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(*Chenopodium Quinoa* Willd.)

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PHARMACOGNOSY
MASTER THESIS

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APPROVAL

This thesis prepared and presented by **Farman Ullah KHAN** entitled as **"Triterpene saponins from the Seed Coat of Quinoa (*Chenopodium Quinoa* Willd.)** has been accepted by the Examination committee for the degree of Master of science in Pharmacognosy.

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According to the relevant articles of the Near East university postgraduate study - Education and Examination Regulations, this thesis has been approved by the members of the Thesis Committee and the decision of the Board of the Directors of the Institute

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ÖZET

Amaranthaceae (Alt familya: Chenopodiaceae) Familyasının bir üyesi olan *Chenopodium quinoa* Willd. Türkiye'de tarımına başlanan bir tarım ürünüdür. Vatanı Güney Amerika'da And dağlarının yüksek platoları olan bu bitki farklı iklim ve toprak koşullarına uyum gösterir. Bu çalışmada, Türkiye'nin Trakya bölgesinde 2017 yılında ekilen bitki materyali kullanılmıştır. *Chenopodium quinoa*'nın tohum kılıfı %80'lik etanolle ekstre edilmiş ve fitokimyasal çalışmalarda bu ekstre kullanılmıştır. Ham ekstreden, Vakumlu Kolon Kromatografisi (VCC), Kolon Kromatografisi (CC) ve Orta Basınçlı Sıvı Kromatografisi (MPLC) gibi bir seri kromatografi tekniği kullanılarak beş oleanan tipi bidesmozidik triterpen saponozitleri (saponinler) (CQ-1, CQ-2&5, CQ-3, CQ-4, CQ-6) izole edilmiştir. 1D ve 2D-NMR spektroskopik ve Yüksek Çözünürlüklü Kütle Spektrometrisi (HRMS) çalışmaları ile saponinlerin yapısı şöyle aydınlatılmıştır: 3β-[(*O*-β-D-glukopiranozil-(1→3)-α-L-arabinopiranozil)oksi]-23-hidroksi-olean-12-en-28-oik-asit-28-*O*-β-glukopiranozit [=3-*O*-β-D-glukopiranozil-(1→3)-α-L-arabinopiranozil hederagenin 28-*O*-β-D-glukopiranozit] (**CQ-3**), 3β-*O*-β-D-glukuronopiranozil)oksi-23hidroksi-olean-12-en-28-oik-asit-28-*O*-β-D-glukopiranozit (= 3-*O*-β-D-glukuronopiranozil-hederagenin-28-*O*-β-glukopiranozil ester) (**CQ-6**), 3β-[(*O*- β-D-glukopiranozil-(1→3)-α-L-arabinopiranozil)oksi]-23,30-dihidroksi-olean-12-en-28-oik-asit-28-*O*- β-D-glukopiranozit (**CQ-1**), 3β-*O*-β-D-glukopiranoziloksi-fitolakkagenik asit-28-*O*-β-D-glukopiranozit [3β-*O*-β-D-glukopiranozil- fitolakkagenik asit -28-*O*-β-D-glukopiranozil ester] (**CQ-4**) and 3β-[(*O*-β-D-glukopiranozil-(1→3)-α-L-arabinopiranozil)oxy]-23-hidroksi-olean-12-en-28,30-dioik-asit-28-*O*-β-glukopiranozit-30-metil ester [= 3-*O*-β-D-glukopiranozil-(1→3)-α-L-arabinopiranozil fitolakkagenik asit 28-*O*-β-D-glukopiranozit] (**CQ-2&5**). Bildiğimiz kadarıyla böyle bir çalışma Türkiye menşeli materyalde ilk kez yapılmıştır.

Gelecekte, saponinler ve onların oleanan-tipi sapogenol grupları üzerinde biyotransformasyon ve biyoaktivite çalışmaları yapılarak, sürdürülebilir doğal kaynaklardan daha etkili biyoaktif doğal bileşiklerin bulunmasına yönelik araştırmalar planlanmaktadır.

Anahtar Kelimeler*: Chenopodium quinoa*; Amaranthaceae; Saponin (=Saponozit); Oleanane-tip Triterpenler

ABSTRACT

As a member of Amaranthaceae *(*Subfamily: Chenopodiaceae*) Chenopodium quinoa* Willd. is one of the new staple crops cultivated in Turkey. C. quinoa is originated freom the Andean highland regions of S. America, adaptable to different types of soil and climatic conditions. In this present study, the plant material was obtained from the Thracian Region of Turkey, from the 2017 cultivars production. The seed coat powder of C. quinoa was subjected to 80% ethanol extraction, which was used for the phytochemical studies. Using a series of chromatographic methods such as Vacuum Column chromatography (VCC), open column chromatography (CC) and Medium pressure liquid chromatography (MPLC), five oleanane- type bidesmosidic triterpene saponosides (saponins) (CQ-1, CQ-2&5, CQ-3, CQ-4, CQ-6) were purifed from the crude extract. Based on 1D- and 2D-NMR spectroscopic studies and High Resolution Mass Spectrometric analysis (HRMS) the structure of the saponosides were identified as: 3β-[(*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl)oxy]-23-hydroxy-olean-12-en-28-oic-acid-28-*O*-β-glucopyranoside[=3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranoside] (**CQ-3**), 3β-*O*-β-D-glucuronopyranosyloxy-23hydroxy-olean-12-en-28-oic-acid-28-*O*-β-D-glucopyranoside (= 3-*O*-β-D-glucuronopyranosyl -hederagenin-28-*O*-β-glucopyranosyl ester) (**CQ-6**), 3β -[(*O*- β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl)oxy]-23,30-dihydroxy-olean-12-en-28-oic-acid-28-*O*- β-D-glucopyranoside (**CQ-1**), 3β-*O*-β-D-glucopyranosyloxy-phytolaccagenic acid-28-*O*-β-D-glucopyranoside [3 β-*O*-β-D-glucopyranosyl-phytolaccagenic acid-28-*O*-β-D-glucopyranosyl ester] (**CQ-4**) and3β-[(*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl)oxy]-23-hydroxy-olean-12-en-28,30-dioic-acid-28-*O*-β-glucopyranoside-30-methyl ester [= 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl phytolaccagenic acid 28-*O*-β-D-glucopyranoside] (**CQ-2&5**), to the best of our knowledge for the first time from Turkish cultivars.

Future studies will be performed on the saponins and as well as on their oleanane-type sapogenol moieties by biotransformation and bioactivity studies improving the structural diversities to discover more efficient bioactive natural compounds from renewable natural resources.

Key words*: Chenopodium quinoa*; Amaranthaceae; Saponins (=Saponosides); Oleanane-type Triterpenes

FIGURES

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SPECTRA

SPECTRA (*Continuing*)

TABLES

Abbreviations

C	Chromatography
CC	Column Chromatography
$CHCl_3$	Chloroform
COSY	^1H-^1H Correlation Spectroscopy
CQ	*Chenopodium quinoa*
DCM	Dichloromethane
EtOAc	Ethylacetate
EtOH	Ethanol
H-ESI	Heated-Electrospray Ionization
HMBC	Heteronuclear Multiple Bond Correlation Long Range ^1H-^{13}C Correlation Spectroscopy
HR-MS	High-Resolution Mass Spectrum
HSQC	Heteronuclear Single-Quantum Coherence Short Range ^1H-^{13}C Correlation Spectroscopy
Me_2CO	Acetone
MeOD	Methanol-d_4
MeOH	Methanol
MPLC	Medium Pressure Liquid Chromatography
MS	Mass Spectrum
NMR	Nuclear Magnetic Resonance
t-BuOH	t-Butanol
NOESY	Nuclear Overhauser Effect Spectroscopy
ROESY	Rotating-Frame Nuclear Overhauser Effect Spectroscopy
VCC	Vacuum Column Chromatography
WSE	Water Soluble Extract

1. INTRODUCTION:

Chenopodium quinoa Willd. (Quinoa) is a member of Amaranthaceae (Subfamily: Chenopodioideae) and found worldwide, with more than 250 species. It is a dicotyledonous annual flowering plant of 1– 2 m tallness with a pompous inflorescence. The seeds (roughly 2.5 mm long and 1.0 mm wide) are yellow, red, dark colored, and dark while the seed coats have a dark colored shading. It is native plant to South America and cultivated by the Andes people for thousands of years because of its high nutritional value. It is cultivated throughout the world including the Thracian Region of Turkey, Northern mountain areas of Pakistan, Egypt, China and India. The seed are rich in proteins consisting of essential amino acids, especially high lysine content. Therefore, this plant is cultivated throughout the world. However, the seed coat of quinoa rich in the bitter-tasting saponins should be removed before consuming as a food material (Kuljanabhagavad & Wink, 2009; Filho et al. 2017).

Medicinal (therapeutic) plants have been utilized since time immemorial for the treatment of disesases, hence Quinoa (*Chenopodium quinoa* Willd) has also been utilized for bioactive triterpenic saponins that it contains. The family Amaranthaceae (previously Chenopodiaceae) is represented with around 175 genera and 2000 species and has many plant species rich the oleanane-, ursane- and lupane-type triterpenoids (Mroczek, 2015). Several bitter-tasting saponins triterpene saponins have been reported from Chenopodium species which are mono- or bidesmosidic derivatives of hederagenin, 30-hydroxyhederagenin, phytolaccagenic acid, serjanic acid, 3β-hydroxy-23-oxo-olean-12-en-28-oic acid and 3β-hydroxy-27-oxo-olean-12-en-28-oic acid (Kuljanabhagavad & Wink, 2009). Saponins accumulated in the seed coat have been the subject of many researches due to their significant biological activities. Saponins have also huge mechanical significance and are utilized in the readiness of cleansers, shampoos, brew, fire quenchers and photography, corrective and pharmaceutical industry.

The aim of this study is confined to phytochemical examination of the bitter tasting outer seed coat of *Chenopodium quinoa* in respect to the triterpene saponosides. Chromatographical methods (VC: Vacuum Chromatography; CC: Column Chromatography; MPLC: Medium Pressure Liquid Chromatography) were used for the fractionation of the alcoholic extract and the isolation of the saponosides. The 1D- (^{1}H &

^{13}C-NMR, DEPT), and 2D-NMR (COSY, HSQC, HMBC, NOESY and ROESY) as well as High Resolution Mass Spectrometric (H-ESI MS) analysis were used in the structure elucidations of the isolated compounds.

2 LITERATURE REVIEW

2.1. Botanical Characters

2.1.1. Amaranthaceae Family

Amaranthaceae is a plant family that is native to Turkey, America and Africa, ranging between tropics and sub-tropics to more temperate regions. The family is believed to have originated in either the southwestern region of the United States, Latin America, or Africa yearly or enduring herbs or bushes, once in a ,while trees or annual or perennial herbs or shrubs, rarely trees or climbers climbers; straightforward, inverse or interchange, whole, without stipules; Inf spikes or head-like or axillary dichasial cymes, or Fl single; Bra and bracteoles frequently bristly, now and then developed and bright; Fl swinger (once in a while unisexual); Per fragments (2-) 3 - 5 (infrequently 0), typically dry and membranous, more often than not. Leaves are generally basic and whole, non-stipulate, phyllotaxy is interchange inverse. Blooms are consistent, cyclic, little, and described by spiked perianth with prominent bracts and bractlets and are either unisexual promiscuous (bisexual), singular accumulated into inflorescence (spikes cymes heads. Calyx presents with 3-5 sepals, corolla with 3-5 petals, androecium with 5 stamens, gynoecium with pistil having 2-3 carpels, 1-3 styles, ovule campylotropus, ovary-better with one chamber walling one in than a few ovules Leaves interchange or inverse, whole, exstipulate. Blossoms little, indiscriminate or unisexual, or sterile and diminished, subtended by 1 membranous bract and 2 bracteoles, single or collected in cymes. Inflorescences extended or consolidated spikes (heads), racemes, or thyrsoid structures of changing unpredictability. Bracteoles membranous or scarious. Tepals 3–5, membranous, scarious or subleathery, 1-, 3-, 5-, or 7(–23)- veined. Stamens the same number of as tepals and inverse these, seldom less than tepals; fibers free, joined into a glass at base or completely into a tube, fiber flaps present or missing, pseudo staminodes present or missing; anthers (1-or)2-loculed, dorsifixed, introrsely dehiscent. Ovary unrivaled, 1-loculed; ovules 1 to many; style relentless, short and indistinguishable or long and thin; shame capitate, penicillate, 2-lobed or shaping 2 fili form branches. Organic product a dry utricle or a meaty case, indehiscent, unpredictably blasting, or circumscissile. Seeds lenticular, reniform, subglobose, or in a matter of seconds cylindric, smooth or verruculose.

Morphology of the androecium, perianth (tepals), and the inflorescence has generally been utilized to delineate genera and clans. Pseudostaminodia are interstaminal limbs with differently molded apices. Fiber extremities are the sidelong members of fibers (one on each side). The essential structure of the inflorescence is the cyme (branchlets emerging from the bracteole axils, the bracteoles filling in as bracts for upper blooms), which can be lessened to one blossom with two bracteoles and a bract. Units of dispersal fluctuate extensively (cases opening with bring down part industrious, bloom and bracteoles falling together, or cymose fractional inflorescences severing above bract) and can be trademark for genera. A few genera have long trichomes serving dispersal at the base of the tepals (Basu, et al., 2014; Ke et al., 2003).

2.1.1.1. Distribution. Cosmopolitan yet packed in tropical and subtropical districts. The family checks with around 70 genera and 750 - 900 species and is firmly identified with the Chenopodiaceae. It incorporates well known garden plants (e.g. Celosia cristata, "Cockscomb") and a few species are utilized as vegetables (e.g. Amaranthus hybridus, leaves utilized as spinach) or pot herbs. Others are troublesome weeds. The monotypic family Arthraerva (Subfamily Amaranthoideae) outfits the main succulent of the family. specially found in Turkey, Pakistan, Afghanistan, Egypt, China, Central America, Australia Mediterranean region.

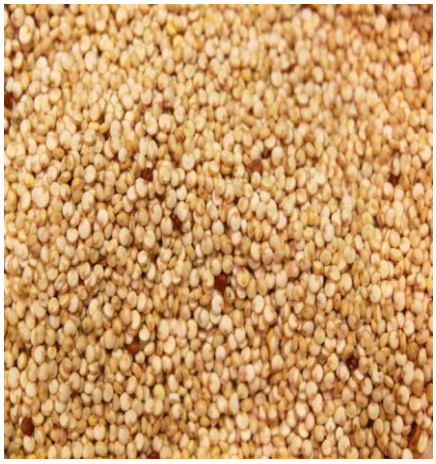
2.1.1.2. *Chenopodium quinoa*

Chenopodium quinoa from Quechua kinwa (kinuwa) is a blossoming plant in the Amaranthaceae. It is an annual herbaceous plant developed as a grain edit essentially for its eatable seeds. Quinoa isn't a grass, yet rather a pseudo cereal naturally identified with spinach and amaranth (*Amaranthus* spp.). *Chenopodium quinoa* Willd. (Amaranthaceae), a staple nourishment of the Andean people group, ordinarily known as quinoa, is a rising grain edit that is developed for its consumable seeds. Quinoa is of impressive logical and business enthusiasm for some districts of the world in view of the healthful structure of their seeds with an astounding proteins content (14-20%), carbohydrates, fats (essential oil composition) and cancer prevention agents, which are something like 5-crease higher than those of oat flours (Galwey, 1993; Jacobsen et al., 2003; Bhargava et al., 2006). The hereditary inconstancy of quinoa is enormous, with cultivars of quinoa being adjusted to development from ocean level to an elevation of more than 4000 meters and from cool, good

country atmospheres to subtropical conditions. This make it conceivable to choose, adjust, and breed cultivars for an extensive variety of ecological conditions, for example, parched or moist territories, chilly or hot situations, acidic or soluble soils (Jacobsen, 2003). The measure of saponins present in the quinoa seeds relies upon genoptype: it is higher in unpleasant flavor assortments than in sweet, or low-saponins, assortments. For saponin content, significant hereditary variety is accessible and a few sweet genotypes with low saponin content have been chosen. Saponin content relies upon the formative phase of the harvest, being low amid spreading and high amid blooming (Bhargava et al., 2006). Dry season diminishes by 45% the amassing of sapogenins in quinoa seeds, in view of one investigation of extreme water shortage led in Southern Europe (Gomez-Caravaca et al., 2012). Saponins of *Chenopodium quinoa* are the fundamental dynamic segments in the concentrate from the grain of *Chenopodium quinoa* seeds. Saponins are substances delivered normally by many plant species that have an extensive variety of bioactive properties, including antimicrobial impacts on different organisms also, microbes. It has likewise been suggested that saponins may prompt fundamental procured opposition (SAR) in treated plants.

https://media3.picsearch.com/is?ypr0X_29QrYGSLmInwP7qpXWBXv28JCCnsVLCVcPAnQ&height=192

Pictures 2.2.a&b. *Chenopodium quinoa*



Pictures 2.3.a&b. The Seeds of *Chenopodium quinoa* (Quinoa)

2.2. Phytochemistry and Nutritional Value of Quinoa

The phytochemical constituents of *Chenopodium* species have been reported together with their ethnopharmacological and pharmacological studies (Kokanova-Nedialkova et al., 2010). As primary metabolites, *Chenopodium* species are rich in carbohydrates and simple monosaccharides, proteins and essential amino acids, aromatic cytokinins and hormones. The genus *Chenopodium* have widely been studied for their secondary metabolites including, mono-, sesqui-, triterpenoids and carotenoids. The flowers, fruits, seed coats and seeds have intensively been studied for the triterpenoid sapogenins and their glycosides (saponins = saponosides). The major groups of optional metabolites revealed in quinoa are triterpenoids (saponins, phytosterols, and phytoecdysteroids), phenolics, betalains, also, glycine betaine.

2.2.1. Saponins

Phytochemical studies performed on the different parts of *Chenopodium* species especially on *Chenopodium quinoa* several oleanane-type saponins have been reported (Dini et al., 2001; Kokanova-Nedialkova et al., 2018; Kuljanabhagavad et al., 2008; Mizui et al., 1998 and 1990; Rastrelli et al., 1996; Woldemichael&Wink, 2001; Zhu et al., 2002). Sapogenol moiety of the isolated saponins are mostly oleanane-type triterpenoids, oleanolic acid, hederagenin, 30-hydroxyhederagenin, phytolaccagenic acid, serjanic acid, 3β-hydroxy-23-oxo-olean-12-en-28-oic acid and 3β-hydroxy-27-oxo-olean-12-en-28-oic acid, phytolaccagenin, 2*β*-hydroxyoleanoic acid, bayogenin, 2*β*-hydroxygypsogenin and medicagenic acid (**Table 2.1**). The saponins (saponosides) are the mono-, bi- or tridesmosidic glycosides containing arabinose, xylose, glucose, galactose and glucuronic acid as sugar units (**Table 2.2**).

From the roots of *Chenopodium bonus-henricus* L., several saponin glycosides have been reported which are phytolaccagenin, 2*β*-hydroxyoleanoic acid, bayogenin, 2*β*-hydroxygypsogenin and medicagenic acid derivatives (Kokanova-Nedialkova et al., 2018).

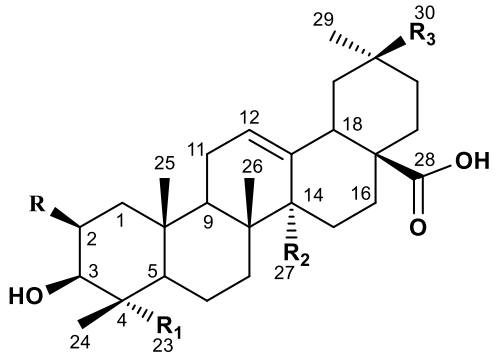
2.2.2. The Other Secondary Metabolites

The chemistry of *Chenopodium* species is recently documented by Kokonova-Nedialkova and her friends, reporting 379 compounds which primary and secondary metabolites. Majority of the secondary metabolites are simple phenolics (benzoic acid and cinnamic acid derivatives), flavonoids (monomeric and dimeric flavanols, flavonols, isoflavones flavanones) and isoprene derivatives; mono-, sesqui-, tri-, and tetraterpenoids, ecdysteroids, ionones, saponins, ecdysteroids and triterpenoids (Kokonova-Nedialkova et al., 2010; Gómez-Caravaca et al., 2012). The flavonoids reported are mostly methoxyflavonols glycosides such as patuletin, 6-methoxykaemferol and spinacetin (Kokanova-Nedialkova, Z. et al., 2016). The same authors reported the hepatoprotective and the antioxidant activity of the flavonoids isolated from *Chenopodium bonus-henricus* which is known in Turkey and Northern Cyprus as "yabani ispanak" (wild spinach). The roots of *C. bonus-henricus* are known as "chuven" in Bulgarian folk medicine and have been applied externally to treat skin inflammations, wounds and boils (Kokanova-Nedialkova, Z. et al., 2017).

The bioactive metabolites have been isolated mainly in the outer layers of the seeds and suggested to be chemical defense compounds against insect and microorganisms. These compounds are of hydrophilic or lipophilic nature. Phenolic compounds, especially phenolic acids as well as saponins are located primarily in the seed coat (Tang & Tsao,2017).

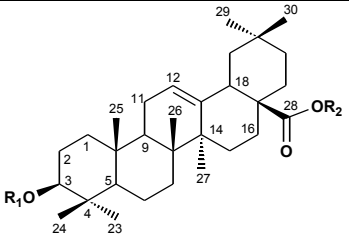
The aerial parts of *Chenopodium murale* collected from the Northern Cyprus have been studied for the volatile constituents (Polatoğlu et al., 2017). As reported in this study, essential oils of *Chenopodium* species are rich in *cis*-ascaridole, limonene, α-terpinylacetate and *cis*-isoascaridole are the main constituents.

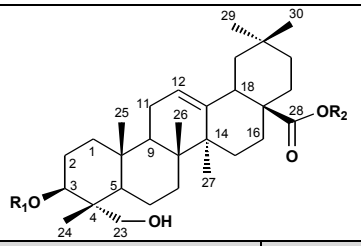
Table 2.1. The Structure of the Oleanane-type Aglycones of the Saponins isolated from *Chenopodium* species

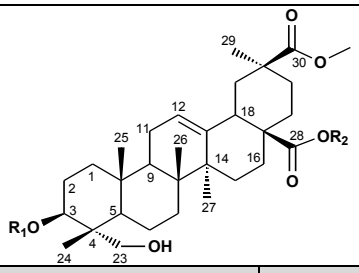


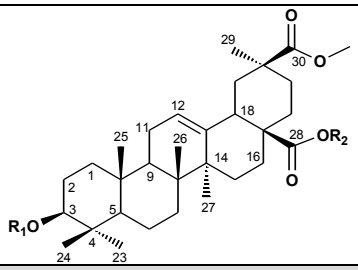
AGLYCONE	R	R_1	R_2	R_3	Formula & Mol. wt.
Oleanolic acid = 3β-hydroxy-olean-12-en-28-oic acid	H	CH_3	CH_3	CH_3	$C_{30}H_{48}O_3$ Mol wt 456
2*β*-hydroxyoleanoic acid	OH	CH_3	CH_3	CH_3	$C_{30}H_{48}O_4$ Mol wt 472
Hederagenin = 3β,23-dihydroxy-olean-12-en-28-oic acid	H	CH_2OH	CH_3	CH_3	$C_{30}H_{48}O_4$ Mol wt 472
30-Hydroxy-hederagenin = 3β,23,30-trihydroxy-olean-12-en-28-oic acid	H	CH_2OH	CH_3	CH_2OH	$C_{30}H_{48}O_5$ Mol wt 488
Phytolaccagenic acid = 3β,23-dihydroxy-olean-12-ene-28,30-dioc acid-30-methyl ester	H	CH_2OH	CH_3	$COOCH_3$	$C_{31}H_{48}O_6$ Mol wt 516
Serjanic acid = 3β-hydroxy-olean-12-en-28-oic acid-30-methyl ester	H	CH_3	CH_3	$COOCH_3$	$C_{31}H_{48}O_5$ Mol wt 500
Gypsogenin 3β-hydroxy-23-oxo-olean-12-en-28-oic acid	H	CHO	CH_3	CH_3	$C_{30}H_{46}O_4$ Mol wt 470
2β-hydroxy-gypsogenin	OH	CHO	CH_3	CH_3	$C_{30}H_{46}O_5$ Mol wt 486
3β-hydroxy-27-oxo-olean-12-en-28-oic acid	H	CH_3	CHO	CH_3	$C_{30}H_{46}O_4$ Mol wt 470
Bayogenin	OH	CH_2OH	CH_3	CH_3	$C_{30}H_{48}O_5$ Mol wt 488
Medicagenic acid	OH	COOH	CH_3	CH_3	$C_{30}H_{46}O_6$ Mol wt 502

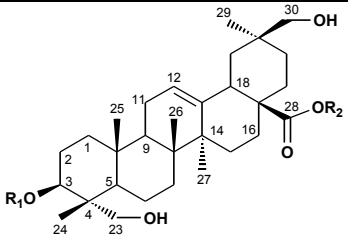
Table 2.2. The Structure of the Mono- and Bi- (**A – G**) and Tridesmosidic (**H**) Saponins (=Saponosides) isolated from *Chenopodium* species*.

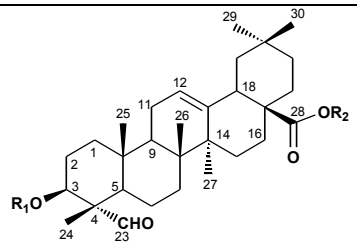
A: **Oleanolic acid Glycosides** = 3β-hydroxy-olean-12-en-28-oic acid Glycosides		
R_1	**R_2**	***Chenopodium* species**
β-D-GlurA	H	*C. quinoa*, *C. album*, *C. ficifolium*
β-D-Glu	H	*C. quinoa*
β-D-Xyl-(1→3)-6-Me-β-D-Glu	H	*C. quinoa*
β-D-Xyl-(1→3)-β-D-Glu	H	*C. quinoa*
β-D-Glu-(1→2)-β-D-Glu-(1→3)-α-L-Ara	β-D-Glu	*C. quinoa*
β-D-Glu-(1→3)-α-L-Ara	β-D-Glu	*C. quinoa*
β-D-Xyl-(1→3)-β-D-Glur A	β-D-Glu	*C. quinoa*
β-D-Glur A	β-D-Glu	*C. quinoa*, *C. album*
3-S1-Glur A	β-D-Glu	*C. album*
α-L-Ara-(1→3)-β-D-Glur A	β-D-Glu	*C. quinoa*
β-D-Glu	β-D-Glu-(1→2)-β-D-Glur A	

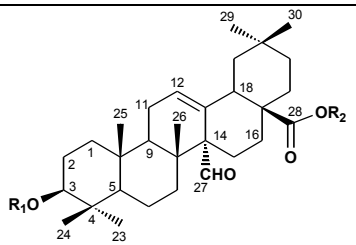
B: **Hederagenin Glycosides** = 3β,23-dihydroxy-olean-12-en-28-oic acid Glycosides		
R_1	**R_2**	***Chenopodium* species**
β-D-Glu-(1→3)-a-L-Ara	H	*C. quinoa*
β-D-Glu-(1→3)- α-L-Ara	β-D-Glu	*C. quinoa*
β-D-Glu-(1→3)-- β-Gal	β-D-Glu	*C. quinoa*
β-D-Glur A	β-D-Glu	*C. quinoa*
α-L-Ara	β-D-Glu	*C. quinoa*
β-D-Xyl-(1→3)- β-D-Glur A	β-D-Glu	*C. quinoa*
β-D-Glu-(1→4)- β-D-Glu-(1→4)- β-D-Glu	β-D-Glu	*C. quinoa*

C : **Phytolaccagenic acid Glycosides** = 3β,23-dihydroxy-olean-12-ene-28,30-dioc acid-30-methyl ester Glycosides		
R_1	**R_2**	***Chenopodium* species**
β-D-Glu-(1→3)-α-L-Ara	H	*C. quinoa*
β-D-Glu-(1→3)-α-L-Ara	β-D-Glu	*C. quinoa*
β-D-Glu-(1→2)- β-D-Glu-(1→3)-α-L-Ara	β-D-Glu	*C. quinoa*
α-L-Ara-(1→3)- β-D-Glur A	β-D-Glu	*C. quinoa*
α-L-Ara	β-D-Glu	*C. quinoa*
β-D-Glu-(1→3)- β-D-Xyl-(1→2)- β-D-Glu	β-D-Glu	*C. quinoa*
β-D-Glu-(1→3)- β-D-Gal	β-D-Glu	*C. quinoa*
β-D-Glu-(1→4)- β-D-Glu-(1→4)- β-D-Glu	β-D-Glu	*C. quinoa*

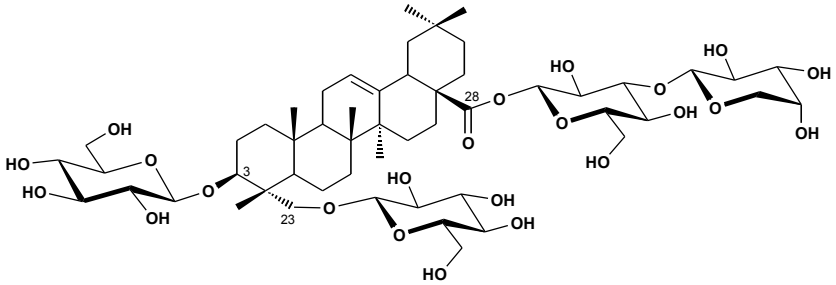
D: **Serjanic acid** = 3β-hydroxy-olean-12-ene-28,30-dioc acid-30-methyl ester Glycosides		
R_1	**R_2**	***Chenopodium* species**
β-D-Glu-(1→2)- β-D-Glu-(1→3)-α-L-Ara	β-D-Glu	*C. quinoa*
β-D-Glu-(1→3)-α-L-Ara	β-D-Glu	*C. quinoa*
α-L-Ara	β-D-Glu	*C. quinoa*
β-D-Glur A	β-D-Glu	*C. quinoa*
α-L-Ara-(1→3)- β-D-Glur A	β-D-Glu	*C. quinoa*

E: **30-Hydroxy-hederagenin Glycosides** = 3β ,23,30-trihydroxy-olean-12-en-28-oic acid Glycosides		
R_1	**R_2**	***Chenopodium* species**
β-D-Glu-(1→3)--L-Ara	β-D-Glu	*C. quinoa*

F: 3β-hydroxy-23-oxo-olean-12-en-28-oic acid Glycosides		
R_1	**R_2**	***Chenopodium* species**
β-D-Glu-(1→3)-α-L-Ara	β-D-Glu	

G: 3β-hydroxy-27-oxo-olean-12-en-28-oic acid Glycosides		
R_1	**R_2**	***Chenopodium* species**
β-D-Glu-(1→3)--L-Ara	β-D-Glu	

H: Tridesmosidic Hederagenin Glycosides



β3,23-bis(*O*-β-D-Glucopyranosyloxy)-olean-12-en-28-oic acid 28-*O*--L-arabinopyranosyl-(1→3)-glucopyranosyl ester

Abbreviations.: **Glur A**: Glucuronopyranosyl; **Glu**: Glucopyranosyl; **Xyl**: Xylopyranosyl; **Ara**: Arabinopyranosyl; **Gal**: Galactopyranosyl; **Me**: Methyl

S1 = HOOC-CH_2-O-CH(COOH)- (*see* A: oleanolic acid Saponins)

*) The saponins presented in Table have been isolated from the seeds of *Chenopodium quinoa*, from the roots of *Chenopodium album* and from the aerial parts of *Chenopodium ficifolium*.

2.2.3. Quinoa and Gluten

Gluten is a composite of the proteins prolamins and glutenin (Food and Drug Administration, 2007). Celiac illness is an insusceptible intervened response to gluten, described by an inadequate T-cell-intervened invulnerable reaction that makes fiery damage the small digestive system. Celiac infection patients must expend sustenance with gluten substitutes or glutenfree grains. A few without gluten foodstuffs contain more salt and fat (immersed fat) yet less minerals and vitamins than their gluten-containing partners. Thus, quinoa is a brilliant without gluten grain, with a high substance of vitamins and minerals that make it a possibly basic piece of any sound, without gluten eat less carbs; additionally quinoa consents to the Codex Alimentarius classification of sans gluten items (gluten content 20 mg/kg) 51.

Gluten free (celiac disease safety*)*

Celiac illness (CD), otherwise called gluten-touchy enteropathy furthermore, nontropical sprue, is a condition that produces aggravation in the small digestive system and is described by harm of the mucosa layer caused by the ingestion of gluten, the significant stockpiling protein of wheat and comparative grains of grain also, rye in hereditarily helpless subjects. The gluten protein is improved in glutamine and proline and is inadequately processed in the human upper gastrointestinal tract. Without gluten eat less (GFD) is the suggested treatment; notwithstanding, following to the unique eating routine is troublesome for CD patients.

As indicated by a 2013 overview, around 33% of the number of inhabitants in United States are either endeavoring to limit or dodge gluten admission. Celiac illness is a hereditary immune system issue where the ingestion of gluten prompts harm in the small digestive tract. It is evaluated to influence 1 of every 100 individuals everywhere throughout the world. In the event that untreated it might prompt different extra medical issues like iron inadequacy paleness, osteopenia, barrenness, lactose narrow mindedness and treatment for this infection is deep rooted adherence to a strict gluten free eating routine (Filho et al. 2017).

2.2.4. Vitamins

Quinoa seeds are a rich wellspring of vitamins, which are required in the human eating routine to go about as enzymatic cofactors in digestion, manage cell development and improvement, ensure against oxidative harm, enhance vision, and assume advantageous jobs in different other physiological procedures (Fitzpatrick and others 2012). Recorded vitamins in quinoa incorporate vitamin A forerunner β-carotene (0.39 mg/100 g), thiamin/vitamin B1

(0.4 mg/100 g), riboflavin/vitamin B2 (0.39 mg/100 g), niacin/vitamin B3 (1.06 mg/100 g), panthothenic corrosive/vitamin B5 (0.61 mg/100 g),pyridoxine/vitamin B6 (0.20 mg/100 g), folic corrosive/vitamin B9 (23.5 to 78.1 mg/100 g), ascorbic corrosive/vitamin C (4.0 to 16.4 mg/100 g), and tocopherols/vitamin E (3.7 to 6.0 mg/100 g) (Filho et al., 2017; Bhargava et al., 2006). Quinoa likewise contains an assortment of carotenoids, transcendently luteins and zeaxanthins, with add up to focuses extending from 1.2 to 1.8 mg/100 g). Centralizations of a large number of these vitamins and provitamins in quinoa are higher than common oat grains.

Table 2.3. Vitamins in quinoa, amaranth and grain (mg/100g dry weight)

Quinoa flour	***Amaranth b flour***	***Barleya***
Thiamin (B1)	*0.29 - 0.36*	*0.07 - 0.10 0.191*
Riboflavin (B2)	*0.30 - 0.32*	*0.19 - 0.23 0.114*
Niacin (B3)	*1.24 - 1.52*	*1.17 - 1.45 4.604*
B6	*0.487*	*0.260*
Folate add up to	*0.18*	*0.023*
Ascorbic corrosive (C)	*4.50 -*	
β-carotene		

2.2.5. Minerals

Quinoa has a higher aggregate mineral (fiery remains) content (3.4%) than rice (0.5%), wheat (1.8%), and different oats (Bhargava et al., 2006). The micronutrients calcium (275 to 1487 mg/kg), copper (2 to 51 mg/kg), press (14 to 168 mg/kg), magnesium (260 to 5020 mg/kg), phosphorus (1400 to 5300 mg/kg), potassium (75 to 12000 mg/kg), and zinc (28 to 48 mg/kg) are present in adequate amounts in quinoa to keep up an adjusted human eating regimen (Repo-Carrasco-Valencia & Serna, 2011; Bhargava et al., 2006; Vega-Galvez et al., 2010).

Quinoa is a phenomenal wellspring of magnesium, meeting in excess of 20 percent of the prescribed every day esteem in a 1/2-glass serving. Magnesium insufficiency is more predominant in individuals with diabetes. One of the essential jobs of magnesium in your body is glucose control. Enhancing magnesium consumption from sustenances, for example, quinoa may help enhance insulin affectability and your glucose numbers.

2.2.6. Carbohydrate and fiber

Quinoa starch includes 58.1% to 64.2% of dry seed weight, in any case, has a low glycemic list (Vega-Galvez et al., 2010). The starch is comprised fundamentally by D-xylose (120 mg/100 g) furthermore, maltose (101 mg/100 g) with low glucose (19 mg/100 g) and fructose (19.6 mg/100 g) content (Bhargava et al., 2006). The starch is exceptionally spread and comprises of little granules (molecule measure under 2 μmin breadth), which are littler than the molecule sizes of basic oat grains.

Quinoa (*Chenopodium quinoa*, Willd.) as a source of dietary fiber

Four assortments of an Andean indigenous harvest, quinoa (*Chenopodium quinoa* Willd.), were assessed as a wellspring of dietary fiber, phenolic mixes and cancer prevention agent action. The yields were prepared by expulsion cooking and the last items were investigated to decide thedietary fiber, add up to polyphenols, radical rummaging action, and in vitro edibility of starch and protein. There were no critical contrasts in the substance of aggregate dietary fiber between assortments of quinoa. In all cases, the substance of aggregate and insoluble dietary fiber diminished amid the expulsion procedure. In the meantime, the substance of solvent dietary fiber expanded.

Dietary Fiber

Dietary fiber is the unpalatable segment of sustenance got from plants, furthermore, has two primary segments: dissolvable and insoluble. Solvent fiber disintegrates in water, is promptly aged in the colon into gases and physiologically dynamic items and has prebiotic properties. Insoluble fiber, which does not disintegrate in water, is either metabolically dormant and gives building mass, or it tends to be prebiotic and metabolically age in the internal organ. Building strands assimilate water, facilitating poop. More prominent utilization of fiber-rich entire grains is related with a bring down danger of sort 2 diabetes 24 and cardiovascular malady. Quinoa is a great wellspring of dietary fiber, including about 2.6%-10% of the aggregate weight of the grain; around 78% of its fiber content is insoluble and 22% solvent.

2.2.7. Protein

The protein amount and nature of quinoa are by and large prevalent to those of oat grains, while offering sans gluten property furthermore, high edibility. Quinoa has a higher

aggregate protein content (12.9% to 16.5%) than grain (10.8% to 11.0%), oat (11.6%), rice (7.5% to 9.1%), and maize (10.2% to 13.4%), and an aggregate protein content equivalent to that of wheat (14.3% to 15.4%) (Repo-Carrasco-Valencia & Serna, 2011). The capacity proteins of quinoa comprise for the most part of globulin and egg whites, with next to zero nearness of prolamins, the real stockpiling proteins in numerous oat crops. Prolamins, such as gliadin from wheat, secalin from rye, and hordein from grain (all things considered alluded to as "glutens.

Quinoa is a standout amongst the most protein rich nourishments we can eat. The natural protein esteem estimates the extent of protein consumed from a sustenance which at that point winds up joined into the proteins of the body. Quinoa has high natural esteem (73%), like that of meat (74%) and higher than those of white rice (56%), wheat (49%) and corn (36%). The protein nature of quinoa is even practically identical to the top notch protein from the dairy items, called casein. Since it contains all the nine fundamental amino acids, it is considered as a total protein rich nourishment.

2.3. Pharmacological Activities

2.3.1. Antioxidant Activity

The antioxidant and antimicrobial properties of quinoa cultivated in Korea have been studied together and the results have been compared with imported quinoa from the USA and Peru. The highest amount of total flavonoid contents with 20.91 mg quercetin equivalents/100 g was found in quinoa seed extract cultivated in Korea, while the total phenolic contents were significantly higher in quinoa from the USA (16.28 mg gallic acid equivalents/100 g). On the other hand, quinoa extracts cultivated in Korea were found to exhibit a superior antioxidant ability. A high correlation have been observed between total flavonoid contents and antioxidant activity and a low correlation between total flavonoid contents and antioxidant activity (Brend et al., 2012; Nsimba et al., 2008; Park et al., 2017).

2.3.1.1. Antioxidant and anticancer activities of *Chenopodium quinoa* leaves extracts

The nutritional value of *Chenopodium quinoa* Leaves has been evaluated through analyses of the phenolic content, elucidation of the effect of phenolic compounds on cancer cell properties and estimation of their antioxidative activity, bioaccessibility and bioavailability in vitro (Gawlick-Dziki et al., 2013). The substantial amounts of phenolic compounds such as ferulic, sinapinic and gallic acids and flavonoids, kaempferol, isorhamnetin and rutin were found in the leaves extract and were linked with its inhibitory effect on prostate cancer cell proliferation, motility and cellular competence for gap junctional communication. By these observations, phenolic compounds have been found responsible for chemopreventive and anticarcinogenic. Because of the relatively high potential bioaccessibility and bioavailability of the compounds, *Chenopodium quinoa* leaves has been suggested for dietary supplementation.

2.3.2. Antimicrobial activity of quinoa

The antioxidant and antimicrobial properties of quinoa cultivated in Korea have been studied together and the results have been compared with imported quinoa from the USA and Peru. The antimicrobial activity of the quinoa extracts has been determined using a disc diffusion assay and optical density method. In both assays, the quinoa seed extracts did not have strong antimicrobial activity against foodborne bacteria, including *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhimurium*,

and *Campylobacter jejuni* (Park et al., 2017). A lectin isolated from the seeds of *Chenopodium quinoa* has been studied for its hemagglutinating activity on human erythrocytes and has also been tested for its antimicrobial activity. It has been demonstrated that antimicrobial activity against three gram-negative bacteria (*Pseudomonas aeruginosa, Escherichia coli* and *Salmonella enterica*), was probably due to their specific bond to sugars that were present at the LPS of those bacteria. It has been proposed the potential of *C. quinoa* lectin as an antimicrobial and biotechnological tool (Pompeu et al., 2015).

2.3.3. Anti-inflammatory activities

A recent review focused on the phytochemical composition of quinoa and amaranth seeds, the antioxidant and anti-inflammatory activities of hydrophilic (e.g. phenolics, betacyanins) and lipophilic (e.g. fatty acids, tocopherols, and carotenoids) nutrients. This study reports that how these compounds play a role as potential health benefits, especially in lowering the risk of the oxidative stress related diseases e.g. cancer, cardiovascular disease, diabetes, and obesity.

Polyphenols separated from quinoa have been accounted for to downregulate IL-1β, IL-8, and TNF cytokines in refined colonic epithelial Caco-2 cells, and to counteract heftiness incited irritation and advance gastrointestinal wellbeing in mice. A gathering of overweight postmenopausal ladies served with quinoa drops for about a month turned around the IL-6 level while those presented with corn pieces did not. IL-6 is a professional incendiary marker hence brought down plasma articulation of IL-6 by quinoa utilization proposes a potential treatment of provocative procedure of postmenopausal ladies. Saponins in quinoa seeds have been shown to be associated inhibiting overproduction of inflammatory mediators like NO, TNF-α, and IL-6. The effect of phytochemicals on the major inflammatory mediators is shown in Figure 2.1 (Tang & Tsao, 2017).

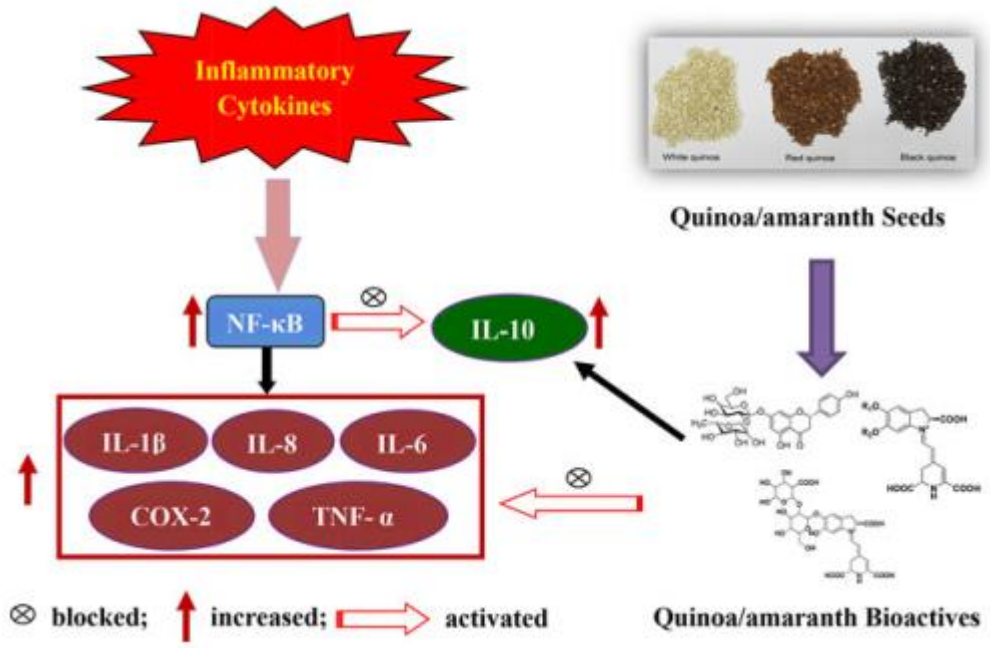


Figure 2.1. Anti-inflammatory Effects of Phytochemical Nutrients in Quinoa and Amaranth

(This figure was taken from a Review reported by Tang & Tsao, 2017)

2.3.4. Anti-obesity and anti-diabetic activities

Diabetes mellitus is ending up progressively pervasive, 8.3% of the U.S. populace has diabetes and an expected 35% have pre-diabetes as per the report of the Centers for Disease Control and Prevention. In excess of 1.9 billion grown-ups were overweight worldwide in 2014, and more than 600 million of them were hefty. Expanded admission of vitality thick nourishments that are high in fat is the primary driver of stoutness and overweight. Sort 2 diabetes is a metabolic issue exemplified by hyperglycemia causing resulting deserts in insulin emission, insulin activity or both, while weight is portrayed by poor quality ceaseless aggravation in fat tissue, liver, and skeletal muscle prompting territories of fat tissue hypoxia. Nourishment treatment and glucose observing including eat less carbs control are proposed as key ways to deal with compose 2 diabetes administration (Tang & Tsao, 2017).

Quinoa Leaves essentially diminished the blood glucose levels in an eating routine incited fat, hyperglycemic mouse demonstrate. Intense investigations in this model have been routinely used to exhibit the in vivo hostile to diabetic impacts of a few pharmacological

operators and botanicals. The intense hypoglycemic impacts of QL, joined with past reports on the counter diabetic and hostile to stoutness impacts of perpetual 20HE organization, bolster QL's capability to treat or forestall hyperglycemia and insulin opposition related with human metabolic disorder. Different segments of QL, including flavonoids (quercetin and kaempferol glycosides), greasy acids and amino acids, may potentiate or synergize (Graf et al., 2014).

2.3.5. Other Activities

In a study performed on twenty-two 18 to 45-year-old students treated daily for 30 days with quinoa in the form of a cereal bar quinoa had beneficial effects in reducing the levels of total cholesterol, triglycerides, and LDL-c. It was concluded that the use of quinoa in diet could be considered beneficial in the prevention and treatment of risk factors related to cardiovascular diseases (Farinazzi-Machado et al., 2012).

2.4. Traditional use

Quinoa has been customarily utilized by a few indigenous people groups of South America, including the Quechua, Aymara, Tiahuancota, Chibcha, and Mapuche (Vega-Galvez et al., 2010; Bhargava & Srivastava 2013). The seeds have been expended likewise to rice, arranged in soup, puffed to make breakfast grain, or ground to flour to deliver toasted and prepared merchandise (treats, breads, bread rolls, noodles, chips, tortillas, flapjacks) (Popenoe et al., 1989; Bhargava et al., 2006). Quinoa leaves have likewise been eaten correspondingly to spinach (Oelke et al., 1992), and the developed quinoa seedlings (quinoa grows) have been consolidated in servings of mixed greens (Schlick & Bubenheim 1996). Moreover, quinoa seeds can be matured to make brew, or a conventional stately alcoholic drink from South America called "chicha" (Healy 2001; FAO 2011). The entire plant has additionally been utilized as a rich wholesome source to encourage animals, including cows, pigs, and poultry (Bhargava and others 2006).

3. Experimental part

3.1. Plant Material

The seed coat material of *Chenopodium quinoa* was provided by Quinoa Producers Association from Thrace Region of Turkey in 2017.

3.2. Material and Methods

3.2.1. Chemical Solid Materials

Revelation (Reagents):

1% Vanillin (A) and 5% H_2SO_4 (B) were used as reagent for TLC studies.

Solvents

Methanol (MeOH), ethanol (EtOH), t-butanol (t-BuOH), acetone (Me_2CO), chloroform ($CHCl_3$), dicloromethane (DCM), ethylacetate (EtOAc) (Merck, Fluka Analytical, Sigma-Aldrich)

3.2.2. Chromatographic Methods

3.2.2.1. Thin Layer Chromatography

<u>Normal Phase TLC</u>:

Adsorbent (Stationary Phase):

Silica gel 60 F_{254}, Aluminium folio 20×20 cm, Merck 1.5554.0001)

Solvent systems (Mobile Phase):

Dichloromethane-Methanol-Water (DCM-MeOH-H_2O; 80:20:2, 70:30:3, 61:32:7 and 65:35:5), Ethylacetate-Methanol-Water (EtOAc-MeOH-H_2O; 100:16,5:13,5) were used as a solvent system.

<u>Reversed phase TLC</u>:

Stationary Phase: Silica gel for 60 RP-18 (Merck1.05559.0001)

Solvent system: Methanol in water was used as a solvent system.

Reagent: 5% H_2SO_4/ETOH was used as a reagent (heated at 105 ºC, 5- 10 min).

TLC was used for the monitoring of isolation studies and the purity control of the isolated compounds.

3.2.2.2. Vacuum Column Chromatography (VCC)

Solvent system (Mobil Phase):

DCM-MeOH-H_2O mixtures (90:10:1, 80:20:1, 80:20:2, … 70:30:3, 65:35:5)

Stationary Phase:

Silica gel 60 (Kieselgel 60, 0.063-0.2 mm, Merck 7734)

Column dimension: 5 x 30cm.

3.2.2.3. Column Chromatography (CC):

Solvent system (Mobil Phase):

DCM-MeOH-H_2O mixtures (80:20:1, 80:25:2.5, 75:30:3, 70:30:3 and 65:35:3)

Stationary Phase:

Silica gel 60 (Kieselgel 60, 0.063-0.200 mm, Merck 7734)

Column dimension: 2 x 35cm.

Fraction Volume: 15 ml

3.2.2.4. Medium Pressure Liquid Chromatography (MPLC)

Stationary Phase: LiChroprep RP-C18 (Merck).

Solvent system: H_2O: MeOH (0%-100% MeOH)

MPLC: Buchi (two pumps C.605 and pump man ger C.615)

Fraction collector: Buchi

Flow rate: 10m/min.

Column dimension: 2.5 x 25cm.

Fraction Volume: 10 ml

3.2.3. Instruments

HR-Mass Spectrometer: UPLC-Quadrupole Orbitrap MS

NMR: Bruker (^{1}H-NMR; 300 MHz; ^{13}C-NMR: 100 MHz)

Lyophilizator: CHRIST Alpha 1-4 LD Plus

Rotary Evaporator: Büchi R-210 and Heidolph 4001

Vaccum pump: Rockk vacuum

Balance: Mettler Toledo PB 1502-S/FACT

Plate Heater: Camag TLC Plate Heater III

UV Lamb: Camag

3.3. Extraction:-

Air dried seed coat material of *Chenopodium quinoa* (100 g) was extracted with 80% EtOH (1000 mL) at 40ºC using continuously mixing by the help of a rotaryevaporator overnight. The extract was filtered with vacuum by using Buchner funnel and the filtrate was concentreated by rotary evaporator under reduced pressure at 40°C to yield 50 ml concentrated extract (water soluble extract, WSE). 2 mL of the concentreated extract was kept for a reference extract.

3.4. Fractionation of WSE and Isolation of the Saponosides (CQ-1 – 6).

3.4.1. Fractionation by Vacuum Column Chromatography (Scheme 3.1)

by TLC (Kieselgel 60 F_{254}) using Concentrated extract in water (50 ml) was subjected to a column chromatography prepared by silica gel (200 g: Silica gel 60) using *DCM-MeOH-H_2O mixtures* [80:20:1 (202 mL), 80:20:2 (510 mL), 75:25:2.5 (256,25 mL), 70:30:3 (515 mL), 65:35:5 (315 mL)] es eluent increasing stepwise the polarity. Thirty fractions were collected (fraction volume: 45 – 50 mL). The fractions were monitored by TLC (**Figure 3.1**) usig DCM-MeOH-H_2O (80:20:2, 70:30:3 or 61:32:7) mixtures. For the revelation 5% H_2SO_4 in EtOH was used as a reagent. They were combined into seven main fractions (A – G) and evaporated to dryness under vacuum at 40ºC and then lyophilized [Frs. 1 – 6 (A: rich in carbohydrates); frs. 7 – 10 (B: 708 mg); frs. 11 – 17 (C: 3023 mg); frs. 18 – 20 (D: 788 mg); frs. 21- 22 (E: 821 mg); frs. 23 – 25 (F: 2485 mg) and frs. 26 – 28 (G: 1213 mg).

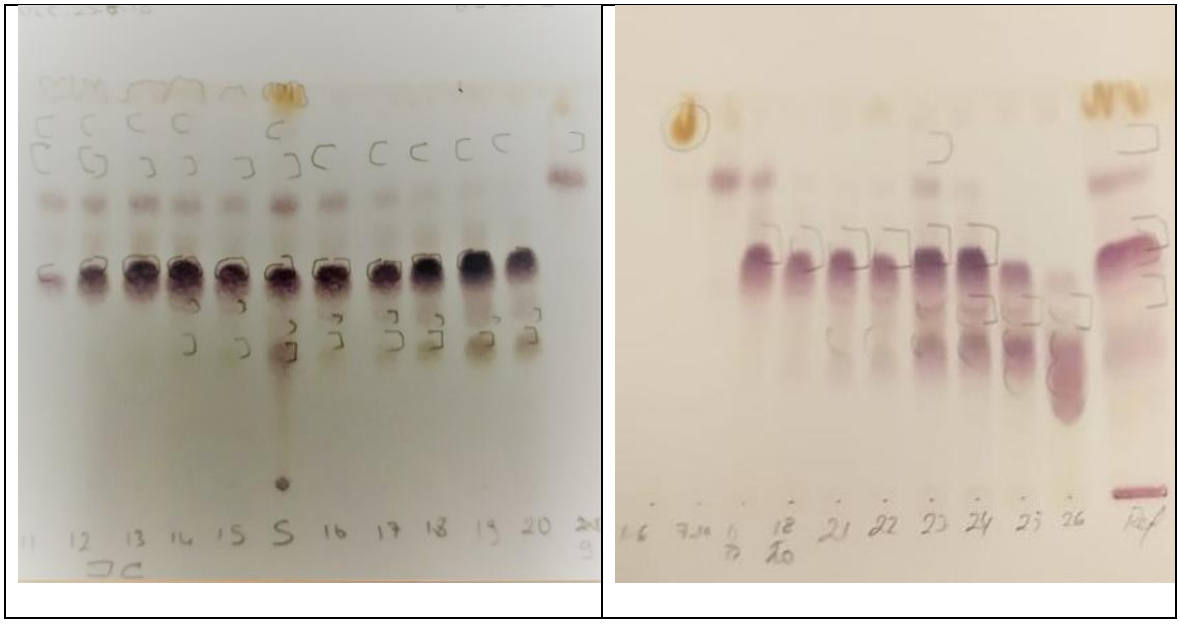
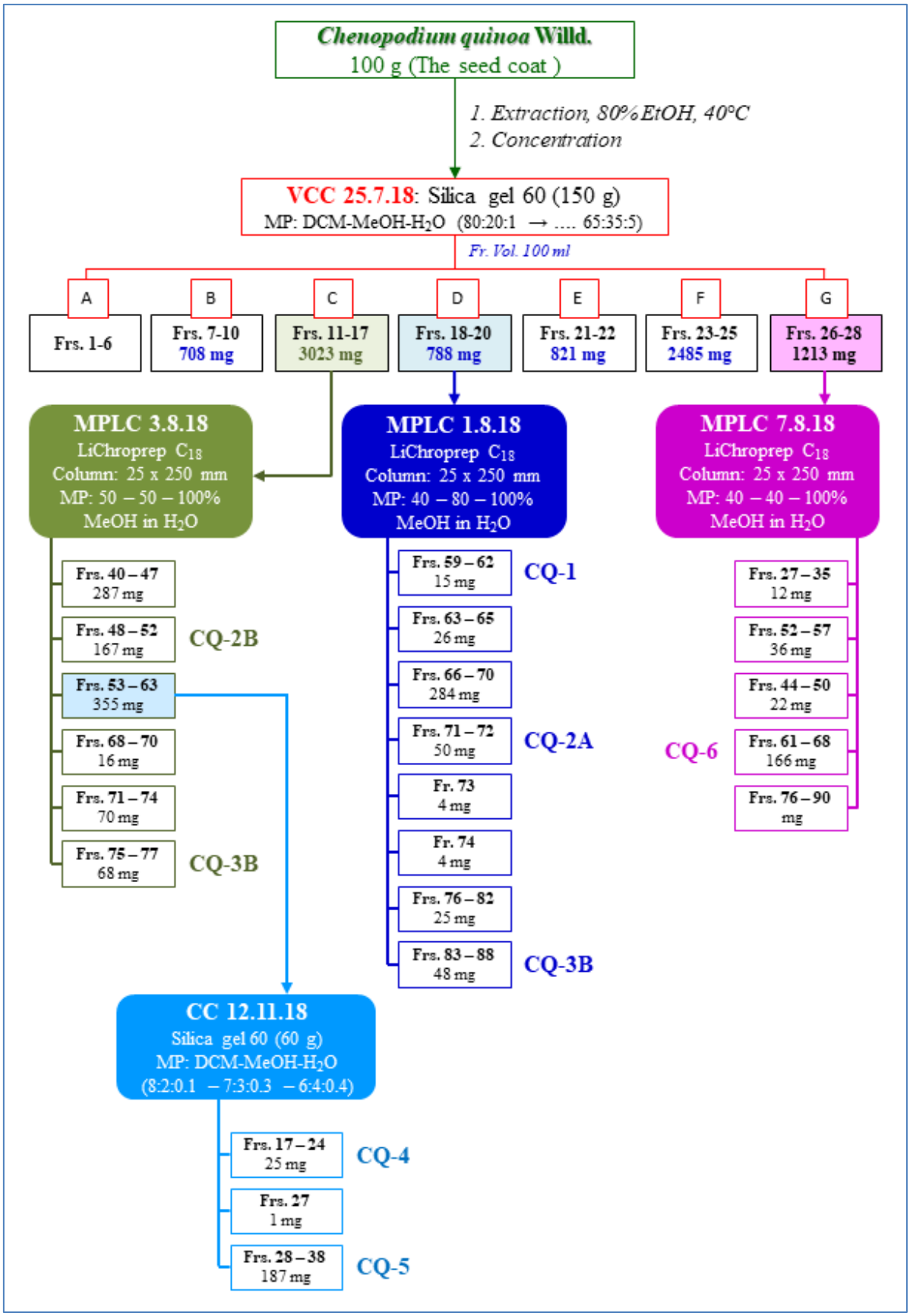


Figure 3.1. TLC of the fractions of VCC (DCM-MeOH-H_2O, 65:35:5)



Scheme 3.1. Fractionation and Isolation of Quinoa Saponosides

3.4.2. Isolation of the Saponosides (Scheme 3.1)

3.4.2.1. Isolation of CQ-1, CQ-2 and CQ-3.

Fr. D (frs.18 – 20; 788 mg) was subjected to MPLC (Scheme 3.1: **MPLC 1.8.18**) over reversed-phase silica gel (LiChroprep RP-18) using H_2O-MeOH mixtures. A gradient elution with increasing amount of MeOH (**Figure 3.2**) afforded 90 fractions (fraction vol. 10 mL). Monitoring of the fractions were made by TLC. Fractions 59 – 62 gave CQ-1 (15 mg) while fractions 71 – 72 yielded CQ-2 (50 mg) in pure state. Fractions 83 – 88 afforded CQ-3 (48 mg). The remaining fractions between the pure saponosides were the mixtures of two or three of them.

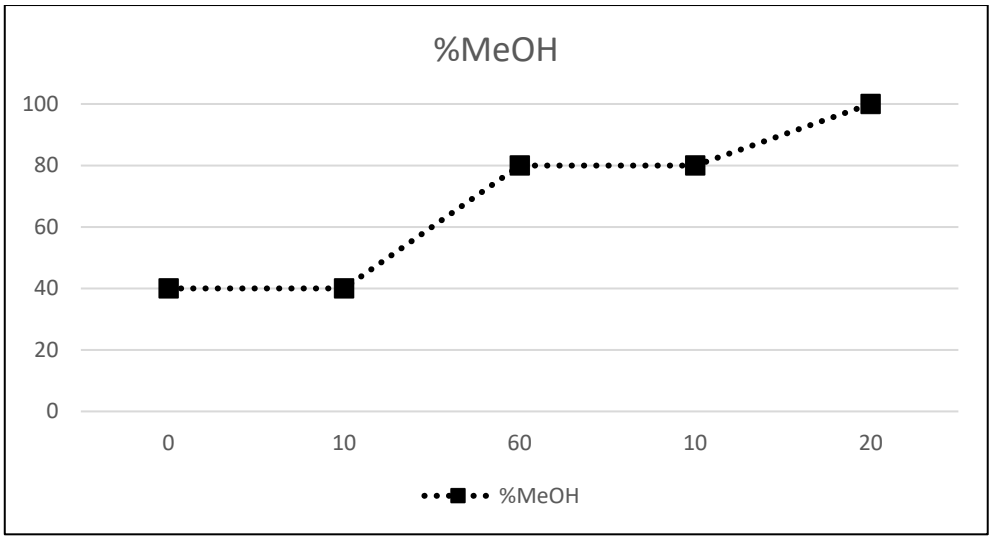


Figure 3.2. Eluent system (% MeOH in H_2O) applied to MPLC 1.8.18

3.4.2.2. Isolation of CQ-2 and CQ-3.

Fr. C (frs. 11 – 17; 3023 mg) was subjected to MPLC (Scheme 3.1: **MPLC 3.8.18**) over reversed-phase silica gel (LiChroprep RP-18) using H_2O-MeOH mixtures. A gradient elution with increasing amount of MeOH (**Figure 3.2**) afforded 150 fractions (fraction vol. 10 mL). Monitoring of the fractions were made by TLC. Fractions 40 – 47 and 48 – 52 afforded pure CQ-2 (278 mg and 167 mg, respectively), while fractions 75 – 77 yielded CQ-3 (68 mg). Fractions 53 – 63 were in rich for CQ-2 (= CQ-5) and CQ-4 (355 mg) which were further applied to a column chromatography (*see* CC 12.11.18).

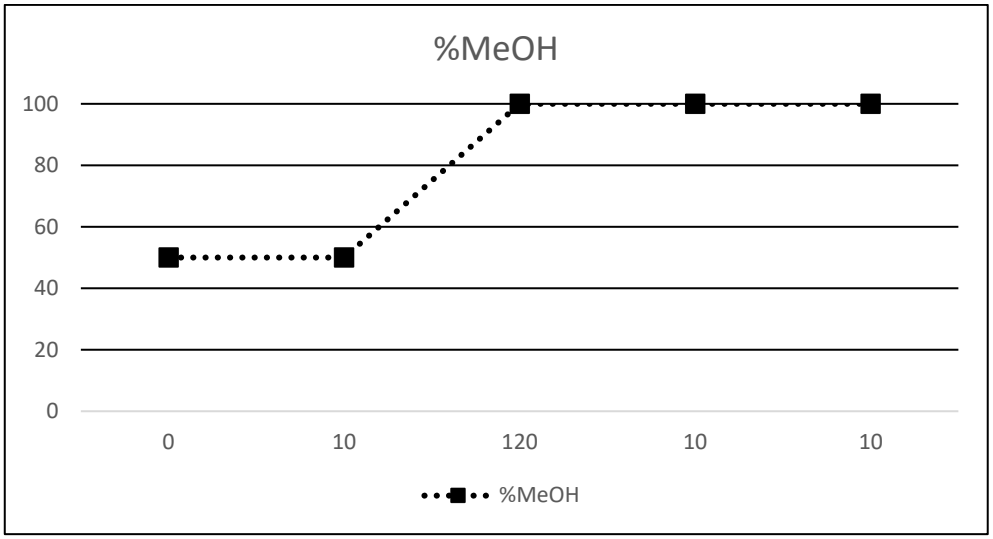


Figure 3.3. Eluent system (% MeOH in H_2O) applied to MPLC 3.8.18

3.4.2.3. Isolation of CQ-6.

Fr. G (frs. 26 – 28; 1213 mg) was subjected to MPLC (Scheme 3.1: **MPLC 7.8.18**) over reversed-phase silica gel (LiChroprep RP-18) using H_2O-MeOH mixtures. A gradient elution with increasing amount of MeOH (**Figure 3.4**) afforded 80 fractions (fraction vol. 10 mL). Monitoring of the fractions were made by TLC. Fractions 61 – 68 yielded CQ-6 (166 mg)

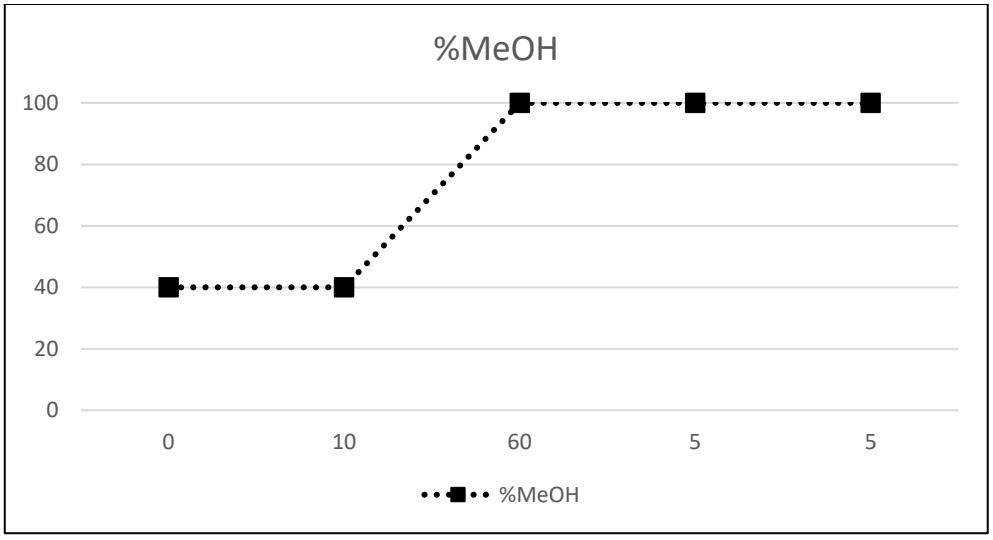


Figure 3.4. Eluent system (% MeOH in H_2O) applied to MPLC 7.8.18

3.4.2.4. Isolation of CQ-4 and CQ-5.

The subfractions **(**Fractions 53 – 63: fr. C_3, 355 mg) of MPLC 3.8.18 was applied a column chromatography (Silica gel 60; 60 g) using DCM-MeOH-H_2O mixtures with increasing polarity [80:20:1 (300 mL), 80:20:2 (200 mL), 70:30:3 (400 mL) and 60:40:4); fraction volume 15 mL] as solvent system. Fractions 17 – 24 yielded pure CQ-4 (25 mg). Additionally, fractions 28 – 35 gave CQ-5 (187 mg) which was identical with CQ-2 isolated from the same fraction C (**Scheme 3.1**).

4. RESULTS and DISCUSSION

The ^{13}C-NMR spectra of the isolated compounds (CQ-1, CQ-2 = CQ-5, CQ-3, CQ-4 and CQ-6) indicated the presence of three anomeric carbon signals for the compounds CQ-1, CQ-2 and CQ-3, while two anomeric carbon signals for the compounds CQ-4 and CQ-6 (Table 1). The ^{13}C NMR spectra of all compounds showed 47 carbon resonances for CQ-1 and CQ-3, 48 for CQ-2, 42 for CQ-4 and 41 for CQ-6. Of which 30 were assigned to a triterpenoid moiety (Mahato & Kundu, 1994) and rest of the signals were attributed to saccharide moieties of the saponosides confirming the number of sugar units. An additional carbomethoxyl resonance was observed at δ 52.4 for CQ-2 and CQ-4. The ^{1}H NMR spectra of the compounds displayed signals arising from five tertiary methyl groups for CQ-1, CQ-2 and CQ-4 while six tertiary methyl groups for CQ-4 and CQ-6 arising from their aglycone moieties (*see* **Table 4.1** and **Spectra 4.1**). A typical carbon signals of C-3 were observed at ca. δ 83.0±1 and corresponding H-3_{ax} at δ 3.64 – 3.69 due to the presence of β-OH group at C-3 position indicating the presence of only one oxygenated carbon resonance on the pentacyclic triterpenoid skeleton except methyl resonances for all compounds. The ^{13}C-NMR data also indicated an oleanene-type sapogenol derivative with two or three of the eight methyl groups substitued by a ±CH_2OH (δ 65.0±0.2: C-23; 66.2: C-30) and ±COOH (δ 178.0±0.8: C-28 and C-30) functions (*see* Table 1). Further features were signals at δ 124.0±0.5 and 144.6±0.3 ascribable to C-12 and C-13 confirmed the Δ^{12} oleanene skeleton for all saponosides (Mahato & Kundu, 1994). A proton signal at δ 5.30±0.5 (1H, dd"t", *J*=3.4 Hz) was also assigned to an olefinic proton for all.

The signals observed at δ 95.6 or 95.7 for all compounds suggested that they have an 28-*O*-glycosidic linkage, which was further confirmed by one of the anomeric protons shifted to downfield. The evidences for the ester linkages between the carboxy group located on C-28 of the aglycone and one of the sugar units were confirmed from the chemical shift values of the anomeric protons observed at δ 5.41 d (*J* = 8.1 Hz) for CQ-1, 5.39 d (8.1 Hz) for CQ-2, 5.41 d (8.1) for CQ-3, 5.39 d (8.0) for CQ-4 and 5.40 d (8.1) for CQ-6. The presence of a 3-*O*-glycosidic linkages was identied by the downfield shifts at δ **83.6, 83.6, 83.7, 83.4 and 82.3** for C-3 of the aglycone moiety, respectively (*See* **Table 4.1**). Thus, all saponosides are bisdesmosides.

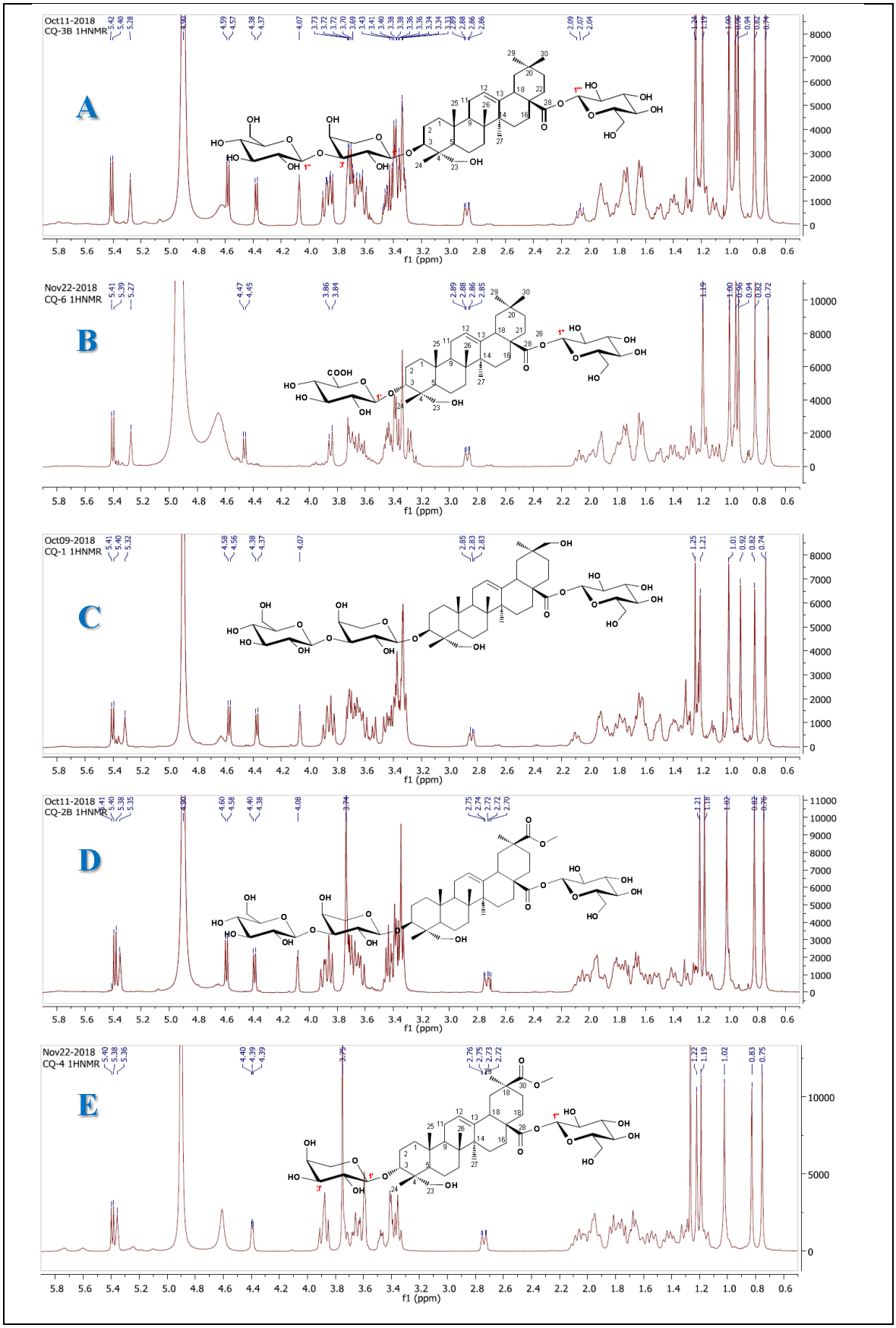
Table 4.1. ^{13}C-NMR Data for compounds CQ-1, CQ-2, CQ-3, C4 and CQ-6 (CD_3OD; δ_C 125 MHz).

Aglycone moiety	**CQ-3**	**CQ-6**	**CQ-1**	**CQ-4**	**CQ-2**	**Oleanolic acid‡**
C/H	δ_C, ppm	δ_C, ppm	δ_C, ppm	δ_C, ppm	δ_C, ppm	δ_C, ppm
1	39.5	39.5	39.5	39.5	39.5	38.5
2	26.2	26.1	26.3	26.26	26.2	27.4
3	**83.7**	**82.3**	**83.6**	**83.4**	**83.6**	78.7
4	43.8	43.0	43.8	47.3	47.2	38.7
5	48.5	48.1	48.3	48.1	48.3	55.2
6	18.9	18.9	18.9	18.8	18.8	18.3
7	33.4	33.3	33.4	33.3	33.3	32.6
8	40.6	40.6	40.7	42.8	42.8	39.3
9	47.9	49.0	49.0	48.9	48.9	47.6
10	37.7	37.6	37.7	37.6	37.2	37.0
11	24.5	24.6	24.6	24.5	24.5	23.1
12	**123.7**	**123.8**	**124.0**	**124.2**	**124.3**	122.1
13	**144.8**	**144.9**	**144.6**	**144.4**	**144.4**	143.4
14	42.9	43.8	43.0	40.5	40.5	41.6
15	28.8	28.8	28.8	28.8	28.8	27.7
16	24.0	24.0	24.3	24.1	24.2	23.4
17	48.8	49.0	47.9	44.9	44.8	46.6
18	42.5	42.6	42.0	43.8	43.7	41.3
19	47.2	47.2	42.4	43.2	43.1	45.8
20	31.5	31.5	36.0	43.8	43.7	30.6
21	34.9	34.9	29.6	31.2	31.2	33.8
22	33.1	33.1	32.7	34.2	34.2	32.3
23	**65.2**	**64.8**	**65.2**	**64.8**	**65.2**	28.1
24	13.4	13.4	13.4	13.4	13.4	15.6
25	16.6	16.5	16.5	16.5	16.6	15.3
26	17.8	17.8	17.8	17.7	17.7	16.8
27	26.4	26.4	26.4	26.32	26.3	26.0
28	**178.0**	**178.2**	**178.0**	**178.6**	**177.4**	181.0
29	33.6	33.5	28.10	28.6	28.7	33.1
30	24.0	24.0	**66.3**	**178.8**	**178.6**	23.6
$COOCH_3$				52.5	52.5	
Saccharide Moiety						
Ara (C-3)						
1′	**106.0**	105.0	**106.1**	106.2	**106.0**	
Glu (C-4′) or (C-3)*						
1″	**105.4**		**105.4**		**105.4**	
Glu (C-28)						
1‴ (1″)**	**95.6**	95.7	**95.7**	95.7	**95.6**	

‡) Mahato & Kundu, 1994

*) For compound CQ-6;

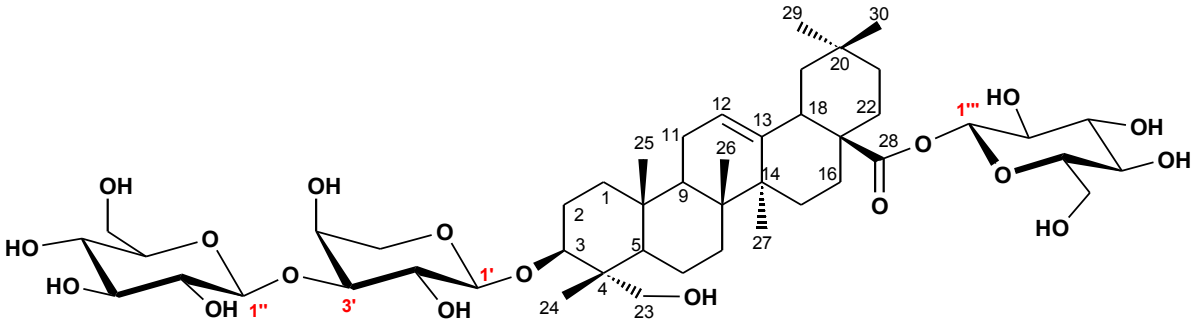
**) For compounds CQ-4 and CQ-5.



Spectra 4.1. The 1H-NMR Spectra of the Saponosides from the Seed Coat of *C. quinoa* (CD_3OD, MeOH: **A**: CQ-3; **B**: CQ-6; **C**: CQ-1; **D**: CQ-2 and **E**: CQ-4)

4.1. Hederagenin Glycosides (CQ-3 and CQ-6)
= 3β,23-dihydroxy-olean-12-en-28-oic acid Glycosides
$C_{30}H_{48}O_4$; Mol wt 472,71

CQ-3: Molecular weight: 928 (calcd. for $C_{47}H_{76}O_{18}$)



The negative ion H-ESI-mass spectrum of CQ-3 (**Figure 4.1.1**) showed quasimolecular ion $[M - H]^-$ at m/z 927.5013, indicating a molecular weight of 928 (calcd for $C_{47}H_{76}O_{18}$). The other significant ion $[M - 163]^-$ was observed at m/z 765.4472 attributed to the loss of one of the terminal molecule glucose unit.

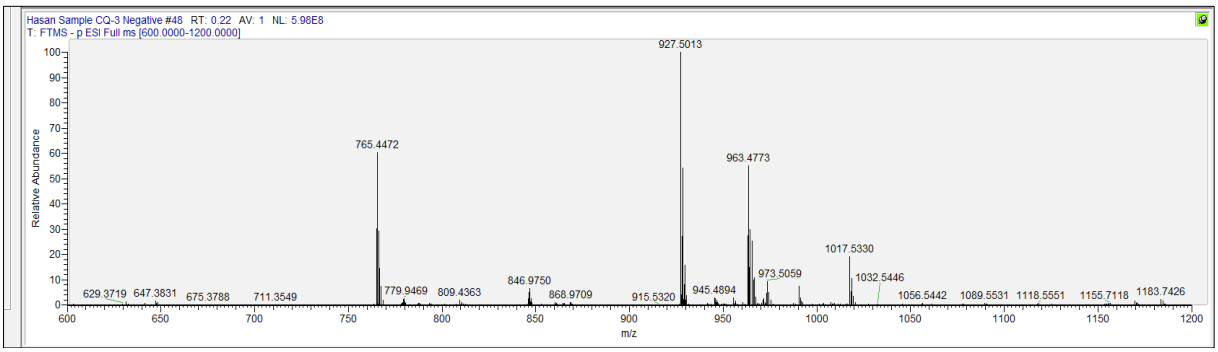


Figure 4.1.1. Negative Ion H-ESI Mass Spectra (HRMS) of CQ-3.

The ^{13}C NMR (**Spectrum 4.1.1**) spectrum showed 47 signals, of which 30 were assigned to a triterpenoid moiety (Mahato & Kundu, 1994) and 17 to a saccharide portion. The ^1H NMR spectrum (**Spectrum 4.1.2**) of the aglycone moiety of **1** showed signals for six tertiary methyl groups (δ 0.74, 0.82, 0.93, 0.95, 1.00, 1.19) and a signal of H-3_{ax} at δ 3.64 due to the presence of an OH group at C-3 position. In the HSQC spectrum (**Spectrum 4.1.3**), the one proton signal at δ5.28 (1H, dd "t", *J*=3.6 Hz), and a pair of signals at δ3.32 and 3.62 (each 1H, d, J=11.5) showed correlations with the carbon resonances at δ 123.7 and 65.2, respectively. These signals were assigned to an olefinic and primary alcoholic function in

the sapogenol moiety. These data also indicated an oleanolic acid derivative with one of the methyl group substitued by a ±CH_2OH function (Mahato & Kundu, 1994).

Furthermore, in the ^{13}C NMR spectrum the signals at δ123.7 and 144.8 ascribable to C-12 and C-13 confirmed the Δ^{12} oleanene skeleton (Mahato & Kundu, 1994). A signal at δ178.0 and the carbon resonances of ring D and E suggested the occurrence of a glycosylated ±COOH group at C-28. Full assignments of the proton and carbon resonances of the aglycone (see Table CQ3-1) were secured by COSY and HSQC spectra (**Spectra 4.1.3** and **4.1.4**). The primary hydroxyl function was found to be located at C-23 on the basis of the downfield shift (+5.1) exhibited by C-4 (δ 43.8) and the highfield shifts (-5.0 ppm; -6.7 ppm; -2.2 ppm) exhibited respectively by C-3 (δ83.7), C-5 (48.5) and C-24 (13.4) in comparison with the same carbon resonances in an oleanene skeleton bearing a Me-23 such as oleanolic acid (*see* Table 1: Mahato & Kundu, 1994). Moreover, the chemical shift of Me-24 in the ^1H NMR spectrum (δ_H 0.74) was diagnostic for a C-23(CH_2OH). The HMBC spectrum of CQ-3 (**Spectrum 4.1.5**) confirmed the position of the alcoholic function showing significative cross-peaks, due to ^1H and ^{13}C long-range correlation between H_2-23 (δ 3.64 and 3.32) and C-3 (δ 83.7), C-4 (δ 43.8) and C-24 (δ 13.4). On the basis of these data the aglycone of CQ-3 was identified as 3β,23-dihydroxyolean-12-en-28-oic acid, known as hederagenin (Mahato & Kundu, 1994).

Glycosylation of the alcoholic function at C-3(OH) and esterification of the C-28(COOH) group were indicated by the downfield shift (+5 ppm) and highfield shift (-3 ppm) observed, respectively. In the ^1H NMR spectrum, three anomeric proton signals (δ4.38, d, J = 7.8 Hz; δ4.58, d, J = 7.8 Hz; δ5.41, d, J = 8.1 Hz). These data and the 17 carbon resonances attributed to the sugar moiety suggested the presence of two hexose and one pentose. The structures of the sugar units were deduced by the help of 2D-NMR experiments (COSY, HSQC and HMBC). The isolated anomeric proton signals between δ4.38 and 5.41 were the starting point for the COSY experiment which helped to establish three different spin systems arising sugar units. HSQC experiment helped for the assignment of corresponding carbon resonances of each spin systems. These experiments allowed the sequential assignments of all of the proton and carbon resonances to the individual monosaccharides as reported in **Table 4.1.1**. Thus the shifts of the sugar resonances, summarized in **Table 4.1.1**, were attributable to two terminal β-glucopyranosyl units. The absence of any ^{13}C NMR

glycosydation shifts for two β-glucopyranosyl units confirmed that these sugars were terminal. The rest of the five carbon resonances and the corresponding proton signals arising from the sugar moieties were evident for the presence of an α-arabinopyranosyl unit. HSQC experiment allowed the assignments of interglycosidic linkages by comparison of the observed carbon chemical shifts with those of the corresponding methylpyranosides. Glycosydation shift was observed only for C-3 of the arabinopyranose (δ84.1). The positions of the sugar residues were unambigously defined by the HMBC experiment (Spectrum 1.5). A cross peak due to long-range correlations between C-3 (δ83.7) of the aglycone and H-1′ of α-arabinopyranosyl (δ4.38) indicated that arabinose was the sugar residue linked to hydroxyl group at C-3 of the aglycone. Further crosspeak between C-3′ of arabinose (δ84.1) and one of the anomeric proton of two glucose units (δ5.41) indicated that glucose was the second unit of the disaccharide chain at C-3 of the aglycone.

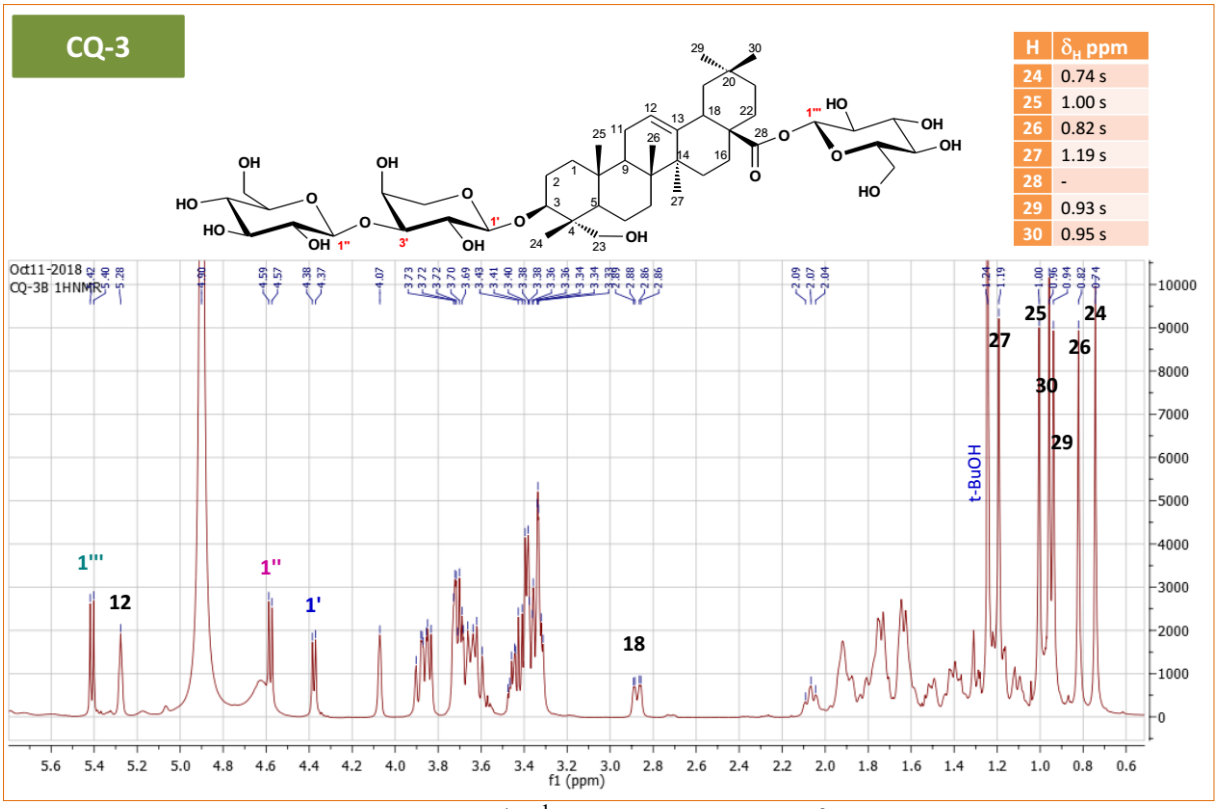
A cross peak between H-1‴ of the second glucose unit (δ5.41) and the ^{13}C NMR resonance of the carbonyl group (δ178.0) of the aglycone moiety provided definitive evidence for an ester linkage between the trisaccharide chain and the aglycone.

Based on the data, the structure of CQ-3 was determined as the 3β-[(*O*-β-D-glucopyranosyl-(1→3)-α-arabinopyranosyl)oxy]-23-hydroxy-olean-12-en-28-oic-acid-28-*O*-β-glucopyranoside [= 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranoside]. The ^{1}H and ^{13}C-NMR data of this saponoside (CQ-3) was also in good aggreement to those reported for same compound isolated from the flowers, fruits, seed coats and seeds of *Chenopodium quinoa* (Dini et al., 2001; Woldemichael&Wink, 2001; Kuljanabhagavad et al., 2008) and from the seeds of *Chenopodium pallidicuale* (Rastrelli et al., 1996) confirming the structure.

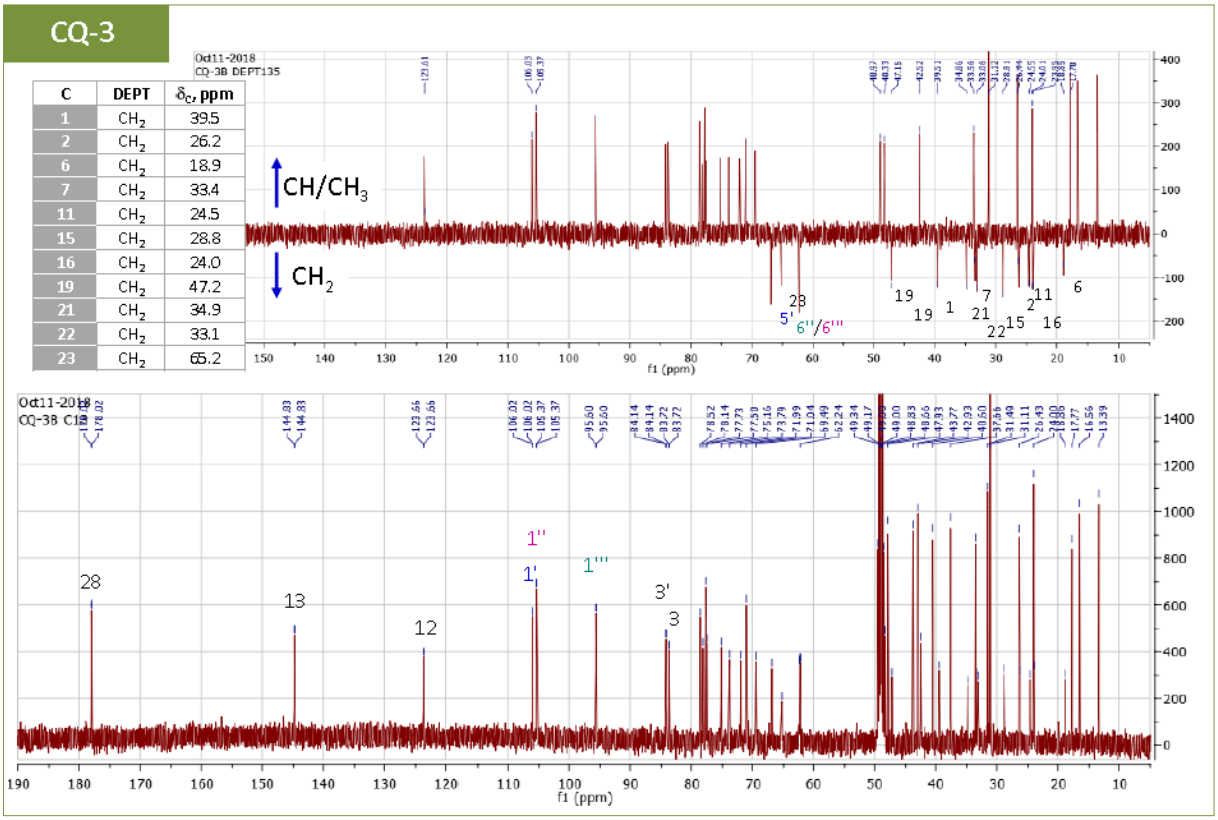
Table 4.1.1. The ^{1}H and ^{13}C-NMR Data for CQ-3 (CD_3OD, δ_H 500 MHz; δ_C 125 MHz)

C/H	DEPT	δ_C, ppm	δ_H ppm, *J* (Hz)	HMBC (from C to H)
1	CH_2	39.5	1.63†/0.97†	Me-25
2	CH_2	26.2	1.88†/1.75†	
3	CH	83.7	3.64†	Me-24
4	C	43.8	-	H_2-23, Me-24
5	CH	48.5	1.63†	Me-24
6	CH_2	18.9	1.50†/1.38†	
7	CH_2	33.4	1.60†/1.29†	Me-26
8	C	40.6	-	Me-26
9	CH	47.9	1.22†	Me-26
10	C	37.7	-	Me-25
11	CH_2	24.5	1.91†	
12	CH	123.7	5.28 dd “t” (3.6)	
13	C	144.8	-	Me-27
14	C	42.9	-	H-12, Me-26, Me-27
15	CH_2	28.8	1.80†/1.11†	Me-27
16	CH_2	24.0	2.07†/1.73†	
17	C	48.8	-	
18	CH	42.5	2.87 dd (13.6/3.6)	H-12
19	CH_2	47.2	1.72†/1.17†	Me-29, Me-30
20	C	31.5	-	Me-29, Me-30
21	CH_2	34.9	1.41†/1.23†	Me-29, Me-30
22	CH_2	33.1	1.74†/1.63†	
23	CH_2	65.2	3.64†/3.32†	Me-24
24	CH_3	13.4	0.74 s	H_2-23
25	CH_3	16.6	1.00 s	
26	CH_3	17.8	0.82 s	
27	CH_3	26.4	1.19 s	
28	C	178.0	-	
29	CH_3	33.6	0.93 s	
30	CH_3	24.0	0.95 s	
Ara (C-3)				
1′	CH	106.0	4.38 d (7.2)	
2′	CH	72.0	3.71†	
3′	CH	84.1	3.69†	H-1″
4′	CH	69.5	4.07 br s	
5′	CH_2	66.8	3.88 br d/3.60 br d (J_{AB} 12.0)	
Glu (C-4′)				
1″	CH	105.4	4.58 d (7.9)	H-4′
2″	CH	73.8	3.36†	
3″	CH	77.5	3.42†	
4″	CH	71.1	3.39†	
5″	CH	77.7	3.34†	
6″	CH_2	62.3	3.85†/3.72†	
Glu (C-28)				
1‴	CH	95.6	5.41 d (8.1)	
2‴	CH	75.2	3.34†	
3‴	CH	78.1	3.45†	
4‴	CH	71.0	3.39†	
5‴	CH	78.5	3.39†	
6‴	CH_2	62.3	3.85†/3.72†	

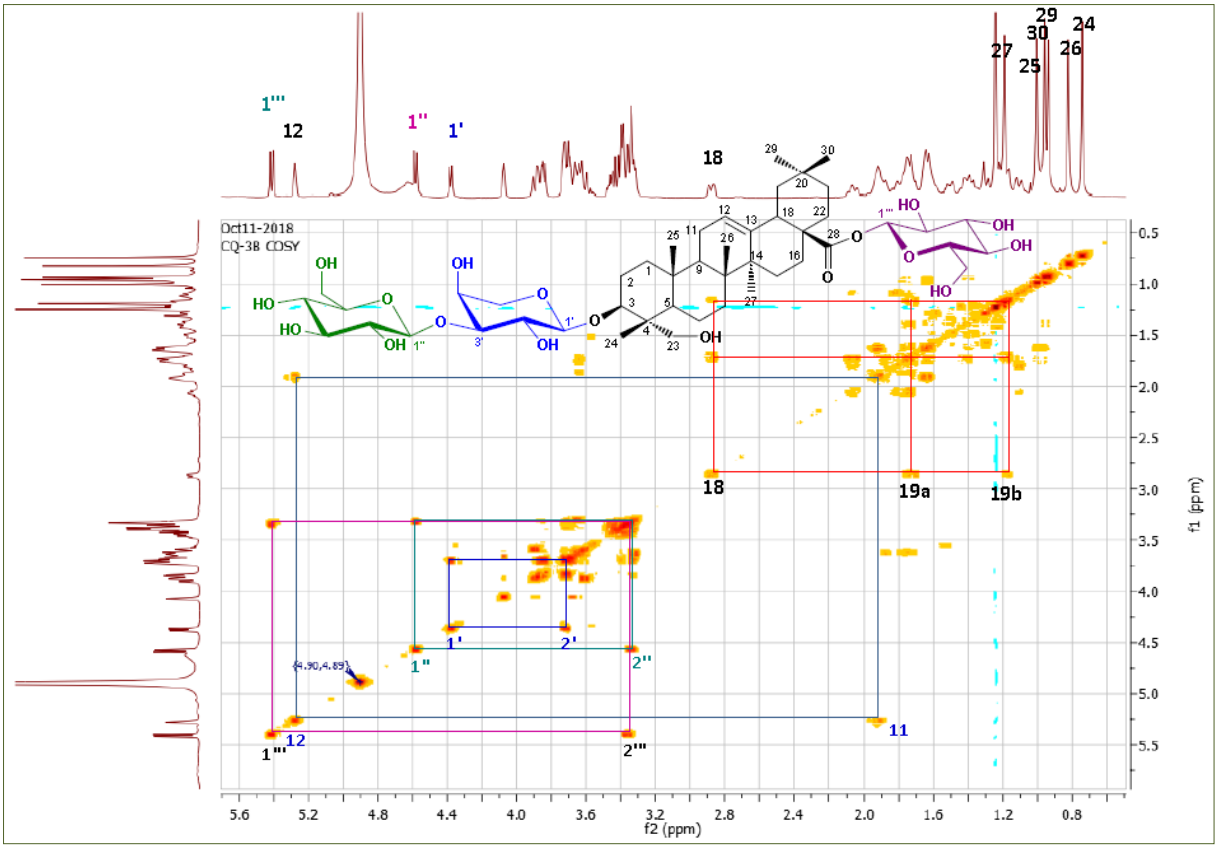
†) *J* values are not clear due to overlapping.



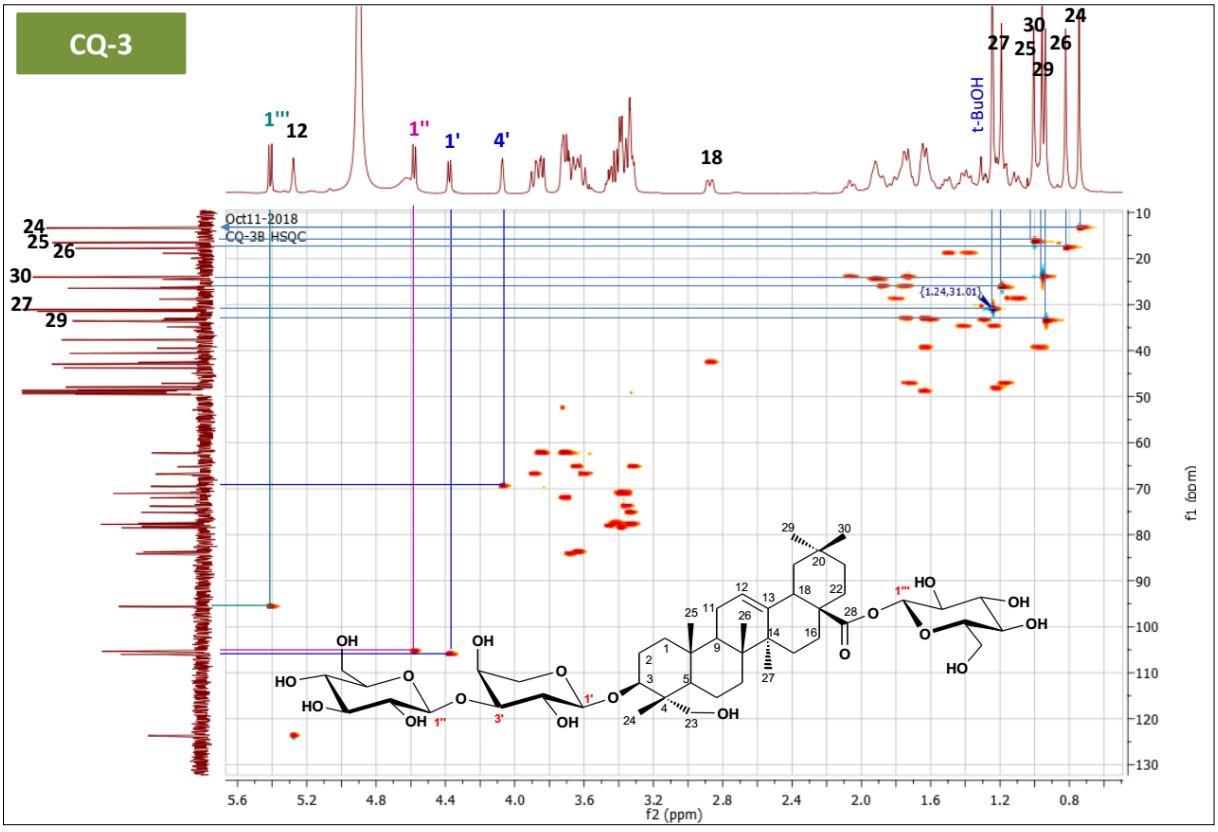
Spectrum 4.1.1. The ^{1}H-NMR Spectrum of CQ-3.



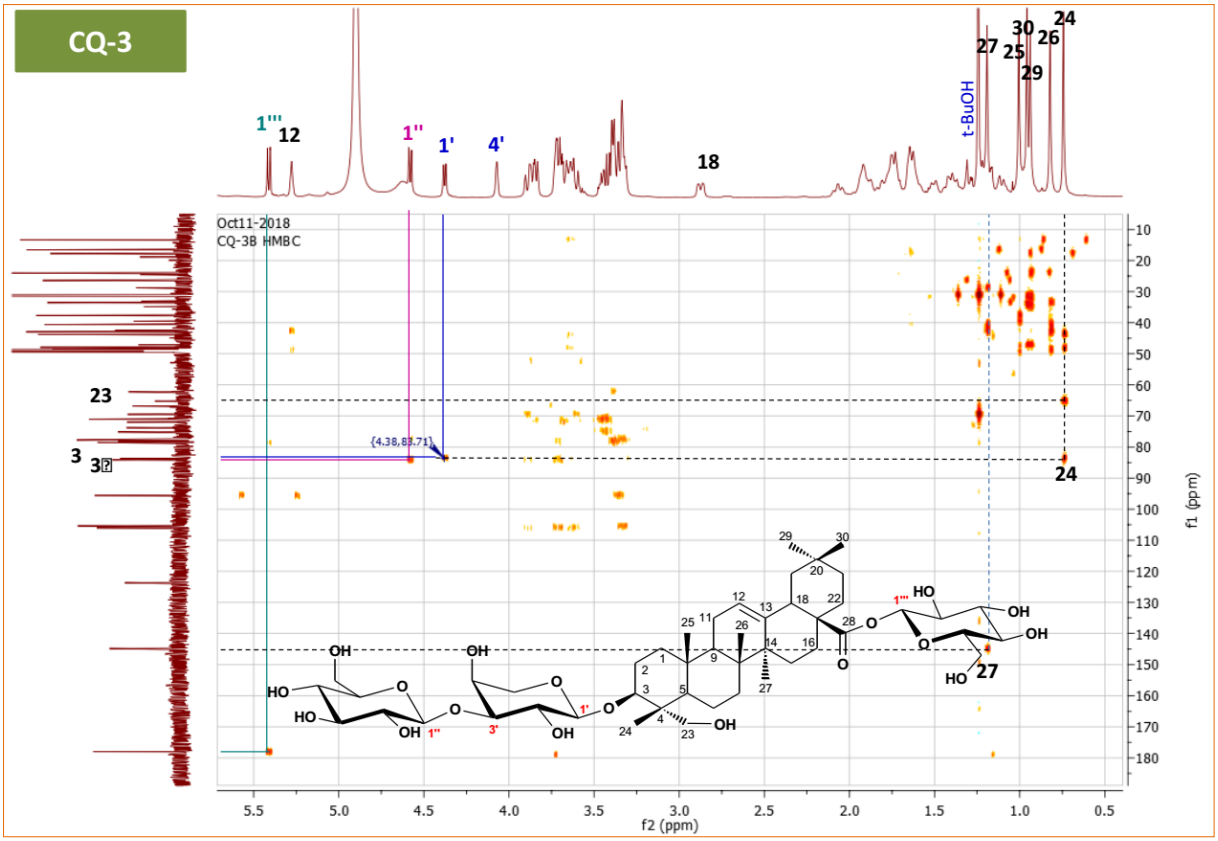
Spectrum 4.1.2. The ^{13}C-NMR and DEPT-135 Spectra of CQ-3.



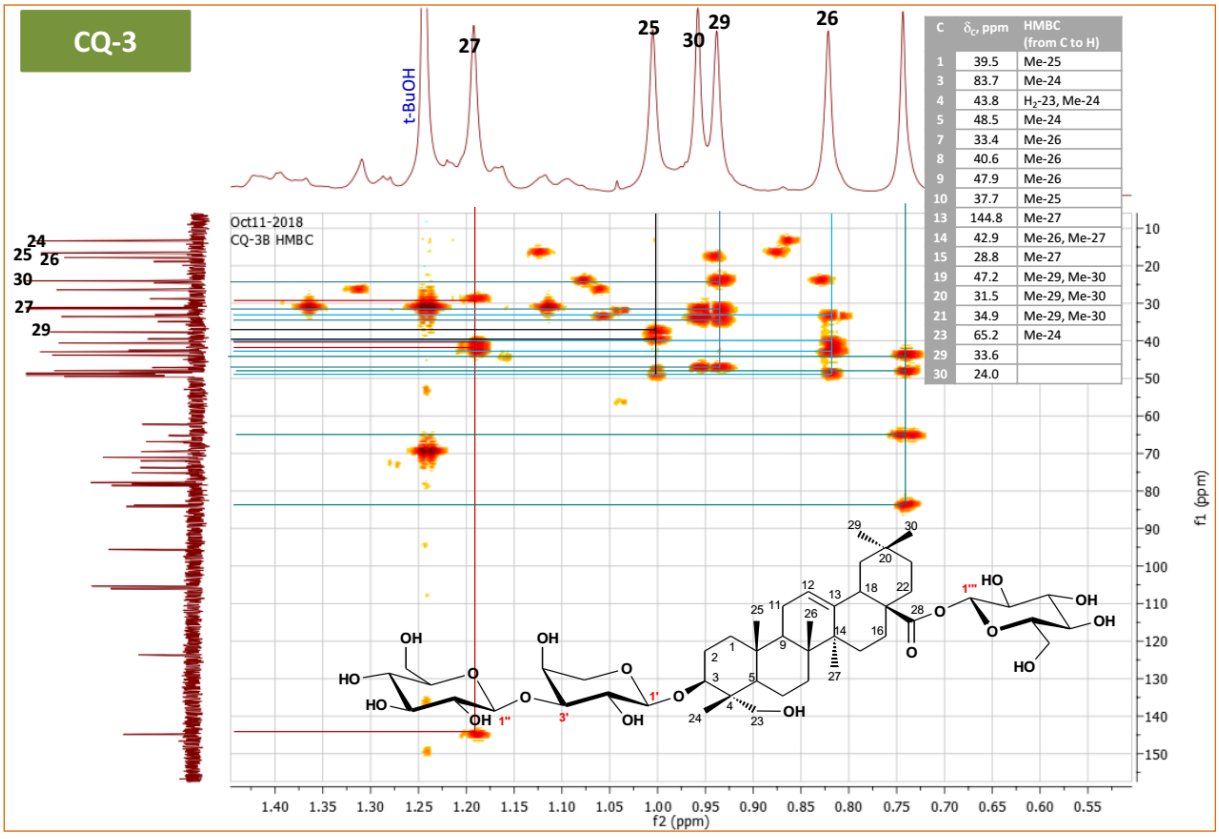
Spectrum 4.1.3. The COSY of CQ-3.



Spectrum 4.1.4. The HSQC of CQ-3.

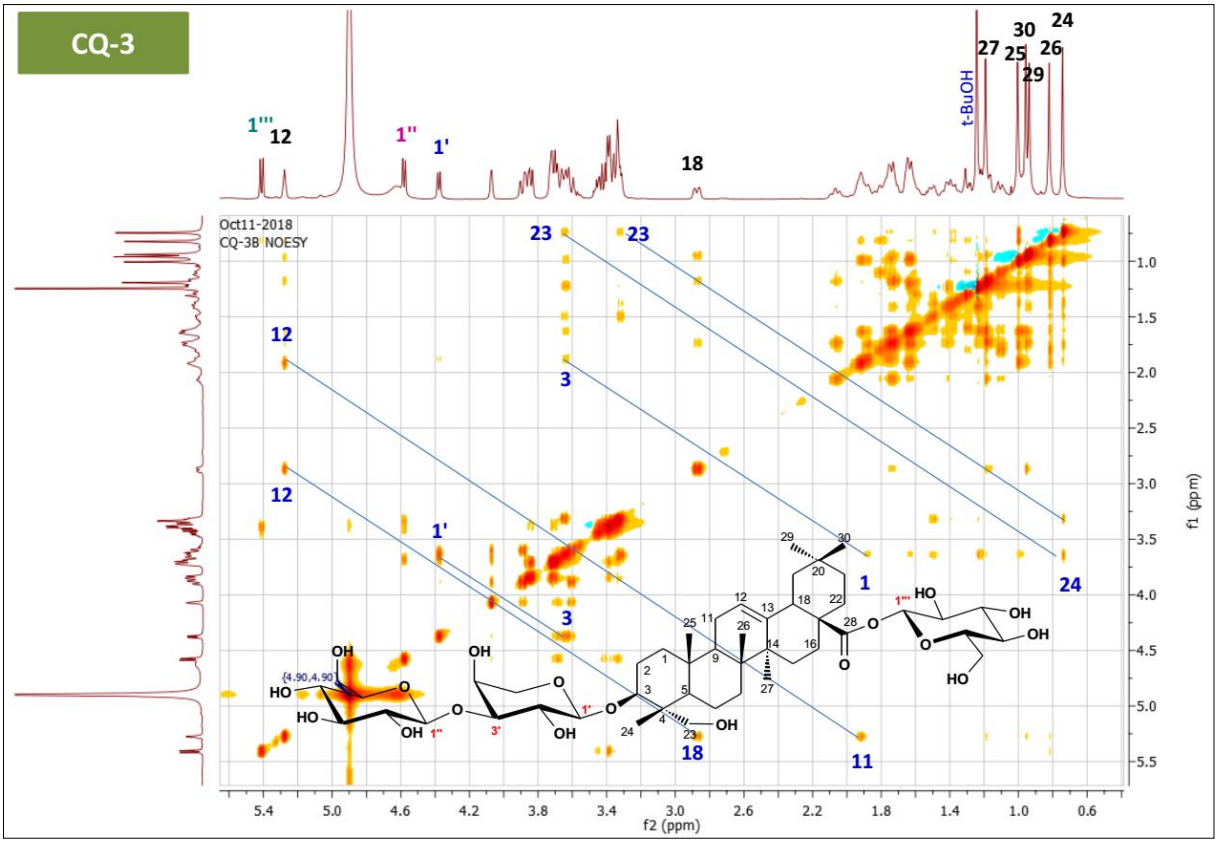


Spectrum 4.1.5a. The HMBC of CQ-3.



C	δ_C, ppm	HMBC (from C to H)
1	39.5	Me-25
3	83.7	Me-24
4	43.8	H_2-23, Me-24
5	48.5	Me-24
7	33.4	Me-26
8	40.6	Me-26
9	47.9	Me-26
10	37.7	Me-25
13	144.8	Me-27
14	42.9	Me-26, Me-27
15	28.8	Me-27
19	47.2	Me-29, Me-30
20	31.5	Me-29, Me-30
21	34.9	Me-29, Me-30
23	65.2	Me-24
29	33.6	
30	24.0	

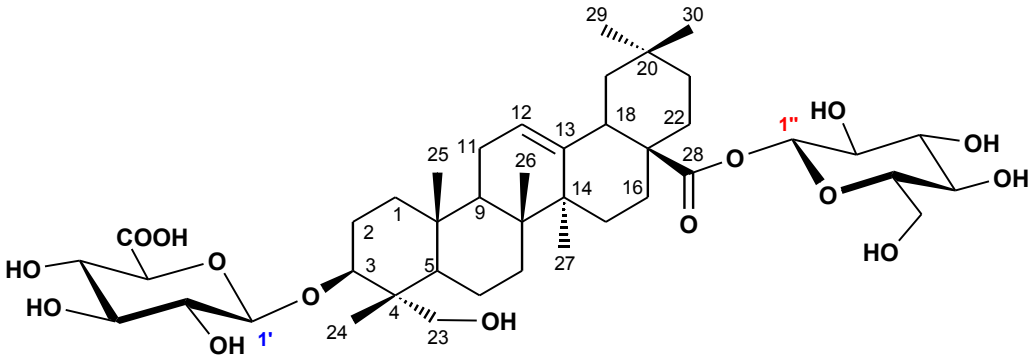
Spectrum 4.1.5b. The HMBC of CQ-3.



Spectrum 4.1.6. The NOESY of CQ-3.

4.2. Hederagenin Glycoside (CQ-6)
= 3β,23-dihydroxy-olean-12-en-28-oic acid Glycoside
$C_{30}H_{48}O_4$; Mol wt 472

CQ-6: Molecular weight 810 (Calcd. For $C_{42}H_{66}O_{15}$)



The negative ion H-ESI-mass spectrum of CQ-6 (**Figure 4.2.1**) showed quasimolecular ion $[M - H]^-$ at m/z 809.4373, indicating a Molecula weight of 810 (calcd for $C_{42}H_{68}O_{15}$).

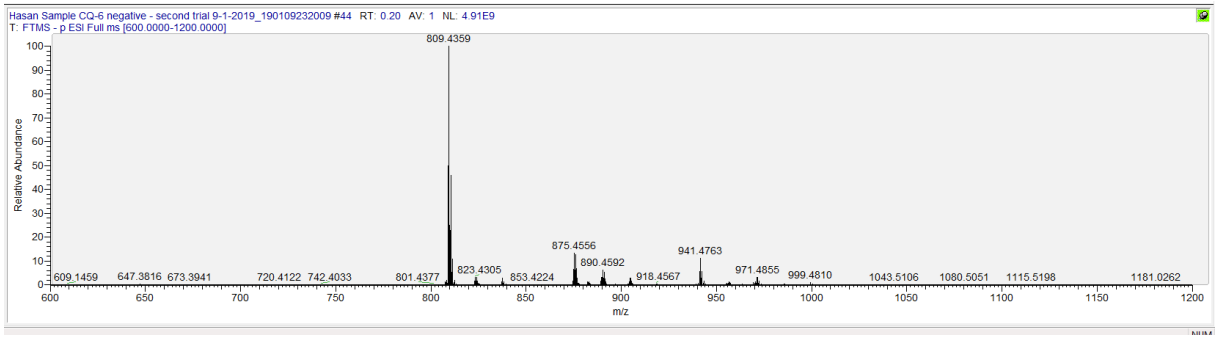


Figure 4.2.1. Negative Ion H-ESI Mass Spectra (HRMS) of CQ-6.

CQ-6 was obtained as a colourless amorphous compound. The ^{1}H-NMR (**Spectrum 4.2.1**) and ^{13}C-NMR spectra (**Spectra 4.2.2a** and **4.2.2b**) of CQ-6 were very similar to those of CQ-3 for the resonances arising from the sapogenol moiety. The ^{13}C-NMR spectrum showed signals corresponding to 42 carbons which were resolved into 6 methyl, 12 methylene, 15 methine and 9 quaternary carbons. The ^{1}H- and ^{13}C-NMR spectra of CQ-6 indicated the lack of the proton and carbon signals arising from a hexose unit in comparison to those of CQ-3. The assignments of all proton and carbon resonances were based on the 2D-NMR (COSY,

HSQC and HMBC) experiments (**Spectra 4.2.3**, **4.2.4** and **4.2.5**). The ^{13}C-NMR data indicated an oleanane-type sapogenol derivative with two of the eight methyl groups substitued by a CH_2O (δ 64.8: C-23) and a COOH (δ 178.2: C-28) functions (**Table 4.2.1**). The signals at δ 123.8 and 144.9 were ascribed to C-12 and C-13 confirming the Δ^{12} oleanene skeleton (Mahato & Kundu, 1994). The chemical shift values observed for six tertiary-methyl resonances at δ 0.72, 0.82, 0.94, 0.96, 1.00 and 1.19 together with their corresponding carbon resonances secured by the help of COSY and HSQC experiments were in good accordance to those of CQ-3 (**Table 4.1.1**) confirming the presence of hederagenin (3β,23-dihydroxy-olean-12-en-28-oic acid) as sapogenol moiety.

Moreover, an olefinic proton at δ 5.27 (1H, dd "t", *J*=3.4 Hz) was assigned to H-12. The chemical shifts of C-3 (δ 82.3) and C-28 (δ 178.2) were indicative for the presence of a bisdesmosidic glycoside in comparison to those of oleanolic acid (*see* **Table 4.1** for oleanolic acid: C-3: δ 78.7; C-28: δ 181.0).

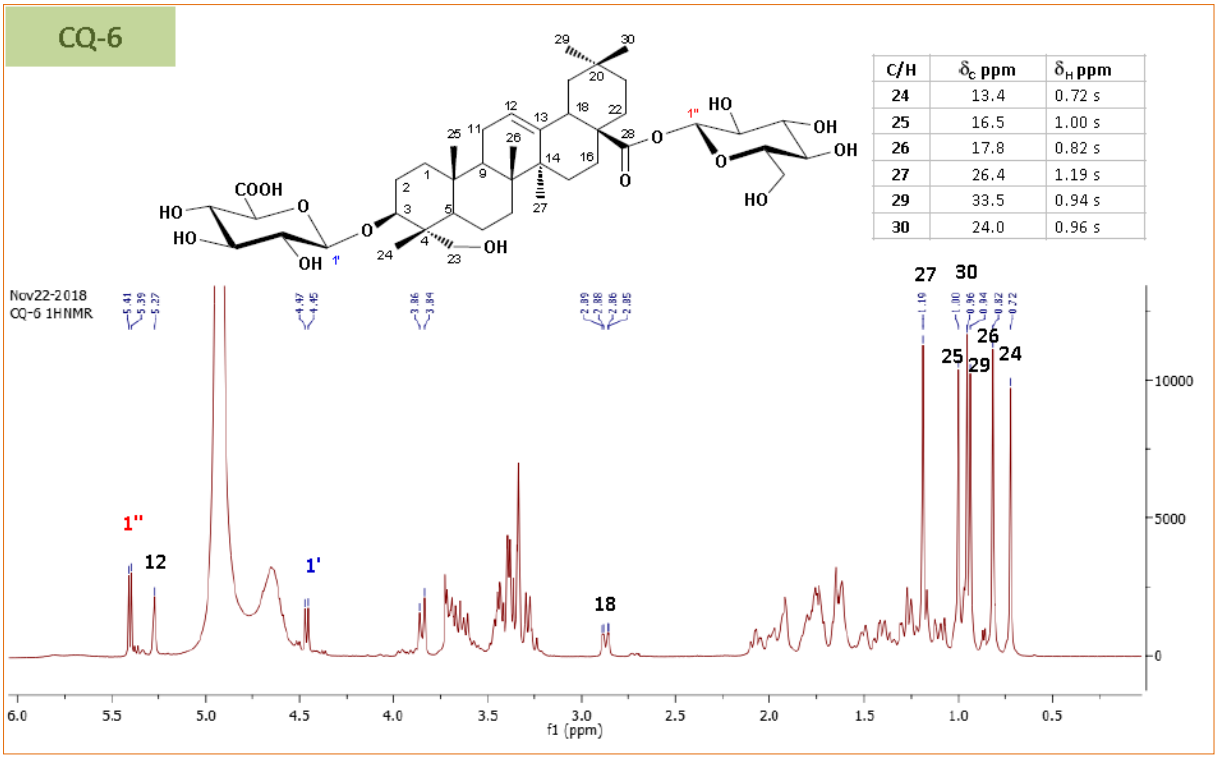
The ^{1}H- and ^{13}C-NMR spectra of CQ-6 exhibited two anomeric protons resonated at δ 4.45 (d, *J* = 7.8 hz; H-1′) and 5.40 (d, *J* = 8.1 Hz; H-1″), which showed correlations with the carbon resonances at δ 95.7 and 105.2, respectively, in the HSQC experiment (Spectrum 2.4). Apart from the anomeric proton at δ 5.40, the chemical shifts of the protons and the corresponding carbon resonances found in the spin system showed that one of the sugar unit was glucose attached to the C-28-COOH function via an ester linkage. The rest of the proton and carbon resonances found in the spin system of the second anomeric proton (δ 4.45) were deduced for the presence of a β-glucuronic acid. The site of glycosidations were established by the HMBC experiment (Spectrum 2.5). HMBC correlations were observed from C-3 (δ 82.3) to one of the anomeric proton at δ 4.45 (H-1′) and from C-28 (178.2) to the second anomeric proton at δ 5.40 (H-1″) confirming the site of glycosidations and the presence of a bidesmosidic diglycosidic structure.

The HRMS and NMR data were evident for a structure of 3β-*O*-β-D-glucuronopyranosyloxy-23hydroxy-olean-12-en-28-oic-acid-28-*O*-β-glucopyranoside (= 3-*O*-β-D-glucuronopyranosyl -hederagenin-28-*O*-β-glucopyranosyl ester). This saponoside has also been previously reported from the bran of *Chenopodium quinoa* (Mizui et al., 1998 and 1990; Kuljanabhagavad et al., 2008). CQ-6 has fistly been reported from *Panax japonicum* and named as 23-hydroxy-chikusetsusaponin IVa (Lin et al., 1976).

Table 4.2.1. The ^{1}H and ^{13}C-NMR Data for CQ-6 (CD_3OD, δ_H 500 MHz; δ_C 125 MHz)

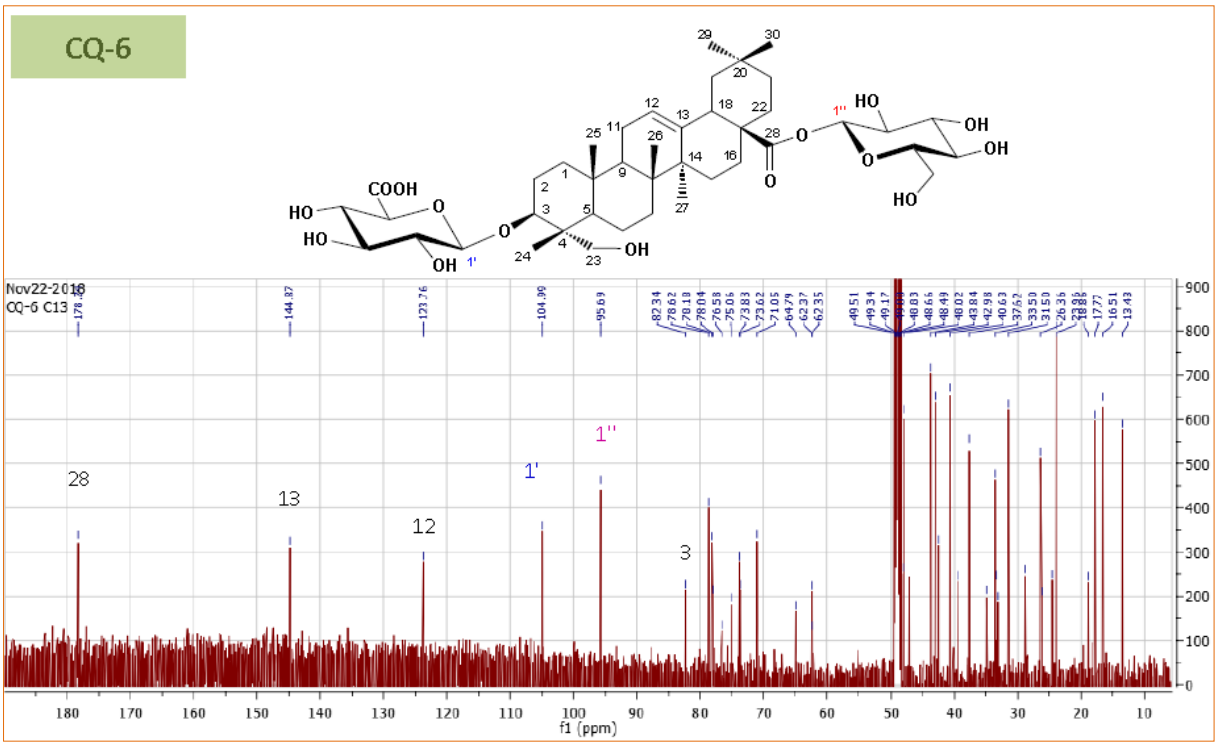
C/H	DEPT	δ_C, ppm	δ_H ppm, *J* (Hz)	HMBC (from C to H)
1	CH_2	39.5	1.63†/0.97†	Me-25
2	CH_2	26.1	1.99†/1.77†	
3	CH	82.3	3.69†	H-1′, Me-24
4	C	43.0	-	H_2-23, Me-24
5	CH	48.1	1.25†	Me-24
6	CH_2	18.9	1.50†/1.38†	
7	CH_2	33.3	1.62†/1.28†	Me-26
8	C	40.6	-	Me-26
9	CH	49.0	1.63†	Me-26
10	C	37.6	-	Me-25
11	CH_2	24.6	1.92†/1.80†	
12	CH	123.8	5.27 dd “t” (3.4)	
13	C	144.9	-	Me-27
14	C	43.8	-	H-12, Me-26, Me-27
15	CH_2	28.8	1.81†/1.09†	Me-27
16	CH_2	24.0	2.06 ddd “dt” (3.9/13.5)/1.73†	
17	C	49.0	-	
18	CH	42.6	2.87 dd (13.6/4.0)	H-12
19	CH_2	47.2	1.74†/1.16†	Me-29, Me-30
20	C	31.5	-	Me-29, Me-30
21	CH_2	34.9	1.41†/1.22†	Me-29, Me-30
22	CH_2	33.1	1.74†/1.63†	
23	CH_2	64.8	3.66 d /3.27 d (J_{AB} 11.6)	Me-24
24	CH_3	13.4	0.72 s	H_2-23
25	CH_3	16.5	1.00 s	
26	CH_3	17.8	0.82 s	
27	CH_3	26.4	1.19 s	
28	C	178.2	-	H-1″
29	CH_3	33.5	0.94 s	
30	CH_3	24.0	0.96 s	
Glur A (C-3)				
1′	CH	105.0	4.45 d (7.8)	
2′	CH	75.1	3.28†	
3′	CH	78.0	3.40†	
4′	CH	73.6	3.42†	H-3′
5′	CH	76.6	3.60 d (9.0)	H-4′
6′	CH_2	n.o.	-	
Glu (C-28)				
1″	CH	95.7	5.40 d (8.1)	H-1″
2″	CH	73.9	3.34†	
3″	CH	78.2	3.43†	
4″	CH	71.1	3.38†	H-2″
5″	CH	78.6	3.38†	H-4″
6″	CH_2	62.4	3.84 br d (12.0), 3.69 dd (12.5/4.5)	

†) *J* values are not clear due to overlapping.
n.o.) Not observed

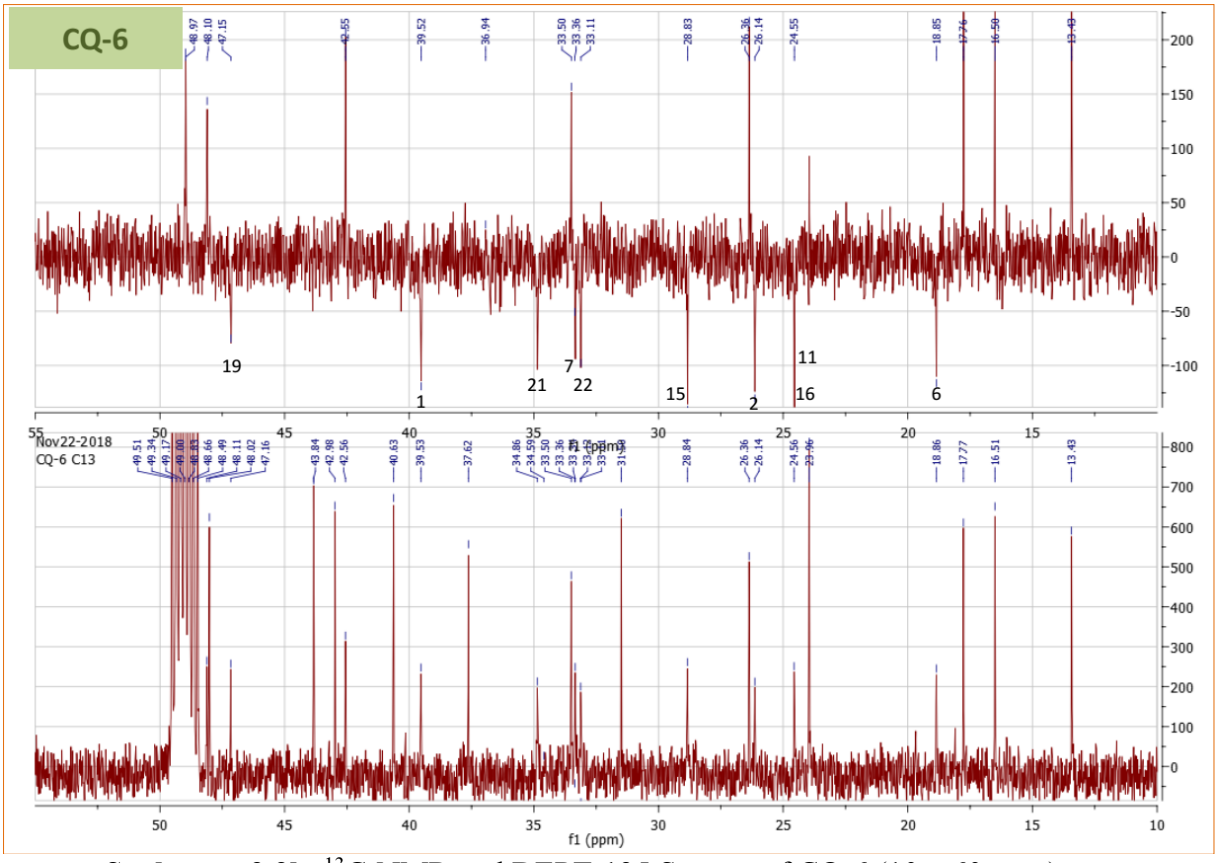


C/H	δ_C ppm	δ_H ppm
24	13.4	0.72 s
25	16.5	1.00 s
26	17.8	0.82 s
27	26.4	1.19 s
29	33.5	0.94 s
30	24.0	0.96 s

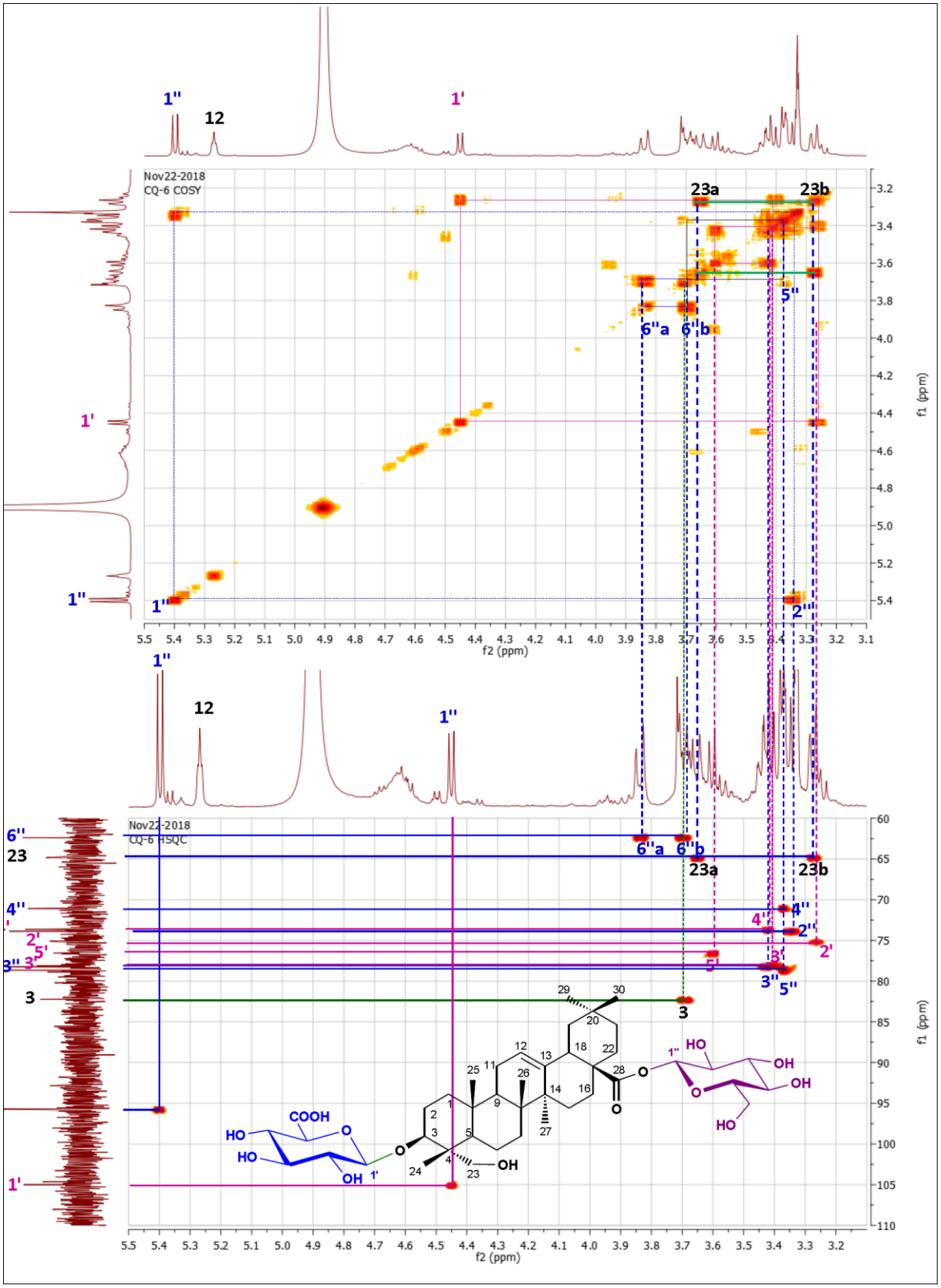
Spektrum 4.2.1. ^{1}H-NMR Spectrum of CQ-6.



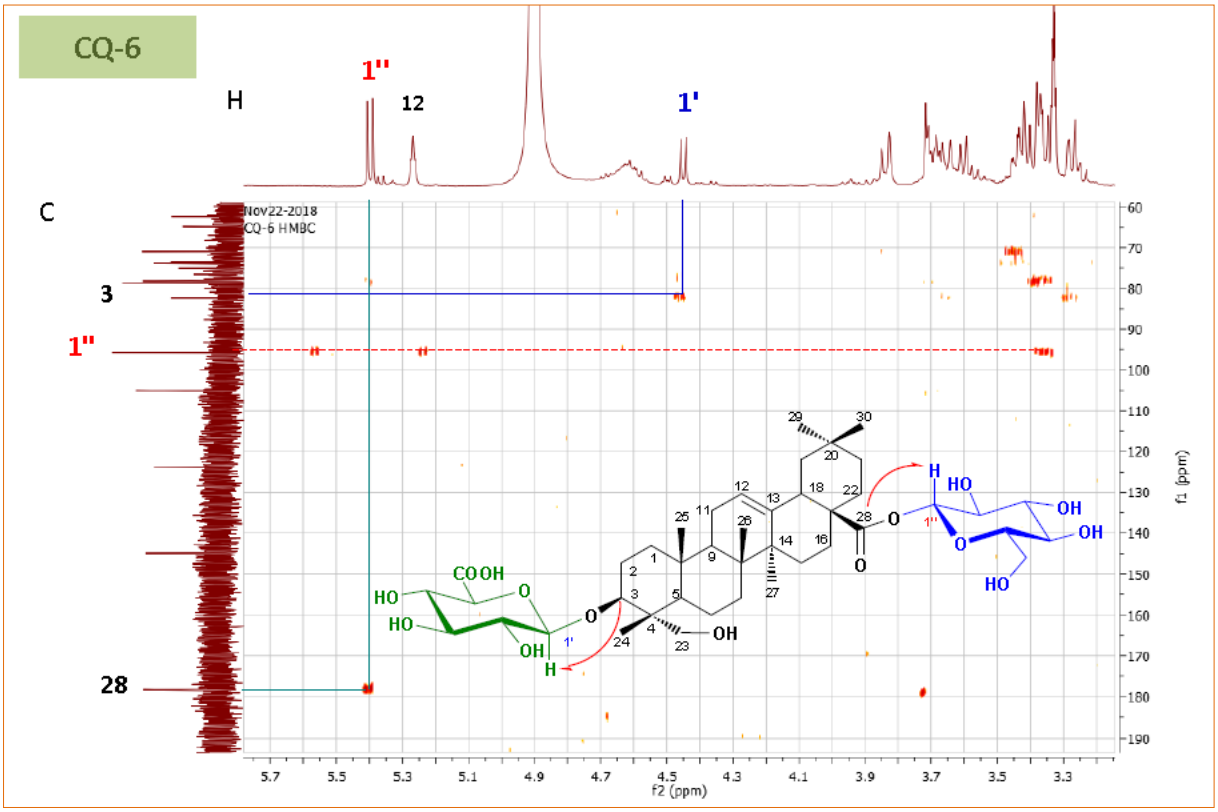
Spektrum 2.2a. ^{13}C-NMR Spectrum of CQ-6 (0 – 180 ppm).



Spektrum 2.2b. ^{13}C-NMR and DEPT-135 Spectra of CQ-6 (10 – 60 ppm).



Spectra 2.3 and 2.4. COSY and HSQC Spectra of
(^{1}H: 3.1 – 5.5 ppm; ^{13}C: 60 – 110 ppm).



Spektrum 2.5. HMBC of CQ-6.

4.3. 30-Hydroxy-hederagenin Glycoside (CQ-1)

= 3β,23,30-trihydroxy-olean-12-en-28-oic acid Glycoside
$C_{30}H_{48}O_5$; Mol wt 488,

CQ-1: $C_{47}H_{76}O_{19}$; Mol. Wt: 944

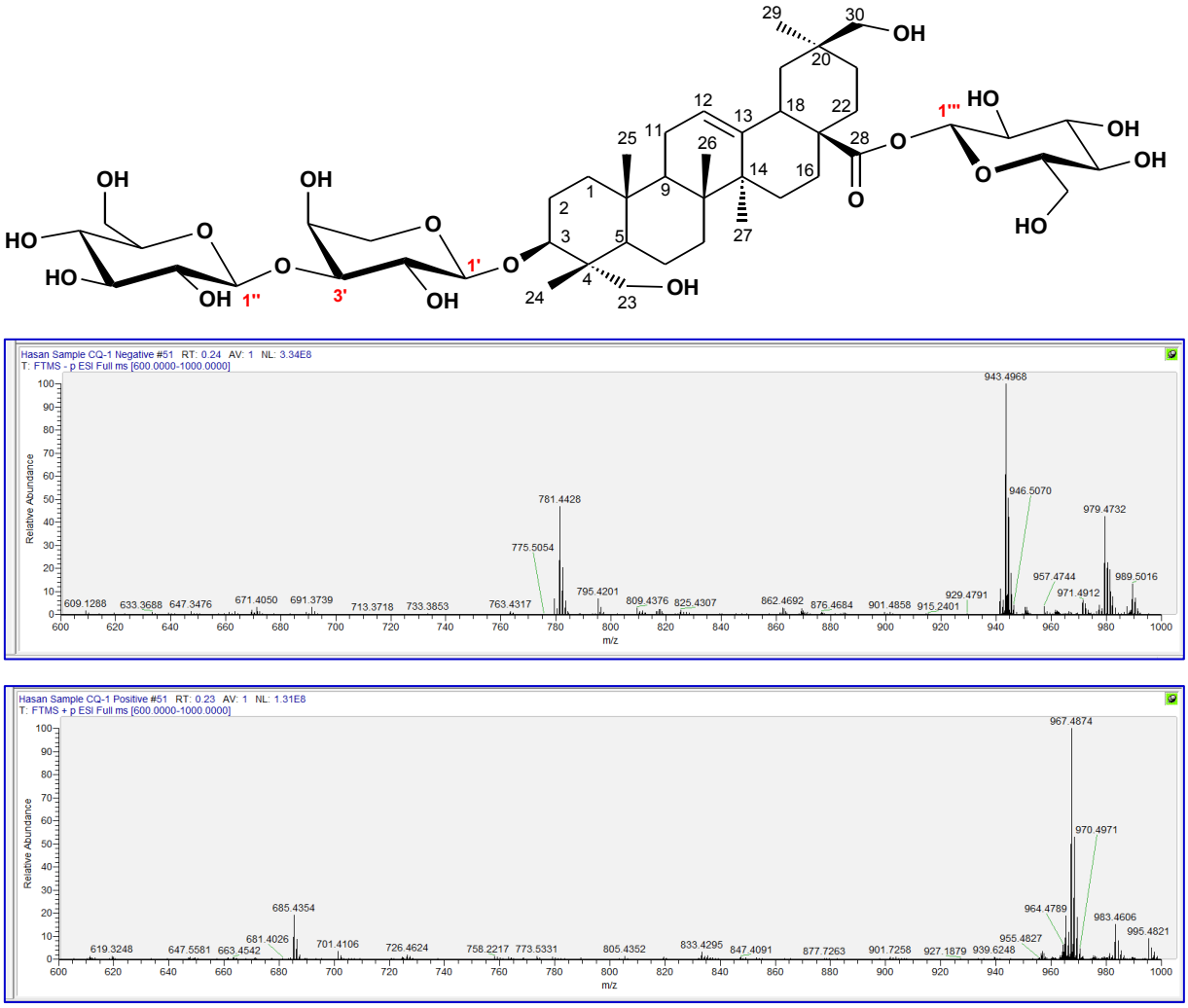


Figure 4.3.1. Negative and Positive ion H-ESI-Mass Spectra of CQ-1.

The negative ion H-ESI-mass spectrum of CQ-3 showed quasimolecular ion $[M-H]^-$ at m/z 943.4968, indicating a Mr of 944 (calcd for $C_{47}H_{76}O_{19}$). The other significant ion $[M-163]^-$ was observed at m/z 781.4428 attributed to the loss of one of the terminal glucose unit. The positive ion H-ESI-mass spectrum exhibited a pseudo-molecular ion peak $[M+Na]^+$ at m/z 967.4874, indicating a Mr of 967 (calcd for $C_{47}H_{76}O_{19}Na$). These results indicated that the molecular weight of CQ-1 to be as 944 ($C_{47}H_{76}O_{19}$) (**Figure 4.3.1**).

Table 4.3.1. The ^{1}H and ^{13}C-NMR Data for CQ-1 (CD_3OD, δ_H 500 MHz; δ_C 125 MHz)

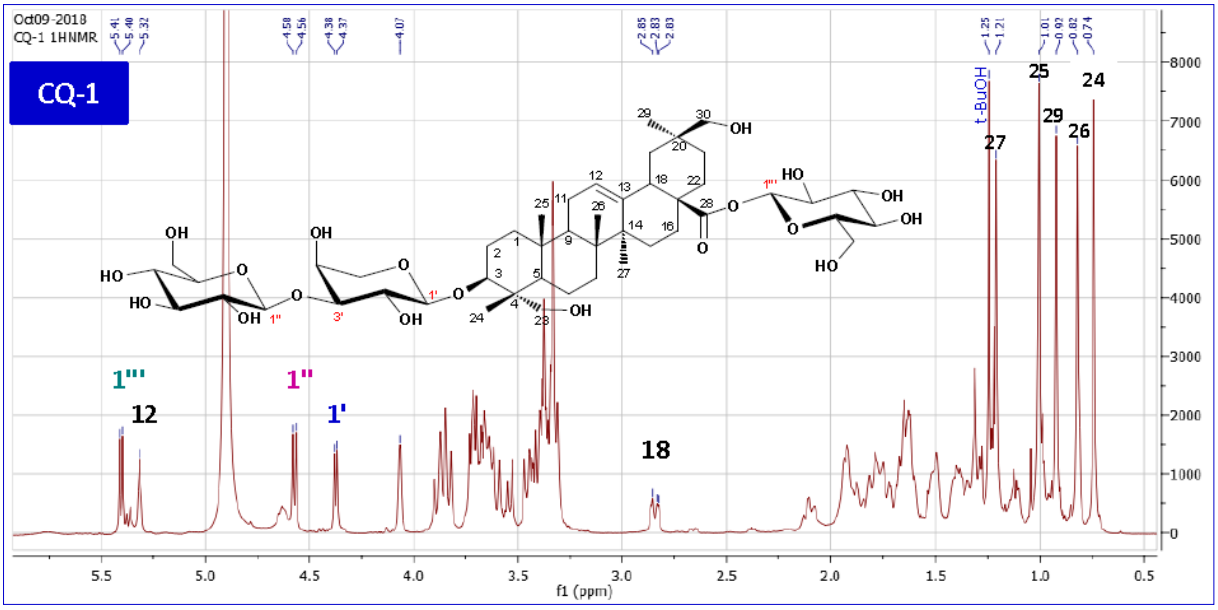
C/H	DEPT	δ_C, ppm	δ_H ppm, *J* (Hz)	HMBC (from C to H)
1	CH_2	39.5	1.64†/0.99†	Me-25
2	CH_2	26.3	1.88†/1.76†	
3	CH	83.6	3.66†	H-1′, Me-24
4	C	43.8	-	Me-24
5	CH	48.3	1.23†	Me-24, Me-25
6	CH_2	18.9	1.50†/1.40†	
7	CH_2	33.4	1.61†/1.29†	Me-26
8	C	40.7	-	Me-26, Me-27
9	CH	49.0	1.65†	Me-25, Me-26
10	C	37.7	-	Me-25
11	CH_2	24.6	1.94†	
12	CH	124.0	5.32 dd “t” (3.5)	
13	C	144.6	-	Me-27
14	C	43.0	-	H-12, Me-26, Me-27
15	CH_2	28.8	1.88†/1.11†	Me-27
16	CH_2	24.3	2.11†/1.77†	
17	C	47.9	-	
18	CH	42.0	2.84 dd (13.4/3.9)	Me-29
19	CH_2	42.4	1.65†/1.40†	
20	C	36.0	-	Me-29
21	CH_2	29.6	1.51†/1.32†	Me-29
22	CH_2	32.7	1.72†/1.60†	
23	CH_2	65.2	3.65†/3.32†	Me-24
24	CH_3	13.4	0.74 s	
25	CH_3	16.5	1.01 s	
26	CH_3	17.8	0.82 s	
27	CH_3	26.4	1.21 s	
28	C	178.0	-	H-1‴
29	CH_3	28.10	0.92 s	
30	CH_2	66.3	3.46 d/3.54 d (J_{AB} = 11.0)	Me-29
Ara (C-3)				
1′	CH	106.1	4.38 d (7.3)	
2′	CH	72.1	3.72†	
3′	CH	84.2	3.68†	H-1″
4′	CH	69.5	4.07 br s	
5′	CH_2	66.9	3.89 br d/3.60 br d (J_{AB} 12.5)	
Glu (C-4′)				
1″	CH	105.4	4.57 d (7.8)	H-4′
2″	CH	73.9	3.35†	
3″	CH	77.6	3.41†	
4″	CH	71.1	3.39†	
5″	CH	77.9	3.34†	
6″	CH_2	62.3	3.84†/3.70†	
Glu (C-28)				
1‴	CH	95.7	5.41 d (8.1)	
2‴	CH	75.3	3.33†	
3‴	CH	78.2	3.43†	
4‴	CH	71.0	3.38†	
5‴	CH	78.6	3.38†	
6‴	CH_2	62.4	3.88†/3.72†	

†) *J* values are not clear due to overlapping.

The ^{1}H-NMR spectrum exhibited five tertiary methyl resonances at δ 0.75, 0.82, 0.92, 1.01 and 1.21 indicated that three of the eight methyl groups were oxygenated (**Spectrum 4.3.1**). The ^{13}C-NMR spectra (**Spectrum 4.3.2**) revealed 47 carbon atom signals, including five tert.-methyl (CH_3) groups, 15 methylene (CH_2), 19 methine (CH) and eight quaternary carbon (C) atoms. The signals at δ 124.0 and 144.6 were in good aggreement to those of CQ-3 and CQ-6 which were depicted to C-12 and C-13, respectively, confirming the Δ^{12} oleanene skeleton (Mahato & Kundu, 1994). An olefinic proton assigned to the H-12 was observed at δ 5.32 (1H, dd "t", J = 3.5 Hz). The chemical shifts of C-3 (δ 83.6) and C-28 (δ 178.0) were also indicative for the presence of a bisdesmosidic glycoside such as CQ-3 and CQ-6. The difference of 16 dalton between the molecular weights of CQ-3 and CQ-1 indicated the presence of an additional oxygene functionality on the Δ^{12} oleanene skeleton.

Five of the methylene and 15 of the methine groups were found to be oxygenated of which three oxygenated methylene and 14 oxygenated methine carbons were depicted to the sugar units. The remaining two oxygenated methylene resonances observed at δ 64.8 and 66.3 were attributed to two CH_2OH groups located at C-23 and C-30, respectively. The presence of a COOH function was evident by a signal at δ 178.0 (C-28). The location of these functional groups were determined by the help of COSY, HSQC and HMBC experiments (**Spectra 4.3.3**, **4.3.4**, **4.3.5a** and **4.3.5b** resp.) (**Table 4.3.1**). In the HMBC experiment, the long range correlations from the carbon resonance at δ 65.2 (C-23: CH_2OH) to the methyl resonance at δ 0.74 (s, Me-24) and from the carbon resonance at δ 66.3 (C-30: CH_2OH) to the methyl resonance at δ 0.92 (s, Me-29) proved the sites of the two CH_2OH groups (**Spectrum 4.3.5a**).

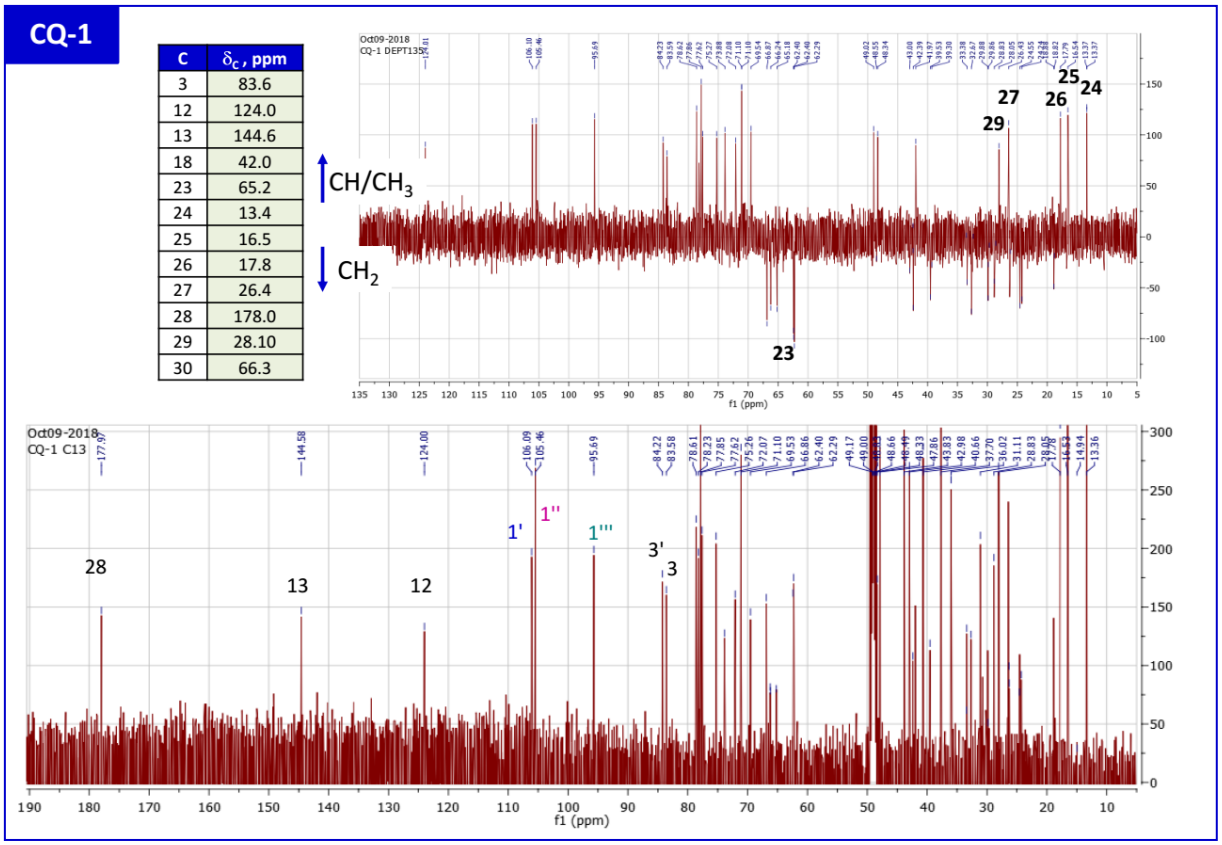
The relative stereochemistry of the CH_2OH group assigned as C-30 (δ 66.3) was determined by the comparison of the chemical shifts of the C-20, C-29 and C-30 with those of the compounds having similar substitutions on the E ring of the sapogenol (Chen et al., 2009; Çalış et al., 2004; Miyokashi et al., 1999; Thuong et al., 2008; Xin et al., 2008). NOESY eperiment clearly confirmed these statements showing correlation between the H-18 (δ 284) and hydroxymethylene protons of C-30 (δ 3.46 and 3.54, AB system, J_{AB} = 12.5 Hz) (**Spectrum 4.3.6**).



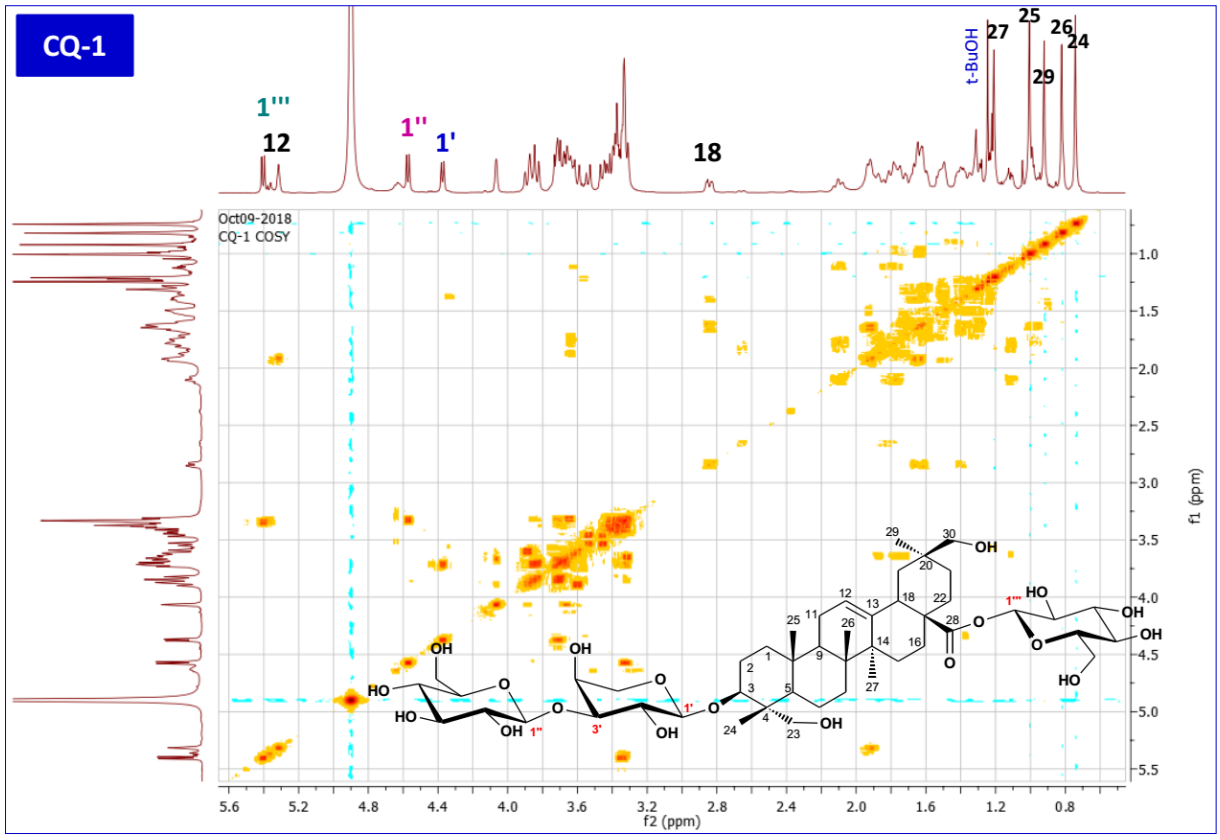
Spektrum 4.3.1. ^{1}H-NMR Spectrum of CQ-1.

The ^{1}H NMR spectrum (**Spectrum 4.3.1**) exhibited three anomeric proton signals (δ4.38, d, J = 7.3 Hz; δ4.57, d, J = 7.8 Hz; δ5.41, d, J = 8.1 Hz). Of 47 carbon resonances in the ^{13}C-NMR spectrum (**Spectrum 4.3.2**), 17 carbon resonances were ascribed to the sugar moiety which were suggested the presence of two hexose and one pentose as observed for CQ-3. The chemical shift values of the proton and the carbon resonances arising from the three sugar units were deduced by the help of 2D-NMR experiments (COSY, HSQC and HMBC). The NMR data given in the **Table 4.3.1** for the sugar moieties were in good accordance to those of CQ-3 showing the presence of same sugar units, an arabinose and two glucose units. The sites of the glycosidations on the sapogenol and the interglycosidic linkage were determined by the help of HMBC experiment (**Spectrum 4.3.5b**). This experiment also showed the same glycosidation pattern as observed for CQ-3.

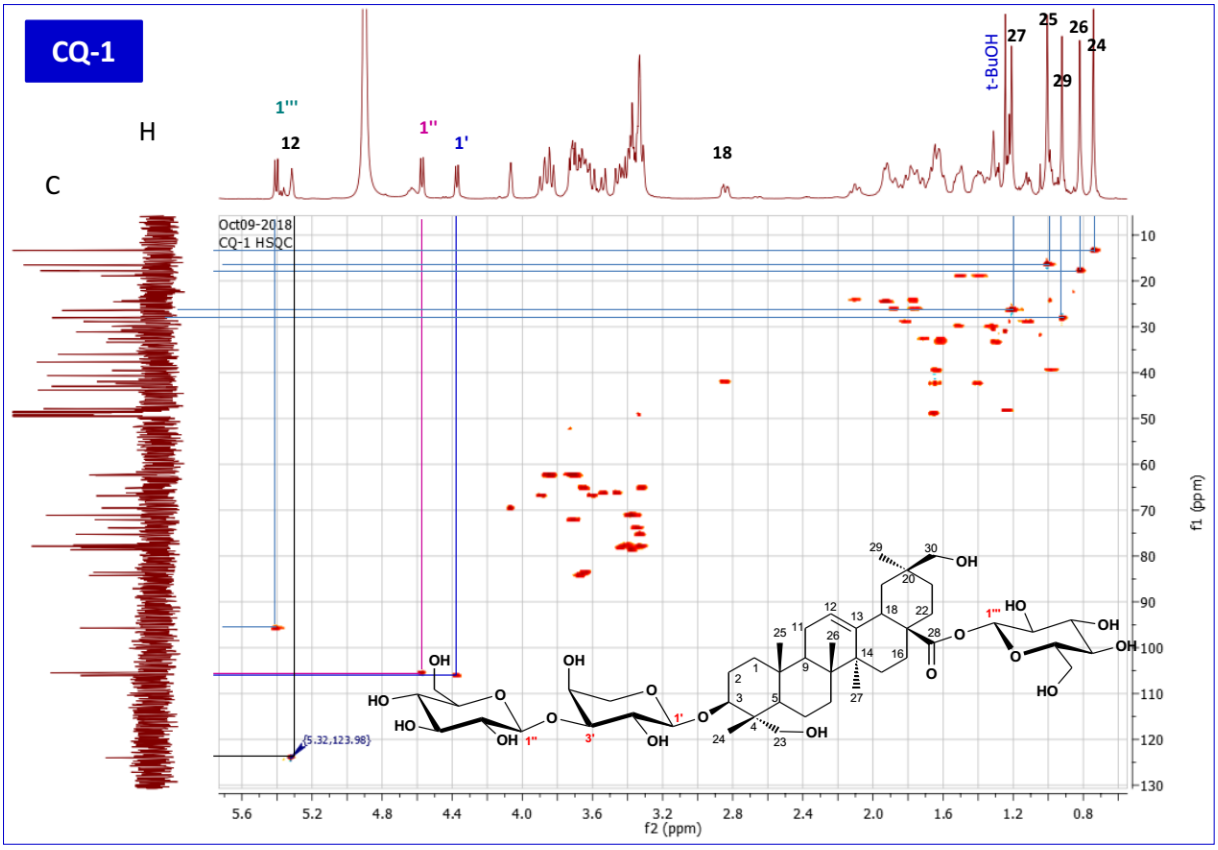
Thus, the structure of CQ-1 was established as 3β-[(*O*-β-D-glucopyranosyl-(1→3)-α-arabinopyranosyl)oxy]-23,30-dihydroxy-olean-12-en-28-oic-acid-28-*O*-β-glucopyranoside. This compound is one of the rare saponin isolated from the *Chenopodium* species (Kuljanaabhagavad et al., 2008).



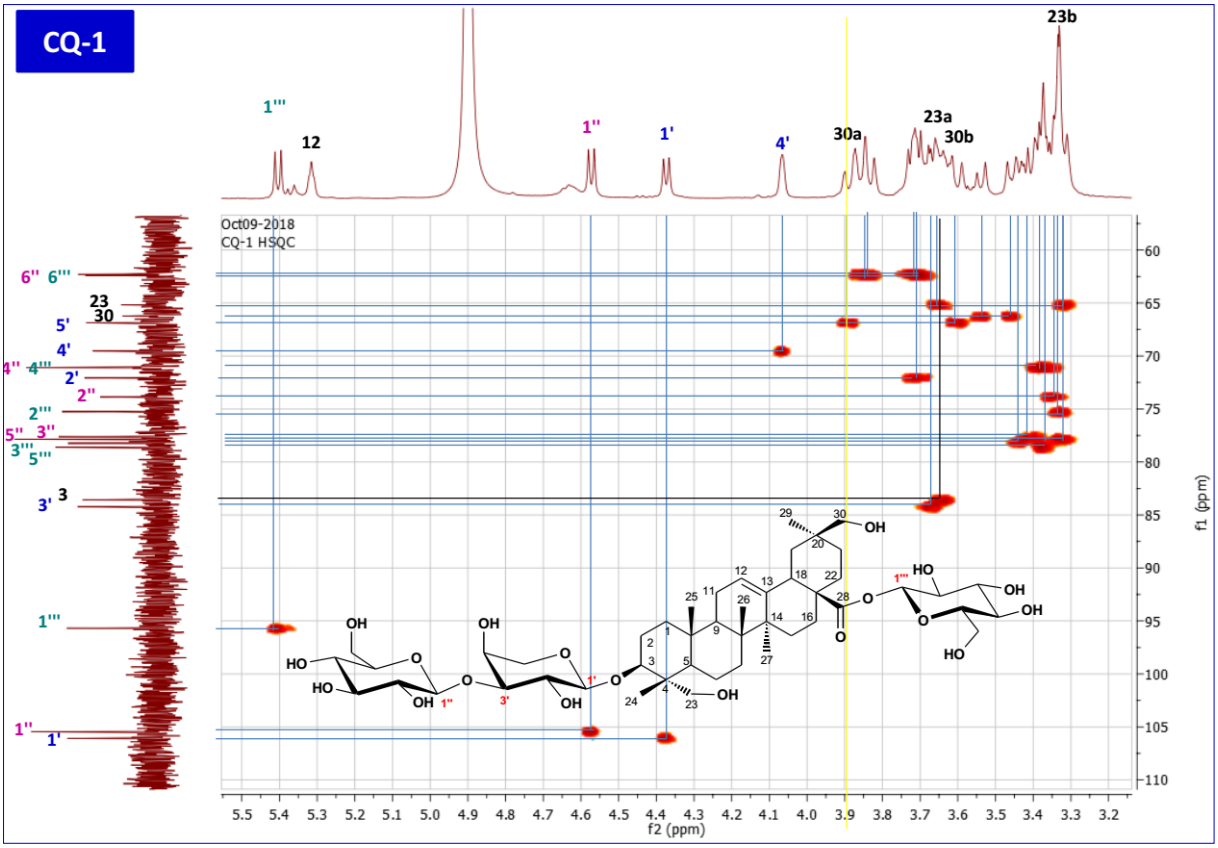
Spektrum 4.3.2. ^{13}C-NMR and DEPT-135 Spectra of CQ-1.



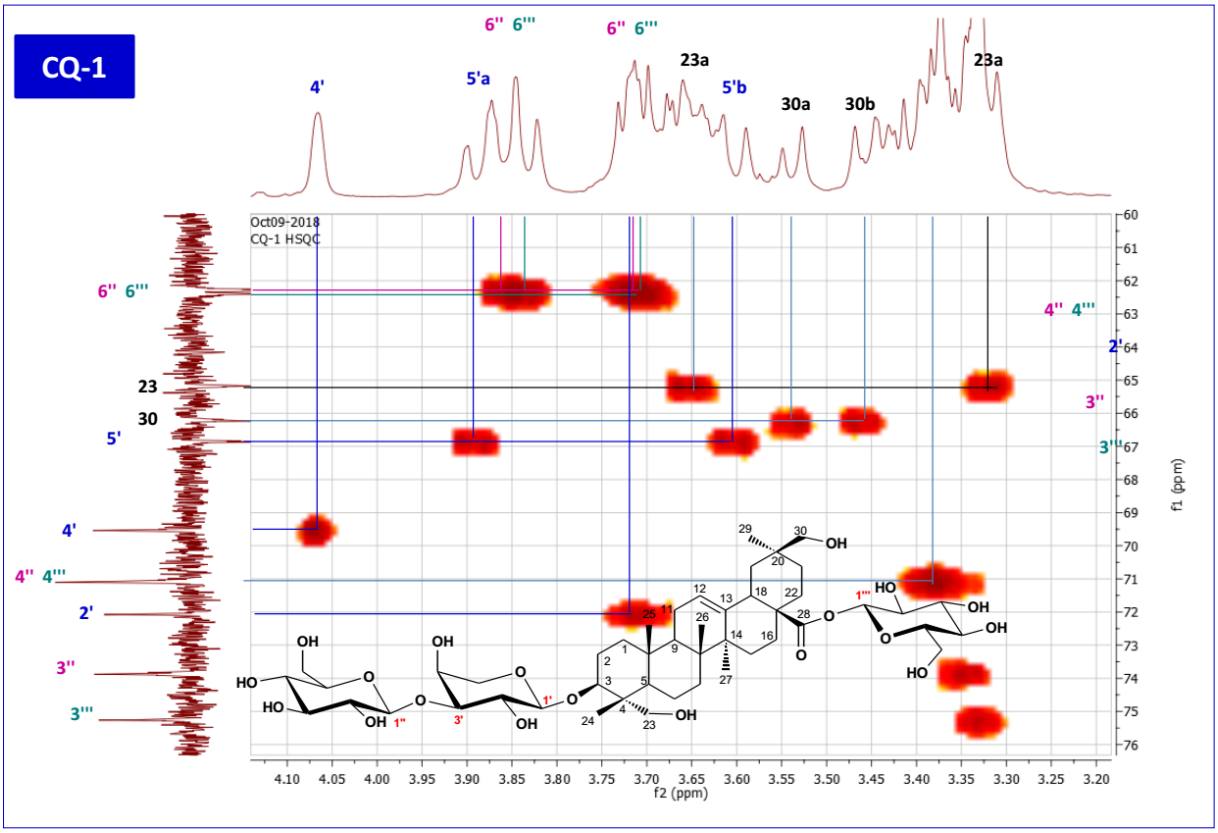
Spektrum 4.3.3. COSY of CQ-1.



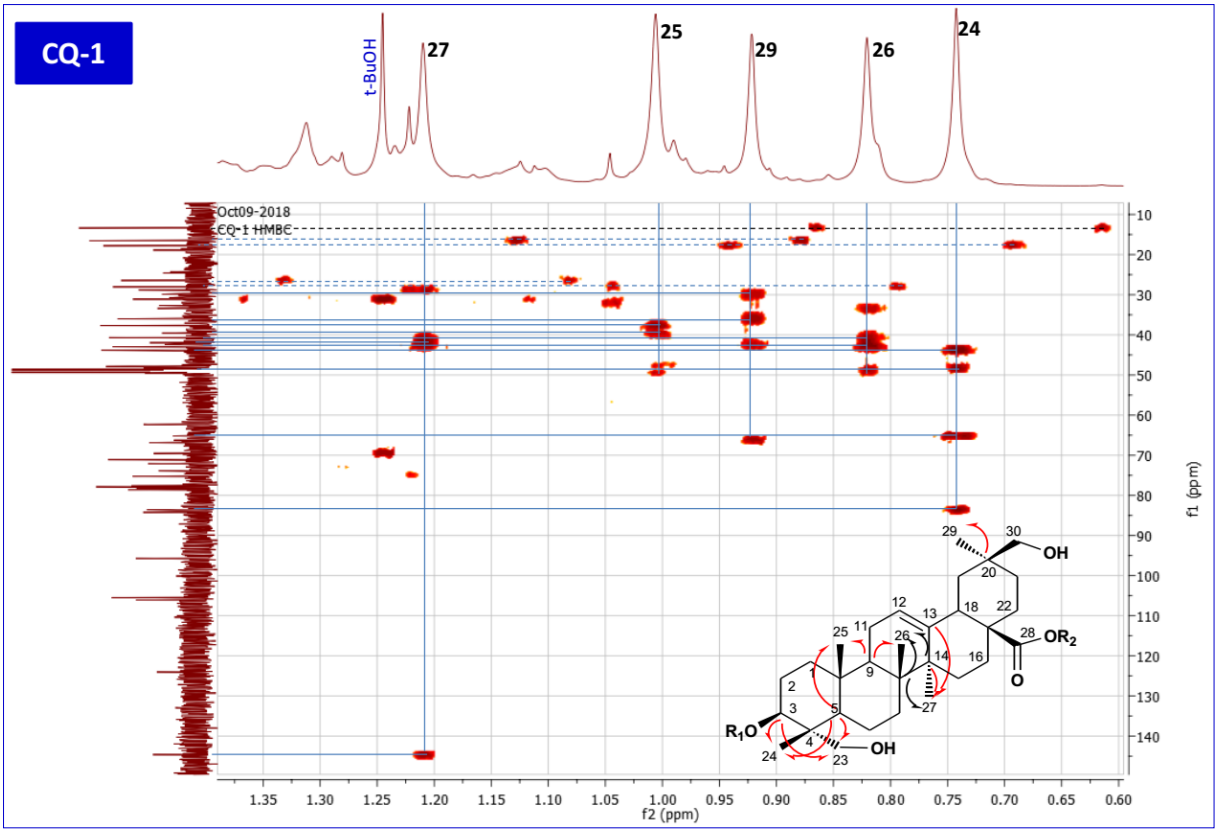
Spektrum 4.3.4a. HSQC of CQ-1.



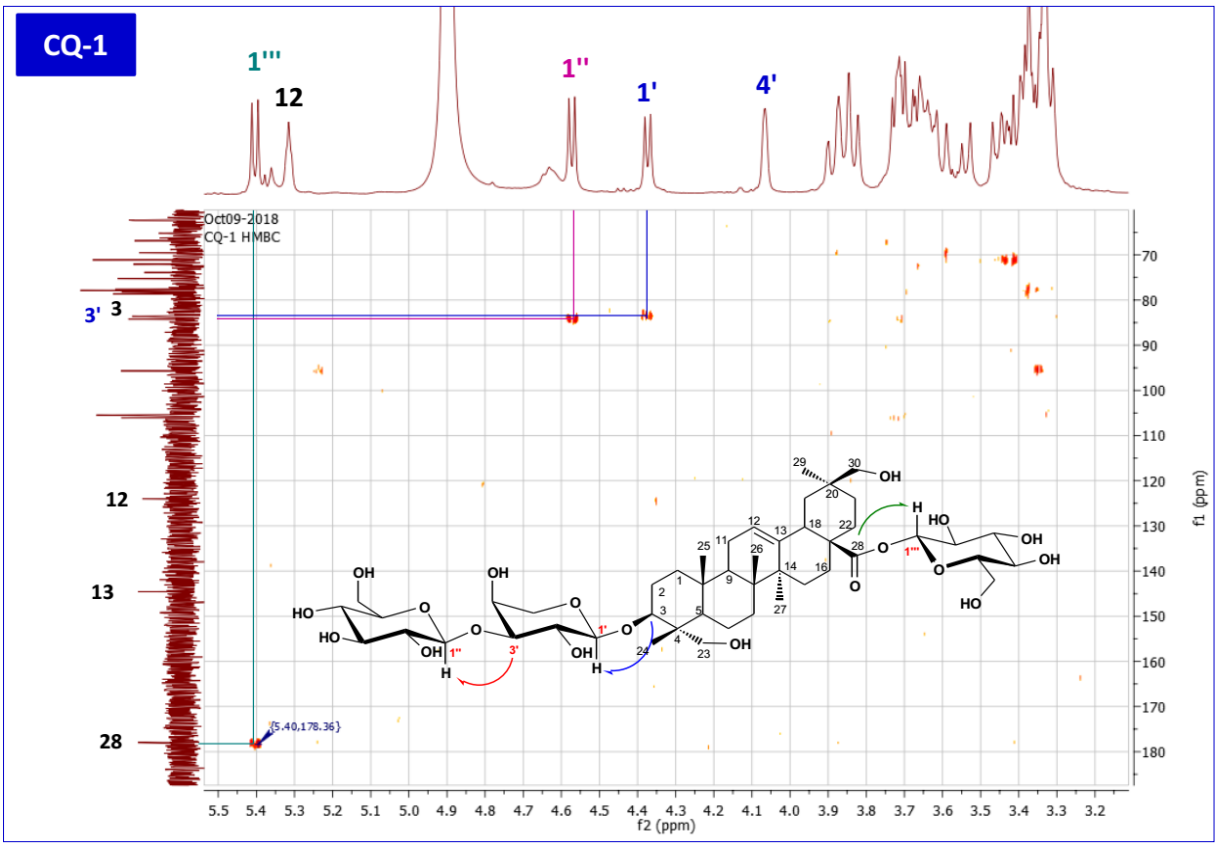
Spektrum 4.3.4b. HSQC of CQ-1.



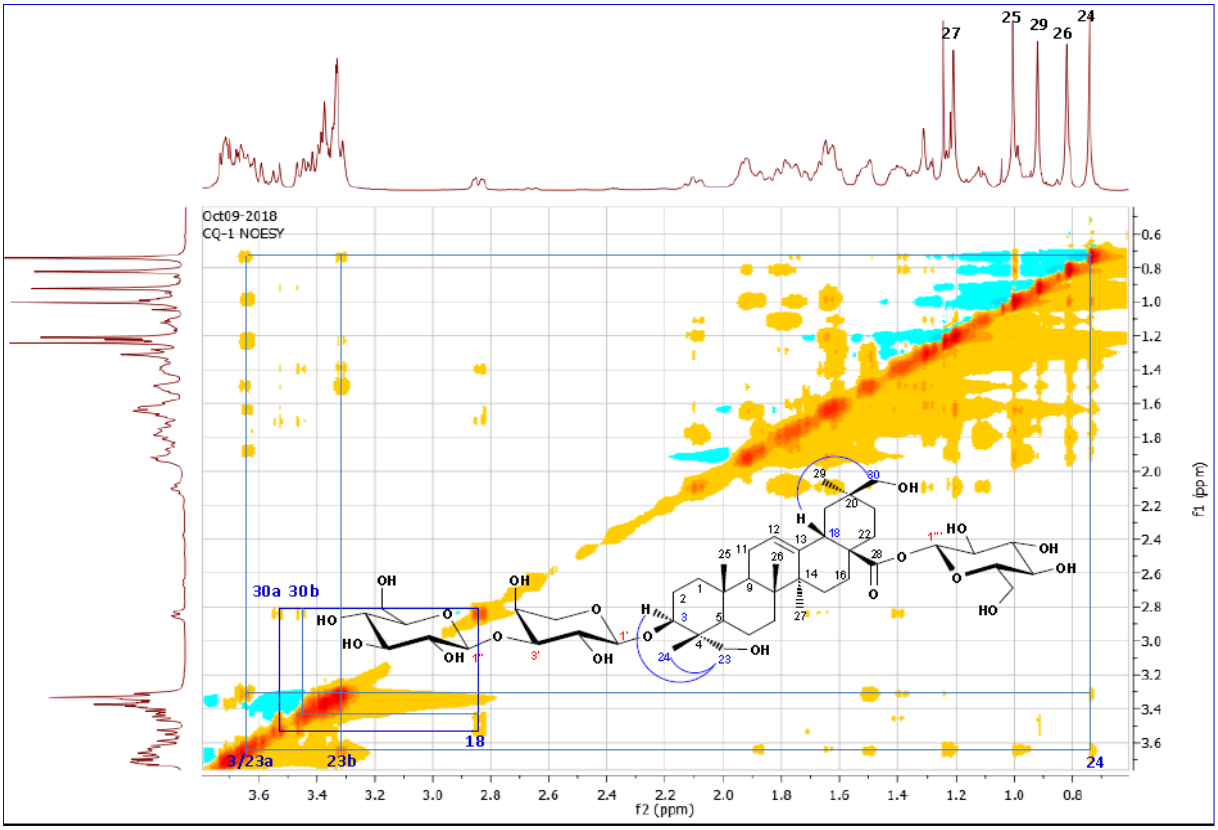
Spektrum 4.3.4c. HSQC of CQ-1.



Spektrum 4.3.5a. HMBC of CQ-1.



Spektrum 4.3.5b. HMBC of CQ-1.



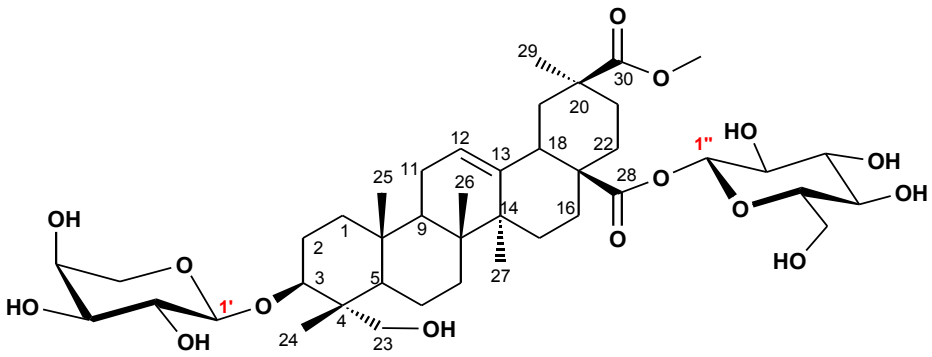
Spektrum 4.3.6. NOESY of CQ-1.

4.4. Phytolaccagenic acid Glycosides (CQ-4 & CQ-2) = 3β,23-dihydroxy -olean-12-ene-28,30-dioc acid-30-methyl ester Glycosides

$C_{31}H_{48}O_6$, Mol wt 516,70

CQ-4: ($C_{42}H_{66}O_{15}$)

Mol. Wt: 810



Compounds CQ-4 has the molecular formula $C_{42}H_{66}O_{15}$, as determined by ^{13}C-NMR, DEPT experiments and H-ESI Mass Spectrum. The negative ion H-ESI Mass Spectra of CQ-4

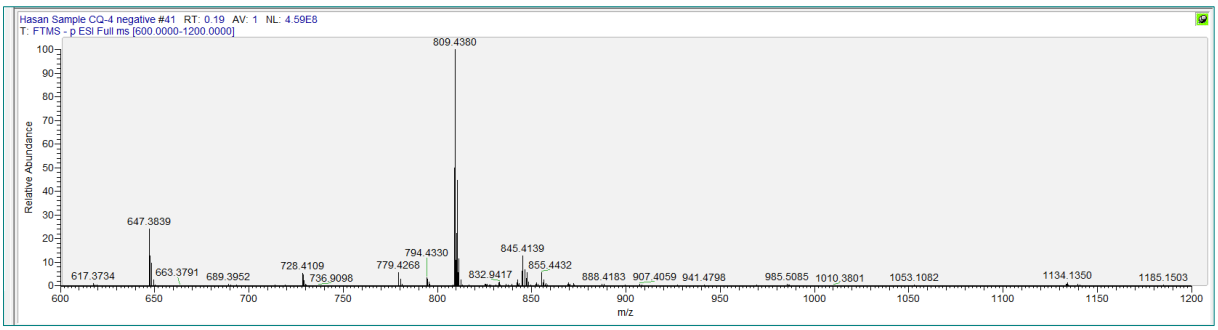


Figure 4.4.1. Negative Ion H-ESI Mass of Spectrum (HRMS) of CQ-4

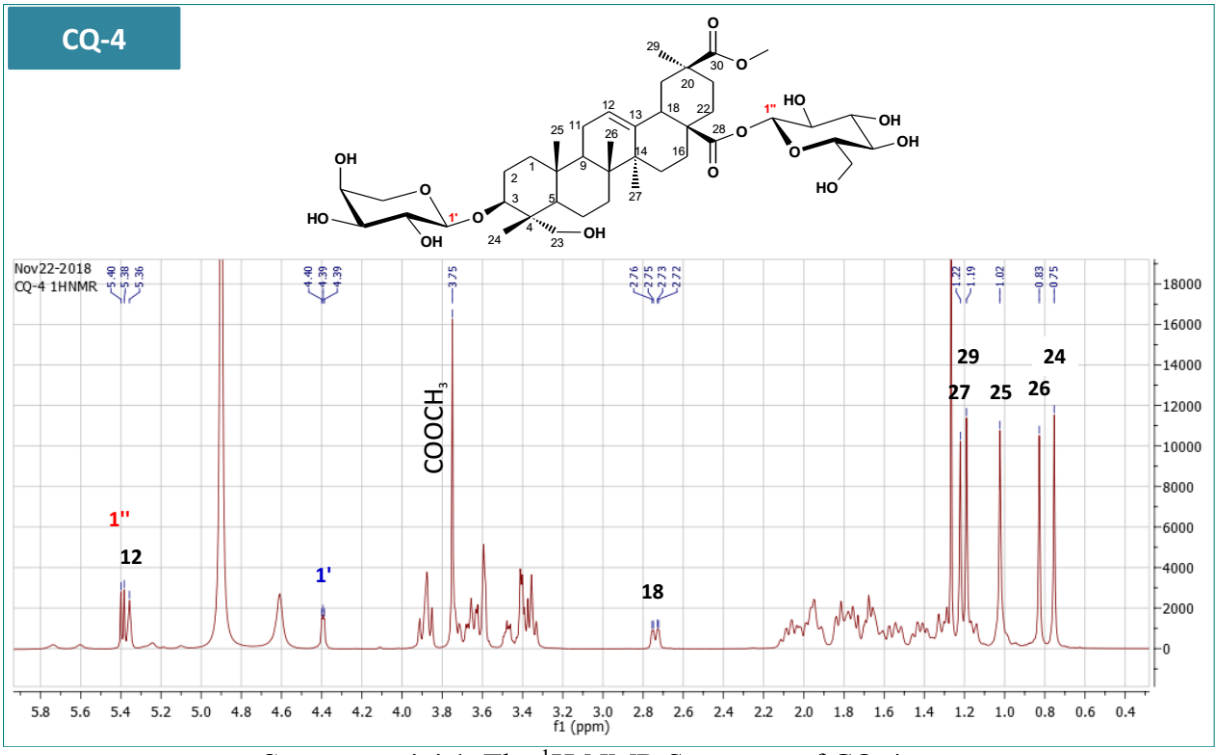
showed a quasimolecular ion at m/z 809.4380 ([M-H]$^-$). Analysis of the ^{13}C-NMR and DEPT spectra (Spectrum 4.2) revealed 42 carbon resonances, including six tertiary methyl (CH_3) (five of them belong to the oleanane skeleton), thirteen methylene (CH_2) (three of them oxygenated), fourteen methine (CH) and nine quaternary carbon (C) atoms (two of them were carbonyl, C=O). Of 42 carbon atoms 31 were attributed to the sapogenol moiety (Table 4.1). In the ^{1}H-NMR spectrum (Spectrum 4.1), the five methyl groups were observed at δ 75, 0.82, 1.02, 1.21 and 1.19 assigned to Me-24, Me-26, Me-25, Me-29 and Me-27,

respectively. The remaining methyl signal was observed at δ 3.75 assigned to an oxygenated methyl group (COOMe).

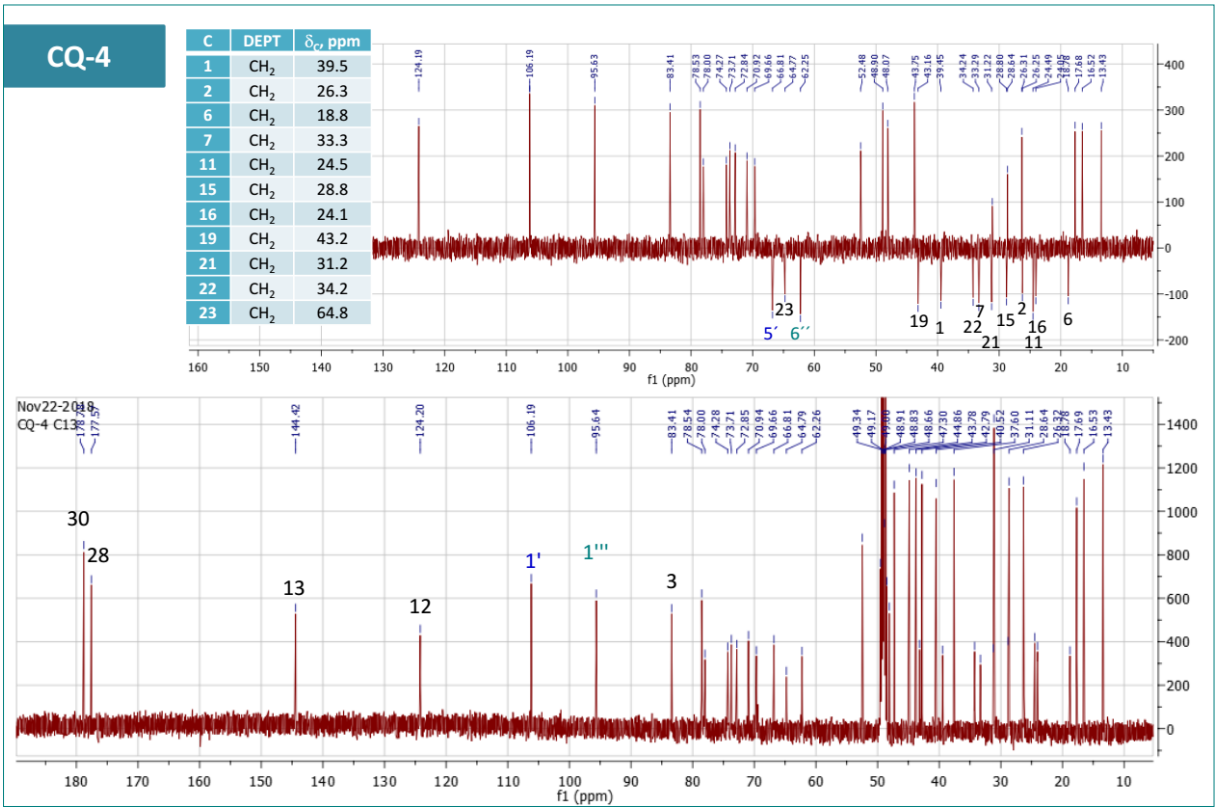
Table 4.4.1. The ^{1}H and ^{13}C-NMR Data for CQ-4 (CD_3OD, δ_H 500 MHz; δ_C 125 MHz)

C/H	DEPT	δ_C, ppm	δ_H ppm, *J* (Hz)	HMBC (from C to H)
1	CH_2	39.5	1.66†/1.00†	Me-25
2	CH_2	26.26	1.92†/1.79†	
3	CH	83.4	3.67 dd (12.4/4.4)	H-1′, H_2-23, Me-24
4	C	47.3	-	H_2-23, Me-24
5	CH	48.1	1.27†	Me-24, H_2-23
6	CH_2	18.8	1.52†/1.40†	
7	CH_2	33.3	1.64†/1.30†	Me-26
8	C	42.8	-	Me-26
9	CH	48.9	1.67†	Me-25, Me-26
10	C	37.6	-	Me-25
11	CH_2	24.5	1.95†	
12	CH	124.2	5.35 dd “t” (3.4)	
13	C	144.4	-	Me-27
14	C	40.5	-	H-12, Me-26, Me-27
15	CH_2	28.8	1.81†/1.13†	Me-27
16	CH_2	24.1	2.08†/1.81†	
17	C	44.9	-	
18	CH	43.8	2.74 dd (13.4/4.1)	H-12
19	CH_2	43.2	2.00†/1.73†	Me-29
20	C	43.8	-	Me-29
21	CH_2	31.2	2.03†/1.43†	Me-29
22	CH_2	34.2	1.75†/1.57†	
23	CH_2	64.8	3.64 d /3.34 d (J_{AB} 12.2)	H-3, Me-24
24	CH_3	13.4	0.75 s	H_2-23
25	CH_3	16.5	1.02 s	
26	CH_3	17.7	0.82 s	
27	CH_3	26.32	1.21 s	
28	C	178.6	-	H-1″
29	CH_3	28.6	1.19 s	
30	C	178.8		Me-29, $COOCH_3$
$COO\underline{C}H_3$	CH_3	52.5	3.75 s	
Ara (C-3)				
1′	CH	106.2	4.39 d (7.8)	
2′	CH	72.9	3.59†	
3′	CH	74.3	3.59†	H-1′
4′	CH	69.7	3.88†	
5′	CH_2	66.8	3.90†/3.61†	
Glu (C-28)				
1″	CH	95.7	5.39 d (8.0)	
2″	CH	73.7	3.38†	
3″	CH	78.0	3.47†	
4″	CH	70.9	3.40†	
5″	CH	78.5	3.40†	
6″	CH_2	62.6	3.86†/3.73†	

†) *J* values are not clear due to overlapping.



Spectrum 4.4.1. The ^{1}H-NMR Spectrum of CQ-4.



Spectrum 4.4.2. The ^{13}C-NMR and dept-135 Spectra of CQ-4.

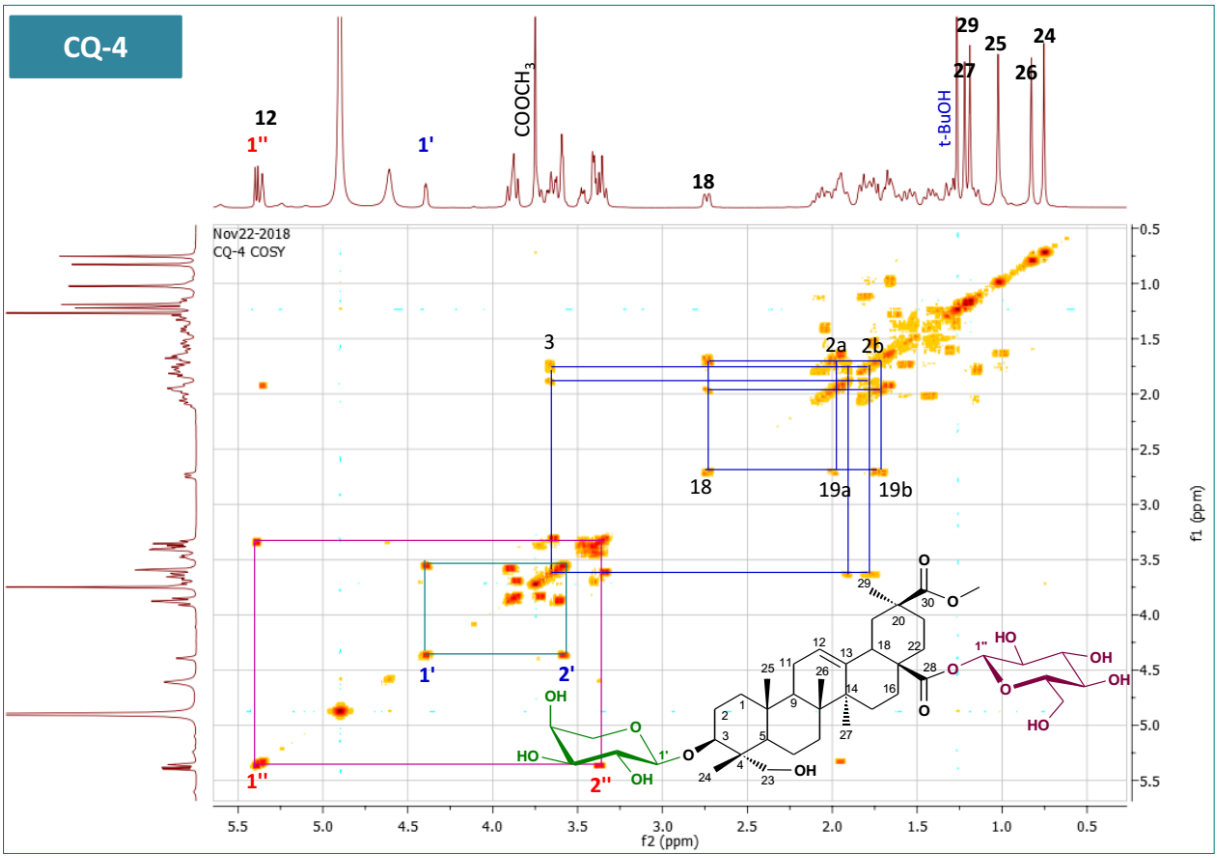
Based on the ^{1}H and ^{13}C-NMR data (**Table 4.4.1**, **Spectra 4.4.1** and **4.4.2**) assigned by the help of 2D-NMR experiments (COSY, HSQC and HMBC; Spectra 4.3a&b, 4.4a&b and 4.5), the existence of a double bond with the carbon signal at δ 124.2 and 144.2 (C-12 and (C-13) together with an olefinic proton resonance at δ 5.35 (H-12: dd "t", *J* = 3.4 Hz) were characteristic for an Δ^{12}-oleanane-type triterpene sapogenol (Mahato & Kundu, 1994).

The rest of the carbon resonances and the corresponding proton signals arising from the sugar moiety were consistent fort he presence of an α-arabinopyranosyl and a β-glucopyranosyl units showing bidesmosidic structure (**Table 4.4.1**). The anomeric carbon resonances at δ 106.2 and 95.6 and the corresponding anomeric proton signals at δ 4.39 (d, *J* = 7. Hz) and 5.39 (d, *J* = 8.0 Hz), respectively, were deduced by the help of HSQC experiment. The other proton and carbon signals secured by the help of COSY and HSQC experiments (**Spectra 4.4.3** and **4.4.4**) unambiguously showed the terminal positions of two sugar units. Because, no glycosidation shifts were observed for the protons and carbon resonances. Furthermore, two of the oxygenated methylene groups were found to be the parts of α-arabinopyranosyl and a β-glucopyranosyl units (C-5′ and C-6″, respectively). The proton signals of the remaining hydroxymethylene group were observed at δ 3.64 and 3.34 as an AB system (J_{AB} = 12.2 Hz). The corresponding carbon resonance was found to be at δ 64.8 in the HSQC experiment (**Spectrum 4.4.4a&b**). The long-range correlations from C-4 (δ 47.3), C-5 (δ 48.1) and Me-24 (δ 13.4) to the hydroxymethylene protons and the reversed correlations clearly showed the location of CH_2OH to be at C-23 as one of the oxygenated methyl groups of β-amyrine-type triterpenes (oleanane) (**Spectrum 4.4.5**). The main differences between the sapogenol part of the CQ-4 and those of other saponosides (CQ-1 and CQ-3) were observed for the E ring.

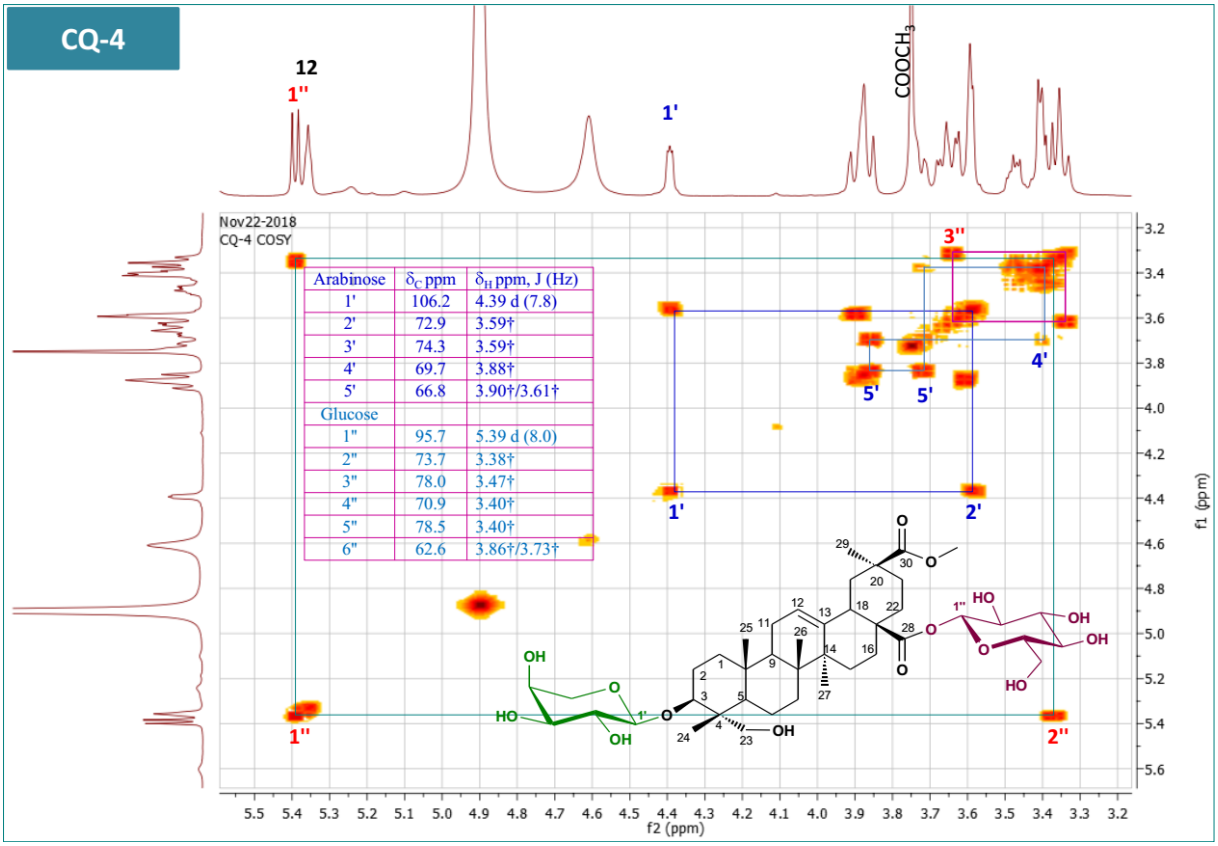
In the ^{13}C-NMR spectrum of CQ-4, two carbonyl resonances were observed at δ 178.6 and 178.8. The location of the second carbonyl group were established by the help of a HMBC experiment. HMBC experiment showed the long-range correlations between the carbonyl group (δ 178.8) and two methyl groups asssigned as C-29 and COOMe (δ 3.75 and 1.19, respectively). These observations were evident for the location of the second carbonyl group being C-30. The relative stereochemistry of the methly group (Me-29) and the carboxymethyl group were determined by the help of NOESY experiment (**Spectrum 4.4.6**). The lack of NOESY correlation between the H-18 (δ 2.74 dd, *J* = 13.4 and 4.1) and Me-29

(δ 1.19 s) was consistent for an equatorial position of Me-29 and axial position of the COOMe at C-20. Based on these results the sapogenol moiety was determined as phytolaccagenic acid (3,23-dihydroxy-olean-12-en-28,30-dioic-acid-30-methyl ester) (Mizui et al., 1988). Furthermore, HMBC experiment showed the long-range correlations between the C-3 (δ 83.4) of the aglycone and one of the anomeric proton at δ 4.39 (H-1′ of the arabinose), and C-28 (δ 178.6) and the second anomeric proton at 5.39 (H-1″ of the glucose). The downfield shift value of the anomeric proton of the glucose confirmed the ester linkage between the phytolaccagenic acid and glucose.

Consequently, the structure of compound CQ-4 was established as 3β-*O*-β-D-glucopyranosyloxy-phytolaccagenic acid-28-*O*-β-D-glucopyranoside [3β-*O*-β-D-glucopyranosyl phytolaccagenic acid-28-*O*-β-D-glucopyranosyl ester] (Mizui et al., 1988). Thic compound was also been isolated from the bran, flowers, fruits, seeds and seed coats of *Chenopodium quinoa* (Kuljanabhagavad et al., 2008).

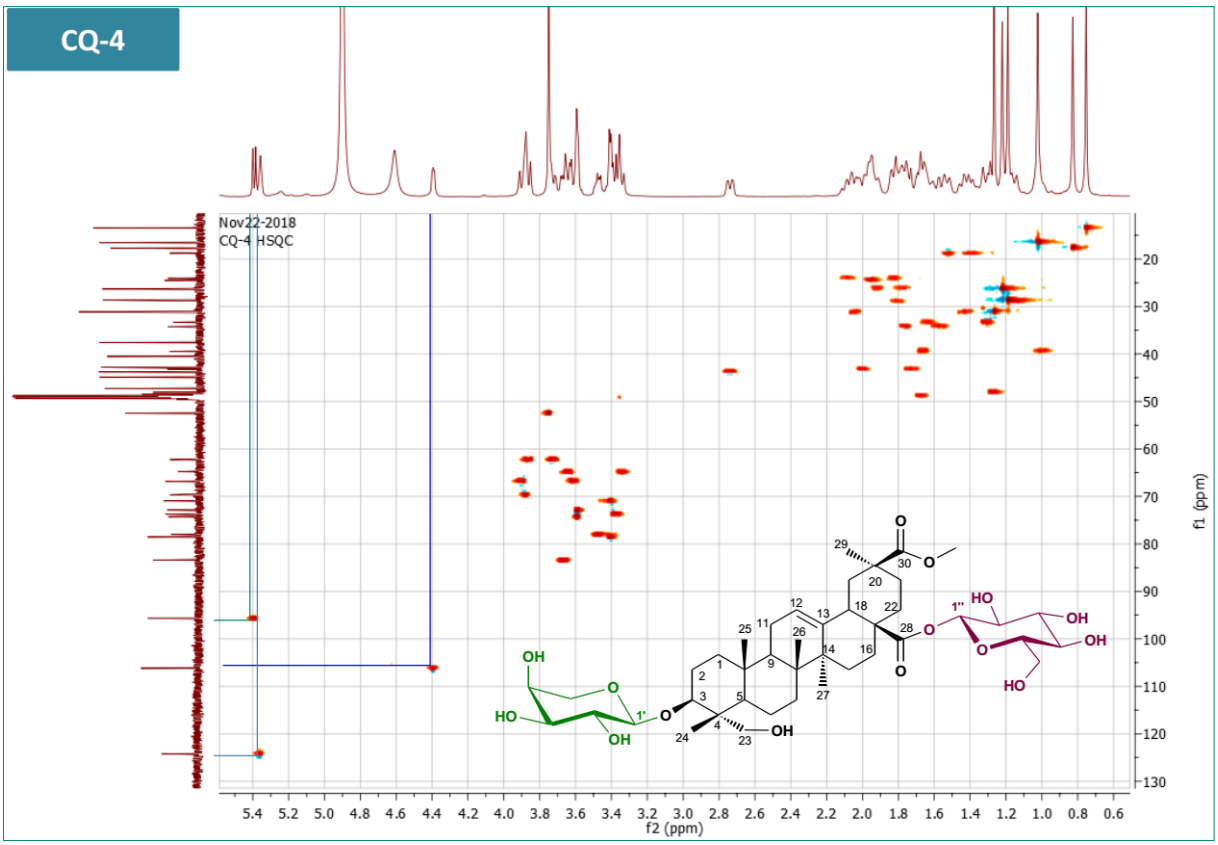


Spectrum 4.4.3a. The COSY of CQ-4.

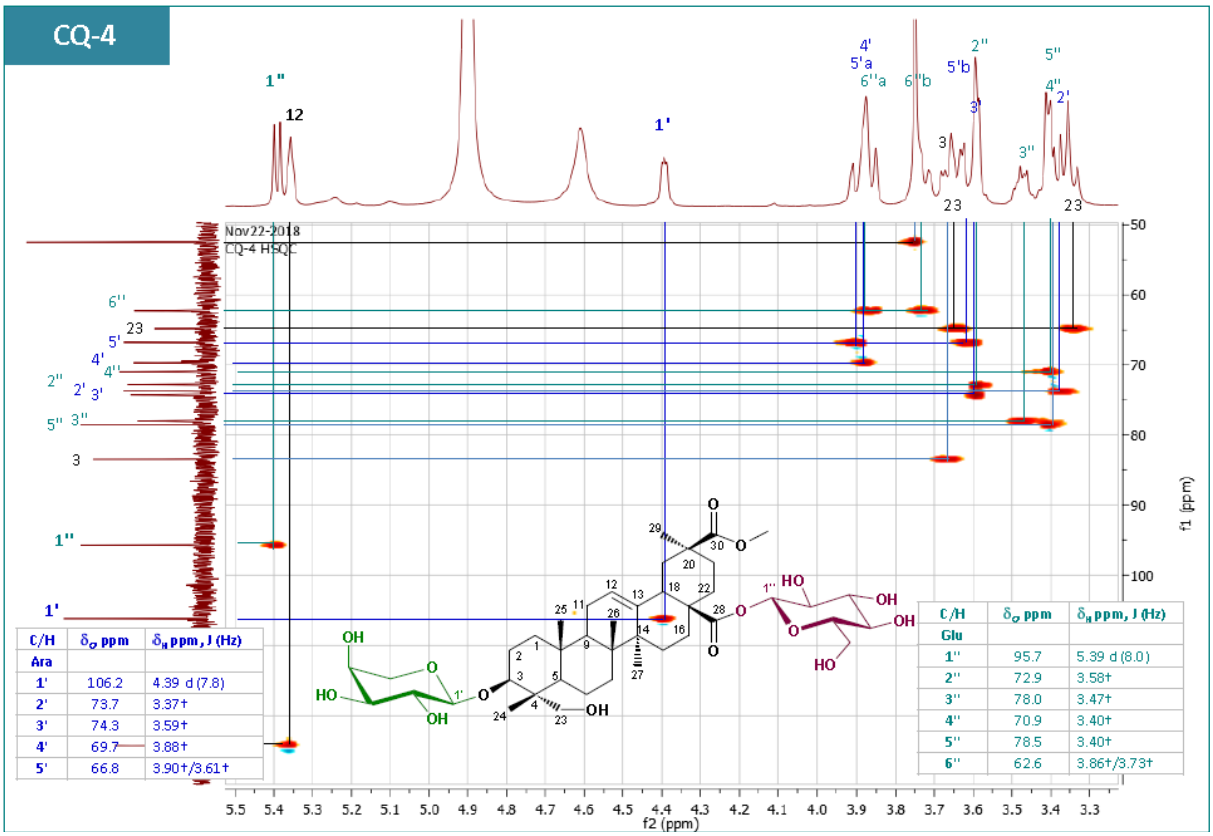


Arabinose	δ_C ppm	δ_H ppm, J (Hz)
1'	106.2	4.39 d (7.8)
2'	72.9	3.59†
3'	74.3	3.59†
4'	69.7	3.88†
5'	66.8	3.90†/3.61†
Glucose		
1''	95.7	5.39 d (8.0)
2''	73.7	3.38†
3''	78.0	3.47†
4''	70.9	3.40†
5''	78.5	3.40†
6''	62.6	3.86†/3.73†

Spectrum 4.4.3b. The COSY of CQ-4.



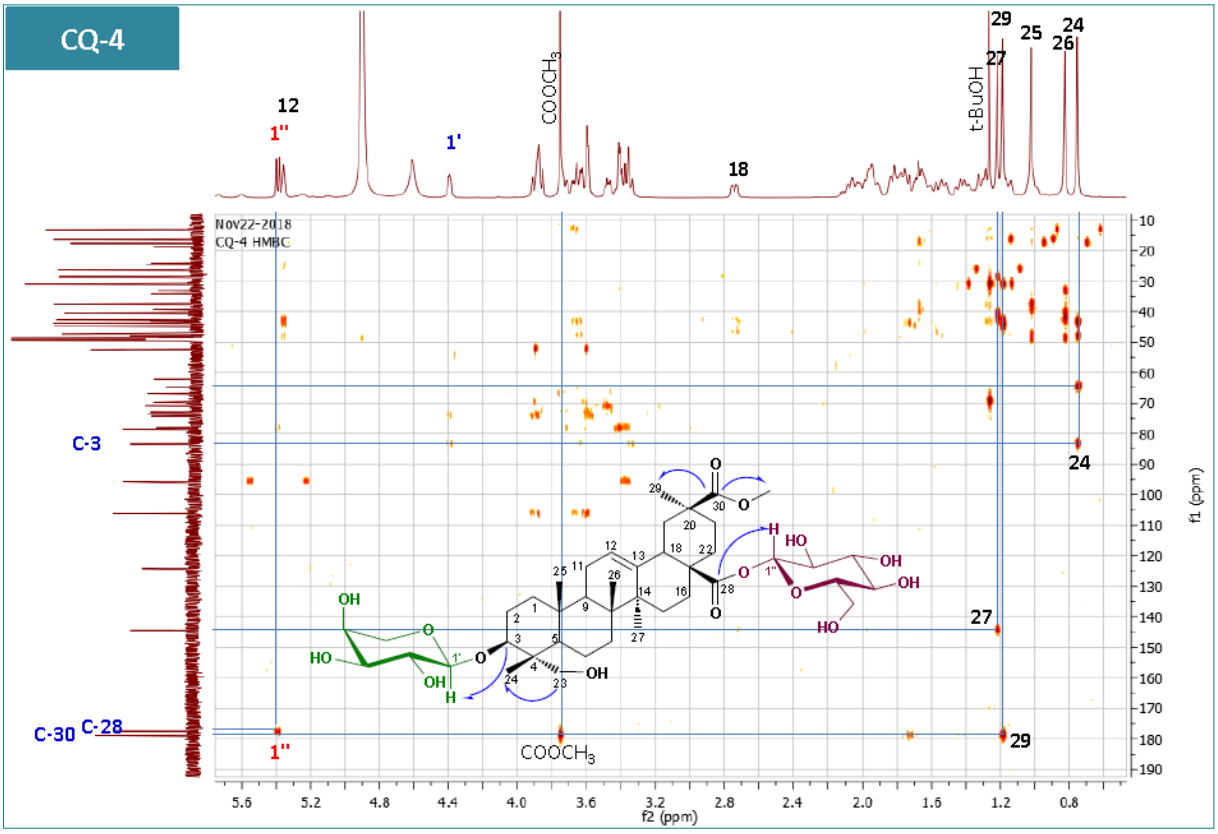
Spectrum 4.4.4a. The HSQC of CQ-4.



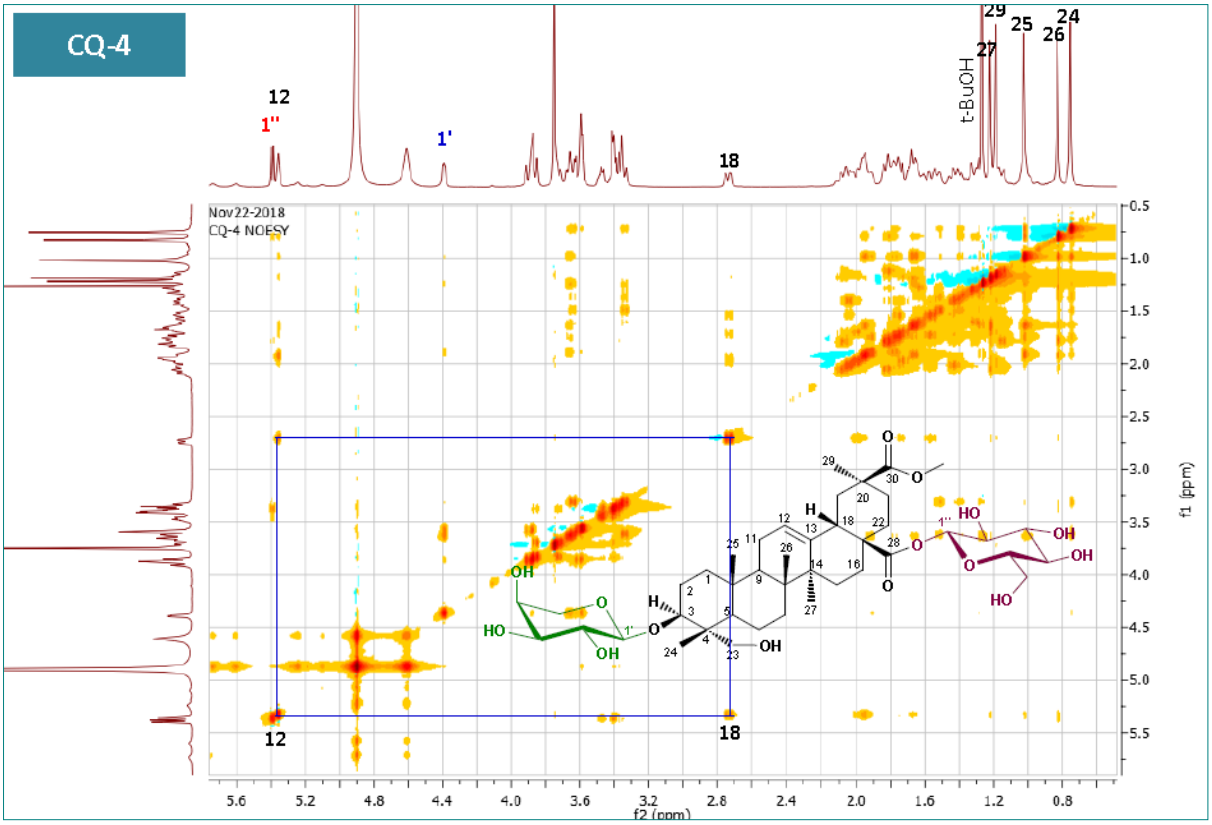
C/H	δ_C ppm	δ_H ppm, J (Hz)
Ara		
1'	106.2	4.39 d (7.8)
2'	73.7	3.37†
3'	74.3	3.59†
4'	69.7	3.88†
5'	66.8	3.90†/3.61†

C/H	δ_C ppm	δ_H ppm, J (Hz)
Glu		
1''	95.7	5.39 d (8.0)
2''	72.9	3.58†
3''	78.0	3.47†
4''	70.9	3.40†
5''	78.5	3.40†
6''	62.6	3.86†/3.73†

Spectrum 4.4.4b. The HSQC of CQ-4.



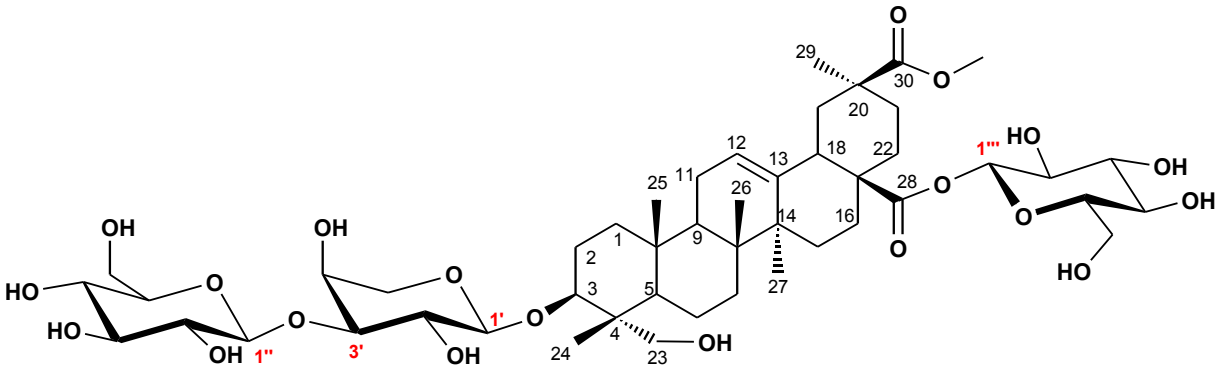
Spectrum 4.4.5. The HMBC of CQ-4.



Spectrum 4.4.6. The NOESY of CQ-4.

4.5. CQ-2 (= CQ-5: $C_{48}H_{76}O_{20}$)

Mol. Wt: 972



The negative and positive ions H-ESI-mass spectra of CQ-2 (Figure 4.5.1) showed quasimolecular ions $[M - H]^-$ and $[M + Na]^+$ at m/z 971.4914 and m/z 995.4809 indicated a molecular weight of 972 (calcd for $C_{48}H_{76}O_{20}$). The other significant ion $[M – 163 (glucose)]^-$ observed at m/z 809.4375 was attributed to the loss of one of the terminal molecule glucose unit.

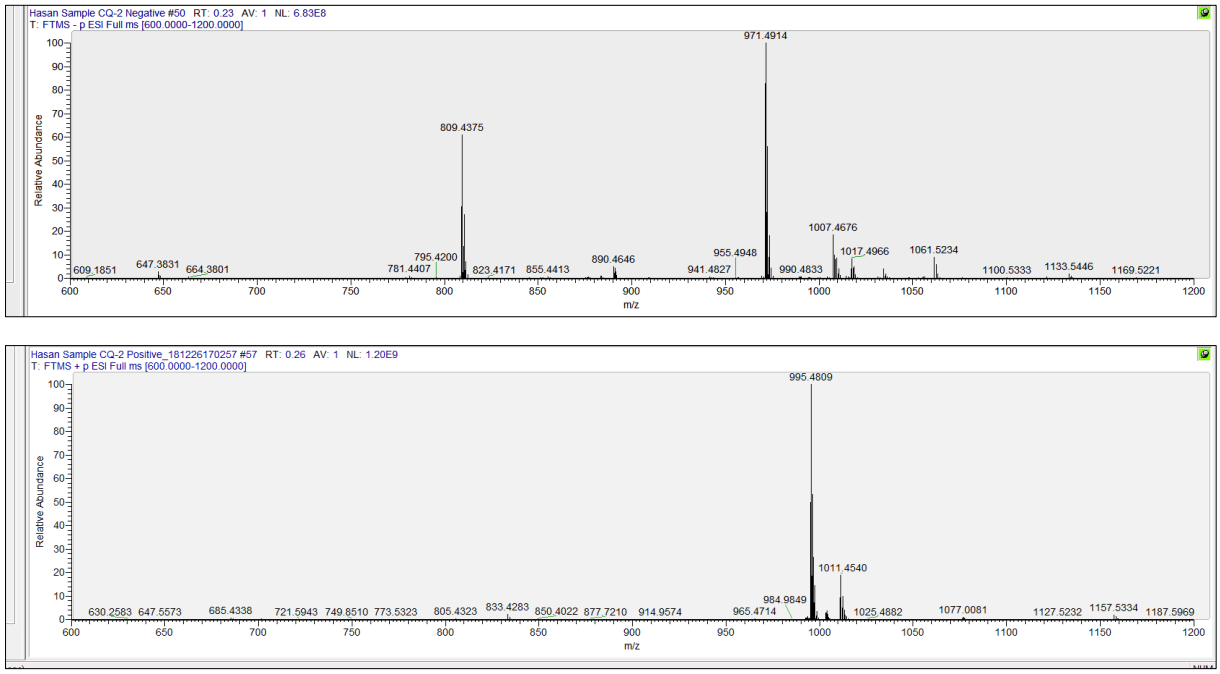
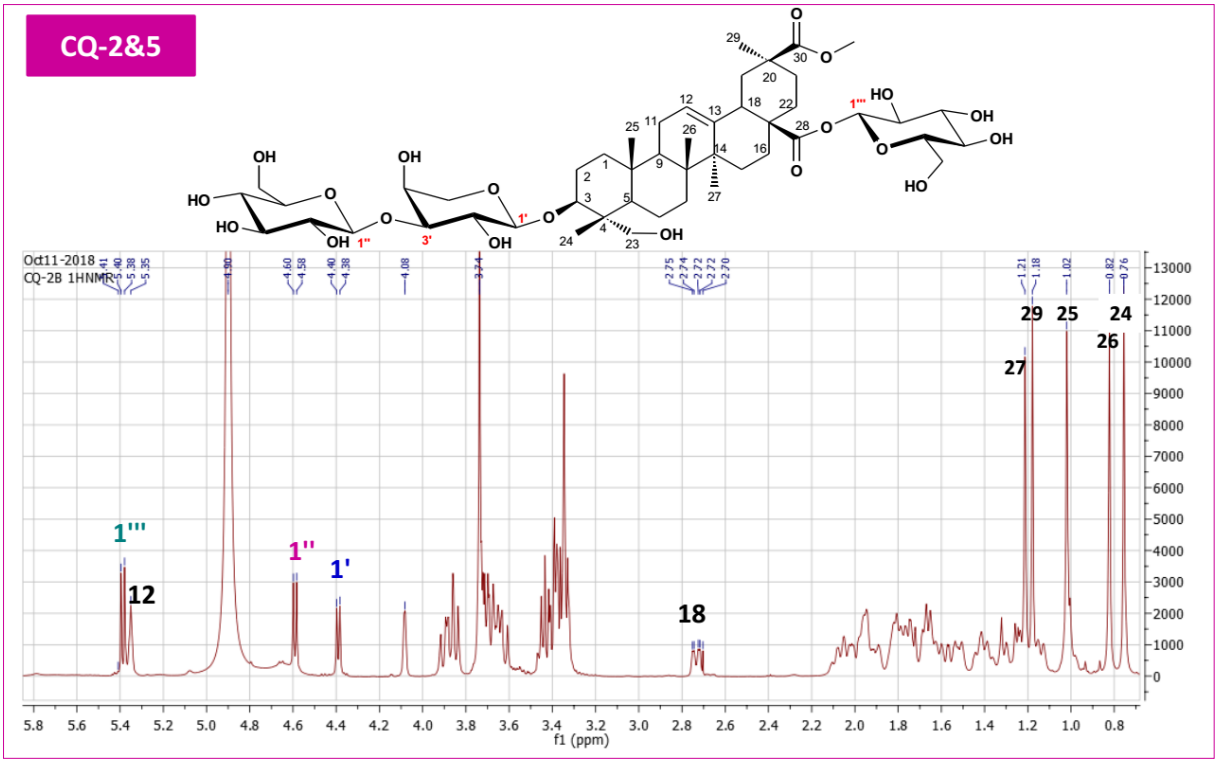


Figure 4.5.1. Negative and Positive ion HR-Mass Spectra of CQ-2.

Table 4.5.1. The ^{1}H and ^{13}C-NMR Data for CQ-2 (CD_3OD, δ_H 500 MHz; δ_C 125 MHz)

C/H	DEPT	δ_C, ppm	δ_H ppm, *J* (Hz)	HMBC (from C to H)
1	CH_2	39.5	1.67†/1.02†	Me-25
2	CH_2	26.2	1.92†/1.79†	
3	CH	83.6	3.65†	H-1′, H_2-23, Me-24
4	C	47.2	-	H_2-23, Me-24
5	CH	48.3	1.26†	Me-24, H_2-23
6	CH_2	18.8	1.54†/1.41†	
7	CH_2	33.3	1.64†/1.32†	Me-26
8	C	42.8	-	Me-26
9	CH	48.9	1.68†	Me-25, Me-26
10	C	37.2	-	Me-25
11	CH_2	24.5	1.97†	
12	CH	124.3	5.35 br s	
13	C	144.4	-	Me-27
14	C	40.5	-	H-12, Me-26, Me-27
15	CH_2	28.8	1.82†/1.14†	Me-27
16	CH_2	24.2	2.09†/1.83†	
17	C	44.8	-	
18	CH	43.7	2.73 br d (13.4)	H-12
19	CH_2	43.1	2.01†/1.74†	Me-29
20	C	43.7	-	Me-29
21	CH_2	31.2	2.05†/1.42†	Me-29
22	CH_2	34.2	1.74†/1.58†	
23	CH_2	65.2	3.68†/3.35†	Me-24
24	CH_3	13.4	0.76 s	H_2-23
25	CH_3	16.6	1.02 s	
26	CH_3	17.7	0.82 s	
27	CH_3	26.3	1.21 s	
28	C	177.4	-	H-1″
29	CH_3	28.7	1.18 s	
30	C	178.6		Me-29, $COOCH_3$
$COO\underline{C}H_3$	CH_3	52.5	3.74 s	
Ara (C-3)				
1′	CH	106.0	4.39 d (5.7)	
2′	CH	72.0	3.73†	
3′	CH	84.1	3.67†	H-1″
4′	CH	69.4	4.11 br s	
5′	CH_2	66.8	3.93 br d/3.65 br d (J_{AB} 12.8)	
Glu (C-4′)				
1″	CH	105.3	4.59 d (7.5)	H-4′
2″	CH	73.7	3.37†	
3″	CH	77.4	3.41†	
4″	CH	71.0	3.37†	
5″	CH	77.7	3.38†	
6″	CH_2	62.6	3.86†/3.73†	
Glu (C-28)				
1‴	CH	95.9	5.39 d (8.1)	
2‴	CH	75.1	3.38†	
3‴	CH	78.0	3.47†	
4‴	CH	70.9	3.39†	
5‴	CH	78.5	3.41†	
6‴	CH_2	62.6	3.86†/3.73†	

†) *J* values are not clear due to overlapping.



Spektrum 4.5.1. The ^{1}H-NMR Spectrum of CQ-2.

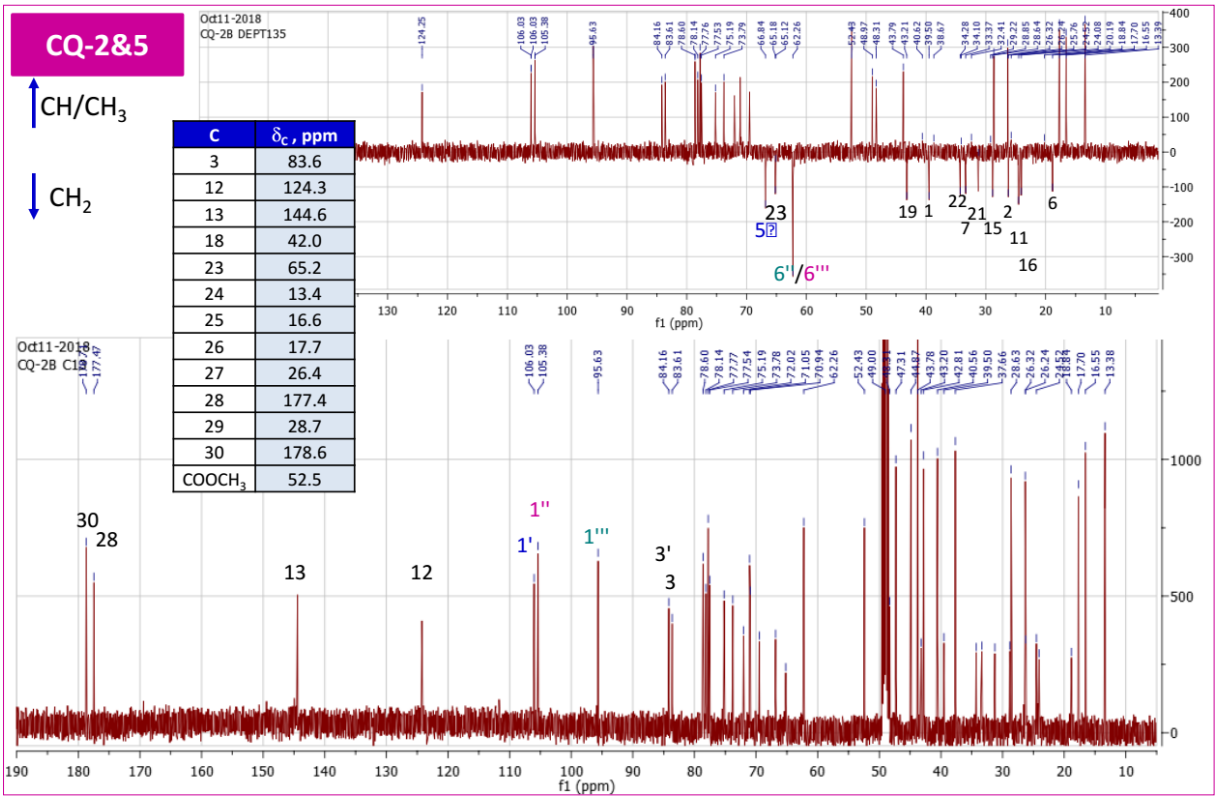
The ^{1}H-NMR spectrum of CQ-2 exhibited most prominent signals of five tertiary methyl, three anomeric protons and a carboxymethyl (δ 3.74) (**Spectrum 4.5.1**). The ^{13}C-NMR spectrum showed signals arising from 48 carbons of which 31 were attributed to the sapogenol moiety (**Spectrum 4.5.2**). The assignments of all proton and carbon resonances were based on 2D-NMR (COSY, HSQC and HMBC) experiments (**Spectra 4.5.3**, **4.5.4**, **4.5.5a** and **4.5.5b**). The long-range correlations from carbon atom resonances belonging to the triterpenoid skeleton (C, CH and CH2) to the tertiary methyl signals helped us for full assignments unambiguously (**Spectrum 4.5.5b**).

The chemical shift values of the methyl groups observed in the ^{1}H- and ^{13}C-NMR were very similar to those of CQ-4 suggesting the presence of same sapogenol, phytolaccagenic acid for CQ-2 (*see* **Tables 4.4.1** and **4.5.1**).

The signals arising from the three sugar units were consistent for presence of an α-L-arabinose and two β-D-glucopyranose units as observed for triglycosidic compounds CQ-1 and CQ-3. The interglycosidic linkage between one of the glucose and the arabinose, as well as site of glycosidations on the phytolaccagenic acid were determined by the help of HMBC experiment (**Spectrum 4.5.5**) confirming the proposed structure. Finally, the relative

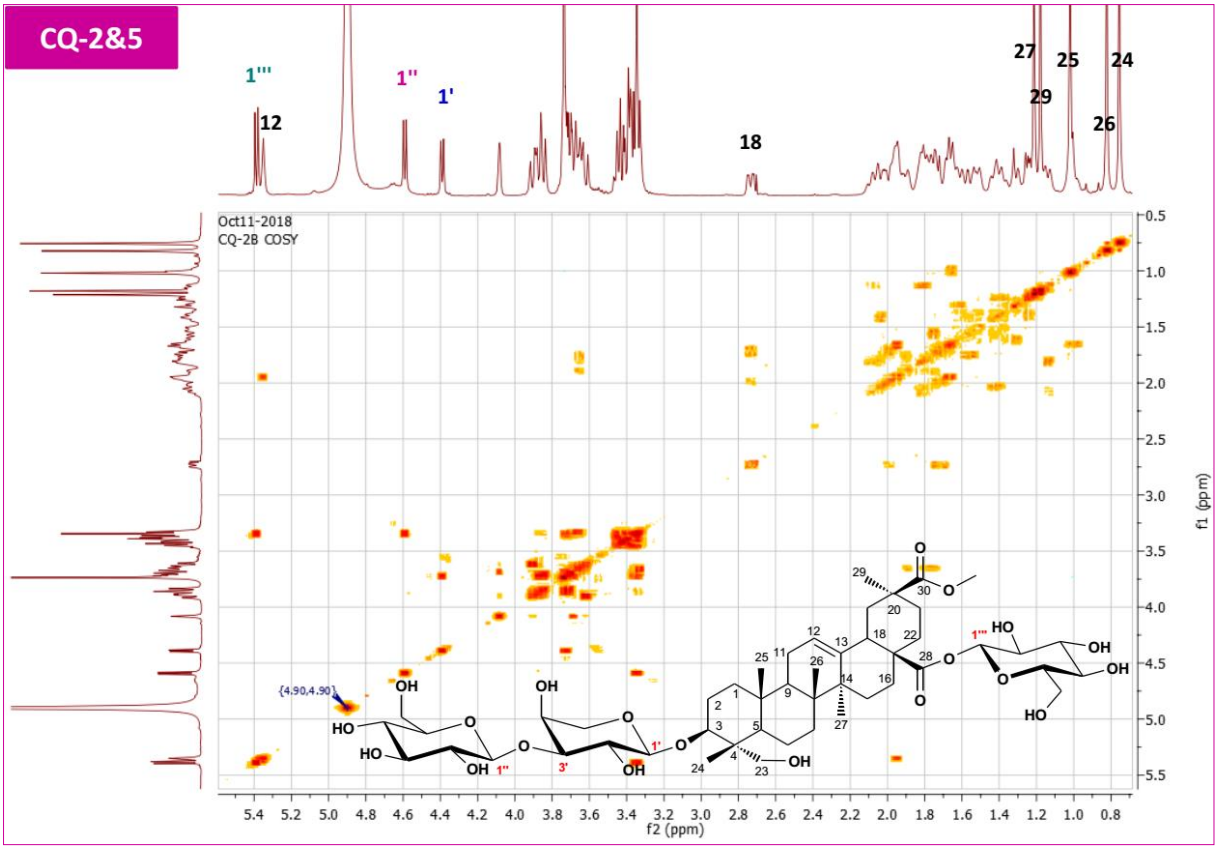
stereochemistry of the CH_2OH and COOMe functionalities were confirmed by a NOESY experiment (**Spectrum 4.5.6**) showing the same substitutions as observed for CQ-4. There was no NOESY correlation between the H-18 and the Me-29 supporting the equatorial position of Me-29. The other significant NOESY correlations were observed between H-1′/H-3 and H-1″/H-3′ confirming the interglycosidic linkage between glucose and arabinose. Moreover, the NOESY correlations were also observed between the protons of hydroxymethylene (H_2-23) and H-3 showed that they are on the α-side of the molecule.

Based on these evidences the structure of CQ-2 was determined as 3β-[(*O*-β-D-glucopyranosyl-(1→3)-α-arabinopyranosyl)oxy]-23-hydroxy-olean-12-en-28,30-dioic-acid-28-*O*-β-glucopyranoside-30-methyl ester [= 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabino-pyranosyl phytolaccagenic acid 28-*O*-β-D-glucopyranoside].

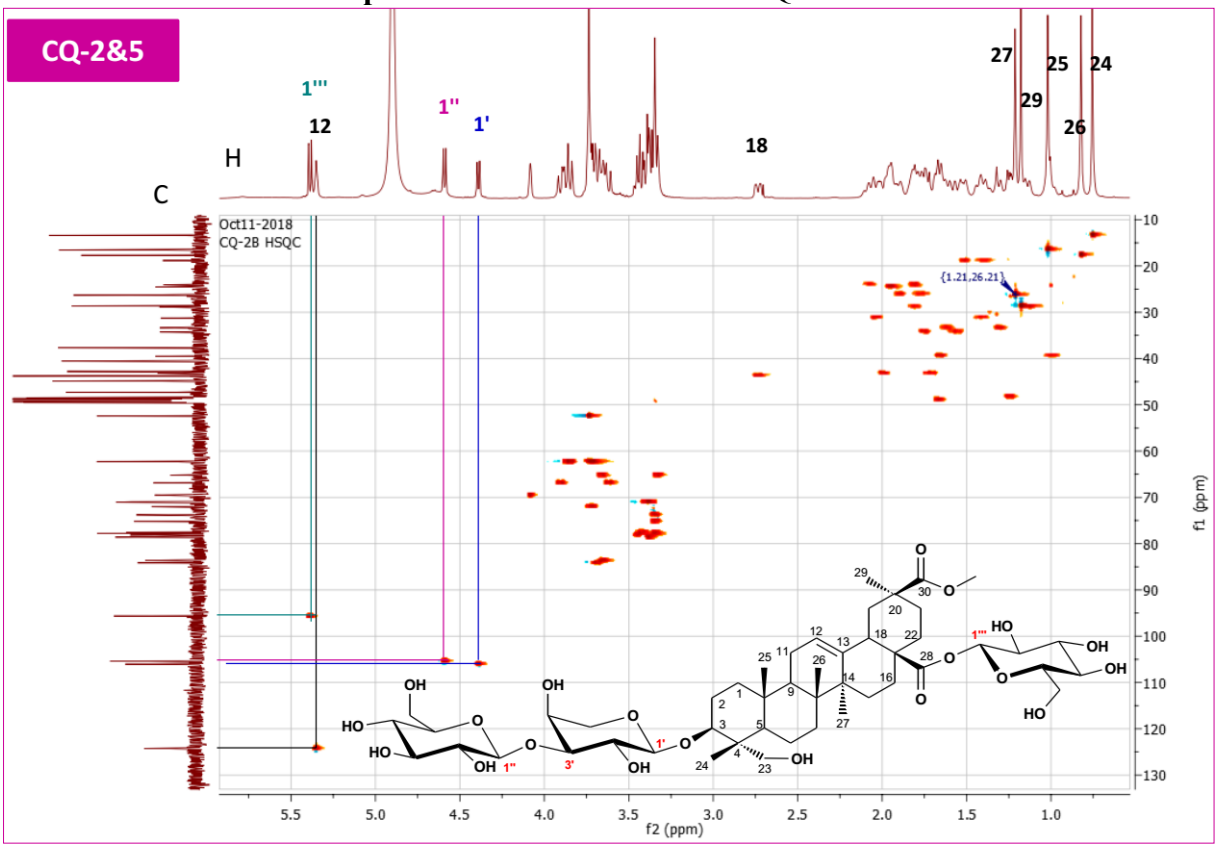


C	δ_C, ppm
3	83.6
12	124.3
13	144.6
18	42.0
23	65.2
24	13.4
25	16.6
26	17.7
27	26.4
28	177.4
29	28.7
30	178.6
$COOCH_3$	52.5

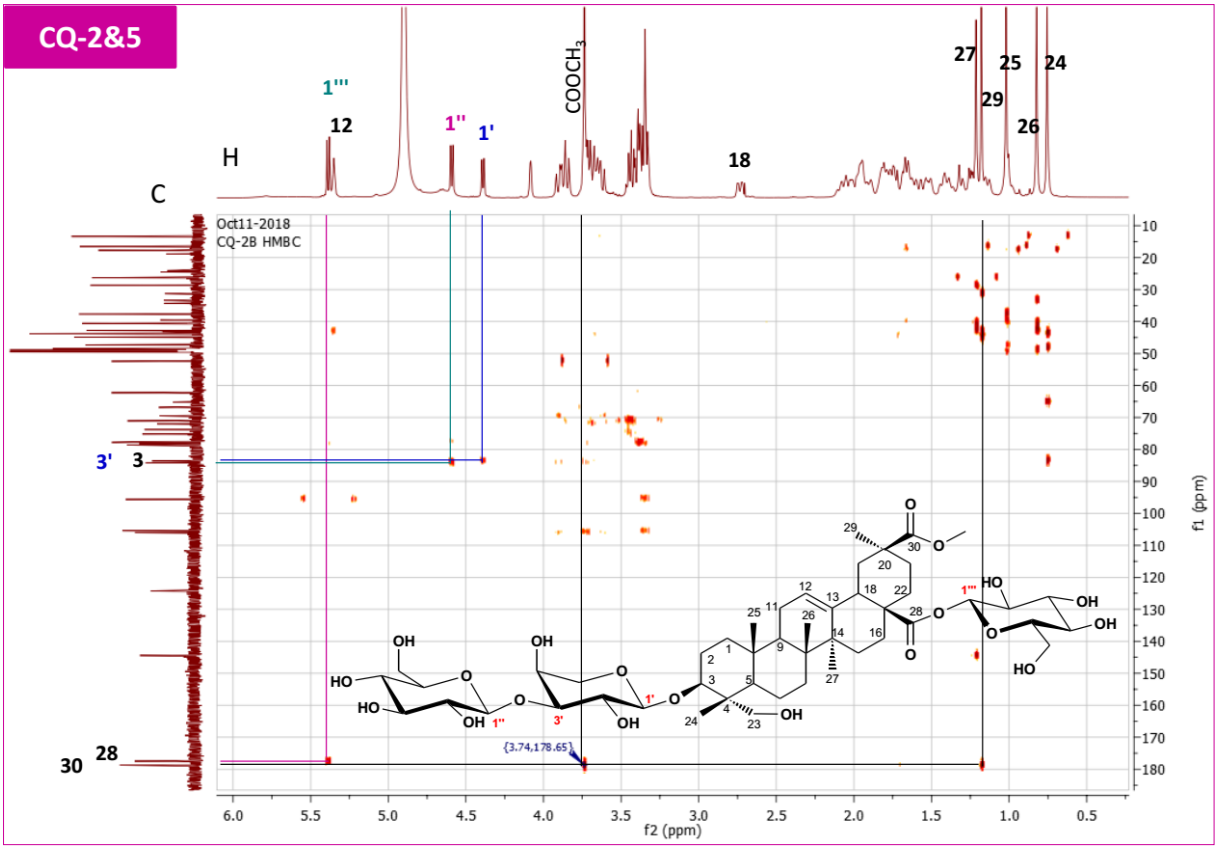
Spektrum 4.5.2. The ^{13}C-NMR and DEPT-135 Spectra of CQ-2.



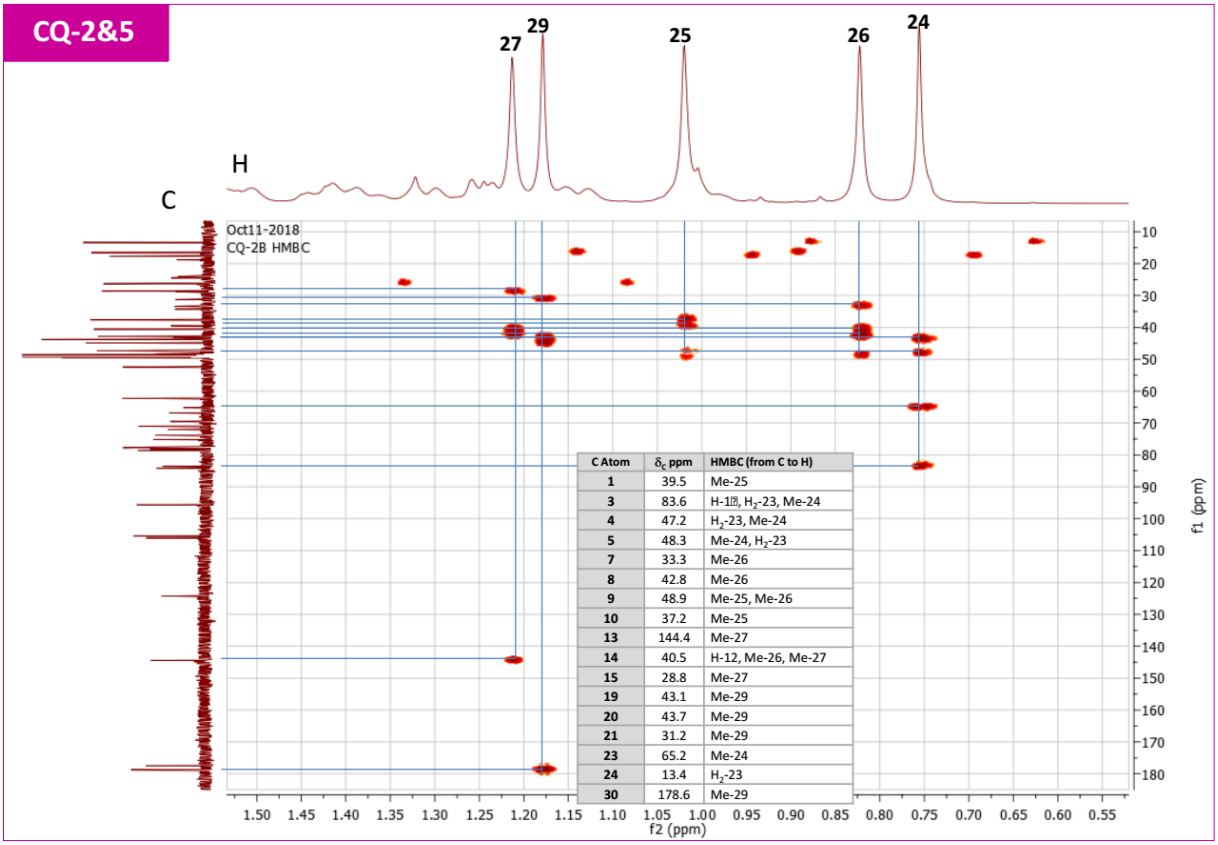
Spektrum 4.5.3. The COSY of CQ-2.



Spektrum 4.5.4. The HSQC CQ-2.

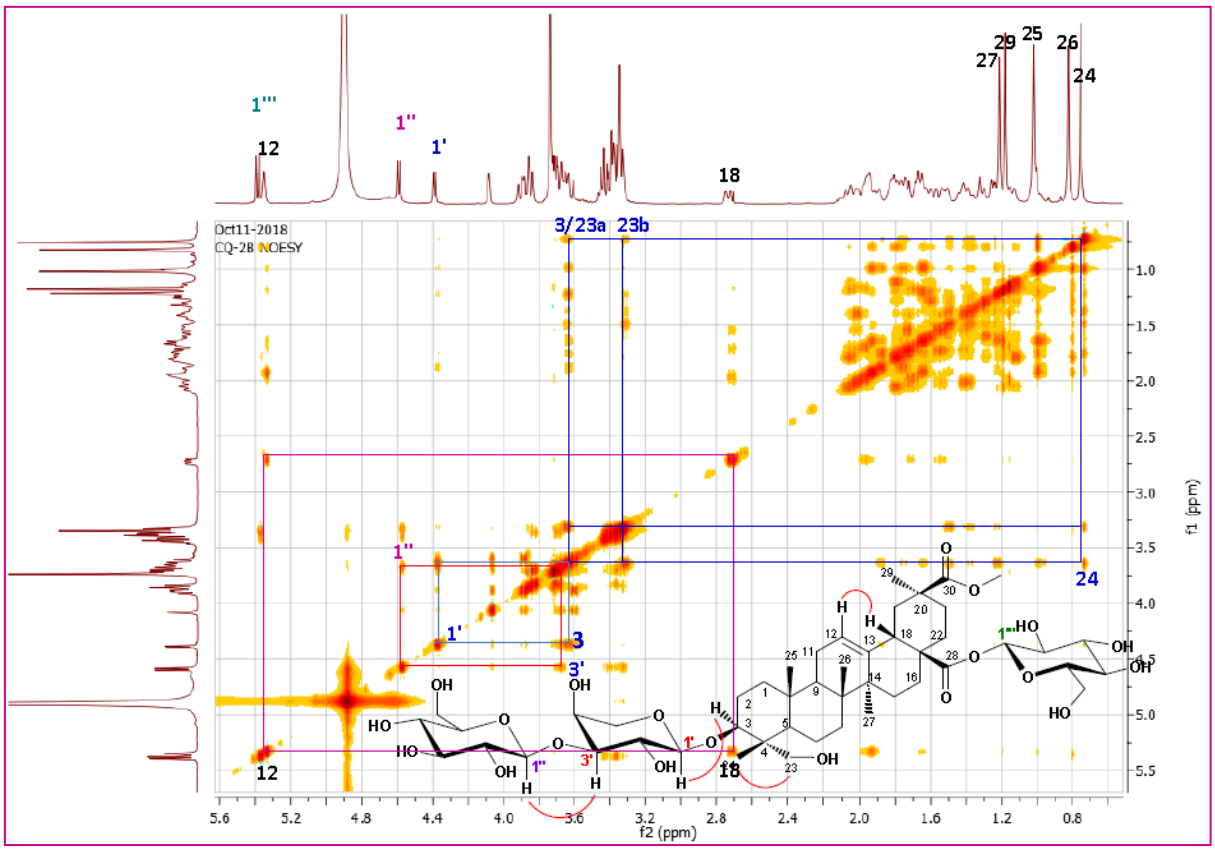


Spektrum 4.5.5a. The HMBC CQ-2.



C Atom	δ_c ppm	HMBC (from C to H)
1	39.5	Me-25
3	83.6	H-1, H_2-23, Me-24
4	47.2	H_2-23, Me-24
5	48.3	Me-24, H_2-23
7	33.3	Me-26
8	42.8	Me-26
9	48.9	Me-25, Me-26
10	37.2	Me-25
13	144.4	Me-27
14	40.5	H-12, Me-26, Me-27
15	28.8	Me-27
19	43.1	Me-29
20	43.7	Me-29
21	31.2	Me-29
23	65.2	Me-24
24	13.4	H_2-23
30	178.6	Me-29

Spektrum 4.5.5b. The HMBC CQ-2.



Spektrum 4.5.6. The NOESY CQ-2.

5. CONCLUSION

As it was already summarized in the Literature Review (Chapter 2) in this study, *Chenopodium quinoa* Willd. (Quinoa) is a member of Chenopodiaceae and is native plant to South America and cultivated by the Andes people for thousand of years because of its high nutritional value, especially for the protein contents rich in essential amino acids. Therefore, this plant is cultivated throughout the world as well aş in Turkey. In this study, the seed coat of quinoa rich in saponins has been provided from the cooperative of farmers in Thrace region.

By the help Chromatographical methods (VCC: Vacuum Column Chromatography; CC: Column Chromatography; MPLC: Medium Pressure Liquid Chromatography) five bidesmosidic oleanane-type triterpene glycosides (saponosides) were isolated from the alcoholic extract of the seed coat of Chenopodium quinoa provided as an agricultural remain. The chemical structures of the isolated saponosides were established by the help of 1D (^{1}H-NMR, ^{13}C-NMR, DEPT-135), and 2D-NMR (COSY, HSQC, HMBC, NOESY and ROESY) as well as High Resolution Mass Spectrometric (H-ESI MS) analysis.

These five glycosides are classified into the three groups according to the structure of sapogenol moieties which are hederagenin (3β,23-dihydroxy-olean-12-en-28-oic-acid), 30-hydroxy-hederagenin (3β,23,30-trihydroxy-olean-12-en-28-oic-acid), phytolaccagenic acid (3β,23-dihydroxy-olean-12-en-28,30-dioic-acid). All of them represent a bisdesmosidic structure and three of them triglycosidic while two are diglycosidic structures.

Based on the data, the structure of the saponosides (= saponins) were determined as the 3β-[(*O*-β-D-glucopyranosyl-(1→3)-α-arabinopyranosyl)oxy]-23-hydroxy-olean-12-en-28-oic-acid-28-*O*-β-glucopyranoside [= 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranoside] (**CQ-3**), 3β-*O*-β-D-glucuronopyranosyloxy-23hydroxy-olean-12-en-28-oic-acid-28-*O*-β-glucopyranoside (= 3-*O*-β-D-glucuronopyranosyl -hederagenin-28-*O*-β-glucopyranosyl ester) (**CQ-6**), 3β-[(*O*-β-D-glucopyranosyl-(1→3)-α-arabinopyranosyl)oxy]-23,30-dihydroxy-olean-12-en-28-oic-acid-28-*O*-β-glucopyranoside (**CQ-1**), 3β-*O*-β-D-glucopyranosyloxy-phytolaccagenic acid-28-*O*-β-D-glucopyranoside [3β-*O*-β-D-glucopyranosyl phytolaccagenic acid-28-*O*-β-D-glucopyranosyl ester] (**CQ-4**) and 3β-[(*O*-β-D-glucopyranosyl-(1→3)-α-arabinopyranosyl)oxy]-23-hydroxy-olean-12-en-28,30-dioic-acid-28-*O*-β-

glucopyranoside-30-methyl ester [= 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabino-pyranosyl phytolaccagenic acid 28-*O*-β-D-glucopyranoside] (**CQ-2**). These compounds have also been isolated from the flowers, fruits, seeds and seed coats.

Hederagenin and its derivatives are widely distributed in nature and have a plethora of biological activities. Hederagenin can cross the blood-brain barrier and distribute into cerebrospinal blood very rapidly. However, its low bioavailability and moderate haemolysis effect limit its clinical application. Therefore, structural modifications are necessary to increase its bioavailability and to reduce its observed toxicity to some animals. Biotransformation or chemical modification of hederagenin and its derivatives can be a good strategy to develop new medicines (Zeng et al., 2018).

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