Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2015, 4 (1): 120-126

(International Peer Reviewed Journal)

Isoquinoline Alkaloid, Flavonoids And A Triol From Leaves of *Annona Cherimola*

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Accepted on 17th December 2014

ABSTRACT

*One new isoquinoline alkaloid (**1**), three quercetin glycosides (**2**-**4**) and a triol (**5**) were isolated from the methanol extract of leaves of Annona cherimola. Quercetin glycosides and a triol were first time reported from this plant. The structures were confirmed by ^{1}H, ^{13}C, 2D NMR spectroscopy and mass spectral data. Compounds **2-4** are found showing prominent antioxidant activity against superoxide radical, DPPH, FRAP, ABTS and moderate anti-inflammatory activity against 5-LOX. The IC_{50} values of compounds **1-4** are 24.29, 5.98, 9.11, 2.87 µg mL^{-1} respectively against DPPH radical showing high antioxidant activity of compound **4**.*

Keywords: *Annona cherimola*, spectral studies, anti-oxidant and anti-inflammatory activities, *in vitro* studies.

INTRODUCTION

From times immemorial, medicinal plants are the centre of attention of both biochemists and organic chemists alike. Herbal medicines are extensively being used, more so in developing countries, because of their precious therapeutic activities and also low toxicity profile. The structures of phytochemicals are often complex and elucidation of structures of them has been and continues to be a major challenge to organic chemists. Hence the phytochemical and pharmacological studies have been constituting a major portion of research of organic chemists and biochemists, especially pharmacologists.

Annona cherimola, belonging to family Annonaceae, is a deciduous or semi-evergreen shrub or small tree native to Andean-highland valleys of Echador and Northern Peru and is distributed widely in the tropical or sub-tropic regions, America, Africa and Asia and even in the south of Europe where it is cultivated for its edible fruits. It has shown good anti-oxidant and anti-inflammatory properties *in vitro* [1]. The methanol extract of *A.cherimola* (EAC) was also evaluated for its anti-hyperlipidamic activity *in vivo* [2].

Its anti-arthritic potential *in vivo* was also studied [3]. The anti-inflammatory and analgesic effects of methanol extract of leaves of *A.cherimola* were also evaluated [4]. The plant has also been used for the treatment of skin diseases, especially boils [5]. The plant on preliminary photochemical screening is reported to contain alkaloids, flavonoids, glycosides, saponins, tannins, carbohydrates, proteins, phenolic compounds, phytosterols and amino acids [6]. Cherimoline, cherinonaine, eleven kauranes, three lignans, eight amides, two acetogenins, two purines, one lactum amide, nine steroids, thirty five alkaloids, one *p*-quinone and nineteen benzinoids were isolated from the stems of this plant. Annocherine C, a new C-α hydroxyl benzyl isoquinoline and some other constituents were isolated from the leaves of *A.cherimola* [7]. Flavonoids are reported wide usage in manufacture of varied medicines [8]. The anti-oxidant capacity of phenolic compounds is reported solvent dependent [9]. Because of the bioactivity of the plant, it is of importance to investigate further to isolate the active principles responsible for the activity hence the present study was carried out to isolate further constituents of leaves of *A.cherimola* and elucidate their structures by using spectroscopic methods and also evaluate their pharmacological activity *in vivo*.

MATERIALS AND METHODS

Melting points were measured in metlar Toledo MP70 instrument and are uncorrected. The ^{1}H NMR spectra were recorded on Bruker Avance AV 400 MHz Spectrometer and ^{13}C NMR spectra were recorded on Bruker Avance AV 100 MHz Spectrometer. Mass experiments were performed on LC-MS system equipped with Agilent 1100 series LC/MSD detector and 1100 series HPLC pump. Normal phase silica gel (ACME, 100-200 mesh) was used for column chromatography. Silica gel pre-coated plates (AlugramSil G/UV_{254}) were used for thin layer chromatography. The plates were eluted with a solvent system containing hexane/ethyl acetate (6:4) and chloroform/methanol (8:2) mixtures and visualized by immersing the plate in sulfuric acid reagent followed by heating at 110ºC. The solvents and other chemicals used were of LR grade and were procured from Qualigens Fine Chemicals, Mumbai (India).

Extraction: *A.cherimola* dried leaves (95g) were pulverized and extracted with methanol (1 x 800 mL, 3 x 600 mL) at reflux temperature for one hour for each extract and filtered on super cell. The filtrate was concentrated on rotavapour under reduced pressure to get 24g of a thick paste.

Fractionation: The above thick paste (24g) was adsorbed on silica gel (65 g, 100 to 200 mesh) and 500 g of silica gel was used for column bed. The column was eluted with hexane, 10%, 20%, 30%, 50%, 60%, 80% ethyl acetate / hexane, ethyl acetate, 50% methanol / ethyl acetate, methanol. All the fractions were collected and monitored by TLC. Depending upon the TLC similar fractions were combined and concentrated to get 11 fractions. 10th fraction (12.8g) was subjected to silica column using chloroform / methanol mixtures as eluents. The fractions obtained were rechromatographed using chloroform/acetone mixtures to get five (Fig 2) compounds viz. Isoquinoline alkaloid (**1**, 40 mg), Quercetin-3-*O*-galacto pyranoside (**2**, 35mg), Quercetin-3-*O*-glucopyranoside (**3**, 20mg), Quercetin-3-*O*-arabinopyranoside (**4**, 22mg) and triol (**5**, 60mg). Compound was acetylated using acetic anhydride and pyridine and purified to get triacetate compound **6**.

RESULTS AND DISCUSSION

Isoquinoline alkaloid (1): brown colour powder; ^{1}H NMR (d_6 DMSO, 400MHz): δ 9.19 (1H, br s, H-OH), 7.56 (1H, s, H-16), 6.75 (1H, s, H-13), 6.58 (1H, s, H-1), 6.12 (1H, s, H-18a), 5.97 (1H, s, H-18b), 3.78 (3H, s, H-OCH_3), 3.05 (1H, dd, J = 4.0, 14.0 Hz, H-11a), 2.97-2.90 (3H, m, H-7a, 8a, 5), 2.57 (1H, d, J = 15.6 Hz, H-8b), 2.43 (3H, s, H-NCH_3), 2.38-2.34 (2H, m, H-7b, 11b).

Quercetin-3-*O*-galactopyranoside(2):yellowpowder ^{1}HNMR (d_6-DMSO,400MHz): δ 12.64 (1H, s, H-5 OH), 7.68 (1H, d, J = 8.4 Hz, H- 6'), 7.54 (1H, s, H-2'), 6.83 (1H, d, J = 8.4 Hz, H-5'), 6.41 (1H, s, H-

8), 6.21 (1H, s, H-6), 5.38 (1H, d, *J* = 7.6 Hz, H-1''), 5.13- 4.44 (4H, br s, H-OHs at 2'', 3'', 4'', 6''), 3.66-3.24 (6H, m, H- 2'', 3'', 4'', 5'', 6'').

Quercetin-3-*O*-glucopyranoside(3): yellow powder; ^{1}HNMR (d_6-DMSO,400MHz): δ 12.63 (1H, s, H-5 OH), 7.57 (2H, m, H- 2',6'), 6.84 (1H, d, *J* = 9.3 Hz, H-5'), 6.39 (1H, s, H-8), 6.19 (1H, s, H-6), 5.46 (1H, d, *J* = 6.8 Hz, H-1''), 5.30- 4.26 (4H, br s, H-OHs at 2'', 3'', 4'', 6''), 3.59-3.07 (6H, m, H- 2'', 3'', 4'', 5'', 6'').

Quercetin-3-*O*-arabinopyranoside(4): yellow powder; ^{1}HNMR (d_6-DMSO, 400 MHz):δ 12.73 (1H, s, H-5 OH), 7.66 (1H, dd, *J* = 2.0, 8.8 Hz, H- 6'), 7.52 (1H, d, *J* = 2.0 Hz, H-2'), 6.85 (1H, d, *J* = 8.8 Hz, H-5'), 6.40 (1H, d, *J* = 1.2 Hz, H-8), 6.19 (1H, d, *J* = 1.2 Hz, H-6), 5.28 (1H, d, *J* = 4.8(Hz, H-1''), 3.77 (1H, t, *J* = 5.6 Hz, H- 2''), 3.65 (1H, m, H- 3''), 3.58 (1H, dd, *J*= 5.2, 9.6 Hz, H-5''), 3.52 (1H, dd, *J* = 3.2, 7.2 Hz, H-4''), 3.23 (1H, d, *J* = 9.6 Hz, H-5'').

Triol(5): pale yellow oil, ^{1}H NMR (d_6- DMSO, 400MHz): δ 5.03 (2H, d, *J* = 1.2 Hz, H-5), 3.98 (1H, m, H-3), 3.96 (1H, d, *J* =12.6 Hz, H-1a), 3.92 (1H, d, *J* =12.6 Hz, H-1b), 3.42 (1H, dd, *J* = 4.4, 10.8 Hz, H-4b), 3.31 (1H, dd, *J* = 6.8, 10.8 Hz, H-4a).

Table 1. HMBC spectral data of Isoquinoline alkaloid (1)

Sl. No.	^{1}H NMR data	^{13}C NMR data	HMBC
1	9.19 (1H,s)		
2	7.56 (1H,s)	111.5	146.3, 128.7, 121.5, 115.3
3	6.75 (1H,s)	115.6	146.4, 121.5, 116.3, 111.5, 33.2
4	6.58 (1H,s)	106.2	146.1, 141.1, 126.6, 116.3, 28.7
5	6.12 (1H,s)	100.5	146.1, 141.1
6	5.97 (1H,s)	100.5	146.1, 141.1
7	3.78 (3H,s)	56.0	146.3
8	3.05 (1H,dd *J*= 4.0, 14.0Hz)	33.2	126.7, 126.6, 121.5, 115.6, 62.0
9	2.97 (1H, m)	52.9	
10	2.94 (1H, m)	28.7	
11	2.90 (1H, m)	62.0	126.6
12	2.57 (1H,d, *J*=15.6Hz)	28.7	
13	2.43 (3H, s)	43.5	62.0, 52.9
14	2.38 (1H, m)	52.9	128.7, 62.0
15	2.34 (1H, m)	33.2	146.2, 128.7, 126.6, 121.5, 115.6, 62.0

Table 2. DEPT spectral data of Isoquinoline alkaloid (1)

-C-	-CH-	CH_2-	-CH_3
146.4	115.6	100.5	56.0
146.3	111.5	52.9	43.5
146.1	106.2	33.2	
141.0	62.0	28.7	
128.7			
126.6			
126.2			
121.5			
116.3			

Table 3. ^{13}C NMR Spectral data of Quercetin glycoside(2-4)

Sl. No.	Position	Compound **1**	Compound **2**	Compound **3**
1	1	--	--	--
2	2	156.3	156.3	156.3
3	3	133.5	133.3	133.7
4	4	177.5	177.5	177.4
5	5	161.2	161.3	161.2
6	6	98.7	98.7	98.8
7	7	164.3	164.2	164.8
8	8	93.5	93.5	93.6
9	9	156.2	156.2	156.2
10	10	103.8	103.9	103.7
11	1'	121.1	121.6	121.9
12	2'	115.9	116.2	115.8
13	3'	144.8	144.8	145.1
14	4'	148.5	148.5	148.7
15	5'	115.2	115.2	115.4
16	6'	121.9	121.2	120.8
17	1''	101.9	100.8	101.5
18	2''	73.2	76.5	71.7
19	3''	71.2	74.1	70.7
20	4''	75.8	77.6	66.0
21	5''	67.9	69.9	64.2
22	6''	60.1	61.0	--

The molecular weight of the compound **1** is 325. It indicates that it may contain an odd number of nitrogen atoms. It was confirmed by the test with Dragendroff's reagent. In proton NMR spectra a broad singlet at δ 9.19 is not correlated with any carbon in HSQC indicating that the proton may be on heteroatom. The proton δ values 7.56, 6.75 and 6.58 are correlated with 111.5, 115.6, 106.2 carbons in HSQC respectively inferring that they are aromatic. The protons 6.12 and 5.97 are correlated with one carbon with δ value 100.5 in HSQC showing that there may be a metylenedioxy group and as the protons are correlated with 146.1 and 141.1 in HMBC indicating that the methylenedioxy group may be present on aromatic ring. The proton signal δ **3.78** (3H) is correlating with 56.0 carbon in HSQC and with 146.3 in HMBC indicating that a methoxy group may be located on aromatic or double bonded carbon. From the ^{13}C NMR, the compound contains nineteen carbons. According to ^{13}C NMR and DEPT 45 spectra the compounds contains nine tertiary carbons. From DEPT 45, 90 and 135 it is learnt that there are two methyl carbons, four methylene carbons and four methyne carbons. In proton NMR one methyl group appeared at 2.43 as singlet. So it may be a methyl group on Nitrogen atom. In HMBC 6.75 correlates 121.5, 146.4, 116.3, 111.5 & 33.2; 6.58 correlates with 146.1, 141.1, 126.6, 116.3 & 28.7 and 7.56 correlates with 146.3, 128.7, 121.5 & 115.3 and all the other correlations are depicted in Fig 1. With all the above correlations it is confirmed that the compound **1** is isoquinoline alkaloid.

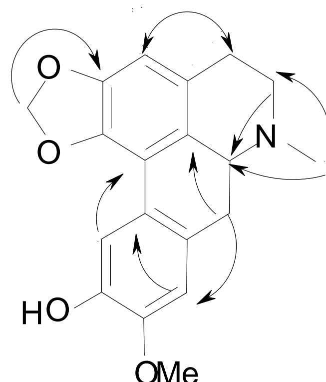
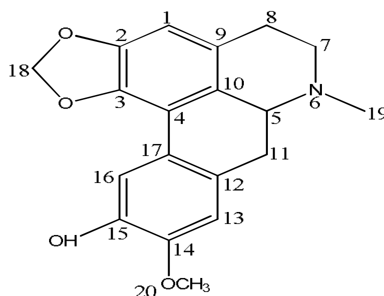


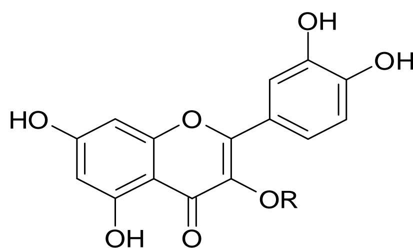
Fig 1 : HMBC correlations of compound 1

The molecular weight of Compound **2** is 464. In the ^{13}C NMR spectra there are 16 peaks with the δ values ranging from 177.5 to 93.5 and they may be aromatic carbons and five peaks in the δ range from 75.8 to 60.1 shows the presence of oxygenated aliphatic or alicyclic carbons in the compound. A peak at 12.64 in ^{1}H NMR indicates chelation through a hydroxyl group. Two singlets at 6.21 and 6.41 indicate protons at 6 and 8 positions of ring A of a flavonoid; peaks at δ = 7.68, 7.54 & 6.83 indicate the presence of 3 protons on the aromatic ring B. All the above data confirms the presence of quercetin. A peak at 5.38 shows the presence of an anomeric proton of carbohydrate. Peaks at 3.66 to 3.24 also show the presence of remaining protons of carbohydrate. From the above data the compound **2** is quercetin-3-*O*- galactopyranoside, the data was also well correlated with literature values [10]. In compound **3** all the ^{1}H & ^{13}C NMR values of the compound are similar to the compound **2** but the difference is only in the range δ 77.6 to 61.0 of carbon spectra. The data of the compound **3** is well correlated with literature data of quercetin-3-*O*-glucopyranoside [10]. Similarly in compound **4** also all the ^{1}H & ^{13}C NMR values are similar to that of compound **2** but the difference is only in the range δ 77.6 to 61.0 of carbon spectra and one carbon lesser than compound **2**. The data is well correlated with literature data of quercetin-3-*O*-arabino pyranoside [11].^{13}C NMR spectral data of compounds (2-4) are comparatively shown in table 3.

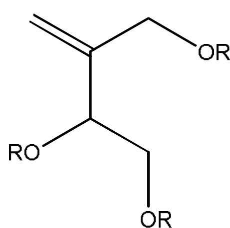
Compound **5** is having five carbons i.e. 150.7, 108.8, 72.8, 65.4, 61.4. It indicates that the compound is having one double bond and three oxygenated carbons. In proton spectra δ 5.03 is having two protons and 3.98 one proton. δ 3.96 and 3.92 are having coupling constant J = 12.6, so they may be geminal protons. The protons 3.42, 3.31 appear as double doublet having coupling constant J = 4.4, 10.8 Hz and 6.8, 10.8 Hz. From all the above data it is concluded that the structure of the compound **5** is as shown in Fig 2. It was acetylated (compound **6**) with acetic anhydride and pyridine and ^{1}H NMR is taken. It gave three acetylated groups and this acetylated product also supports the structure of compound **5** as 2-methylenebutane-1,3,4-triol. The data was also well correlated with literature values[12].



1. Isoquinoline alkaloid



2. R= *β*-D-galactopyranoside
3 . R= *β*-D-glucopyranoside
4. R= *β*-D-arabinopyranoside



5. R = H, **6**. R = Ac

Fig 2: Structures of Isolates of *A. cherimola*

APPLICATIONS

Bioactivity Studies *In Vitro:* In view of the structures, especially the presence of hydroxyl groups in isolates of *A.cherimola*, the antioxidant and anti-inflammatory activity of the compounds was carried out *in vitro* by Superoxide radical scavenging activity (NBT) [13,14] , DPPH free radical scavenging activity [15], Ferric reducing antioxidant power (FRAP) assay [16], ABTS antioxidant assay[17,18] and 5-Lipoxygenase inhibition assay [19-21] methods. The results are summerised in Table 4.

Table 4: *In vitro* activity studies of compounds 1-5 of *A. cherimola*

S.NO	Compound	IC_{50} µg/Ml				
		Anti-oxidant activity				Anti-inflammatory activity
		NBT	DPPH	FRAP	ABTS	5-LOX
	1	--	24.29	275.62	--	--
	2	27.35	5.98	>500	5.67	6.1
	3	34.22	9.11	>500	7.39	7.3
	4	13.60	2.87	257.94	2.62	21.5
	5	--	64.45	>500	--	--
	Gallic acid	0.52	-	-	-	-
	Vitamin-C	-	3.57	113.96	1.63	-
	NDGA	-				5.3

The Compounds **2**-**4** are showing prominent antioxidant activity against superoxide radical, DPPH, FRAP, ABTS and moderate anti-inflammatory activity against 5-LOX. The IC_{50} values of compounds **1**-**4** are 24.29, 5.98, 9.11, 2.87 µg mL^{-1} respectively against DPPH radical indicating their moderate antioxidation potential. The antioxidation potential IC_{50} of compound **4** is 2.87 against DPPH radical and 2.62 against ABTS and hence its activity is comparable to that of vitamin C, the standard.

CONCLUSIONS

In this investigation one new isoquinoline alkaloid, three quercetin glycosides and a triol (1-5) were isolated from the methanol extract of leaves of A.cherimola and the structures of the isolates were elucidated by spectroscopic methods. In vitro activity studies revealed that the compounds 2-4 are having prominent anti-oxidant activity and moderate anti-inflammatory activity.

ACKNOWLEDGEMENT

The authors thank Dr G. Ganga Raju, Chairman, Laila Group of companies, Mr. G. Rama Raju, Director and Mr. B. Kiran, CEO, Laila Impex Research and Development centre, Vijayawada for their encouragement.

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