and direct comparison with authentic samples (m.p. CO-TLC and Superimposable IR). All the above compounds are being reported for the first time from this plant.

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