Isolation of Urosilic Acid from Knoxia corymbosa

Mrutyunjaya Rao R1, Ramakrishna K2, Suresh Babu K3 and Surya Kumar MV1

1Department of Chemistry, VSM College, Ramachandrapuram, East Godavari District, Andhra Pradesh, India
2Scientist, IICT, Hyderabad, Telangana, India
3Corresponding author: Mrutyunjaya Rao R, Department of Chemistry, VSM College, Ramachandrapuram, East Godavari District, Andhra Pradesh, India, Tel: 08986769830; E-mail: rmj.rao@rediffmail.com

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Abstract

Air-dried, milled leaves of Knoxia corymbosa (220 gm) were extracted repeatedly with dichloromethane. After removal of solvent in vacuo, the CH2Cl2-solvent residue was fractionated by using hexane and ethyl acetate solvents. After fractionation, all fractions are submitted for NMR spectroscopy. Out of all fractions interesting fractions are subjected to column chromatography, so that we isolated one pure compound as Urosilic acid which is a known compound as it is characterized by using reference data. This is the first time to be isolated the above compound from this species. Urosilic acid is a five-membered cyclic triterpenoid compound. A structure of the isolated compound has been assigned on the basis of their analytical data. By surveying the literature, we came to understand that the compound urosilic acid is a cyclic five-member triterpenoid first to be isolated from Knoxia corymbosa. And also, it is first time to isolate the above compound from this species Knoxia corymbosa.

Keywords: Knoxia corymbosa; CH2Cl2; Urosilic acid; Column chromatography; Spectroscopy

Introduction

Pharmacological activities of plants and plant derived drugs necessitate for the search of new and useful drugs globally. India is the largest producer of medicinal herbs. These values are shown vast and tremendous biodiversity potential in India, which can be utilized in drug industry. Knoxia corymbosa is assumed to containing some medicinal values [1-6] because girijans of the forest area are using for fevers and skin diseases. Some authors reported that they isolated some chromone glycosides [7,8] from Knoxia corymbosa. Knoxia species reported to contain herbal medicine, β-sitosterol which is one of its main components was isolated. β-sitosterol is known to control cholesterol levels, reduce the activity of cancer cell, promote prostate gland health enhance immunity in the human body. The plants of family Rubiaceae is an important source of medicinal natural products, particularly alkaloids and triterpenes, quinovic acid glycosides, flavonoids and coumarins have been isolated from this family. Pharmacological studies are described according to cytotoxicity, anti-inflammatory, antiviral, immune stimulation, antioxidant, CNS-related response, vascular, hypertensive, mutagenicity and antibacterial properties. The compounds obtained from this family are used as immunomodulatory, anti-inflammatory and vascular-related conditions. The information summarized here is intended to serve as a reference tool to practitioners in the fields of ethno pharmacology and natural products chemistry.

Various natural products occur in Rubiaceae plants. Extensive phytochemical investigation has been realized regarding the natural occurrence of triterpenoids [10-16], antirheumquines and indole alkaloids [17-20] in the family. Rubiaceae family plants exhibited antimalarial, antimicrobial, antihypertension, antidiabetic, antioxidant, and anti-inflammatory activities. Bioactive compounds including indole alkaloids, terpenoids, anthraquinones and anti-tumors have been isolated from these plants.

Materials and Methods

220 gms of shade dried powder leaves of Knoxia corymbosa were filled in the thimble and extracted successively with n-hexane, dichloromethane, ethyl acetate and methanol solvents in soxhlet extractor for 48 hours intervals. The solvent extracts were concentrated under pressure and preserved at 40°C in an airtight bottle for further use. After fractionation, all fractions are submitted to NMR Spectroscopy. Out of all fractions dichloromethane fraction is subjected to column chromatography. By thin layer chromatography (TLC) method Dichloromethane extract seems to be containing more compounds. So, the extract from dichloromethane is subjected to column chromatography with n-hexane and ethyl acetate solvents. The purity of fractions was tested with the help of TLC.

Figure 1: Structure of Urosilic acid. IUPAC Name of ursolic acid is: 3-beta-3-hydroxy-urs-12-ene-28-oic-acid, or 3-β-hydroxy-urs-12-en-28-oic acid, urson, prunol or malol.
Out of all fractions, the fractions having similar Rₚ values were combined together and isolated three samples. The obtained samples were submitted to UV, IR, NMR and Mass Spectroscopy. The obtained data is compared with reference data and confirmed that, out of three samples one sample i.e., sample-3 (sample-1 and sample-2 are not pure so rejected), seems to be pure one and confirmed the structure. The obtained data is compared with reference data and confirmed the structures and the compound is identified and confirmed as ursolic acid (Figure 1).

**Spectral data of Knoxia corymbosa**

UV spectral data is at 474, 442 and 422.

FT-IR KB Absorption bands assigned to the compound are C-O stretching is (1036.04 cm⁻¹) olefinic system (C=C) (1458.48 cm⁻¹ and 2860.52 cm⁻¹), carboxylic acid stretching is (2928.24 cm⁻¹), carbonyl system (1690.38 cm⁻¹), and hydroxyl group (3444.99 cm⁻¹).

1H NMR: (400 MHz, CD3OD): δH=0.728 (3H, s, CH₃), 0.768 (3H, s, CH₃), 0.884 (3H, CH₃), 1.05 (3H, , CH₃), 0.965(H, d, CH₃), 1.28 (3H, s, CH₃), 1.106 (3H, s, CH₃), 1.24, 1.352-2.025 (13H, m, [1, 2, 5-7, 9, 11, 15, 16,19-22]), 1.218 (1H, d, H-1), 3.303 (1H, d, H-3) and 5.217(1H, m, H-12).

13C NMR: (400 MHz, CD3OD): δC=38.14 (C-1), 27.88 (C-2), 79.69 (C-3), 38.14 (C-4), 54.40 (C-5), 17.83 (C-6), 31.8 (C-7), 39.83 (C-8), 48.14 (C-9), 34.33 (C-10), 21.60 (C-11), 23.80 (C-12), 139.71 (C-13), 40.76 (C-14), 28.7 (C-15), 24.08 (C-16), 43.24 (C-17), 54.40 (C-18), 39.83 (C-19), 39.99 (C-20), 30.76 (C-21), 34.33 (C-22), 27.88 (C-23), 16.06 (C-24), 16.38 (C-25), 17.66 (C-26), 24.08 (C-27), 181.98 (C-28), 17.83 (C-29), 21.60 (C-30).


**Results and Discussion**

Dichloromethane extract to column chromatography with n-hexane and ethyl acetate as eluting solvent mixture and obtained 198 fractions.

Plants with possible antibacterial activities should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it.

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**References**


