

Bioassay Guided Search For Active Fraction/S Of *Annona Muricata* With Reference To Diabetes

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Abstract:

Annona muricata is a member of the *annonaceace* family and it is known as soursop, Graviola, is an evergreen plant that is mostly distributed in tropical and subtropical regions of the world. A wide array of ethnomedicinal activities is contributed to different parts of *A.muricata* and indigenous communities in Africa and South America extensively use this plant in their medicine. Phytochemical studies reveal that annonaceousacetogenins are the major constituents of *A. muricata*. More than 100 annonaceousacetogenins have been isolated from the leave, stem, barks, seed, fruits, roots, of *A.muricata*. Numerous investigations have revealed these hepatoprotective and antidiabetic activities. This study focuses on the identification of the active fraction/s of *A.muricata* plant parts leaf, stem and fruit with reference to diabetes and to report the NMR, LC-MS data of pure isolated compounds.

Keywords: Acetogenins, diabetes, soursop.

1. INTRODUCTION

A Natural product is a chemical compound or substance produced by a living organism that is found in nature which usually has a pharmacological or biological activity for use in pharmaceutical drug discovery and drug design. Natural products may be extracted from tissues of terrestrial plants, marine organisms or micro organism fermentation broths. The term natural products today is quite commonly understood to refer to herbs, dietary supplements, traditional Chinese medicine or alternative medicine.

2. AIM AND OBJECTIVES

2.1 Aim

The main aim of the project is to find active fraction/s, from the medicinal plant *Annona muricata*, commonly known as soursop using bioassay guided fractionation approach with respect to diabetes.

2.2 Objectives

1. Extract preparation of plant material using suitable solvents
2. Fractionation of crude extract by using suitable solvent system
3. Biological assays (α -glycosidase assay and antioxidant assay) performance for fractions
4. Isolation and purification of compounds from the fractions by employing TLC and Column chromatographic techniques
5. Characterization of pure phyto-molecules by UV-VIS, IR, Nuclear magnetic Resonance (NMR) and Mass spectroscopic studies

3Plan of work

Literature survey of the plant *Annona muricata* revealed that is a medicinally very important plant exhibiting various pharmacological activities. Thus, it was taken up for present study.

The plan of work involved following steps:

- Identification, Collection and Authentication of the plant material.
- Drying and powdering of the collected material
- Extract preparation of plant material by using suitable solvent
- Fractionation of crude extract
- Biological assays
- Bioassay guided purification of fractions by using column chromatography and identification of the compounds
- Characterization of purified bio-molecules by UV-VIS, IR, Nuclear Magnetic Resonance (NMR) and Mass Spectroscopic studies

4. MATERIALS AND METHODS

Isolation, fractionation and purification of phytochemical constituents from the different parts (leaf, stem, fruit) of *Annona muricata*

4.1 Materials, Chemicals, and Instruments

Silica gel columns of different sizes, conical flasks, beakers, Weighing balance, test tubes, Merck Millipore PLC Silica gel 60 F₂₅₄ plate, 5% H₂SO₄ in Methanol charring solution, TLC chamber, UV chamber, Waters HPLC instrument, twin trough plate development chamber. All the chemicals and reagents used were obtained from Ranbaxy Fine Chemicals Ltd, Punjab.

4.1.1 Collection and Authentication

The plant sample (*Annona muricata* Linn.) was collected from Sri Venkateshwara University, Tirupathi, Andhrapradesh. The plant was authenticated by Dr. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateshwara University, Tirupathi, Andhrapradesh.

4.1.2 Preparation of powder of *Annona muricata* Leaves

The leaves were washed thoroughly in tap water and air dried in the shade for 4weeks. The dried leaves were grinded into coarse powder with the help of milling machine and weighed (about 10kgs).

4.1.3 Small scale extraction by Cold maceration

About 100gm of sample was taken into 5 small conical, added sufficient quantity of different solvents into each conical (hexane, methanol, acetone, chloroform, water) and kept for

4.1.4 Fractionation by column chromatography

Silica gel column of 60-120 mesh size was packed with hexane as a solvent and added with leaf ethyl acetate extract. Gradient column was done for fractionation with hexane and ethyl acetate solvents. A gradient ranging from 95% hexane: 5% ethyl acetate to 20% hexane: 80% ethyl acetate was run into column. 20% methanol: 80% chloroform system was also run at the last and fractions were collected. 120 Fractions were collected and based on TLC results; they were combined into 15 main fractions labelled as KSB-AML-1, 2, 3..., etc.

Figure 8: Fractionation by Column chromatography

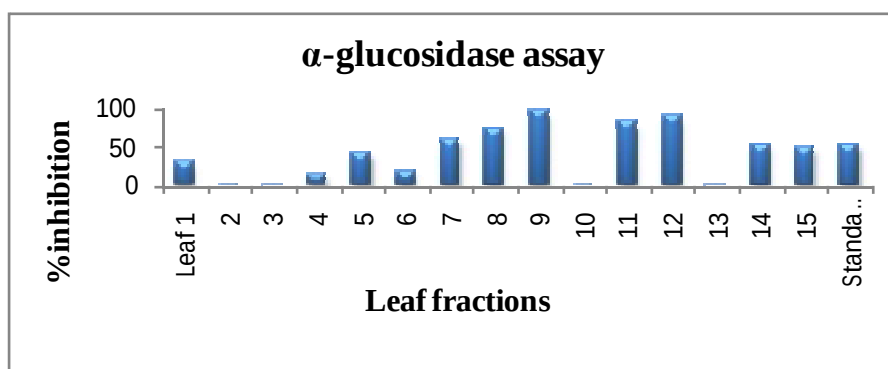


Figure 1: α-glucosidase assay Results of leaf fractions

5 Biological results of fruit fractions

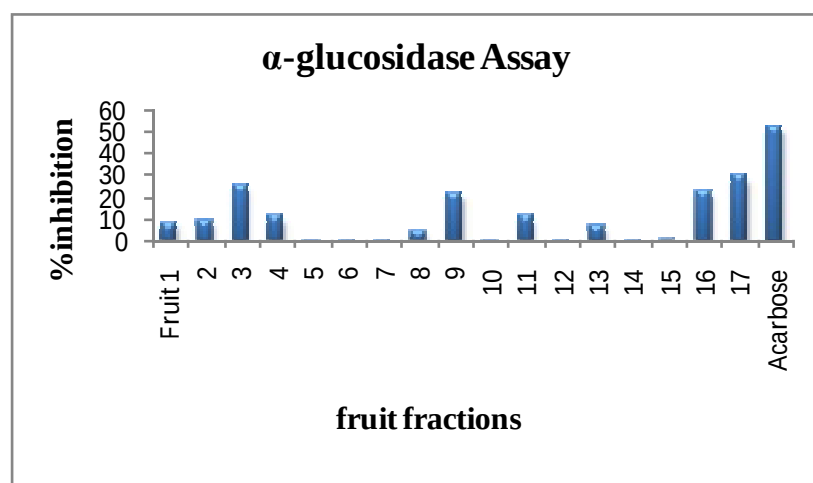


Figure 2: α-glucosidase assay results of fruit fractions

¹H-NMR spectrum of compound -1

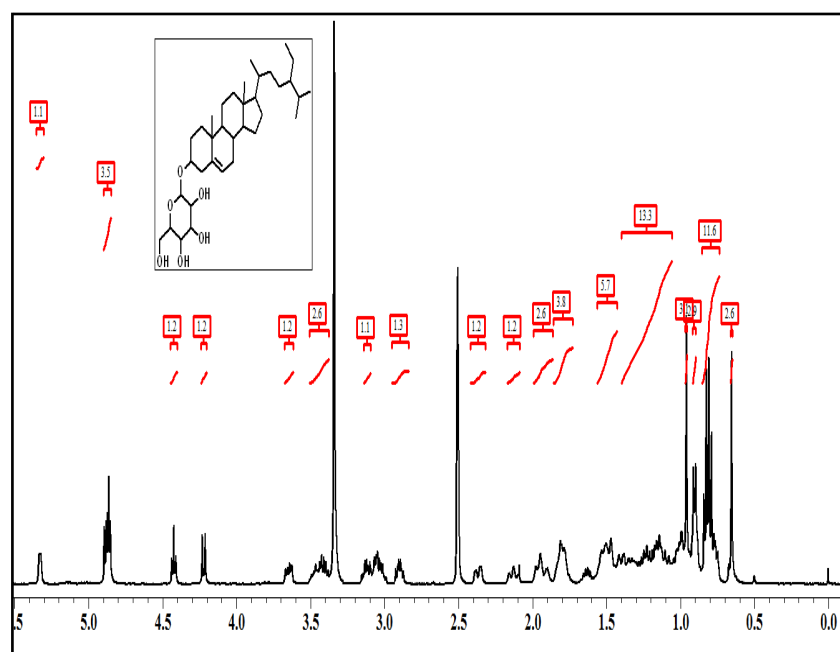


Figure 3: ¹H-NMR spectrum of compound -1(DMS O-d₆ 400MHz)

Discussion: From the above ¹H-NMR spectral data concluded that 6methyl groups groups and olefinic proton and comparison with literature data it was confirmed the compound Daucosterol.

^{13}C -NMR spectrum of compound -1

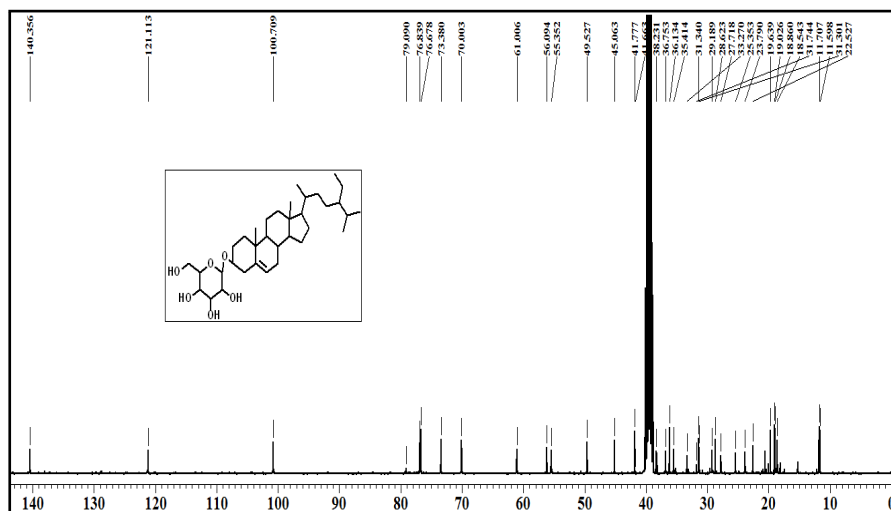


Figure 4: ^{13}C -NMR spectrum of compound -1($\text{DMSO}-d_6$ -100MHz)

Discussion: From the above ^{13}C -NMR spectral data concluded that indicates the presence of olefinic carbon, oxygen attached to carbon and hydroxyl group and comparison with literature it was confirmed as Daucosterol.

HRMS of compound -1

Minimum:		5.0	5.0	-1.5				
Maximum:		50.0	50.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	1-FIT	Norm	Conf(%)	Formula
599.4202	599.4208	1.4	2.2	5.5	529.1	n/a	n/a	$\text{C}_{28}\text{H}_{46}\text{O}_6\text{Na}$

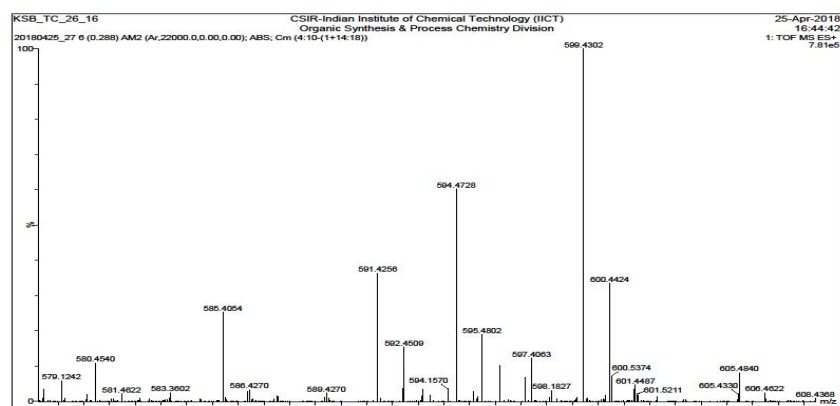


Figure 5: HRMS spectra of compound -1

Discussion: From the HRMS spectra the mass was obtained at $m/z 599(M^+ + Na^+)$ by compare with reported value and this corresponding to the molecular formula $C_{35}H_{60}O_6$ it was confirmed as Daucosterol.

IR data of compound -1

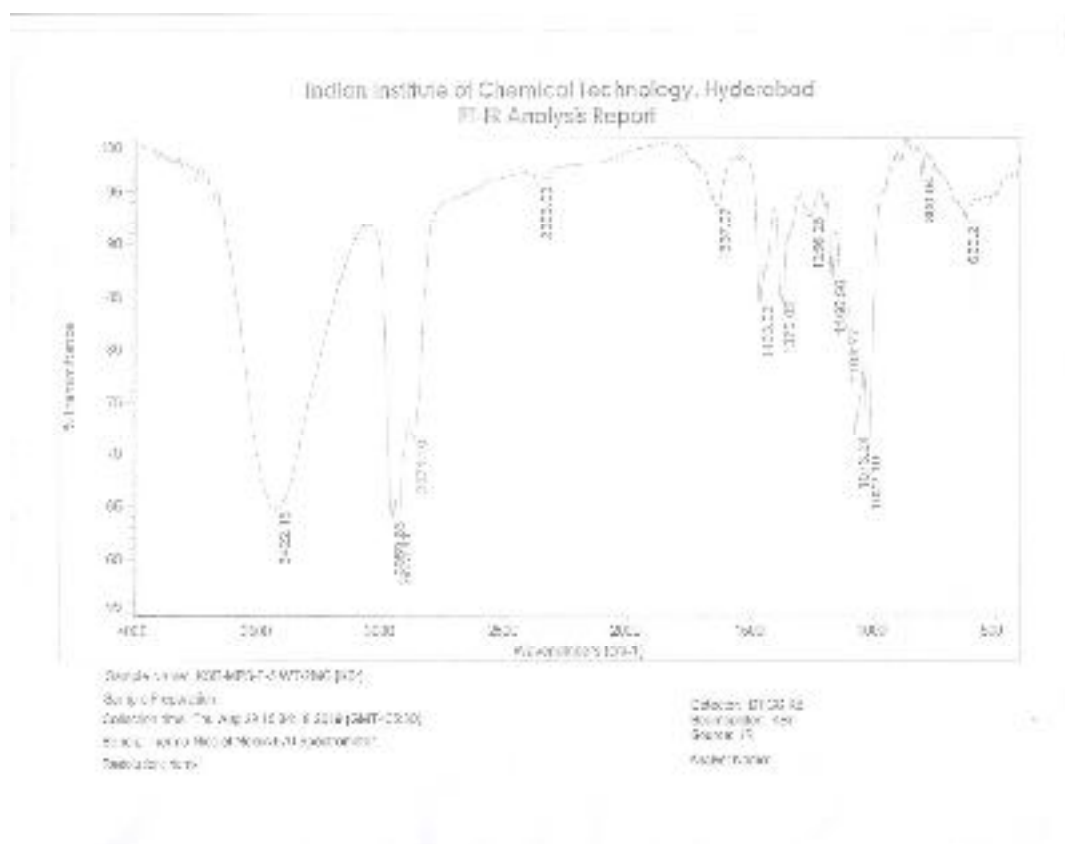


Figure 6: IR spectra of compound -1

Discussion: From the obtained IR bands relating to their functional groups the compound was confirmed to be as Daucosterol from the literature study.

5.5.1 Biological activity results of compound1 (Daucosterol)

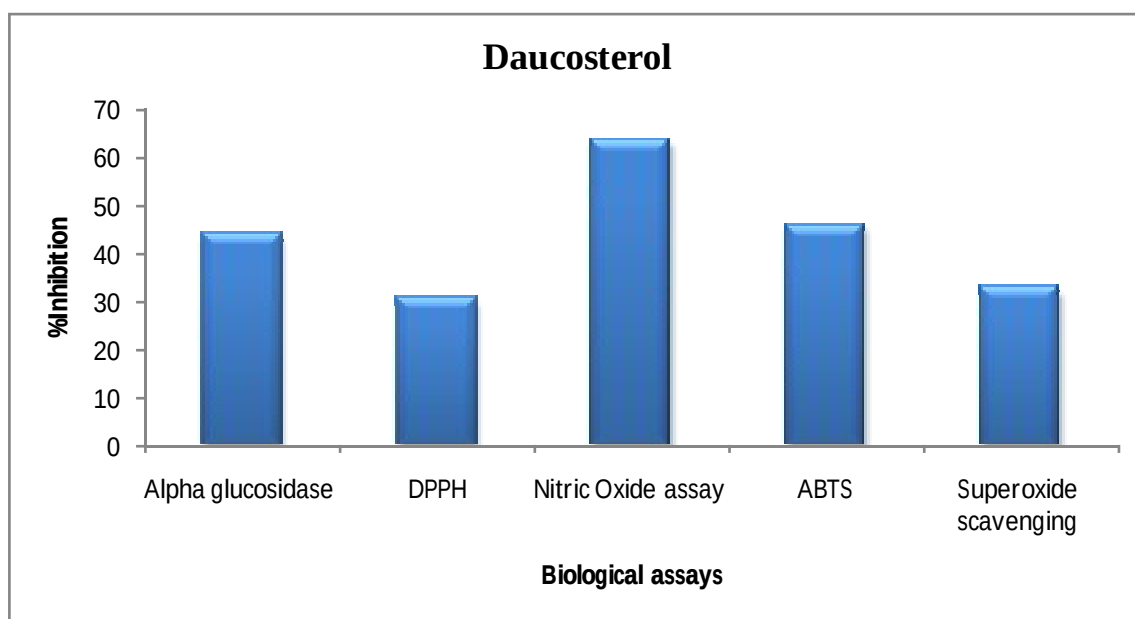


Figure7: Biological assay results of Compound1 (Daucosterol)

Conclusion:

As per the results of biological assays, stem main & sub-fractions KSB-AM-S-Fr2, 6, 8 are showed better α -glucosidase activity comparable with standard acarbose (52.9). KSB-AM-S-Fr2, 6, 8 sub-fractions showed good antioxidant activity revealed by DPPH, nitric oxide and superoxide assay. Also leaf main & sub-fractions KSB-AM-L-Fr7, 8, 9,11,12,14 Showed better α -glucosidase activity and antioxidant activities comparable to standard acarbose (52.9). Also fruit main fractions KSB-AM-F-Fr3, 9, 16, 17 Showed better α -glucosidase activity comparable to standard acarbose (52.9). As per overall comparison stem fractions are much better in activity than leaf, fruit fractions. Daucosterol isolated from fruit (Fr9) showed overall better α -glucosidase activity comparable to standard acarbose (52.9). From the results it can be concluded that stem fractions has acetogenins responsible for these activities and can be used for future studies against diabetes.

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