

Chemical Examination of *Morinda Pubescens* Var. *Pubescens*. (Rubiaceae) and Isolation of Crystalline Constituents

U. Viplava Prasad¹, B. Syamasunder², Anuradha. G³ and J. Sree Kanth Kumar⁴

¹Department of Organic chemistry, Andhra university, Visakhapatnam. A.P

²Principal, College of sciences, Acharya Nagarjuna University, Guntur. A.P

³Department of chemistry, Acharya Nagarjuna University, Guntur. A.P

⁴ Department of Humanities and Sciences, Swarna Bharathi Institute of Science and Technology, Khamman, A. P

Email: viplav_31049@yahoo.com, profbsyamsundar@yahoo.co.in, anuradha.mythri@gmail.com, sreekanth.mythri@gmail.com

Abstract

Morinda pubescens var. *pubescens* (Rubiaceae) is a tropical plant whose leaves, bark, roots and fruits have been used as traditional remedy for various diseases. In our study we identified four known compounds as two pentacyclitriterpenoids Ursolic acid and Taraxerol, two sterols β -Sitosterol and Stigmasterol from *Morinda pubescens* var. *pubescens* (Rubiaceae). Solvent extraction, column chromatography were major techniques used for isolation of compounds, while structures were elucidated by integration of data from IR, UV, ¹H NMR AND ¹³C NMR analysis. We are reporting presence of Taraxerol a pentacyclitriterpenoid first time from the leaves of *Morinda pubescens* var. *pubescens* of Rubiaceae family.

Introduction

The history of medicinal plants goes back to the history of human civilization. The ancient medical men through their trial and error mechanism identified herbs which are useful in healing various diseases and the same knowledge was passed on to the generations [1]. Even in recent times the plant products play very vital role in all the forms of medicine like Ayurveda, Siddha and Unnani. As the time proceeded the use of herbs transformed from raw form to purified form and extraction of chemical compounds leads to the synthesis of modern drugs [2]. The present study focuses on the Chemical Examination of *Morinda pubescens* var. *pubescens* of Rubiaceae family and isolation of crystalline constituents.

Identification of the Plant Material

The plant was collected from Acharya Nagarjuna University Campus, Guntur. Andhra Pradesh, India. Based on Morphological and the Anatomical data [3]

the plant was identified by B.S.I. Coimbatore as *Morinda pubescens* (J.E. Smith) var. *pubescens* of Rubiaceae family.

Chemical Composition of *Morinda* Species

The various chemical compounds were extracted from *Morinda* species like Anthraquinones [4, 10], Flavonoids [5], Iridoids [6, 9], Steroids [7] and Terpenoids [8]. The molecular formula, Structure was elucidated based on melting point; I.R, U.V and ¹H NMR data in the present work.

Extraction Procedure Adopted

The leaves were dried at room temperature. Dried and powdered leaves are weighed. The weight of the material is 690 Grams. The powdered leaves are taken in a 5-liter Soxhlet apparatus and 4 liter of acetone is added. The mixture is kept for 24 hours. The first 3 liters cold fraction is collected into 5 liters round bot-

tom flask. The acetone extract-I was kept for distillation. The remaining solvent about 2.5 liters collected after distillation of cold extract-I is again added to the Soxhlet jar. The cold acetone fraction-I after dryness under reduced pressure yielded a residue of 7.770 grams. The acetone cold extract-II was collected next day about 3 liters in round bottomed flask and kept for distillation under reduced pressure yield around 3 grams of residue.

The remaining material after cold extraction, taken into the Soxhlet apparatus under controlled temperature the hot acetone extract -I was collected after successive 12 siphons. The methanol hot extract also collected after 6 siphons. The hot acetone extract yield 1.200 gms and the hot methanol extract give 3gms residue.

The cold acetone extract I, II and hot acetone extract-I mixed up; the substance weighted around 11.97 grams. The column is setup with 200 grams column silica gel (acme) 100 – 200 mesh in 1250ml of n-Hexane and Benzene in 9:1 ratio. The residue (around 12 grams) is taken and dissolved in acetone and impregnated in silica gel and the acetone is evaporated on water bath. The powdered substance with silica gel is further fine powdered (without granules). The fine powder is mixed with 9:1 Hexane: Benzene solvent. The column thus set is run with n-hexane, Benzene, ethyl acetate, ethyl alcohol (9:1 to 1:1) and finally with ethyl acetate.

Materials and Methods

General procedure: – TLC was carried out on percolated silica gel 60 F254 plates (Acme). Spots were detected under UV (254 and 366nm) before and after spraying with 1% methanol sulfuric acid spray reagent, followed by heating the plate at 110°C for 5 min. Preparative TLC was performed on percolated silica gel 60 F254 plates, layer thickness 0.5mm (Merck) unless indicated otherwise. Column chromatography was carried out on silica gel 60 (acme 100–200mesh) and sephadex LH20.

Chemical Examination of *Morinda Pubescens* Var. *Pubescens* Leaves:

The air-dried leaves powder was soxhleted with, n-hexane, Acetone and methanol. The hot and cold

extracts of Acetone and n-hexane were found to be similar on TLC these extracts were combined and chromatographed extensively to yield eight compounds which are designated as AMP-1 to AMP-8 (Table 1) only four of them were isolated in a pure form for this present study. They are AMP-1 (Fig 1), AMP-4 (Fig 2), AMP-5 (Fig 3) and AMP-8 (Fig 4). The methanolic extract was in the form of greenish gummy substance. Hence the work was not pursued further.

Table 1: TLC of compounds AMP1–8

Compound Code	Yield	TLC Solvent System	R _f value
AMP – 1	70 mg	n-Hexane : Ethyl acetate 9:1	0.59
AMP – 2	30 mg	n-Hexane : Ethyl acetate 8:2	Long Streaks with no promising spots
AMP – 3	60 mg	n-Hexane : Ethyl acetate 8:2	No promising spot
AMP – 4	150 mg	n-Hexane : Ethyl acetate 4:1	0.78
AMP – 5	120 mg	n-Hexane : Ethyl acetate 4:1	0.71
AMP – 6	40 mg	n-Hexane : Ethyl acetate 1:1	0.61, Long streaks green color
AMP – 7	50 mg	n-Hexane : Ethyl acetate 1:1	0.66, Long streaks green color
AMP – 8	75 mg	n-Hexane : Ethyl acetate 9:1	0.59 with shot tailing

Examination of AMP – 1 :- (Urosolic Acid XCI)

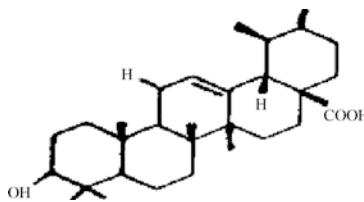


Fig. 1: structure of urosolic acid XCI

AMP-1 was colorless solid and recrystallized from ethylacetate gave a colorless compound. Indicated it is as triterpene. It give effervescences with NaHCO₃ and Na₂CO₃, [α]_D²⁴ + 72.2°C (methanol) soluble in hot CHCl₃, MeOH, ethyl acetate. Melting point of the compound is 285.5°C. L.B. Test gave +ve result with pink to violet coloration. Molecular formula C₃₀H₄₈

O₃Element analysis Found C, 83.72%, H, 11.78% Requires: C, 83.82%; H, 11.82%. I.R.: 3421, 1620 Cm⁻¹. ¹H (400, MHz, CDCl₃): δ0.8 (3H, S 24-Me), 0.84 (3H, S, 28-Me), 0.90 (6H, S, 25, 29-Me), 0.95 (3H, S, 30-Me), 0.98 (3H, s, 23-Me), 1.20 (6H, S, 26, 27-M2), 3.15 (1H, M, H-3), 5.2 (1H dd, 15-H).

Examination of AMP – 4 :- (β-Sitosterol LXXXVI)

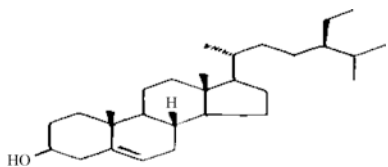


Fig. 2: structure of β-Sitosterol LXXXVI

AMP-4 isolated as white colored compound and recrystallized from methanol as colorless needles (150mg) melting point is 135–136°C, $[\alpha]_D^{27} -35^\circ$ (C, 1.0, CHCl₃) positive L.B. test for sterols (violet → blue → green)

Molecular formula C₂₉ H₅₀ O. Element analysis Found : C, 83.86%, H, 11.90% Requires: C, 83.98%; H, 12.16% Rf : 0.67 (n-hexane, ethyl acetate-4:1). U.V.: No prominent absorption was noticed. I.R.: ν_{max}^{Nujol} : 3436, 1690, 1607, 1460, 1366, 985 Cm⁻¹. ¹H NMR (400, MHz, CDCl₃) : δ0.6 (3H, S 18-Me), 0.81 (6H, d, J = 7 Hz, 26, 27-Me), 0.84 (3H, t, 29-Me), 0.89 (3H, d, J = 6.3 Hz, 21-Me), 0.99 (3H, s, 19-Me), 3.2 (1H, m, 3_α-H), 5.3 (1H, m, 6-H). ¹³C NMR (125.70, MHz, CDCl₃) : δ36.1 (C-1), 39.7 (C-2), 71.8 (C-3), 56.8 (C-4), 140.7 (C-5), 121.1 (C-6), 29.1 (C-7), 28.9 (C-8), 50.0 (C-9), 37.2 (C-10), 31.8 (C-11), 36.4 (C-12), 42.2 (C-13), 42.1 (C-14), 11.7 (C-15), 28.2 (C-16), 56.8 (C-17), 11.8 (C-18), 18.9 (C-19), 29.6 (C-20), 0.98 (C-21), 21.1 (C-22), 28.2 (C-23), 51.2 (C-24), 24.2 (C-25), 21.0 (C-26), 31.6 (C-27), 19.4 (C-28), 19.3 (C-29).

Examination of AMP – 5 :- (Stigmasterol XCVII)

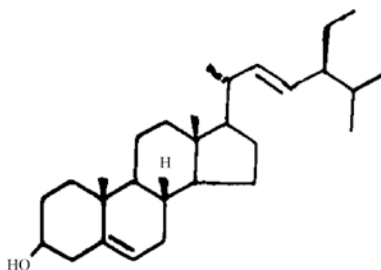


Fig. 3: Structure of Stigmasterol XCVII

AMP-5 was crystallized from chloroform-methanol as needles (120 mg). Melting point was 168 – 170°C, $[\alpha]_D -45^\circ$ (C, 1.2 CHCl₃). It showed pink color, which finally turned to green. It gave positive L.B. test for steroids. Molecular formula: C₂₉ H₄₈ O. Elemental analysis: Found: C, 83.78 %, H, 11.60 % Requires: C, 84.40 %; H, 11.72 % Rf: 0.65 (n-hexane, ethyl acetate-4:1) U.V.: No prominent absorption was noticed. I.R.: ν_{max}^{Nujol} 3442, 2928, 1693, 1457, 1387, 1031, 996 Cm⁻¹. ¹H NMR (400, MHz, CDCl₃) : δ0.70, 0.80, 0.90, 1.00, 1.05, 1.30, (18H, 6xMe), 3.25 (1H, br, 3_α-H), 5.1 (2H, m, 22, 23-H), 5.3 (1H, m, 6-H). ¹³C NMR (125.70 MHz, CDCl₃): δ37.4 (C-1), 31.7 (C-2), 71.8 (C-3) 42.4 (C-4), 140.9 (C-5), 121.7 (C-6), 31.9 (C-7), 31.9 (C-8), 50.3 (C-9), 38.6 (C-10), 21.1 (C-11), 39.8 (C-12), 42.4 (C-13), 57.0 (C-14), 24.4 (C-15), 28.9 (C-16), 56.0 (C-17), 12.2 (C-18), 19.4 (C-19), 40.5 (C-20), 21.1 (C-21), 138.4 (C-22), 129.4 (C-23), 51.3 (C-24), 31.9 (C-25), 19.0 (C-26), 21.1 (C-27), 25.4 (C-28), 12.0 (C-29).

Examination of AMP – 8:- (Taraxerol XCVIII)

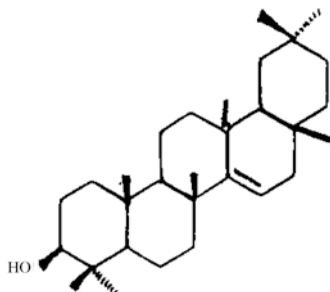


Fig. 4: Structure of Taraxerol XCVIII

AMP-8 was crystallized from chloroform-methanol, as crystalline solid (75 mg). Melting point 279–280°C, $[\alpha]_D^{20} + 0.72$ (C, 0.97, CHCl₃). It showed pink color in L.B. test for triterpenes. Molecular formula: C₃₀ H₅₀ O. Element analysis: Found: C, 87.32%, H, 11.63% Requires: C, 84.45%; H, 11.82% Rf : 0.59 (n-hexane, ethyl acetate-9:1) I.R.: $\nu_{\max}^{CHCl_3}$ 3442, 2933, 1694, 1458, 1376, 1033 Cm⁻¹. ¹H NMR (500, MHz, CDCl₃) : δ 0.80 (3H, S, 24-Me), 0.84 (3H, S, 28-Me), 0.90 (6H, S, 25, 29 – Me), 0.95 (3H, S, 30 – Me), 0.98 (3H, S, 23-Me), 1.05 (6H, S, 26, 27 – Me), 3.15 (1H, m, H-3), 5.33 (1H, dd, J = 8.1 and 4.0 Hz, 15-H). ¹³C NMR (150.8 MHz, CDCl₃): δ 39.97 (C-1), 27.11 (C-2), 79.07 (C-3), 38.96 (C-4), 55.49 (C-5), 18.77 (C-6), 35.07 (C-7), 38.96 (C-8), 48.70 (C-9), 37.97 (C-10), 17.49 (C-11), 35.78 (C-12), 38.74 (C-13), 158.05 (C-14), 116.85 (C-15), 36.65 (C-16), 37.70 (C-17), 49.25 (C-18), 41.29 (C-19), 29.36 (C-20), 33.67 (C-21), 33.07 (C-22), 27.98 (C-23), 15.45 (C-24), 15.45 (C-25), 29.91 (C-26), 25.89 (C-27), 29.81 (C-28), 33.34 (C-29), 21.30 (C-30).

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