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Phytochemical investigation of the roots of *Wattakaka volubilis*

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ABSTRACT

Phytochemical investigation of the roots of Wattakaka volubilis led to the isolation of steroid like β-sitosterol; a triterpenoid aglyconedrevogenin A; fatty acid 9, 12 – octadecadienoic acid; a phenolic compound quinic acid; aromatic ester 1, 2 – benzenedicarboxylic acid diisooctyl ester; a flavonoid 5, 7 – dihydroxy – 6, 8 – dimethoxyflavone; an alkaloid N-[4-bromo-n-butyl]-2-piperidinone and a desoxy sugar digitoxose from the ethanolic extract. IR, ^{1}HNMR and EI-MS spectrometric data were used for the characterization of above mentioned compounds. All these eight compounds have been reported for the first time from the roots of Wattakaka volubilis.

Key words: Asclepiadaceae, Digitoxose, Drevogenin A, Ethanol.

INTRODUCTION

Wattakaka volubilis (Linn.f.)Stapf., (Syn. *Dregea volubilis* (L.f.) Benth. ex Hook.f., *Marsdenia volubilis* Cooke) (Family: *Asclepiadaceae*) is a tall woody climber, 11m high & 95cm in girth, with densely lenticellate&pustular branches[1]. It is found distributed in Bengal, Assam, Konkan, Maharashtra, Deccan, Bangalore, Mysore, Sri Lanka and districts of Madras[2-5]. The leaves are much employed as an application for boils and abscesses. The roots and tender stalks are considered emetic and expectorant[2]. It is also used in eye diseases and snake bites[6]. A pentacyclic triterpenoid designated as taraxerol, characterized as D-friedoolean-14-en-3-ol has been reported from the fruits [7]. Unusual novel triterpenoid ether, multiflor-7-en-12, 13-ether from hexane extract and a new multiflor-7-en-12α-ol has been reported from acetone extract of the leaves of *Wattakaka volubilis*[8]. Antitumor effect has been reported from petroleum extract of fruit in Ehrlich ascites carcinoma bearing mice [9]. This species has been extensively investigated and a number of phytoconstituents have been isolated[10, 11]. The present study deals with the isolation and characterization of phytoconstituents from the ethanolic extract of the roots of *Wattakaka volubilis*.

MATERIALS AND METHODS

All the melting points were recorded on Model No. BT2-38 melting point apparatus and were uncorrected. IR spectra of the compounds were recorded using the KBr pellet method on Bruker α – T Spectrophotometer and Shimadzu FTIR 8700 Spectrophotometer. ^{1}HNMR spectroscopic data of the compounds were carried out on Amx-200 liquid state PMR spectrophotometer using $CDCl_3$ and DMSO as a solvent. Mass spectra were recorded on ECMS 2010 PLUSH 200 MHz Shimadzu. TLC was carried out using Aluchrosep Silica Gel 60/$UV_{254}$and Silica Gel (200-400 mesh) was used for column chromatography.

Plant material:
The roots of *Wattakaka volubilis* were collected from GomantakAyurvedicMahavidyalaya and Research Centre, Shiroda - Goa by Dr. S. K. Das during November, 2011. It was authenticated by Prof. G. I. Hukkeri, Associate Professor, Department of Botany, Dhempe College of Arts and Science, Miramar – Goa.

Extraction and Isolation:
The dried roots were powdered (500g) and exhaustively extracted by maceration with ethanol for three days. After three days, the ethanolic layer was decanted off. The process was repeated thrice. The solvent from the total extract was distilled off and the concentrate was evaporated to a syrupy consistency and then evaporated to dryness (25g).

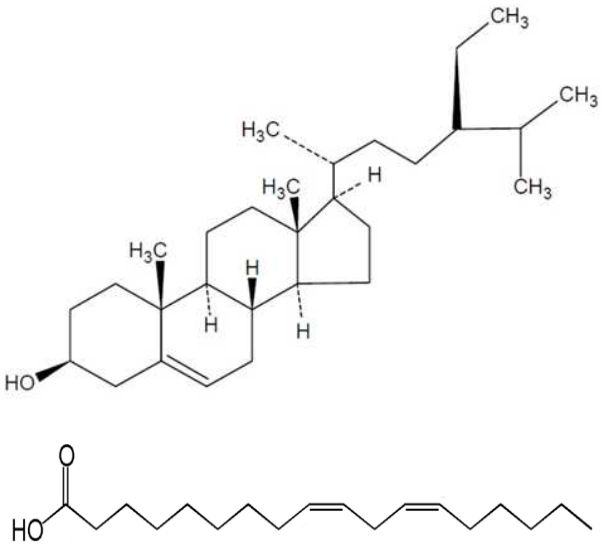
The ethanol soluble fraction (10 g) was taken and subjected to column chromatography over silica gel prepared in petroleum ether (60-80). The column was eluted with petroleum ether (60-80) (100%) followed by petroleum ether (60-80) : chloroform graded mixtures (95:5, 90:10, 80:20, 70:30, 50:50); chloroform 100% followed by chloroform : ethyl acetate graded mixtures (95:5, 90:10, 80:20, 70:30, 50:50); ethyl acetate 100% and finally ethyl acetate : methanol graded mixtures (99:1, 98:2, 97:3, 96:4, 95:5, 90:10, 80:20).

The elution's were monitored by TLC (Silica gel-G); visualization by UV 254nm, 366nm and Vanillin - Sulphuric acid reagent heated at 110°C). Each time 10 ml were collected and identical elutes (TLC monitored) were combined and concentrated to 5 ml and kept in a refrigerator. Eight compounds were isolated as pure components. These were designated as compound I (petroleum ether (60-80) : chloroform – 90 : 10) (25 mg), compound II (petroleum ether (60-80) : chloroform – 80 : 20) (35 mg), compound III (petroleum ether (60-80) : chloroform – 60 : 40) (45 mg), compound IV (chloroform : ethyl acetate – 60 : 40)(0.9 ml),compound V (chloroform : ethyl acetate – 50 : 50) (33 mg), compound VI (ethyl acetate 100%), compound VII (ethyl acetate : methanol – 95 : 5) and compound VIII (ethyl acetate : methanol – 80 : 20)

RESULTS AND DISCUSSION

Compound I (9, 12- octadecadienoic acid): Brown semi solid, b.p 230 °C. IR (KBr pellet): ν_{max}3411.83 cm^{-1} (br, OH), 2932.27 cm^{-1} (C-H str. in CH_3), 1731.69 cm^{-1} (C=O str in COOH), 1453.93 cm^{-1} (C-H deformation in CH_3). ^{1}HNMR ($CDCl_3$, 200 MHz) δ 0.93 (s, 3H, terminal methyl), δ 2.28 (s, 2H, H-2), δ 2.31 (s, 4H, H-8,14), δ 2.37 (s, 2H, H-11), δ 5.39 – δ 5.47 (t, 4H, vinylic protons), δ 1.00 – δ 1.33 (m, 14H, 7xCH_2), δ 1.79 (s, 2H, H-3, 1x CH_2). EI-MS *m/z* $[M]^+$ 280 ($C_{18}H_{32}O_2$), 267, 252, 238, 224, 222, 207, 191, 177, 161, 149, 132, 120 (100%), 105, 91, 83, 65, 55, 39, 27, 14.From the above evidences compound I was determined as **9, 12- octadecadienoic acid.**

Compound II (β - sitosterol): It resulted in development of red colourbySalkowski's test and a green colourbyLibermann-Burchard's test for presence of steroids.Pearl white powder, m.p 138-140°C. IR (KBr pellet): ν_{max}3411.83 cm^{-1} (OH), 2932.27 cm^{-1} (C – H str. in CH_3), 2832.62 cm^{-1} (C-H str. in CH_2), 1637.83 cm^{-1}(C=C str.), 1082.63 cm^{-1} (C – O str. of secondary alcohol). ^{1}HNMR ($CDCl_3$, 200 MHz)δ 0.85 (s, 3H, H-18), δ 1.040 (s. 3H, H-19), δ 0.9122 (s, 3H, H-21), δ 0.86(s, 3H, H-26), δ 1.0079 (s, 3H, H-27), δ 0.87 (s, 3H, H-29), δ 1.136 to δ 1.549 (d, 22H, 11 × CH2), δ 1.5648 to δ2.0013 (m, 7H, methine protons), δ 2.3202 (m, 1H, OH), δ 3.63 (s, 1H, H-3), δ 5.69 (d,1H, vinylic proton). EI-MS *m/z*$[M]^+$ 414 ($C_{29}H_{50}O$), 301, 273 (M+- side chain), 255 (M+ -side chain + H2O), 231, 213, 161, 145, 133, 107, 97 and 83, 71, 57(100%).From the above evidences compound II was determined as**β –sitosterol.**

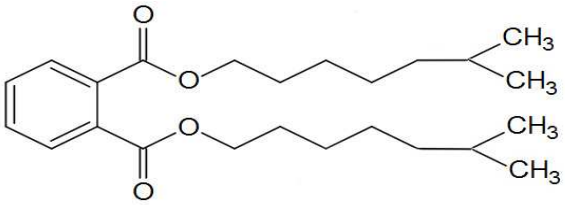


Compound III (Drevogenin A): It resulted in development of green color by Liebermann-Burchard test for presence of triterpinoids. Pale brown powder, m.p 188°C.IR (KBrpellet): ν_{max}3411.83 (OH), 2932.27 (C – H), 1731.69 (C = O),1082.63 (C – O) cm^{-1}. ^{1}HNMR ($CDCl_3$, 200 MHz) δ 3.440 (m, 1H, H-3), δ3.6580 (s, OH, H-3),

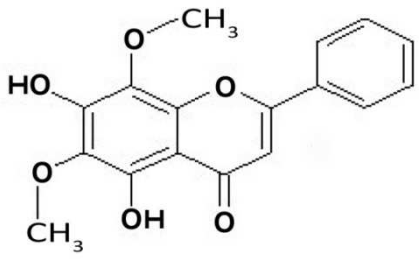
δ5.469 (m, 1H, vinylic proton), δ1.6346 (s, 1H, H-9), δ5.386 (s, 1H, H-11), δ4.849 (s, 1H, H-12), δ3.7318 (s, OH, H-14), δ2.901, δ1.9381 (s, 1H, 3H, H-17), δ1.0711(s, 3H, H-18), δ1.1397 (s, 3H, H-19), δ1.9561,δ2.275(s, 1H, 3H, H-11 Acyl proton), [δ2.3088(s), δ2.0499 (s), δ1.0277 (s)] (9H, 12-Isoval) and δ1.1397 – δ1.8991 (m, 6 x CH_2). EI-MS*m/z*$[M]^+$ 490 ($C_{28}H_{42}O_7$), 474, 396, 345, 311, 294, 279, 261, 251, 235, 209, 189, 171, 159, 145, 131, 113(100%), 87, 74, 43. From the above evidencescompound III was determined as **drevogenin A**.

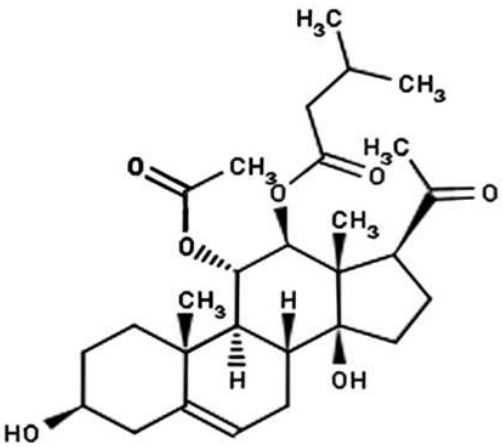
Compound IV (1,2-Benzenedicarboxylic acid, diisooctyl ester): Pale yellow oily liquid, b.p231^oC. IR (KBr pellet): ν_{max}2942.51 cm^{-1} (C – H str. in CH_3), 2832.62 cm^{-1} (C – H str in CH_2), 1718.92 cm^{-1} (C=O str.), 1659.45 cm^{-1} (C – O str.). ^{1}HNMR ($CDCl_3$, 200MHz) δ 0.9 - δ 0.98 (m, 12H, 4xCH_3), δ 1.13 - δ 1.33 (m, 18H, 9xCH_2), δ 4.31 (m, 2H, 1xCH_2), δ 1.69 (s,2 H, 2x CH), δ 7.68 - δ 7.73 (m, 4H Aromatic protons).). EI-MS *m/z* [M+1] 391 ($C_{24}H_{38}O_4$), 340, 311, 279, 167, 149 (100%), 113, 71, 57, 41, 27.From the above evidences compound IV was determined as**1,2-Benzenedicarboxylic acid, diisooctyl ester**.

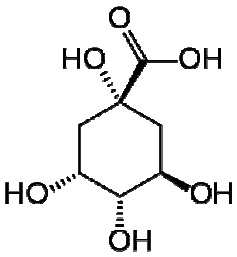
Compound V (5,7-dihydroxy-6,8-dimethoxyflavone): It gave a positive Shinoda test. Yellow powder, m.p 210°C.IR (KBr pellet): ν_{max}3353.29 (OH), 2942.51 and 2832.62 (C – H) stretching in CH_3 and CH_2, 1718.92 (C = O), 1659.45 (C – O)cm^{-1}. ^{1}HNMR ($CDCl_3$, 200MHz)δ 7.68 - δ 7.73 (m, 5H, ArH), δ7.50 - δ 7.55 (m, 1H, C-3), δ3.432 (s, 6H, OCH_3), δ5.453 (s, 2H, OH).EI-MS *m/z*[M+1]315($C_{17}H_{14}O_6$), 312 (100%), 300, 294,285, 279, 261, 267, 249, 225, 206, 192, 175, 159, 147, 137, 124, 105, 83, 57, 41.From the above evidences compound V was determined as **5,7-dihydroxy-6,8-dimethoxy-flavone**.



Compound VI (Quinic acid): Creamish powder, m.p 108°C.IR (KBr pellet): ν_{max}3464.27 cm^{-1} (br, OH), 2968.55, 2933.83cm^{-1} (C-H str. in CH_2), 1647.26 cm^{-1} (C=O str.), 1459.2 cm^{-1} (C-H deformation), 1258.59 cm^{-1} (C-OH), 1084.03 cm^{-1} (C-O str. of secondary alcohol). ^{1}HNMR ($CDCl_3$, 200MHz) δ 1.5782 – δ 1.9878 (m, 4H, H-2,6), δ 3.237 – δ 3.7717 (m, 3H, H-3,4,5), δ 4.571 – δ 4.779 (m, 3H, OH), δ 5.475 (d,1H, H-1). EI-MS *m/z* [M – l]191($C_7H_{12}O_6$),175, 167, 149, 127, 123, 111, 85, 71, 57, 43 (100%), 41, 27.From the above evidences compound VI was determined as **quinic acid**.



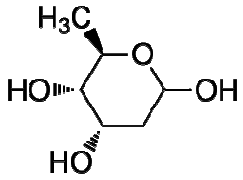


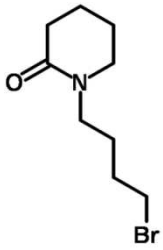


Compound VII (N-[4-bromo-n-butyl]-2-piperidinone): It gave a positive test for Dragendorff's test, Hager's test, Wagner's test and Mayer's test for alkaloids. Light brown powder, m.p 102°C.IR (KBrpellet): ν_{max}2968.55 and

2931.90 (C – H), 1739.85 (C = O), 607.60 (C-Br) cm^{-1}. ^{1}HNMR ($CDCl_3$, 200MHz) δ 2.187 (s), δ1.950 (s), δ1.936 (s) and δ3.419 (s) [8H, heterocyclic CH_2], δ3.440 (s), δ1.790 (s), δ1.837 (s) and δ3.523 (s) [8H, CH_2].EI-MS *m/z* $[M]^+$ 234 ($C_9H_{16}BrNO$), 154, 135, 125, 111, 99, 97, 81, 74 (100%), 69, 67, 57, 55, 43, 41. From the above evidences compound VII was determined as **N-[4-bromo-n-butyl]-2-piperidinone**.

Compound VIII (Digitoxose): It gave a positive test for Killer – Killani. m.p 102°C. IR (KBr pellet): ν_{max}3239.04 (OH), 2937.42 (C – H), 1649 (C – O) cm^{-1}. ^{1}HNMR (DMSO, 200MHz) [δ 5.1022 (s), δ3.40 (s), δ3.46 (s)] (4H, C-2,4,5,6), δ1.19 (s, 3H, CH_3), δ2.50 (t, 2H, C-3), δ4.44 – δ4.94 (m, 3H, OH). EI-MS *m/z* [M+1] 149 ($C_6H_{12}O_4$), 133, 121, 99, 86, 73, 57, 43, 41, 18(100%).From the above evidences the compound VIII was determined as **digitoxose**.





CONCLUSION

The chemical investigation led to the isolation of 8 compounds from the ethanolic extract of the roots of *Wattakaka volubilis*β-sitosterol; drevogenin A; 9, 12 – octadecadienoic acid; quinic acid; 1, 2 – benzenedicarboxylic acid diisooctyl ester; 5, 7 – dihydroxy – 6, 8 – dimethoxyflavone; N-[4-bromo-n-butyl]-2-piperidinone and digitoxose. The phytoconstituents have been isolated for the first time from the roots of *Wattakaka volubilis*.

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