

Research Article

Scopoletin – An Anti-hyperglycemic Coumarin from the Fruit of *Averrhoa carambola* L. (Star fruit)Wijayabandara M.D.J^{1*}, Choudhary M.I², Wijayabandara M.D.L.O³, Adhikari A²

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Abstract

Purpose: *Averrhoa carambola* L. has a long history of its use in traditional medicine for the treatment of various diseases such as diabetes, intermittent fevers, intestinal worms, scabies and inflammatory conditions. The fresh fruit of *A. carambola* is very popular in Sri Lanka for its glucose-lowering effect and is taken as good remedy for diabetes. The present study was carried out to isolate and characterize anti-hyperglycemic principles of the fruit of *A. carambola* to obtain additional scientific evidence for its usage in traditional medicine in the management of diabetes.

Methods: The dried and powdered fruits of *A. carambola* were successively extracted with n-hexane and CH₂Cl₂ using soxhlet apparatus. The CH₂Cl₂ extract was subjected to silica gel column chromatography, eluting in a stepwise gradient with hexane and ethyl acetate (EtOAc) mixtures. The fraction eluted with hexane:EtOAc (60:40) yielded a mixture which with preparative thin-layer chromatography afforded a pure compound designated as JW-AC-3. The structure of JW-

AC-3 was elucidated on the basis of its Ultra-violet spectroscopy (UV), Infra-Red spectroscopy (IR), Mass Spectroscopy (MS), and Nuclear Magnetic Resonance spectroscopy (NMR) including Distortionless Enhancement Polarization Transfer (DEPT), Homonuclear Shift-Correlation Spectroscopy (COSY), Nuclear Overhauser Spectroscopy (NOESY), Hetero Multiple Bond Connectivities (HMBC) and Heteronuclear Multiple-Quantum Coherence (HMQC) experiments and direct comparison with reported data.

Results: JW-AC-3 was identified as scopoletin by direct comparison of its spectral data with reported data of scopoletin. To our knowledge, scopoletin has not been reported from *A. carambola*. Scopoletin has been shown to possess significant anti-hyperglycemic activity by previous workers. The results of this study clearly indicate that the majority of the anti-hyperglycemic activity of the fruits of *A. carambola* could be attributed to scopoletin. The results also provide for the use of scopoletin as a bio-marker in the standardization and quality



control of products derived from the fruits of *A. carambola*.

Conclusion: The results of this study demonstrate potential use of scopoletin in the development of drugs of natural origin in the treatment of diabetes. The results could also be used in the rationalization of ethnomedical use of the fruits of *A. carambola*.

Keywords: *Averrhoa carambola*, Oxalidaceae, Scopoletin, Anti-hyperglycemic activity.

Introduction

Averrhoa carambola L. is an evergreen tree commonly known as “kamaranga” or “star fruit”, belonging to the family oxalidaceae. Etymologically the species name carambola is derived from the Sanskrit name “kamaranga” meaning “food appetizer”. *A. carambola* is believed to have originated in Ceylon and the Moluccas.(1) However it is widely cultivated throughout tropical countries for their fruit.

People in both developing and developed countries use medicinal plants in the treatment of various diseases. *A. carambola* has various medicinal uses viz. anti-pruritic, antipyretic, anti-diabetic, anthelmintic, anti-inflammatory, anti-ulcer, antimicrobial etc.(2,3). Various parts of the tree have been used in traditional medicine while the fruits are very popular in Sri Lanka for its glucose-lowering effect. Hence the fresh fruit is commonly taken as a good remedy for diabetes. Several chemical constituents including tannins, terpenoids, saponins, alkaloids, flavonoids have been reported from this plant. Fruits of *A. carambola* are a good source of natural antioxidants due to the presence of L-ascorbic acid, (-)epicatechin,

gallic acid and they are also particularly rich in insoluble dietary fibers.(2)

Diabetes mellitus (DM) is an important medical problem throughout the globe and the number of those affected is on the increase. Though there are various drugs available along with insulin for the treatment of DM, they are insufficient and possess adverse side effects. The treatment of diabetes without any side effects is still a challenge to the allopathic medical system and there is a need for discovering more effective and safe oral hypoglycemic agents. Several traditional medicinal plants are claimed to have safe antidiabetic potentials and use of these plants as hypoglycemic is a major avenue in Sri Lankan perspectives particularly for treating diabetes.(3) On the other hand, natural products have been used as sources for pharmacologically active compounds and have potential for developing some novel therapeutic agents. Although numerous studies have been conducted on different parts of *A. carambola*, there is a need for the characterization of active principles in order to explore scientifically the hidden medicinal potential of this plant. As a continuation of our studies on the pharmacologically active constituents of Sri Lankan medicinal plants, this paper deals with the isolation and structure elucidation of scopoletin as an anti-hyperglycemic agent of the fruits of *A. carambola*.

Methods

Equipment

Infra-Red (IR) spectrum was recorded on a Bruker VECTOR 22 Fourier Transform Infra-Red (FTIR) spectrophotometer and Ultra-Violet (UV) spectrum was recorded on

Shimadzu UV Spectrophotometer. Melting point was recorded on SMP 10 digital melting point apparatus. EI-MS was recorded on MAT 312 mass spectrophotometer. The ^1H and two-dimensional COSY, NOESY and J-resolved NMR spectra were obtained in CDCl_3 on Bruker Avance AV-600 NMR spectrometer operating at 600 MHz and ^{13}C NMR, DEPT and two-dimensional HMQC and HMBC spectra were recorded in CDCl_3 on Bruker Avance AV-600 NMR spectrometer operating at 600 MHz. Tetramethyl silane (TMS) was used as internal standard. The chemical shift values are reported in ppm (δ) units and the coupling constants (J) are given in Hz.

Chromatographic conditions

Pre-coated aluminum sheets (silica gel G-60F₂₅₄ E. Merck) were used for thin-layer chromatography (TLC). Visualization of the TLC plates was achieved under UV at 254 and 365 nm and by spraying with ceric sulphate reagent. Dichloromethane (CH_2Cl_2): Ethyl acetate (EtOAc) (9:1) solvent system was used.

Plant material

The fresh fruits of *A. carambola* were collected from Mathugama area (Southern province) in July 2014. Voucher specimens have been deposited in the laboratory of B.Pharm Degree program, Faculty of Medical Sciences, University of Sri Jayewardenepura. Identification was confirmed by comparison with herbarium specimens housed at Department of Plant Science, University of Colombo.

Extraction and isolation of scopoletin from fruits of *A. carambola*

The fresh fruits were cut into small pieces and oven dried at 40°C. The dried and powdered fruits (2kg) of *A. carambola* were extracted

successively with n-hexane and CH_2Cl_2 in a soxhlet apparatus for 6h for each solvent. The solvent in each extract was evaporated *in vacuo* to obtain dried solid residues. The dried CH_2Cl_2 extract (7 g) was subjected to silica gel column chromatography (CC), eluting in a stepwise gradient of 100 ml of hexane and ethyl acetate (EtOAc) mixtures to afford 40 fractions ($F_1 - F_{40}$). The fractions ($F_{16}-F_{17}$) eluted with hexane: EtOAc (60:40) on evaporation yielded a mixture which with preparative thin layer chromatography (CH_2Cl_2 : EtOAc – 9:1) afforded a pure compound (20 mg) designated as JW-AC-3.

Structure elucidation of JW-AC-3.

The structure of JW-AC-3 was elucidated on the basis of its UV spectroscopy, IR spectroscopy, Mass spectroscopy (MS), Nuclear Magnetic Resonance (NMR) spectroscopy including DEPT, COSY, NOESY, Heteronuclear Multiple-Quantum Coherence (HMQC), Hetero Multiple Bond Connectivities (HMBC) experiments and direct comparison with reported data. JW-AC-3 was identified as scopoletin by direct comparison of its spectral data with reported data of scopoletin.(4) The structure of scopoletin is given in Figure 1.

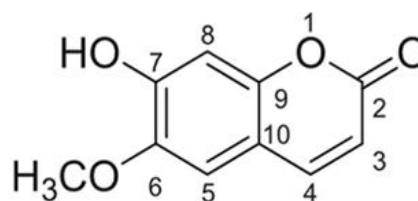


Figure 1: Structure of scopoletin (7-hydroxy-6-methoxy coumarin)

^1H and ^{13}C NMR data: see Table 1.

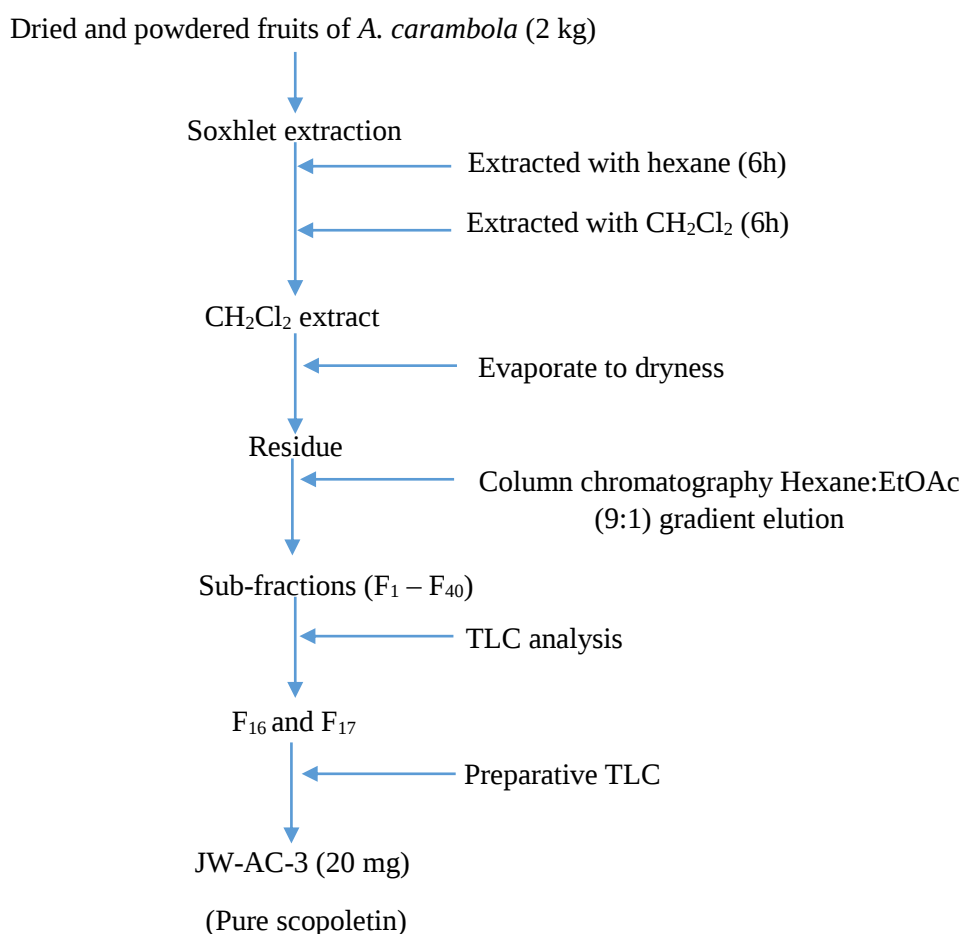
Table 1: ^1H and ^{13}C NMR spectroscopic data for JW-AC-3 in CDCl_3 (600MHz for ^1H and ^{13}C)

Carbon atom	^{13}C (δ)	^1H , δ (J, H_z)
CH_3	56.4	3.93, s
2	161.4 (C)	
3	113.4 (CH)	6.25, d, 9.5
4	143.3 (CH)	7.58, d, 9.5
5	107.4 (CH)	6.89, s
6	149.6 (C)	
7	143.9 (C)	
8	103.1 (CH)	6.82, s
9	150.2 (C)	
10	111.5(C)	

The ^1H - ^{13}C Connectivities and ^{13}C multiplicities were deduced from HMQC and DEPT experiments

JW-AC-3 (Scopoletin) $\text{C}_{10}\text{H}_8\text{O}_4$, pale yellow crystals, m.p. 180-182 $^{\circ}\text{C}$; EIMS m/z (rel.int); 192(M^+ , 100), 164(63.5), 177(80.0), 149(83.8), 121(68.9), 92(19.7), 79(64.9), 69(98.7); UV λ_{max} nm (EtOH) 229.2, 253.7, 260.0, 298.8, 346.5; IR ν_{max} ; 3300 (OH), 1670 (CO), 1615 (C=C), 1595, 1550, and 1490 (aromatic C=C) Cm^{-1}

Scheme 1: Extraction and isolation of scopoletin



Results

The structure of the compound JW-AC-3 was determined by spectroscopic analyses and it was identified as scopoletin (Figure 1). Scopoletin was isolated from the fruits of *A. carambola* by extracting dried and powdered fruits with n-hexane, removing fatty non-polar constituents in a soxhlet apparatus for 6h. The fruit material was then extracted with CH₂Cl₂ to obtain extract enriched with scopoletin. This extract was then subjected to CC on silica gel using gradients of n-hexane-EtOAc followed by preparative thin layer chromatography to obtain pure scopoletin as shown in Scheme 1. Although scopoletin has previously been isolated from various plant species, to our knowledge, it has not been reported from *A. carambola*.

Discussion

Scopoletin has been shown to possess significant anti-hyperglycemic activity in streptozotocin induced diabetic rats.(5) It has also been shown to possess antioxidant activity. Potent α -glucosidase inhibitory activity of this compound when compared to the positive control acarbose has also been reported previously.(6) As α -glucosidase inhibitors are widely used in the treatment of type II diabetes mellitus, the results of this study also suggest that scopoletin can be used as a lead in the development of novel medicines for treating type II diabetes.(7,8) The results also provide for the use of scopoletin as a bio-marker in the standardization and quality control of products derived from the fruits of *A. carambola*.

The results of this study clearly indicate that the majority of the anti-hyperglycemic

activity of the fruits of *A. carambola* could be attributed to scopoletin. It also contributes to the total antioxidant activity of the fruit. As the fruits are used in traditional medicine for anti-hyperglycemic effects, the results obtained in this study could be used in the rationalization of ethnomedical use of the fruits of *A. carambola*.

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