SMGroup

SM Journal of Biology

Research Article

Isolation and Identification of Diterpenes Extracted from *Annona Squamosa*

Abdul Mushin M Shami*

Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia

Article Information

Received date: Apr 19, 2016
Accepted date: Jun 15, 2016
Published date: Jun 17, 2016

***Corresponding author**

Abdul Mushin M Shami, Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia, Email: aashbio@yahoo.com

Keywords *Annona Squamosa*; Diterpenes; TLC; IR

Abstract

The purpose of the study was to isolate and identified the diterpenes fractions of *Annona squamosa*. TLC of diterpenes extracts from the plant used in this study revealed the presence of these compounds to reveal characteristic blue bands of diterpenes. IR spectra of diterpenes extract from the fruit of *A. squamosa* exhibited at band 3268.62 cm^{-1} by O-H stretching. C-H stretching group was detected at 2925.25 and 2853.14 cm^{-1}. The C=O functional group was detected at band 1716.57 cm^{-1}. C-H group bonds were detected at bands 1512.54, 1454.41 and 1325.53 cm^{-1}. The C-O functional group was detected at band 1233.33 cm-1. It could be concluded that the diterpenes of the plant can be a new source of antimicrobials against pathogenic bacteria and antioxidant source.

Introduction

A. squamosa is belongs to the Annonaceae family. Its common names are Nona, sugar apple, ata, gishta and sweet sop plant [1,2]. The genus *Annona* comprises 120 species. An economically significant species is *A. squamosa* which belongs to the Annonaceae family. Its specific native range is indefinite because of widespread commercial cultivation but is generally deemed to originate from the Caribbean region [3]. Common names for this plant are Nona, sugar apple, ata, gishta and sweet sop [1,2]. It is a small semi-evergreen tree/shrub, 3-7 m tall, with irregular or crown branches. The leaves are oblong-lanceolate and pale green on both surfaces. The flowers are greenish-yellow and produced in single or short lateral clusters [4]. The petioles are green and 0.6-1.3 cm in length. The fruit of this plant is round, heart shaped, ovate or conical. It is green-yellow in colour initially. The ripe fruit is white with the sweetly aromatic pulp also white [1]. The seeds are shiny, numerous, and blackish or dark brown in colour [5]. It is used as a medicine for a general tonic, enriches blood, relieves vomiting, cancer, vermicide, skin complaints and also used for applied wounds and ulcer [4,6,7].

Terpenoids are very important compounds found in the fruit and stem of this plant. These terpenoids include 16 α hydroxy-(-)-kauran-19-oic acid, kauran-16-en-18-oic acid, annomosin A, annosquamosin C, annosquamosin D, annosquamosin E, annosquamosin F, annosquamosin G and annosquamosins B. Previous studies have reported that 16α hydroxy-(-)-kauran-19-oic acid extracted from the fruit of this plant has antibacterial activity against strains of *S. aureus* and *Streptococcus pneumonia* [8]. The aim at this study is to isolate and determine of diterpenes this plant.

Materials and Methods

Plant collection

The fresh ripe fruit *A. squamosa* was collected in November 2010, from Juasseh, Kuala Pilah. This plant was identified with the herbarium under the registration numbers KLU 047368. All samples were washed under tap water and dried in an oven at 40 °C for 3 days. The plant materials were then put through a grinder with a mesh size of 2 mm.

Diterpens extract from *A. squamosa* fruit

This method is based on [9]. One kg of the dried fruit was extracted five times from methanol. The combined methanolic extracts were evaporated under reduced vacuum at 40 °C. Chloroform solution containing 3% HCl was added and then the extracts were dried by evaporation under reduced vacuum at 40 °C. The product yield was 0.01% of the original sample.

Thin layer chromatography and IR spectrometry

TLC chromatography based on the method [10]. Diterpenes fractions of the plant were

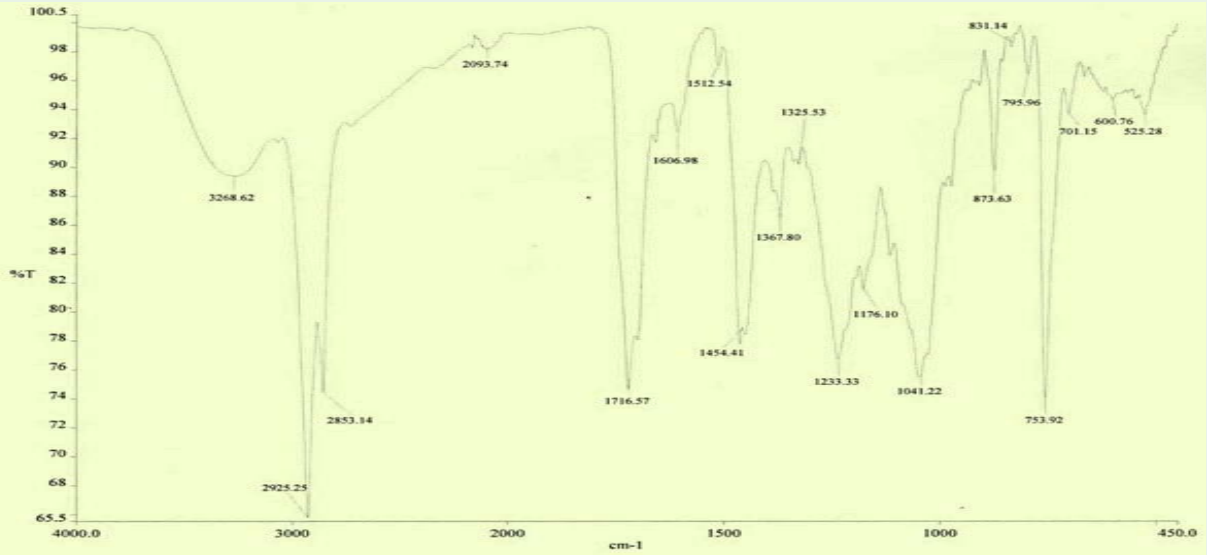

Figure 1: IR spectra of diterpenes extract from the fruit of *A. squamosa*.

loaded onto TLC plates 60 F254 (Merck, Germany). The mobile phase dichloromethane: methanol (9:1) and spray by the reagent anisaldehyde H_2SO_4 to get the blue or violet spot for diterpenes compounds. All TLC plates were visualized under UV light at wavelength 245 nm and 356 nm. Then, the IR spectrum of these compounds was recorded by FTIR (Perkin Elmer spectrum 400 FT-IR, UK) at room temperature from 400 to 4000 cm^{-1} for scanning directly.

Results and Discussion

These results from diterpenes extract from the fruit of *A. squamosa* revealed the presence of the components by using anisaldehyde H_2SO_4. The fruit of the plant is known to contain ditepenes compounds [11,12]. TLC results of diterpene extract from *A. squamosa* fruit was observed at band 3268.62 cm^{-1} by O-H stretching. A C-H stretching group was detected at 2925.25 and 2853.14 cm^{-1}. The C=O functional group was detected at band 1716.57 cm^{-1}. C-H group bonds were detected at bands 1512.54, 1454.41 and 1325.53 cm^{-1}. The C-O functional group was detected at band 1233.33 cm^{-1} (Figure 1). IR spectra of diterpenes extracted from the fruit of *A. squamosa* indicated the presence O-H stretching, C-H stretching, C=O functional group and Bond C-H groups. Schulz and Baranska [13] have previously reported similar results for diterpenes extracted from different plants. Diterpenes extracted from *A. squamosa* fruit identified six major compounds: kuaran-18-al, 16,17,19-kauranetriol, kauren-18-ol, kaur-16-ene, stigmasterol and annosquamosin.

In conclusion, this is the first report that studied isolation and identification of diterpenes extracts from the plant. Diterpenes extracted from these plants identified important compounds which may be used to develop biopharmaceuticals against infectious diseases with antioxidants source in future.

Acknowledgment

The author would like to thank University of Malaya for the financial and lab facilities support for this study from IPPP grant (PV034/2011A).

References

1. Lim T. *Annona squamosa*: Edible medicinal and non-medicinal plants. Springer. 2012; 207-220.
2. Pareek S, Yahia EM, Pareek OP, Kaushik RA. Postharvest physiology and technology of *Annona* fruits. Food Research International. 2011; 44: 1741-1751.
3. Egydio A, Catarina C, Floh E, Santos D. Free amino acid composition of *Annona* (Annonaceae) fruit species of economic interest. Industrial Crops and Products. 2013; 45: 373-376.
4. Shah R. Pharmacognosy and pharmacology of *Annona squamosa*: A review. International Journal of Pharmacy &Life Science. 2011; 2: 1183-1189.
5. Pino JA. *Annona* fruits. Handbook of Fruit and Vegetable Flavors. 2010; 231.
6. Pandey N, Barve D. Phytochemical and pharmacological review on *Annona squamosa* linn. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2011; 2: 1404-1412.
7. Saelee C, Thongrakard V, Tencomnao T. Effects of thai medicinal herb extracts with anti-psoriatic activity on the expression on nf-κb signaling biomarkers in hacat keratinocytes. Molecules. 2011; 16: 3908-3932.
8. Wiart C, Au TS, Mohd Y, Hamimah H, Sulaiman M. 16 hydroxy-(-)-kauran-19-oic acid: An antibacterial diterpene from sweet apple (*Annona squamosa l.*, annonaceae). International Journal of Pharmacology. 2005; 1: 296-298.
9. Wu Y, Hung YC, Chang FR, Cosentino M, Wang HK, Lee K. Identification of ent-16β, 17-dihydroxykauran-19-oic acid as an Anti-HIV principle and isolation of the new diterpenoids annosquamosins A and B from *Annona squamosa*. Journal of Natural Products. 1996; 59: 635-637.
10. Smita N, Sushma M. Preliminary physicochemical and phytochemical evaluation of *Morinda citrifolia* fruit extractives. International Journal of Pharmacy and Pharmaceutical Sciences. 2010; 2: 150-154.
11. Lai HY, Lim YY, Kim KH. Blechnum orientale linn-a fern with potential as antioxidant, anticancer and antibacterial agent. BMC Complementary and Alternative Medicine. 2010; 10: 15-23.
12. Hassan H, Hanifah YA, Wiart C, Kamaruddin MY. Antibacterial activity of *Annona squamosa* linnaeus (Annonaceae). Investing in Innovation. 2003; 3: 7-10.
13. Schulz H, Baranska M. Identification and quantification of valuable plant substances by IR and Raman spectroscopy. Vibrational Spectroscopy. 2007; 43: 13-25.