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Investigation of chemical constituents and biological activities of some native halophytes

6D072100 − Chemical Technology of Organic Substances

Dissertation submitted in fulfillment of the requirements for the degree
Doctor of Philosophy (PhD)

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STANDARD REFERENCES

In the present dissertation used the following standards:

GOST 2237-75	Raw medicinal plant. Flowers, leaves and grass. Part 1 (Collection) - Moscow-Pubi standards, 1994, 159 p.
GOST 24027. 1-80	Raw medicinal plant. Methods of determining the authenticity, storage pests' infestation, grinding and impurity content.
GOST 24027.2-80	Raw medicinal plant. Methods for determination of moisture content, ash content, extractives and tannins, essential oil.
GOST 4564-79	Raw medicinal plant. Smoke tree leaf.
GOST 4517-87	Reagents. Methods of preparation of auxiliary reagents and solutions used in the analysis.
GOST 6709-72	Distilled water.
GOST 23932-90 E	Glassware and equipment, laboratory glass.
GOST 25336-82	Glassware and equipment, laboratory glass. Types, basic parameters and dimensions.
GOST (TU) 258-2021-003-88	Mercury glass laboratory thermometers.

ABBREVIATIONS

arab	Arabinose
ATCC	American Type Culture Collection
br.s.	broad singlet
BAW	Butanol-acetic acid-water
CA	*Candida albicans*
CC	Column chromatography
CCl$_4$	Carbon tetrachloride
CDCl$_3$	deuterated chloroform
COSY	Homonuclear correlation spectroscopy
d	doublet
dd	double doublet
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO-d_6	deuterated dimethyl sulfoxide
EAs	essential amino acids
EI-MS	Electron Impact Mass Spectroscopy
ESI	electrospray ionization
EtOAc	Ethyl acetate
GLC	Gas Liquid Chromatography
GC	gas chromatography
HCT-116	Colon carcinoma cells
GC/MS	Gas Chromatography-Mass Spectroscopy
Hep G2	Human hepatocarcinoma cell line
^{1}H-NMR	Proton nuclear magnetic resonance
HMBC	Heteronuclear Multiple Bond Correlation spectroscopy
HMQC	Heteronuclear Multiple-Quantum Correlation spectroscopy
HPLC	High performance liquid chromatography
HR-MS	High resolution mass spectrometry
HR-ESI-MS	High resolution Electron spray ionization mass spectrometry
Hz.	Hertz
hr	Hour
hrs	Hours
IC$_{50}$	The half maximal inhibitory concentration
IR	Infra-Red
J	Coupling constant
Kg	Kilogram
L	Length
LB	Luria-Bertani
μ	micron
m	multiplet
MCF-7	breast adenocarcinoma cells
MS	Mass spectroscopy
m.p.	melting point
m.m.p.	mixed melting point

MHz	Mega Hertz
mm	millimeter
mM	Millimolar
m/z	Mass/charge units
MUFA	Monounsaturated fatty acid
NCC	nitrogen-containing compounds
NEAs	Non-essential amino acids
NMR	Nuclear Magnetic Resonance
PC	Paper chromatography
PUFA	Polyunsaturated fatty acid
ppm	Part per million
PTP1B	protein tyrosine phosphates1B
q	quartet
R.A.	Relative amount
rel. int.	Relative intensity
R_f	Retention factor
rha.	Rhamnose
RP-18	Reversed Phase
R_t	Retention time
s	singlet
SA	*Staphylococcus aureus*
SDA	Sabaurauds agar
SPME	solid-phase micro-extraction
SIM	selected-ion-monitoring
SPE	Solid phase extraction
t	triplet
TLC	Thin Layer Chromatography
TMS	Tetra Methyl Silane
TSF	Total saturated fatty acid
UV	Ultra violet
xyl.	Xylose

INTRODUCTION

General characteristics of the work: This work is dedicated to the investigation of the chemical composition and biological activity of some native halophytic plants *Camphorosma lessingii* and *Zygophyllum fabago* belonging to the family *Chenopodiaceae* and *Zygophyllaceae* in order to establish new sources for biologically active substances to be used in medicine, agriculture and industrial processes.

Urgency of the work: Many plants such as halophytes have not yet received much attention as sources of bioactive molecules due to limited popularity or lack of commercial applications. Halophytes and their derivatives have properties that make them safer and alternatives to commercial drugs in many countries. These plants are not only available at an affordable cost, but are widely distributed and can be propagated by the local population. It is known that a significant part of land in Asia and Africa affected by salinization, in which a perfectly different halophytic plants grow. While about 2000 higher plants worldwide including in Central Asia, around 700 species are known to possess some salinity tolerance, only few species have been investigated for their antioxidant capacities, antimicrobial, anti-inflammatory and antitumor activities, etc. In this regard, the study of the chemical composition and biological activity of halophytes for their further use in the practice of medicine, and it is known that herbal drugs recently occupy an important place in the treatment of several cancer diseases, infectious, gastrointestinal, and other disease is of practical and scientific interest. Furthermore, over the last decades, well-being and health promoting substances have aroused a huge interest among consumers, food and pharmaceutical industries. Actually, the chemistry of halophytes tends to establish that they may be a source of novel compounds along with providing a new source for many already known biologically active compounds. Halophytes are exposed to unfavorable environmental conditions and many of them are able to withstand and quench toxic reactive oxygen species due to their natural powerful antioxidant system. This performance is very likely related to halophyte content in some vitamins, terpenoids (carotenoids and essential oils), phenolic and other secondary metabolites. Amid plant kingdom, halophytes represent a virtually untapped reservoir of a new generation of foods and drugs awaiting imaginative and progressive organizations. Furthermore, public health authorities consider prevention and treatment with nutraceuticals as a powerful instrument in maintaining health and to act against nutritionally induced acute and chronic diseases, thereby promoting optimal health, longevity and quality of life. There is a compelling argument for expanding the exploration of halophytes as source of novel active agents.

The choice of these species due to the fact that on the territory of the Republic of Kazakhstan salt tolerant plants have a huge reserves and in depth studies not performed before which promotes the study of their chemical composition and biological activity for the purpose of development of new drugs needed for the domestic pharmaceutical industry which is one of the main priorities of socio-economic policy of the Government of Kazakhstan.

Plants of the genus *Camphorosma* used in folk medicine as diuretic, diaphoretic, and for treatment of lung diseases and rheumatism and many plants belonging to the genus *Zygophyllum* have been shown to cause various biological effects and are used in folk medicine in Mediterranean, Arabic and Asiatic countries and are used for very diverse illnesses.

Kazakh species (*Camphorosma lessingii* f: *Chenopodiaceae* and *Zygophyllum fabago* f: *Zygophyllaceae*) are belonging to the obligate halophytes plants have not been subjected to in depth studies before for their chemical constituents and biological activities, in addition the *Camphorosma lessingii,* generally not subjected to in depth studies before all over the world. Therefore, the study of the chemical composition, the development of methods for isolation of biologically active substances from these plants and the study of their biological activity in order to create new drugs is an urgent task.

The purpose and the objectives of the study: investigation of the chemical composition and biological activity of plants (*Camphorosma lessingii* and *Zygophyllum fabago*) which growing on the salt soils of the territory of Kazakhstan. To achieve the intended objective we will study the following **tasks**:

1. Comparative investigation and quantitative determination of the chemical composition of complex biologically active substances of the two salt-tolerant species.
2. Development the technological scheme of optimal methods for the determination and isolation of biologically active substances.
3. Identification and structure elucidation of individual metabolites with the assistance of modern chemical and physico-chemical methods of analysis.
4. Conduct biological screening of total extracts on different types of activity.

Degree of the problem: The phytochemical studies of the Central Asian species of genus *Camphorosma* were reported by (Sokolov P.D.) that there is evidence of the accumulation of glycine, betaine and phytol in leaf of *Camphorosma annua* (Murakcory E.P.), whereas (Goryaev M.I.) proved essential oils (0.21%) in *Camphorosma monspeliacum* and *C. songorica*. The study of the Kazakh species of the genus *Camphorosma* started at the department of chemistry and technology of organic substances, natural compounds and polymers, Al-Farabi Kazakh National University by Abilov Zh.A., Sultanova N.A., Umbetova A.K. and they isolated and identified a number of phenolics and other compounds.

While the investigation of the chemical composition of the genus *Zygophyllum* were performed by many scientists in Egypt, Pakistan, China and other countries and highlighted the presence of phenolic acids, alkaloids, triterpenes, and other compounds (Ahmad V.U., Khan S.S., Amin E., El-Hawary, S.S., Elgamal, M.H., Hussein S.R., Marzouk M.M., Cong-Jun L.).

Scientific novelty of the work
• Study the polyphenolic, triterpenoid, amino acids, fatty acid and mineral composition of the Kazakh species; *Camphorosma lessingii* family *Chenopodiaceae* and *Zygophyllum fabago* family *Zygophyllaceae*.

- Investigation of lipophilic constituents of the *Camphorosma lessingii* and *Zygophyllum fabago* and identification of twelve and seven components in *Camphorosma* and *Zygophyllum* respectively.
- The essential oil contents of *Camphorosma lessingii* analyzed by GC-MS and clarified the presence of 81 components and identification of 37 of them.
- The scheme of extraction and separation of individual substances by different new techniques (Solid phase extraction, Vacuum liquid chromatography, and Flash chromatography) allocated 18 substances from *Zygophyllum fabago*, and 12 from *Camphorosma lessingii*.
- 3-O-*β*-D-xylopyranosyl-urs-12-ene-27, 28-dioic acid (3-O-*β*-D-xylopyranosyl quinovic acid) and 28-Nor-quinovic acid-3-O-*β*-D-quinovopyranoside were reported as new compounds from *Zygophyllum* together with 16 known compounds. While from *Camphorosma* isolated 12 compounds. Structures of the compounds were proved by chemical and physico-chemical (IR, UV, ^{13}C-NMR, ^{1}H-NMR, 2D NMR: COSY-45°, HMQC, HMBC, HRESI-MS, FAB, ECD, EI and FD mass spectrometry) methods of analysis.
- The biological activity of six extracts from both plants and one new individual compound (3-O-*β*-D-xylopyranosyl quinovic acid) reported new anti-leishmanial, anti-diabetic, phytotoxic, antimicrobial and cytotoxic activities.

Novelty of the results is protected by publications in international and local journals and conferences.

The practical value of the work: Revealed plant sources of biologically active substances- *Camphorosma lessingii* of the family *Chenopodiaceae* and *Zygophyllum fabago* of family *Zygophyllaceae*. Investigation of the biological activities established anti-leishmanial, anti-diabetic, phytotoxic, antimicrobial and cytotoxic activity. One triterpenoid glycoside isolated from such extracts has a significant cytotoxic activity.

Statements submitted for defense:
- Analysis of the major groups of biologically active substances of the aerial parts of the investigated species of the genus *Camphorosma* (*C. lessingii*) *Zygophyllum* (*Z. fabago*) families *Chenopodiaceae* and *Zygophyllaceae*.
- Optimal methods for the isolation and separation of biologically active substances of the studied halophytic species by different chromatographic methods.
- Establishment of the structure of the essential oil constituents, lipophilic contents, triterpenoids, flavonoids, phenolic acids, and chromones of the *Camphorosma lessingii* and *Zygophyllum fabago*.
- The results of the tested biological activities of six extracts and one individual substance obtained from the studied halophytic species.

The personal contribution of the author: The collection, processing materials, experimental part, working hours, preparations for study of biological activity,

analysis of the results, discussion, interpretation and formulation of conclusions and preparing publications.

Approbation of the work: The main findings and results of the dissertation were reported/discussed in the following international/state conferences:
- International conference of students and young scientists "World of Science" Faculty of chemistry and chemical technology, Al-Farabi Kazakh National university, Almaty, Kazakhstan, April 17-18, 2013.
- International conference of students and young scientists «World of Al-Farabi», Faculty of chemistry and chemical technology, Al-Farabi Kazakh National university, Almaty, Kazakhstan, April 9-10, 2014.
- 21st Young researcher meeting -SCT, Montpellier, France, March 24-25, 2014.

Correlation of the dissertation topic with the planned research projects: this Work performed as part of research programs RK: program «Isolation, purification and generation of biologically active substances from plants of Kazakhstan (*Salicornia*, *Camphorosma*» State registration number 0112RK02107 (2012).

Publications: Based on the findings and results of the dissertation, 10 articles were published in international/state journals and conferences proceedings, 3 of them was published in international journal has impact factor (two in Thomson Reuter's database), 3 articles in local editions journals recommended by the Committee for Control of Education and Science of Ministry of education and science of Republic of Kazakhstan, and 4 thesis were published in the proceedings of the international/state conferences listed above.

Structure and volume of the dissertation: The dissertation is consists of standard references, abbreviations, introduction, review of literature, materials and methods, results and discussion, conclusion and list of 218 references used. The work is formulated from 147 pages, contains 47 figures and 29 tables.

1 LITERATURE REVIEW

Some of the plants have the ability to grow under salinity due to the presence of different mechanisms in them for salt tolerance such plants are known as salt resisting plants, salt tolerating plants or halophytes which represent only 2% of terrestrial plant species but they represent a wide diversity of plant forms. About half of the higher plant families consist of halophytes and they have a polyphyletic origin. The largest number of halophytes is included in *Chenopodiaceae* and it consists of about 550 halophyte species, while other families that include halophytes are *Poaceae, Fabeaceae* and *Asteraceae* however less than 5% of the species in these families are halophytes. One of the basic differences between halophyte and glycophytes is that halophytes have the ability to survive under a salt shock as for example due to tidal or rainfall events this capacity allows the halophytes to develop a metabolic steady state for growth in a saline environment as compare to glycophytes. Halophytes respond to salt stress at three levels i.e. cellular, tissue and whole plant level [1].

1.1 Classification and distribution of halophytes

A halophyte is a plant that naturally grows and completes their life cycle where it is affected by salinity in the root area or by salt spray, such as in saline semi-deserts, mangrove swamps, marshes and sloughs, and seashores. Adaptation to saline environments by halophytes may take the form of salt tolerance or salt avoidance. Plants that avoid the effects of high salt (e.g. completes its reproductive life cycle during rainy season) even though they live in a saline environment may be referred to as facultative halophytes rather than obligate halophytes. Obligate halophytes (xerohalophytes are the desert species of halophytes) are plants that thrive when given water having greater than 0.5% NaCl. Halophytes are often classified as secretor/recretor versus succulents or as excluders versus includers. A small number of plant lineages in numerous, related families have evolved structural, phenological, physiological, and biochemical mechanisms for salt resistance [2].

Many halophytes have potential agricultural value and can be grown in the degraded saline areas. Mangroves and many associate species thrive well in tidal zone. Many tropical plants, which occur in highly saline water, can be grown in coastal areas irrigating with seawater. Many halophytes not only survive in these conditions but also produce considerable biomass. Many of the salt tolerant species are of industrial application and may be grown as commercial crops [2].

Based on the basis of adaptability of saline soils, Sen et al. (1982) classified the halophytes in to the following three categories:

True (obligate) halophytes: Plants mainly attaining optimal growth on the saline soil (above 0.5% NaCl level). For example, *Suaeda fruticosa, Cressa cretica, Aeluropus lagopoides, Salsola baryosma, Haloxylon recurvum, Zygophyllum simplex and Camphorosma annua* [3, 4].

Facultative halophytes: Those plants, which obtain optimal growth on saline soil (at 0.5% NaCl level) like true halophytes, and can also, grow on non-saline soil

e.g. *Trianthema triquetra, Tamarix dioica, Launaea nudicaulis, Eragrostis ciliaris, E. pilosa, Salvadora persica, Pulicaria wightiana* and many others [3].

Glycophytes or transitional halophytes: Plants growing only at the transition of saline and non-saline areas and achieve optimal growth at non-saline niches of the salt basin. e.g. *Sporobolus marginatus, S. helvolus, Haloxylon salicornicum* and *Datyloctenium sindicum* [3].

1.2 Cellular adaptations of the plants against salt stress Synthesis of compatible solutes (Osmolyte production)

Osmolytes are the organic compounds they affect osmosis and play role in maintaining fluid balance as well as cell volume for example a cell may burst as a result of external osmotic pressure under this situation certain osmotic channels, may get open which allow the efflux of certain omolytes through them as they move outside they carry water with themselves preventing the cell from bursting out. Sugars, alcohols, amino acids, polyols, tertiary and quaternary ammonium and euphonium compounds are different examples of osmolytes. Due to the increase of salt contents of the soil, the flow of water towards the roots of the plants is decreased causing a decrease of the cell membrane permeability. Under such a situation, osmotic adjustment of the plant cells is required. Plants carry out this adjustment by the synthesis of compatible solutes called osmolytes, which play role in the reduction of oxidative damage that may occur due to the production of ROS under salinity stress as well as they protect sub-cellular structures. Some osmolytes and their roles in stress are given subsequently:

Proline analogues: Naidu (2003) reported that some of the halophytes are able to cope with the high salinity of the soil due to the production of proline analogue as it happens in Australian. *Melanleuca* species as for example *Melanleuca bracteata*, which accumulate the proline analogue 4-hydroxy-N-methyl proline (MHP). Such proline analogues increase the ability of plants to survive during salinity stress due to their ability to cause regulation, compartmentalization, and production outlay [1].

Aquaporin: Another type of osmolyte is named as aquaporin, which believed to be involved in intracellular compartmentalization of the water. These pore forming proteins in halophytes conduct the water molecules. It indicates that the gating of water channels could have an impact on inter compartmental movement of water. Such aquaporins are believed to play some role in salt tolerance by maintaining osmotic homeostasis and turgor of the plant cells under salt stress [1].

Glycine betaine (GB): GB is a quaternary ammonium compound, which acts as an osmo-protectant and can offset the high salinity concentration in the vacuole. It is a stabilizing osmolyte and has the role of protection of macromolecule of the plant under dehydration stresses. GB is not found to accumulate in crops during stress but is generally found in halophytic members of *Poaceae* and *Chenopodiaceae* [1].

1.3 Diversity of Halophytes

Halophytes are considered to be rare plant forms that arose separately in unrelated plant families during the diversification of angiosperms; in this they

resemble epiphytes, saprophytes, xerophytes, aquatics, and marsh plants. No comprehensive list of halophyte species exists, due partly to the problem of defining the lower salt-tolerance limit at which a plant should be considered a halophyte [5].

Aronson (1989) compiled a partial list of halophytes containing 1560 species in 550 genera and 117 families. His list was drawn from literature reports and interviews with researchers as part of a program to assemble a world halophyte collection to screen for new crops. He used a broad definition of halophyte that included any plant that was reportedly more tolerant than conventional crops, for which the upper salt content of irrigation water was taken to be 5 g/l total dissolved solids (TDS) (85 mM as NaCl). However, his list only included plants that had potential as food, forage, fuel wood, or soil stabilization crops [5].

Based on a comparison of Aronson's entries with the known number of species in selected halophytic genera, Le Houerou (1993) estimated that Aronson's list probably included 20 to 30% of the terrestrial halophytic flora, which would then reach 5000 to 6000 species, or 2% of world angiosperm species. Of the species in the list, 57% came from just 13 families [5].

The largest numbers of halophyte species are in the *Chenopodiaceae*; over half of its 550 species are halophytic. The three superfamilies, *Poaceae* (grasses), *Fabaceae* (legumes), and *Asteraceae* (composites), also have large numbers of halophytes, although they represent fewer than 5% of the species in these families [5].

These families proliferated through radiative evolution into many diverse niches, including saline habitats, during the early evolution of angiosperms. Flowers et al. (1977) plotted the occurrence of halophytes in the major orders of flowering plants in a dendogram showing probable relationships between orders. Halophytes occurred throughout the dendogram in both primitive (e.g., *Laurales, Nymphales*) and advanced (*Asterales, Orchidales*) orders [5].

1.4 General characters of the genus *Camphorosma*

Chenopodiaceae are usually divided into two subfamilies *Cyclolobeae* S.A.M. and *Spirolobeae* S.A.M., In the Soviet Union found 53 genus and 390 species of them in Kazakhstan, 47 genera and 218 species. Members of this family occupy a huge territory of deserts and semi-deserts of Kazakhstan mainly on saline and alkaline soils [6, 7]. They are particularly successful in dry, saline or disturbed habitats of temperate and subtropical climates in the northern and southern hemisphere. the *Chenopodiaceae* comprise about 1250 species in about 100 genera worldwide with the center of diversity in the Old World desert belt from the Canary Islands to Central Asia, where all tribes and nearly two-thirds (approximately 60%) of the genera are present. The second center is Australia, followed by North America, South America, and South Africa [8]. *Chenopodiaceae* family is extremely versatile; the members of this family play a predominant role in the lowland landscapes spaces and deserts, they acquire a decisive economic importance as food plants of the desert zone. Chemical analysis of most plants indicates their high nutritional value. Fresh halophytes well eaten only by camels, other are eaten by animals often in the fall and

winter, after the rains and frosts, when the bulk of the leached salts. Also used as a source of high energy fuel necessary to specify the leafless saksaul, harvested in large quantities for the needs of the population of the southern cities of Kazakhstan. Some are good sand strengthener; others are of interest for planting desert localities. Used also as vegetables of great importance table beet, spinach, etc. But the most important is the culture of sugar beet. Some members contain in its chemical composition potash and other alkalis which used in soap making while others of great importance like Anabasis which is alkaloids containing plant yield anabasine-sulfate, which is a valuable insecticide. From other saltworts of the family members isolated alkaloid salsolin which used in lowering blood pressure, also some members of *Chenopodiaceae* give vegetable ink [6, 7].

Camphorosmeae: belong to subfamily *Salsoloideae* and are centered in the Old World desert belt from the Canary Islands to Asia. A few species grow in N America and Africa [8]. It represents a species-rich tribe of *Chenopodiaceae* comprising 19 genera and 190 species. Most species are dwarf shrubs or annuals, or more rarely perennial herbs. They have alternate leaves, inconspicuous, sessile flowers and variously appendaged nut-like fruits. *Camphorosmeae* are confined to arid, saline or disturbed (ruderal) environments. They are adapted to their habitats by a dense indumentum of dead hairs and leave which are reduced in size and often succulent. The distribution is centered in Australia, Eurasia and North Africa but also extends to other continents [9].

Camphorosma: the genus contain about 10 species, in Kazakhstan three species reported; *C. Sonngoricum* Bge., *C. lessingii* Litw. and *C. monspeliacum* L. [10, 11]. Most species are xerophytes or obligate halophytes in open plant communities of dry steppes, semi deserts or in drier types of saltmarshes, preferably on soils with slightly or distinctly increased contents of soluble salts, in particular of sodium chloride, soda, or gypsum [4, 9]

Camphorosma monspeliacum L: is an ever green shrub growing up to 0.6 m and named in some countries as (KAFURI) and flowered in October. The scented flowers are hermaphrodite and are pollinated by insects. The stems and leaves are emitted a powerful camphor-like scent. The plant prefers light (sandy) and medium (loamy) soils and requires well drained soils also prefers acidius, neutral and basic soils and can grow in high alkaline and saline soils and can tolerate to drought [12]. It grows in salt marshes along the banks of rivers and lakes of salt complex steppes and in depressions of hilly sands, less on gravelly slopes. Found in Europe, part of the USSR, the Caucasus, Western Siberia, Western Europe, Asia Minor, Iran and Mongolia, in Kazakhstan reported in Tob.-Ishim., Irt., Semip. boron., Kokchet., Caspian., Aktobe., EMB., Turgay., west and east melkosop., Says., north Ust-Urt, Priaral., Kz. Ord., Betpakd., Muyun kum., Balh.-Alak., and Altai. [10, 11, 13]. It Used as fodder plant for camels, sheep and goats, but of low quality; it also has been a traditional medicinal plant in East Mediterranean countries since medieval times due to its content of volatile oils about 0.2 % with the smell of essential oil of bitter almonds which upon distillation with KOH yields propylamine (Vemer), it has camphor smell and had known before in medicine as an aphrodisiac, antiasthmatic,

expectorant, diuretic, emmenagogue, stimulant and diaphoretic, in the present time used in some areas as a folk medicine [9, 12].

Chemical analysis of Kazakh specimen of dry weight gave the following results: 1) for flowering: Percentage: 8.13 hygroscopic water, ash, 14.31-15.58, fiber 26.60-28.30, crude protein 10.74-11.69, crude fat 2.51-2.73, nitrogen-free extractives 38.31-41.70, starch equivalent 28.90-31.46; 2) for fruiting 6.38 hygroscopic water, ash 15.68-16.75, fiber 20.55-21.95, crude protein 7.97-8.51, crude fat 3.37-3.60, nitrogen-free extractives 46.05-49.19, starch equivalent 83.46-85.07 [10, 11, 13].

Camphorosma lessingii Litw.: Found in Lower Volga, central Asia, Caucasus, Western China, and Mongolia. Grows on salt marshes, chalks, and sandy soils, on stony slopes and depressions of sand, on clayey and rubbly areas to height 2500 meters, it found in groups, sometimes forms pure sparse thickets. Occurs in Syrt, Tob. - Ishim ., IRT., Semip. boron., Kok -Thu., Caspian, Aktobe, EMB, Turgay, I. west and east Small - const., North Ust -Urt, Priaral, Kz. Ord., Betpak, Murong - kum., Balh.- Alak.,Turkestan, Altai, Jung. Alat., Zail. Kunt. Alat., Chu, CL. mountains, Kirgh. Alat., and Karat [14].

Camphorosma Sonngoricum Bge.: Found in Europe, part of the USSR, North Caucasus, Western Siberia and China. Grows in wet salt marshes, salt meadows, salt marshes meadow, in the steppe zone. Found in Tob.-Ishim., IRT., Semip. boron, Kokchet., Caspian., Aktobe, west and east melkosop, Says., Priaral [14].

### 1.5 General characters of the genus *Zygophyllum*

Zygophyllaceae R. Br, in the classification by Sheahan and Chase (1996, 2000), are a family of approximately 285 species subdivided into five subfamilies and about 27 genera. They consist of Annual and perennial grasses, trees, shrubs and herbs with opposite or ordinary fleshy leaves, mostly restricted to arid and semi-arid areas in the tropics and subtropics. In the USSR reported 6 genera and 48 species, While in Kazakhstan found 6 genera and 35 species [15-18].

Zygophylloideae: as proposed by Sheahan and Chase (1996, 2000), are the largest subfamily in *Zygophyllaceae* and consist of about 180 species of shrubs, subshrubs and herbs currently grouped in four genera, monotypic *Augea* (South Africa), monotypic *Tetraena* (China), widespread *Fagonia* (c. 30 species), and likewise widespread *Zygophyllum* (c. 150 species) [18].

Zygophyllum: plants of the genus *Zygophyllum* are the most widely distributed of the *Zygophyllaceae* family. This genus includes about 100 representatives in the world flora, most species belonging to obligate halophytes and found mostly in the desert countries of Arabia, the Mediterranean, Asia, and Australia. In the USSR the genus *Zygophyllum* includes 40 species and in Kazakhstan 29 of which 13 are endemics. [19-21]. Species belonging to this genus represent a group of succulent plants that are drought resistant and/or salt tolerant, living under severe, dry climatic conditions; moreover, it is recorded by many authors as one of the important components of the desert vegetation. The abundance of species related to this genus could be attributed to their high tolerance to environmental stresses in addition to their unpalatability. The growth and distribution of *Zygophyllum* species are

(A)
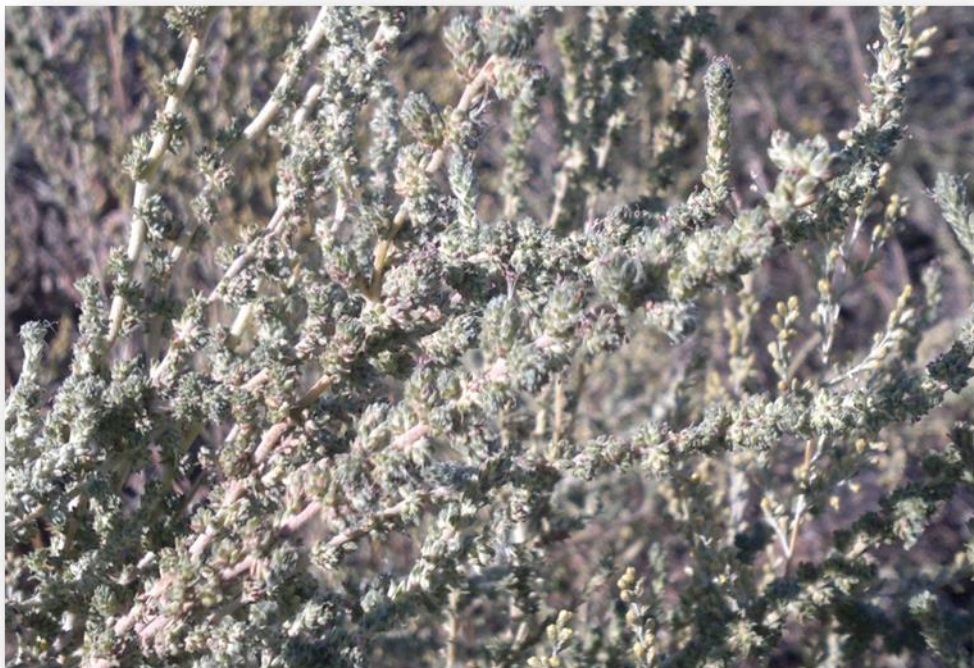
(B)

Figure 1 – Photos of the investigated halophytes; *Zygophyllum fabago* (A), *Camphorosma lessingii* (B)

attributed to their dependence on the chemical nature of the soil of their habitats [22]. Many plants belonging to the genus *Zygophyllum* have been shown to cause various biological effects and are used in folk medicine in Mediterranean, Arabic and Asiatic countries and are used for very diverse illnesses; notably, *Zygophyllum coccineum* is recommended against rheumatism, asthma, gout, hypertension, is also used as a diuretic, anthelminthic and antidiabetic agent, extracts from *Zygophyllum dumosum* (BOISS.) are used in Egypt to treat rheumatism, gout, asthma and hypertension, the leaf juice of *Zygophyllum simplex* acts as a skin cleanser. It was also reported for the horny patches on skin in the North Africa and Arabia region and *Zygophyllum gaetulum* is known as an antidiabetic, antispasmodic, anti-eczema, and as a remedy for stomach and liver pain [23-26].

Zygophyllum fabago L.: also known as Syrian bean-caper, is a native of the region extending from the steppes of Russia southeast to Afghanistan, found widely in the Mediterranean area, Europe, part of the USSR, Caucasus, Wed Asia, Balkans, Asia Minor, Iran, Iraq, Syria being typical of the Middle East, North Africa and in the south-western United States, where it is considered an invasive plant. It is widespread in Kazakhstan and represented by three subspecies: ssp. *Typicum* M. Pop., fruits aimed down, long, cylindrical (eastern coast of the Caspian Sea); ssp. *orientale* Boriss., Fruits short, 10-15 mm long, Ovate or oblong-ovate (east of the river. Amu Darya) ssp. *dolichocarpum* M. Pop., fruits are longer, flowers larger, more powerful plant (b. OrKhorgos), found in Caspian, Aktobe, EMB, Turgay, west Melkosop, Kz. Ord, Betpakd, Murong-kum, Balh. - Alak, Turkestan, Jung Alat, Zail, Kung, Alat, Ketmen, Terek, Alat, Karat [27-30].

The plant *Zygophyllum fabago* is one of the important herbs with known anti-rheumatic, anthelminthic, cathartic, and anti-asthmatic properties. It is also used as a part of drug for rheumatism and gout; also used externally as poultice to cure skin diseases, external wounds, septic, and injuries. In China *Z. fabago* is used as antitussive, expectorant, and as anti-inflammatory agent [23, 28, 31].

1.6 Chemical review of halophytes

Plants produce high diversity of natural products or secondary metabolites with a prominent function in the protection against predators and microbial pathogens on the basis of their toxic nature and repellence to herbivores and microbes and some of which also involved in defense against abiotic stress (e.g. UV-B exposure) and also important for the communication of the plants with other organisms, and are insignificant for growth and developmental processes. There are three major groups of secondary metabolites viz terpenes, phenolics and N and S containing compounds [32].

1.6.1. Triterpenes and saponins

Triterpenoids are groups of natural products containing about thirty carbon atoms. They have a common origin and their structures can be considered as being derived from that of squalene by various cyclisation or other changes [33].

Glycosides of triterpenes, also named saponins, commonly occur in higher plant. They are highly polar, non-volatile and thermally labile compounds [34]. They are mainly produced by plants, but also by lower marine animals and some bacteria. They derive their name from their ability to form stable, soap-like foams in aqueous solutions. This easily observable character has attracted human interest from ancient times. Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be triterpenoid (fig 2a) or steroid (fig 2b) in nature. The aglycone may contain one or more unsaturated C–C bonds. The oligosaccharide chain is normally attached at the C_3 position (monodesmosidic), but many saponins have an additional sugar moiety at the C_{26} or C_{28} position (bidesmosidic). The great complexity of saponin structure arises from the variability of the aglycone structure, the nature of the side chains and the position of attachment of these moieties on the aglycone [35].
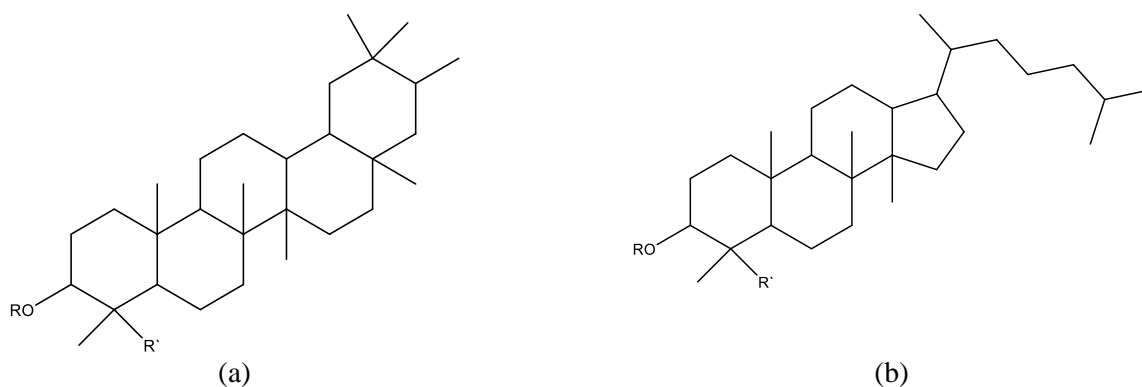
Figure 2 – Basic structures of sapogenins: a triterpenoid (a) and a steroid (b).

Saponins are secondary plant metabolites that occur in a wide range of plant species. They are stored in plant cells as inactive precursors but are readily converted into biologically active antibiotics by plant enzymes in response to pathogen attack. Saponins are glycosylated compounds that are widely distributed in the plant kingdom and can be divided into three major groups; a triterpenoid, a steroid, or a steroidal glycoalkoloid [33, 36].

Triterpenes and saponins with oleanolic acid nucleus have been isolated and identified from different species belonging to the halophytes plants for example; oleanolic acid 3-O-*β*-D-glucopyranoside (1) and oleanolic acid acyl-*β*-D-glucopyranoside (2) were isolated from *Camphorosma monspeliacum* by adsorption-distribution chromatography of the EtOAc fraction over silica gel (CHCl$_3$:MeOH) and polyamide (MeOH:H$_2$O) The structures of the isolated compounds were established by chemical (acid hydrolysis, alkaline destruction, anthocyanidine test) and spectral methods (mass, ^{1}H-NMR, ^{13}C-NMR, and 2D NMR spectroscopies) and by comparison with the literature. The attachment sites of the carbohydrate units were determined by 2D heteronuclear correlation spectroscopy HMBC [37].
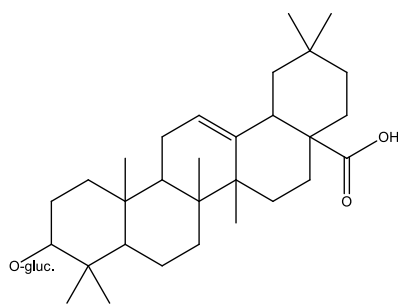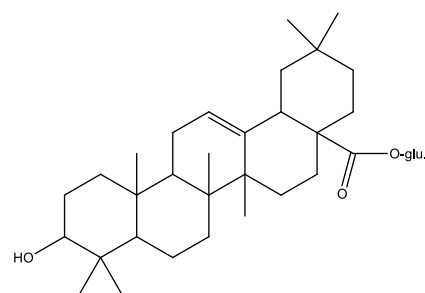
(1)   (2)

While from another genus four new triterpenoid saponins were isolated and identified from the aerial parts of *Fagonia cretica*, they were characterized as 3-O-[*β*-D-glucopyranosyl (1→2)-α-L-arabinopyranosyl] hederagenin 28-O-*β*-D-glucopyranosyl ester (3), 3-O-[*β*-D-glucopyranosyl (1→2)-α-L-arabinopyranosyl] oleanolic acid 28-O-[*β*-D-glucopyranosyl (1→6)-*β*-D-glucopyranosyl] ester (4), 3-O-[*β*-D-glucopyranosyl (1→2)-α-L-arabinopyranosyl] 27-hydroxy oleanolic acid 28-O-[*β*-D-glucopyranosyl (1→6)-*β*-D-glucopyranosyl] ester (5) and 3*β*-O-[*β*-D-glucopyranosyl (1→2)-α-L-arabinopyranosyl] olean-12-en-27-al-28-oic acid 28-O-[*β*-D-glucopyranosyl (1→6)-*β*-D-glucopyranosyl] ester (6). The structures of the saponins were assigned by spectral analyses (FABMS,^{1}H,^{13}C-NMR,^{1}H-^{1}H COSY, TOCSY, HMQC and HMBC spectra) and NOE experiments 38][.
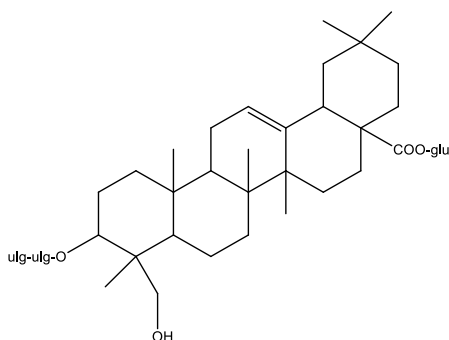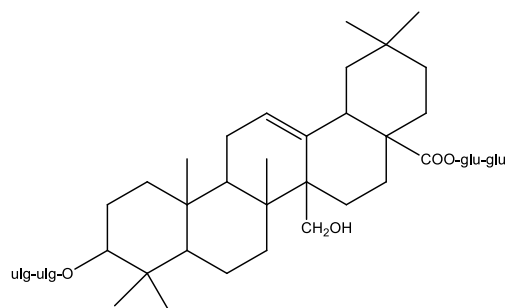
(3)   (4)
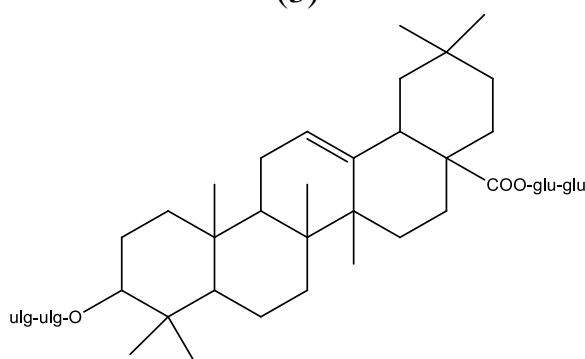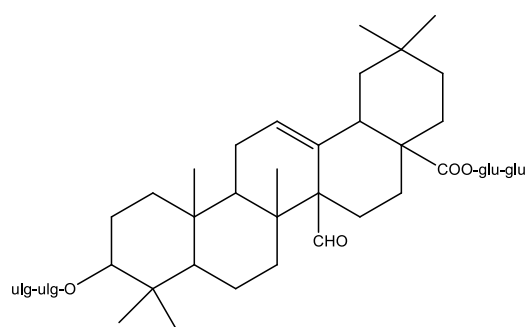

(5)   (6)

Also from the aerial parts of *Kalidium foliatum* collected from the Almaty region isolated three new olean-12-ene-23,28-dioic acids in their either partly or fully esterified forms (Me or glucosyl (Glc) esters), which were named kalidiumoside A (7), kalidiumin (8), and kalidiumoside B (9). Their structures were elucidated through

spectral studies including 2D-NMR experiments (HMQC, HMBC, COSY, NOESY) and *J*-resolved spectra [39].
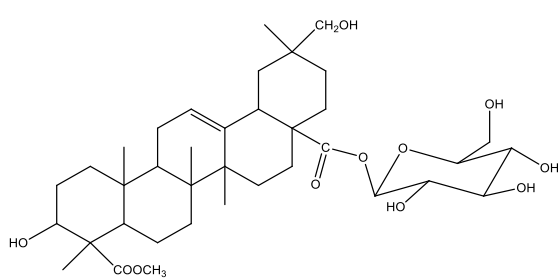
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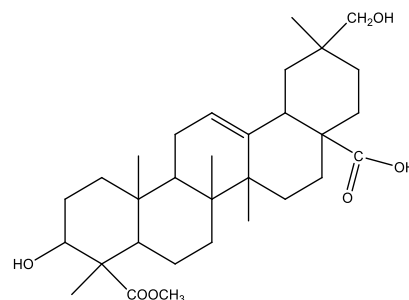
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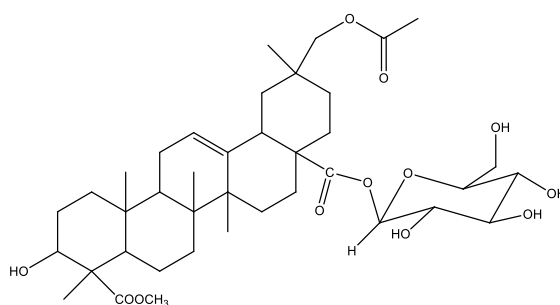
(9)

Another investigation done by Ch. Annaev et al. 1983, revealed the identification of new triterpene glycosides from the aerial part of *Climacoptera transoxana* (Iljin) Botsch. named Copterosides E (10) and F (11). According to chemical transformations and physicochemical characteristics, Copteroside E has the structure of oleanolic acid 28-O-*β*-D-glucopyranoside 3-O-[[O-*β*-D-xylopyranosyl-(1→2)]-[O-*β*-D-xylopyranosyl-(1→4)]-*β*-D-glucuronopyranoside} and Copteroside F that of hederagenin 28-O-*β*-D-glucopyranoside 3-O-[[O-*β*-D-xylopyranosyl-(1→2)]-[O-*β*-D-xylopyranosyl-(1→4)]-*β*-D-glucopyranoside] [40].
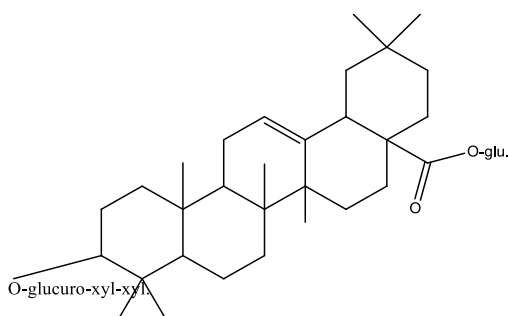
(10)
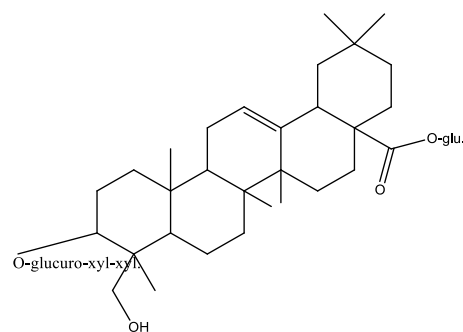
(11)

Continuing the study of the *Climacoptra transoxana* clarified the presence of another two triterpene glycosides, hedaragenin 3-O-*β*-D-glucopyranosiduronic acid and hederagenin 3-O-[O-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranoside] named Copterosides B (12) and C (13) respectively [41] and two new bisdesmosidic glycosides; gypsogenic acid 28-O-*β*-D-glucopyranoside 3-O-*β*-D-glucuronopyranoside (Copteroside G) (14) and gypsogenic acid 28-O-*β*-D-glucopyranoside 3-O-[O-*β*-D-xylopyranosyl-(1→2)-*β*-glucuronopyranoside] (Copteroside H) (15) [42].

(12)   (13)

(14)   (15)
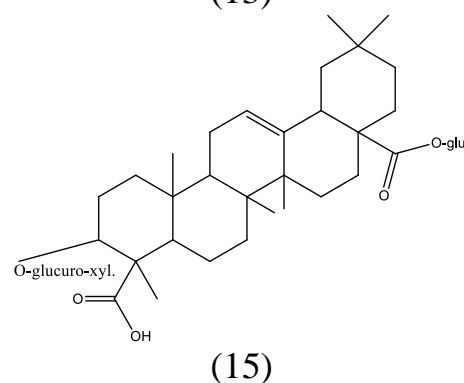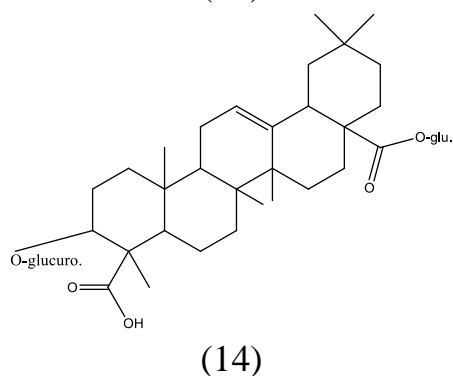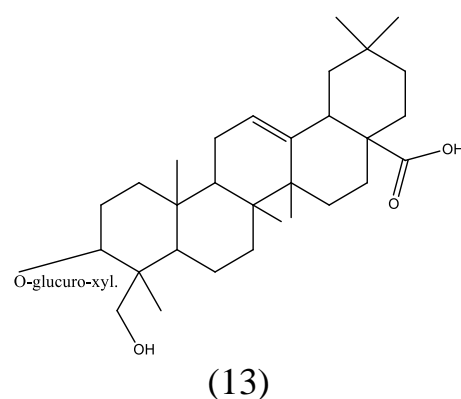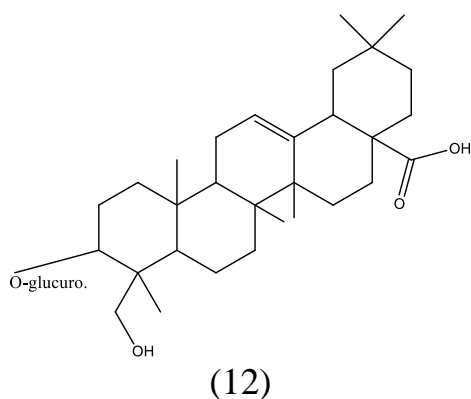
Further study of the genus *Climacoptera* revealed the isolation and identification of another oleanolic acid derivatives identified as oleanolic acid 3-O-{[*β*-D-xylopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosido-28-O-*β*-D-glucopyranoside (16) from aerial part of *C. lanata* and *C. aralensis,* the plant material was collected from the Aral region, this compound isolated by column chromatography over silica gel followed by Gel chromatography over Sephadex LH-20 and identified by spectral methods (mass, ^{1}H-NMR, ^{13}C-NMR and HMBC) [43].
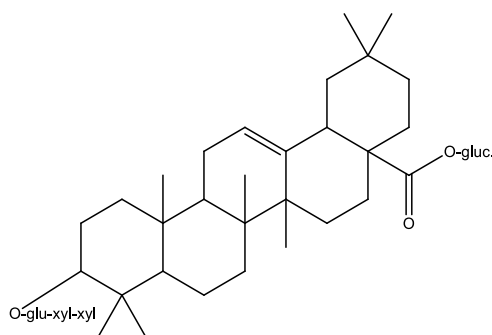
(16)

While the two new bidesmosidic saponins; gypsogenin 3-O-[β-D-xylopyranosyl-(1→3)-β-D-glucopyranoside]-28-O-{β-D-glucopyranosyl} ester (17) and hederagenin 3-O-[β-D-xylopyranosyl-(1→3)-β-D-glucopyranoside]-28-O-[β-D-glucopyranosyl} ester (18) were isolated from *Climacoptera obtusifolia* [44].
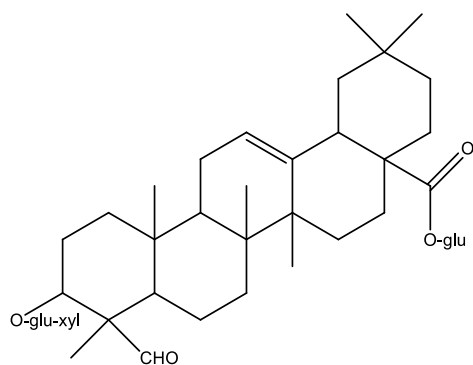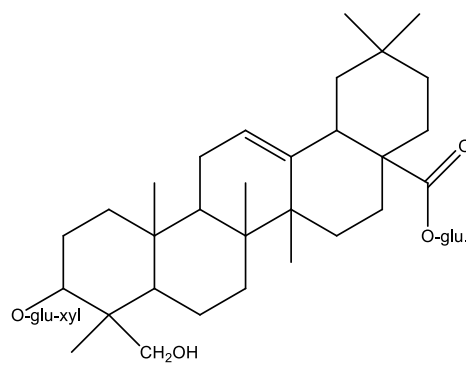
(17)   (18)

Because the saponins are a large group of the biologically active compounds, different nucleuses are found in different plant species from which the friedoolean nucleus reported also from the halophytes; Isotamarexin (19) is one of these compounds which is identified as 3-α-(3",4"-dihydroxy- trans-cinnamoyloxy)-D-friedoolean-14-en-28-oic acid reported as new compound from *Tamarix hispida* [45], 3-β-al-D-friedoolean-14-en-28 carboxylic acid methyl ester (20) is also another new compound isolated from *Tamarix laxa* and *Tamarix elongate;* this plants are indigenous in Aral and Almaty regions [46].
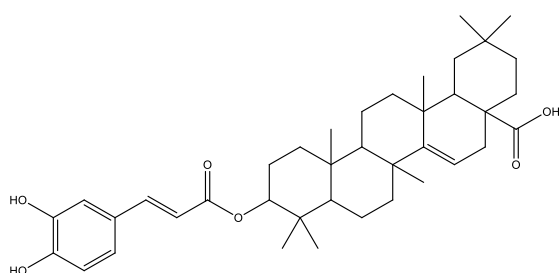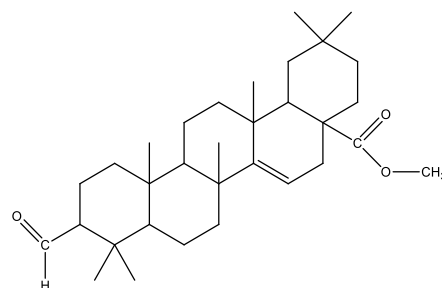
(19)   (20)

The *Tamarix chinensis*, has shown a friedoolean triterpene compounds identified as tamarixol (21) and its 3-ketone tamarixone (22) [47]. The bark of *Tamarix aphylla* contains the new triterpene D-friedoolean-14-en-3α, 28-diol (isomyricadiol) (23), its 3β isomer (myricadiol) and its 3-ketone, the structure of isomyricadiol was established by comparing its ^{13}C-NMR data with those of its 3β isomer and 3-epibetulinic aldehyde. The published ^{13}C-NMR spectrum of myricadiol has been partly reassigned via a standard and an inverse H, C-COSY experiment [48].

(21)

(22)

(23)
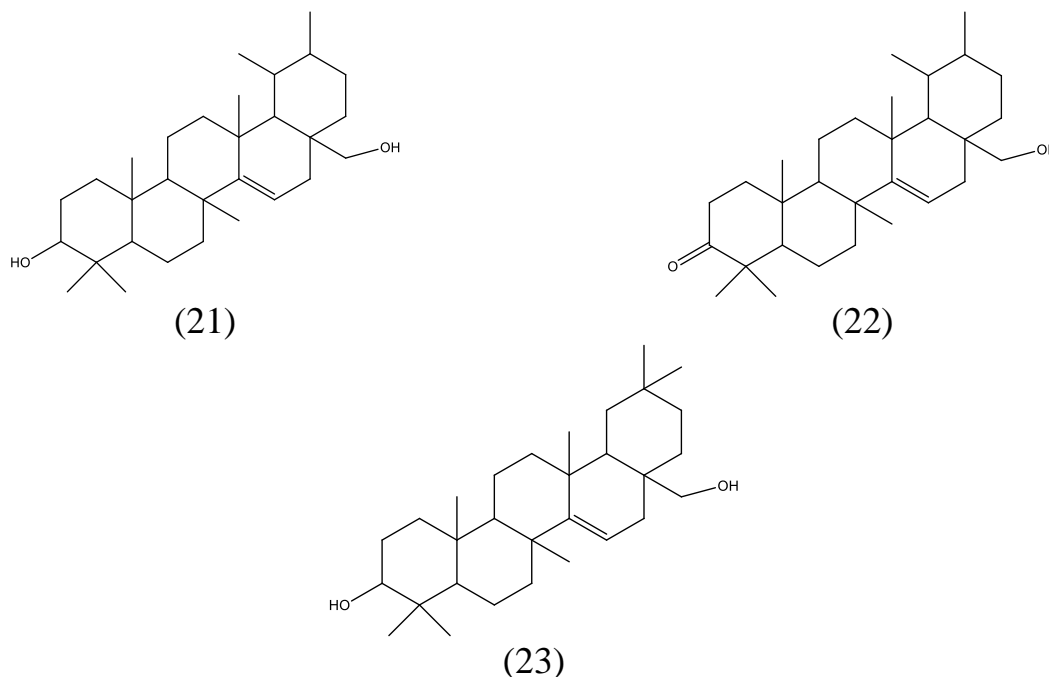
1.6.1.1 Isolation and characterization of saponins

The unique chemical nature of saponins demands tedious and sophisticated techniques for their isolation, structure elucidation and analysis. The task of isolating saponins from plant material is complicated also by the occurrence of many closely related substances in plant tissues, and by the fact that most of the saponins lack a chromophore. Thus, for many years, the complete characterization of saponins from even well-known saponin-containing plants was not achieved. However, recently renewed interest in medicinal plants and foods alongside the dramatic evolution of analytical tools has resulted in a burst of publications presenting numerous novel saponins. There are several modern strategies available for the isolation of saponins. As a general rule, they begin with the extraction of the plant material with aqueous methanol or ethanol. Further processing of the extract is carried out after evaporation under reduced pressure, dissolution in a small amount of water and phase separation into *n*-butanol. It is currently recognized that this step is sometimes undesirable, since only those saponins with short oligosaccharide side chains will eventually be extracted into the butanolic phase [35]. Precipitation method (an initial purification method) was also used in the extraction of saponins. Saponins would precipitate when a large quantity of diethyl ether or acetone was added to the methanol or ethanol extract rich in saponins. Ultrasound-assisted extraction was evaluated as a

simpler alternative to conventional extraction methods for the isolation of saponins from various types of plants. It was found that sonication-assisted extraction of saponins was about 3 times faster than the traditional extraction method. In addition, it can be carried out at lower temperatures, which are favorable for thermally unstable compounds [49]. A further purification is then carried out, which involves liquid chromatography over a silica gel column or a gradient elution from a polymeric support or liquid–liquid partition chromatography or as most commonly employed HPLC separation. In most cases, certain of the above steps have to be repeated with a change of support or eluent to achieve high purity. Once the saponin has been purified it may be subjected to analytical methods including MS, proton and carbon NMR, and infrared spectroscopy. Other classical methods are used to ascertain the presence of saponins in a crude plant extract, and to elucidate their composition throughout purification steps. TLC and staining with dehydrating reagents containing aromatic aldehydes (such as anisyl aldehyde in sulfuric acid) are commonly used. The pure saponin may also be hydrolyzed to verify the nature of its glycosidic moieties [35].

Immunoassays are non-chromatographic techniques which use monoclonal antibodies (MAbs) against drugs and low molecular weight natural bioactive compounds are becoming an important tool in saponin analyses. They show high specificity and sensitivity for receptor binding analyses, enzyme assays and qualitative and quantitative analytical techniques. They find an application in new Eastern blotting and in immuno-affinity column chromatography, for diagnosis, therapy and drug monitoring. Enzyme-linked immunosorbent essay (ELISA) based on MAbs are in many cases more sensitive than conventional HPLC methods. Number of MAbs was also developed for saponins. These include ginsenoside Rb1 (measuring range of ELISA assay from 20 to 400 ng/ml), ginsenoside Rg1 (measuring range 0.3–10μg/ml), glycyrrhizin (measuring range 20–200 ng/ml), saikosaponin measuring range 26 ng/ml to 1.5μg/ml), oleanolic acid (lowest concentration 100–200 nmol/μl) and solamargine [50].

Thin-layer chromatography (TLC) is becoming rather a supporting technique in analysis of saponin fractions obtained from column chromatography. This has been also used for confirmation of purity and identity of isolated compounds. Some trials of using TLC for qualitative and quantitative analysis of multicomponent saponin mixtures were also reported. The TLC separation of 18 saponins of *Medicago sativawas* developed. The technique was based on two-dimentional (2D) TLC with a sorbent gradient. First development was performed on RP-18 plate and in the perpendicular direction on silica gel plates. Since the use of RP-18 and silica gel as a bilayer is rather complicated due to possibility of modification of stationary phases by first solvent system, two single layer TLC plates were used. Such a sorbent gradient 2D TLC gave substantially better separation of saponins as compared to normal 2D TLC on one sorbent [50].

A preparative separation of individual saponins for structure determination and biological activity evaluation was performed by combinations of low-pressure liquid chromatography (LPCC). For this purpose proper careful selection of stationary and

mobile phases was essential for successful work. There were still a limited number of possibilities as regard to stationary phases. Most frequently, the first step of crude extract separation employed Sephadex LH-20 molecular filtration, which allowed preliminary separation of complex matrix of the extract into saponins and into accompanying impurities. In some cases during this preliminary separation, simultaneous partition of mixtures of saponins into simpler subfractions could also be achieved. Similar goal could also be accomplished by passing water solution of plant crude extract trough a porous polymer gel column Diaion HP-20 (synthetic polyaromatic adsorbent with pore volume 1.3 ml/g, surface area 500 m^2/g, pore radius >200). Washing this column with water removed some impurities and adsorbed saponins which were eluted with MeOH. Plant extract could also be separated to different classes of phytochemicals by selective solid phase extraction/fractionation on RP-18 short column.

Next step of saponin isolation includes column chromatography (CC) on silica gel with mobile phase composed of different combinations of $CHCl_3$–MeOH–H_2O and EtOAc–MeOH–H_2O, or on reversed phase silica gel RP-18, with mobile phase composed of MeOH–H_2O or MeCN–H_2O and their acidified (HOAc, TFA) modifications. In most cases, one column separation was not efficient enough and combination of normal and reversed phase separation were needed. Final purification could also be accomplished using HPLC systems with normal or reversed phase columns, but this procedure was rather tedious and did not allow obtaining bigger amounts of pure saponins. Higher quantities of separated compounds could be obtained from high-speed counter-current chromatography, the system working without any solid support, with separation based on fast partitioning effects of the analytes between two immiscible liquid phases. With this technique irreversible absorption effects and artifact formation was minimal [50].

The absence of a chromophore in saponins hampers their detection in ultraviolet light and allows non-specific detection at 200–210 nm. Thus, most of published data are based on recording HPLC profiles at 200–210 nm. But at this wavelength other than saponin components of the analyte may overlap with saponins making determination difficult. To overcome these problems and to be able to develop validated analytical methods for quality control of some products; several trials were performed to apply evaporative light scattering detection (ELSD) for detection of saponins. Validated HPLC method with ELSD was also developed for determination of major ginsenosides in samples of Chinese traditional medicine. Saponins were successfully separated on Spherisorb ODS2, C18 column in MeCN–H_2O gradient and quantified using calibration curves, with detection limits of 50 ng [50].

Difficulties in detection of saponins by the LC/UV methods encouraged development of hyphenated techniques combining liquid chromatography and mass spectrometry (LC/ESIMS). Applying MS to structural and analytical problems has become increasingly common over last few years, and MS has been extensively used to characterize, to confirm and to determine saponins in plant extracts. Most extensive work was performed on commercially important plants. This technique is

still a challenge and has several limitations. For the development of reliable routine method the availability of appropriate standards obtained by classical separation methods is essential. Even structural confirmation is not absolutely certain by this technique [50].

Sephadex LH-20: is a cross-linked dextran gel used in liquid chromatography. The separation is based on molecular size. In addition, it has the ability to separate according to selective absorption, as long as the compound has a molecular mass below 1000 g/mol. Sephadex LH-20 has been specifically developed for gel filtration of natural products, i.e. terpenoids, lipids, steroids and low molecular weight peptides, in organic solvents. It is also widely used for initial fractionation of crude extracts of highly polar compounds. The hydroxypropylated dextran gel forms a straightforward, universal and powerful separating system with pure solvents like methanol, acetone, methylene chloride, chloroform, ethyl acetate and N-methyl-2-pyrrolidone as mobile phases. The name "Sephadex" stems from separation, pharmacia dextran [51].

Reverse phase chromatography: When separating natural products, reverse phase (RP) flash chromatography is commonly used. This technique enables easy separation of saponins from other polar components, such as glycosides and oligosaccharides. In reverse phase chromatography the stationary phase is non-polar (lipophilic) and the mobile phase polar (hydrophilic). The stationary phase is usually modified silica with a surface bound to long-chained or substituted alkyl groups, or to other hydrocarbons. Solvents used as mobile phase are usually mixtures of water and water-miscible organic solvents such as MeOH, ACN or THF. The mechanism of retention varies depending on the properties of the RP material used. Retention is partially caused by direct interactions with the surface of the stationary phase or parts of the surface, and partially due to partition chromatography in a stationary phase containing solvent-solvated ligands. The most important interactions between the compound and the stationary phase are van der Waal forces. These relatively weak forces increase with molecular size. RP materials with long-chained hydrocarbon groups, such as C-18, give greater retention than hydrocarbons with shorter chains. In liquid chromatography, systems involving RP are commonly used. Polar molecules will have less affinity for the stationary phase and therefore elute faster. When increasing the polarity of the mobile phase, the elution becomes slower. It is also worth emphasizing the importance of the mobile phase being an aqueous solution. The general increase in retention with increasing solute size and reduced retention of polar solutes and ions, establish favorable intermolecular interactions with water. This is due to the high cohesive energy, hydrogen-bond acidity and dipolarity of water. This is the inverse of what is seen in normal phase chromatography. There are strong intermolecular interactions between water molecules that tend to promote self-association over interactions with different solvent or solute molecules [51].

1.6.1.2 Identification of triterpenes and saponins by NMR and Mass spectrometry

Triterpenes and Saponins have a very complex structure, which is hard to elucidate. The benefit of using nuclear magnetic resonance (NMR) in combination with mass spectrometry (MS) is that this allows an examination of the intact saponin, instead of using cleavage reactions to cleave off sugar moieties from the sapogenin and analyze them separately.

^{1}H and ^{13}C-NMR spectrometry is widely used to determine the structure of triterpenes and saponins. Where the glycosidic linkages to the aglycone are positioned; the number, sequence and nature of monosaccharide units; configuration of the interglycosidic linkages; presence of acyl glycosides in the chains; what kind of aglycone the saponin has, and the structures of eventually attached esters. By use of various 2D-NMR techniques, in a specially adapted procedure, we can determine the structure of a triterpene saponin. Methods such as H, H-COSY and TOCSY can be used to establish the nature of the monosaccharide. TOCSY also confirmed the nature of the oligosaccharide. NOESY, ROESY and HMBC can be used to find the sequence of the oligosaccharide chain and the linkage site of glucuronic acid [51].

The location of the primary hydroxyl group at C_{23}, C_{24}, C_{29} and C_{30} in oleanenes may be determined from the chemical shifts of the hydroxymethylene carbons, as the equatorial hydroxymethylenes (C_{23} and C_{29}) are less shielded than their axial counterparts (C_{24} and C_{30}). Configurational determination of 2,3-dihydroxy and 2,3,23- and 2,3,24-trihydroxy substituents in oleanenes and ursenes by ^{1}H-NMR spectroscopy has been reported. However, ^{13}C-NMR data of these triterpenes are also extremely useful for the determination of configurations of 2,3-; 3,23-; 3,24- dihydroxy and 2,3,23 and 2,3,24- trihydroxy substituents. Comparison of the ^{13}C data of two triterpenes containing equatorial and axial hydroxyl groups at C_3, reveals that not only the carbonyl carbon of the equatorial isomer is less shielded (δ 79.0) than the axial one (δ 76.4), but also the axial C_4 methyl and C_1 methylene groups are shifted by about 6.5 ppm and 2.0 ppm, respectively of one triterpene, in comparison to that of the other due to γ-gauche interaction. In triterpenes containing 2α,3β-, 2α,3α -, 2β,3α- and 2β,3β-hydroxyl groups, the C_2 and C_3 carbons resonate at δ 68.8,83.8; 66.5,78.9; 68.9,78.2; 71.0 and 78.4 ppm, respectively. Evidently the hydroxyl bearing C_2 or C_3 is less deshielded by the adjacent axial hydroxyl than by the equatorial one. In triterpenes bearing 3β,24 and 3α,24 hydroxyls the C_3 of the former is only slightly affected but the latter is shielded by about 6 ppm. Comparison of the ^{13}C data of triterpenes indicates that the C_3 carbon containing the equatorial hydroxyl is shielded by about 5 ppm by the 23-hydroxyl group but for the axial hydroxyl, it remains more or less unaffected. In triterpene containing lα,3β-hydroxyls, C_3 is shielded by about 7 ppm and in triterpene bearing 1β,3β-hydroxyls, the C_{25} methyl carbon is shielded by about 3 ppm due to γ-gauche interaction. In triterpenes containing axial and equatorial hydroxyl groups, respectively, the C_6 carbons resonate at δ 68.9 and 67.3 ppm i.e. the axial bearing carbon is less shielded than the equatorial counterpart. As already mentioned, this may be attributed to the 1,3-syn diaxial interaction [52].

The configuration at C18 in an oleanene triterpene, namely whether the oleanene triterpene belongs to the 18α- or 18β-series, can be recognized by inspecting the chemical shift of some characteristic carbons. The geometry of the D/E ring junction does not cause a significant alteration in the shielding of carbons in the A and B rings. In ring C; C_{12} and C_{13} of the 18α-series exhibit diagnostically valuable upfield shifts of about 5 and 2.5 ppm, respectively, with respect to the corresponding carbons of the 18β-series. For C_{12}, the upfield shift is attributed to the strong steric interaction with C_{19}. In ring D, C_{16} exhibits a significant downfield shift (~ 11 ppm) caused by the absence of two γ-gauche interactions with C_{19} and C_{21} in the triterpenes of 18α-series. The chemical shift of C18 is also sensitive to the change of absolute configuration at C18. The signals appear at about 7-8 ppm higher field in the 18α-series than in the 18β-series. This is ascribed to a new γ-gauche interaction with the C_{27} methyl group. The C_{28} signals in the 18α-series appear at a higher field of about 11 ppm, in comparison to those of the 18β-series, due to two γ-gauche interactions with the axial hydrogens at C_{19} and C_{21} [52].

Many of the pentacyclic triterpenoids occur as sugar conjugates, called glycosides. The sugar moiety of these glycosides is generally oligosaccharide, linear or branched, attached to a hydroxyl or a carboxyl group or both. The site of attachment may be one (monodesmoside), two (bisdesmoside) or three (tridesmoside). The glycosylation of a hydroxyl group, depending upon its nature (alcoholic and carboxylic), causes a change in chemical shifts at α and β-carbons and, rarely, γ-carbons to the OH group, in which the glycosylation takes place. These glycosylation shifts, i.e. chemical shift changes from aglycone and methylglycoside to triterpene glycoside, are characteristic of chemical and steric environments of the hydroxyl group in which the glycosylation takes place. The α-effect for sterically unhindered secondary alcoholic glycosides varies from 5 to 10 ppm and shows dependence upon its stereochemical relationship to the pyranose ring oxygen. The β-effect is always larger *(ca* 4 ppm) for the anti-related β-resonance, in comparison to *syn*-related β -resonance, which shows an upfield shift of *ca* 2 ppm. For the sterically hindered secondary alcoholic glycosides, the α-effect is usually greater (8-12 ppm) and the β upfield shift effect is relatively lower (1-3 ppm), or almost negligible, depending upon the magnitude of substitution. The quaternary β –carbons/either show little or negligible upfield shift and in some cases, small downfield shifts. The downfield shifts of α-resonances and upfield shifts of β -CH_2 resonances are useful for determination of the glycosylation sites [52].

Glycosylation of a carboxyl group causes a downfield shift (2-5 ppm) of the resonance of the carboxyl carbon, along with an upfield shift (0.5 2.0 ppm) of the β-carbon resonance. The anomeric carbon of the sugar linked to the carboxyl group resonates at a remarkably upfield position (93-97 ppm). These characteristics are helpful in identifying the sugar involved in glycosylation of the carboxyl group [52].

Mass spectrometry has some unique advantages over other physicochemical techniques. It has been found that, in general, the mode of fragmentation of triterpenoids is controlled by the position of double bonds (retro-Diels-Alder fragmentation) either originally present or generated by the elimination of water or

acetic acid and, therefore, characteristic features appear in the MS which frequently allow the assignment of a given triterpene to one of the major classes. In addition, the location of functional groups can often be narrowed down by consideration of the fragmentation pattern. The MS of C-7, C-8 and C-9 unsaturated compounds with the oleanane or ursane skeleton present an entirely different fragmentation pattern. These, compounds showed a base peak at M^+- 179 with strong peaks variously at m/e M^+ - 167 and 205. Furthermore, the C-12 oxygenated derivatives of C- 9 unsaturated oleanane compounds give base peaks which are believed to arise by retro-Diels-Alder decomposition of ring C [53].

Djerassi and his co-workers firmly established the retro-Diels-Alder reaction to be responsible for the prominent fragmentation of triterpenoids with the oleanane and ursene skeletons leading to the formation of the base peak ion at m/e 218 in which the charge remains with the diene. For example, each of commit acids A, B, C, D and E shows a base peak at m/e 218 (203 + Me). However, the position of the base peak in compounds in which there are substituents in rings D and E does not appear to follow a regular pattern. For example, whereas methyl ursolate and 2α-hydroxyuval show a base peak at m/e 203 as a result of loss of COOMe and COOH from rings D and E during electron impact. Thus with this class of compounds, the diagnostic base peak is at m/e 218, when the compounds contain no substituents in rings D and E. However, any other base peak which can be easily related to 203 by the addition of the masses of simple functional groups, like COOH, COOMe, OAc, OH, etc., are equally indicative of C-12 unsaturated pentacyclic triterpenoids with the oleanane or ursane skeleton. This later operation has a useful application because it may be used to detect the presence of such functional groups in rings D and E. Thus, a pentacyclic triterpenoid carboxylic acid which shows a base peak at m/e 248 could probably be a C-12 unsaturated compound containing a COOH group in ring D or E [54].

The ionization method in MS depends on the polarity, liability and molecular weight of the compound to be analyzed. Previously, ionization techniques such as fast atom bombardment (FAB) and chemical ionization (CI) have been applied to find important structural information, like molecular weight and sugar sequence, for naturally occurring glycosides. These techniques enabled analysis without derivatization of the glycosides. However, the matrix background generates a chemical noise, which reduces the sensitivity of the FAB method. For the most common MS method, electron impact (EI), samples need to be volatile and thermally stable. Saponins require conversion to permethyl or peracetyl derivatives in order to be analyzed by EI. This derivatization also has its limitations, since it is not applicable to saponins containing more than three sugar moieties. Electrospray ionization (ESI) has been reported as a powerful tool in determine the molecular weight of saponins due to its high sensitivity, rapid analysis time and low levels of sample consumption. This ionization technique combined with collision-induced dissociation (CID) can aid in identification of the backbone and glycosidic linkage sites of the saponins. CID is a process where energy is transferred to an ion through collision with a neutral collision gas (He, N_2, Ar). This energy transfer is sufficient to

result in bond cleavages and rearrangements of the selected ion. Fragmentation will be possible for gaseous ions that are otherwise perfectly stable [51].

1.6.2 Polyphenolic compounds

Plants produce a large variety of secondary products that contain a phenol group, a hydroxyl functional group on an aromatic ring called Phenol, a chemically heterogeneous group also. They could be an important part of the plants defense system against pests and diseases including root parasitic nematodes, the phenolic compounds include Coumarin, Ligin, Flavonoids, Isoflavonoids and Tanins [32].

Polyphenolics represent the most common phytochemically investigated compounds. These different phenolic compounds include flavonoids, chromons, coumarins, organic acids and lignans.

The unique sulphated phenolics, gallic acid 3-methyl ether 5-potassium sulphate (24), isoferulic acid 3-potassium sulphate (25), and ellagic acid 4,4`-dimethyl ether 3-potassium sulphate (26) have been isolated from the flowers of *Tamarix amplexicaulis* Ehrenb. (*Tamaricaceae*). The hitherto unknown natural phenolic acid, gallic acid 3-methyl ether (27), together with the known phenolic, gallic acid, gallic acid 4-methyl ether, isoferulic acid, ferulic acid, ellagic acid, and ellagic acid 4,4`-dimethyl ether have been also separated and characterized. The structures were established by conventional methods, including electrophoretic analysis and confirmed by ESIMS, ^{1}H- and ^{13}C-NMR [55].

(24)   (25)

(26)   (27)
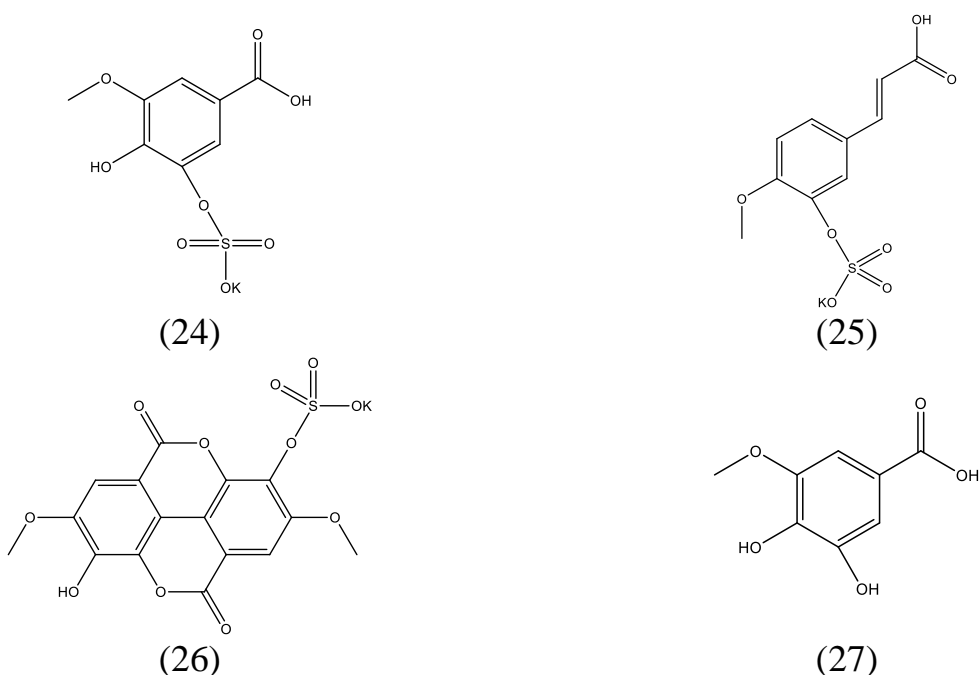
Also Ferulic acid was isolated from *Eurotia ceratodes* [56], and Gallic acid, 3-methoxy-5-O-sulfate were reported from *Frankenia laevis* [57]. Furthermore Dehydrodigallic acid isolated also from *Tamarix nilotica* [40], Dehydrotrigallic acid from *Tamarix aphylla* [58], and Ellagic acid; 3,8-Di-Me ether, 2- O –sulfate from *Tamarix tetragyna* [59] Also from the root of *Limonium gmelinii* isolated (-)-Epigallocatechin-3-O-gallate, (-)-Epigallocatechin, and (+)-Gallocatechin [60].

1.6.2.1 Chromones

Two 2,3-unsubstituted chromones were isolated from the reddish leaves and stems of glasswort (*Salicornia europaea* L.) and, on the basis of chemical and spectral evidences and syntheses of both of the compounds, they were identified to be 6,7-methylenedioxychromone (28) and 6,7-dimethoxychromone (29), respectively. This is the first report, which shows the natural occurrence of these two chromones [61] together with one known chromone; 7-O-beta-D-glucopyranosyl-6-methoxychromone (30) [62].
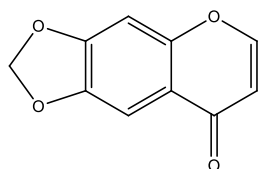
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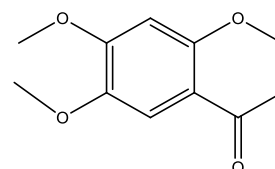
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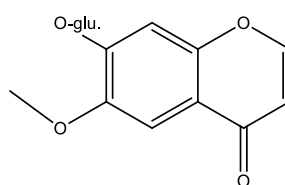
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The genus *Tamarix* also showed the presence of chromones which identified as 6-hydroxy-7,8 dimethoxy-4-methyl coumarin (Troupin) (31) from *Tamarix troupii* [63] *and* 3,4,8,9,10-Pentahydroxy-6 H -dibenzo[b,d]pyran-6-one (32) from *Tamarix nilotica* [64].
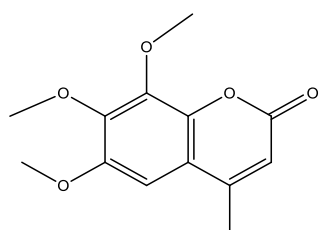
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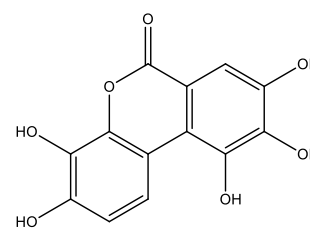
(32)

Camphorosma monseliacum is another halophyte plant which revealed the presence of chromones, from it isolated three chromones which identified as 7-methoxy-2-phenoxychromone (33), 5,7 dimethoxy-2-(4`-hydroxyphenoxy) chromone (34) and 5,7 dihydroxy-2-(4`-methoxyphenoxy) chromone (35) and this was the first report of isolation of these chromones from genus *Camphorosma* [65].

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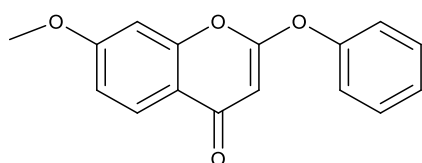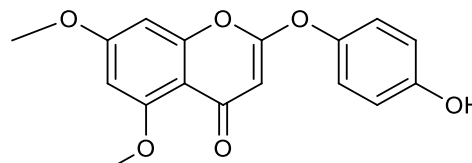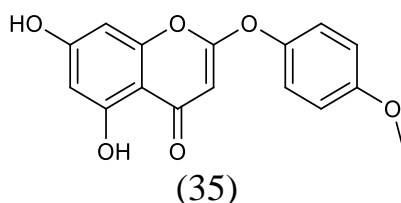
1.6.2.2 Flavonoids

Flavonoids: One of the largest classes of plant phenolic, perform very different functions in plant system including pigmentation and defense [32]. Examples of flavonoids which reported from halophytic plants were 3,5,7,3′,4′,6′-Hexahydroxyflavane which isolated by fractional extraction and adsorption-distribution chromatography from roots of *Limonium gmelinii* [60]. Isorhamnetin (36), rhamnocitrin (37), afzelin (38) and 5,3′-Dihydroxy-7,4′-dimethoxyflavone 3-O-*β*-D-glucopyranoside were also isolated from *Tamarix hispida* [66]. The flavonoid, tamarixetin (39) (3',3,5,7-tetrahydroxy-4'-methoxyflavone) isolated from *Tamarix ramosissima*, *Tamarix troupeiiand and Tamarix dioica* (an Indian shrub) [67-69].

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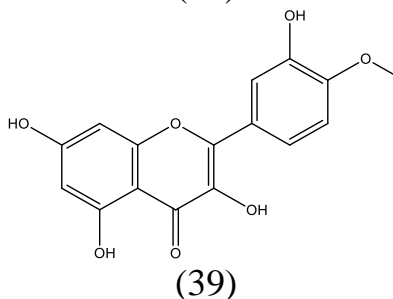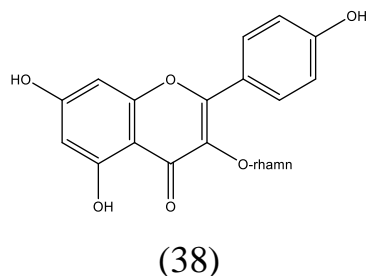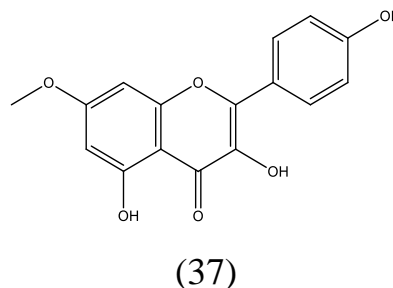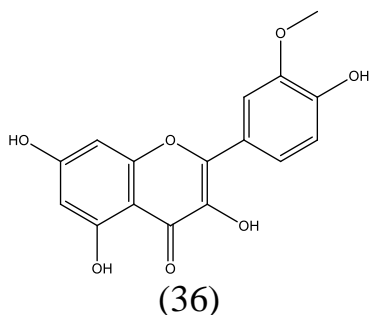
In addition to tamarixetin, various highly oxygenated bioactive flavones were isolated from the aerial parts of *Tamarix dioica namely*; (5,7,2'-trihydroxy-6,4'-dimethoxyflavone (tamaridone) (40); 5,2`,4`-trihydroxy -6,7,8-trimethoxy flavone (tamadone) (41); apigenin, gardenins A (42), B (43), C (44) and E (45) and nevadensin A (46) [67].

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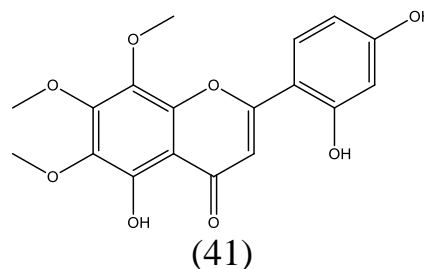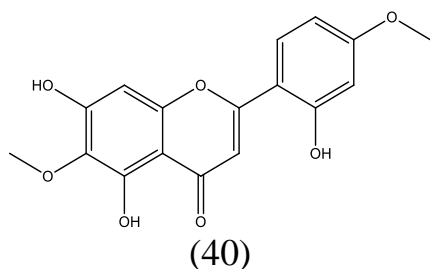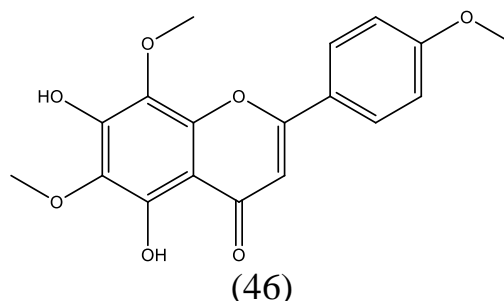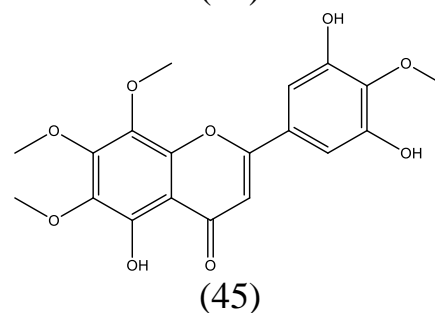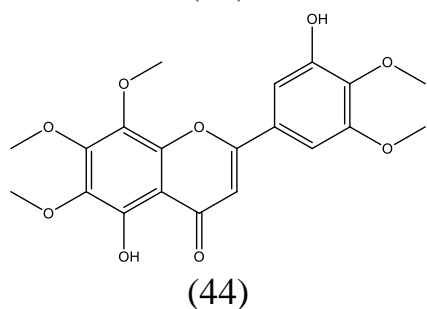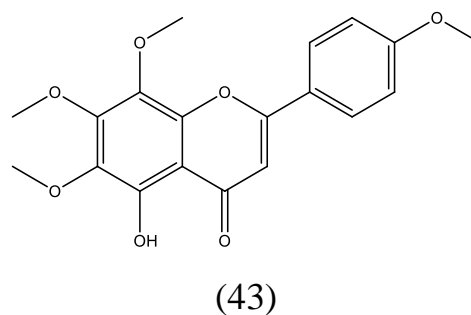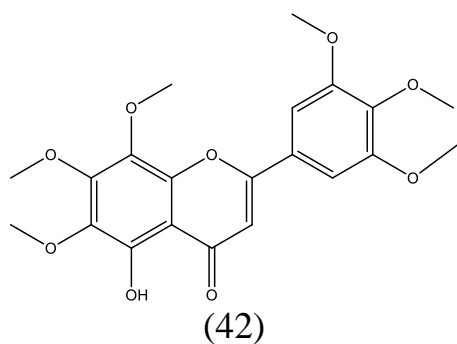
The aglycone Isorhamnetine reported also from *Tamarix laxa, Tamarix elongate, Camphorosma monseliacum* and *Tamarix* hispida [37, 66, 70]. *Furthermore,* Tamarixetine, Quercetine and Quercetine-3-O-*ß*-D-glucopyranoside (Isoquercetin), Tamarixetine-3-O-α-L-rhamnopyranoside, Tamarixetine-3-O-α-L-arabinopyranoside, Chrysoeriol were also isolated from *Tamarix laxa* and *Tamarix elongate,* these compounds isolated for the first time from plants of the genus *Tamarix* and the *Tamaricaceae* family [70, 71]. The Isorhamnetine-3-O-*ß*-D-glucopyranoside isolated from different plants belonging to halophytes such *Camphorosma monseliacum, Tamarix laxa, Tamarix elongate* and also isolated for the first time from *Climacoptera obtusifolia* together with isorhamnetin 3-O-[α-L-rhamnopyranosyl-(1→6)-*ß*-D-glucopyranoside (Narcissin) (47) [37, 44, 71], this flavonoid (Narcissin) with Quercetin-3-O-*ß*-D-galactopyranoside (Hyperin) (48) were also isolated from two *Climacoptera* species; *Climacoptera lanata* and *Climacoptra aralensis* [43].

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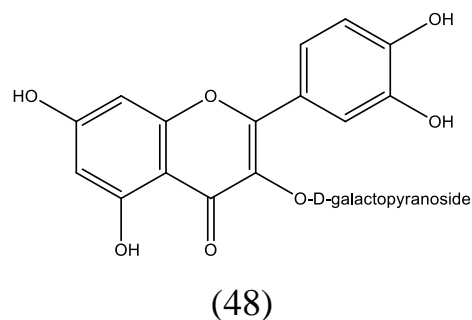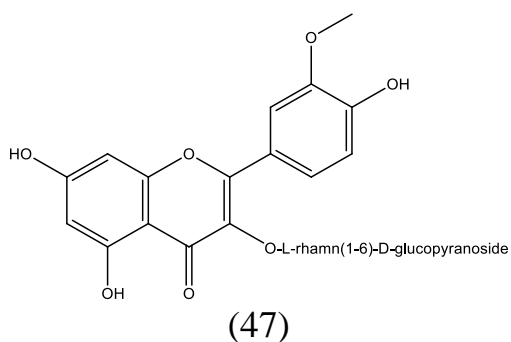
Isorhamnetine-3-O-*β*-D-galactopyranoside is another flavonoid glycoside isolated from *Camphorosma monseliacum* and were isolated for the first time from a plant of the *Camphorosma* genus [37]. The genus *Limonium* one of the largest halophytes containing genus, from many plants of this genus also reported many polyphenolic compounds for example 3,3',4',5,5',7-Hexahydroxyflavone; 3-O-*β*-D-Xylopyranoside, Myricitine-3-O-[α-L-Rhamnopyranosyl-(1→2)-α-L-rhamnopyranoside] and 3,3',4',5,5',7-Hexahydroxyflavone; 7-O-α-L-Rhamnopyranoside (Myricitin-7-rhamnoside) (49) isolated from *Limonium gmelinii* [72],while 3,3',4',5,5',7-Hexahydroxy flavone; 3-O -[4-Hydroxybenzoyl-(1→2)-α-L-rhamnopyranoside] [2``-O-(4-hydroxy benzoyl) myricitin] (50) were isolated from *Limonium sinense* [73], in addition to a new *flavonol glycoside* 3,3',4',5,5',7-Hexahydroxyflavone; 3-O-*β*-D-xylo–Hexulopyranoside (Myricitine 3-*β*-D-sorboside) (51) was identified from *Limonium axillare* [74], and from *Limonium sinuatum* isolated two flavonoids; 3,4',5,7,8-Pentahydroxyflavone; 3,8-Di-O-*β*-D-glucopyranoside (Herbacetin-3,8 diglucoside) (52) and 3,4',5,5',7-Pentahydroxy-3'-methoxyflavone; 7-O-*β*-D-Glucopyranoside (Laricitrin-7-O-glucoside) (53) [75].

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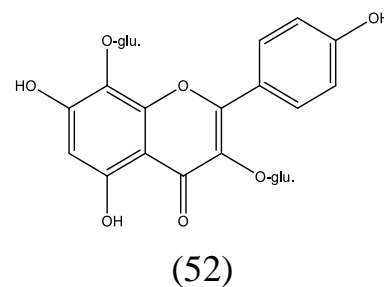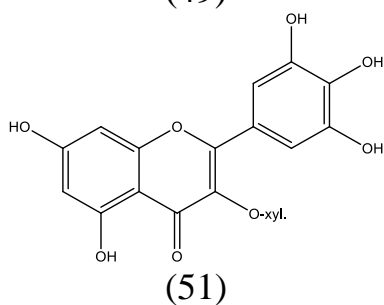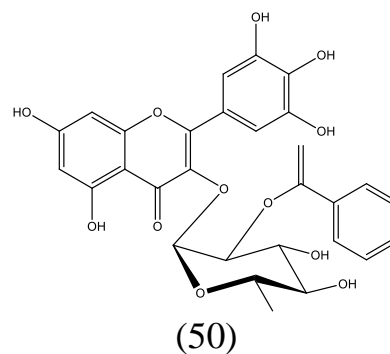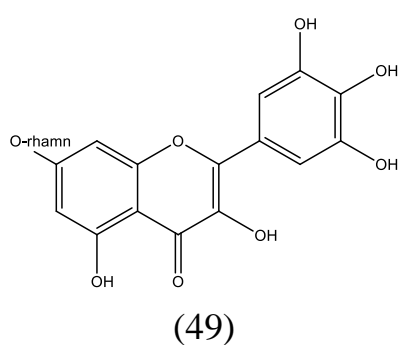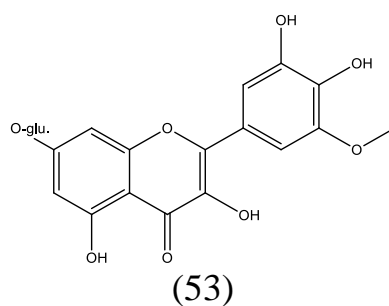
Because the halophytes plants characterized by the presence of many salts, also the flavonoids reported from it contains some salt derivatives; sulphated flavonoids were the most abundant and reported in many halophytic plants; as 3,4',5,7-tetrahydroxyflavone; 3,7-di-O –sulfate (Kaempferol-3,7 disulfate) (54) which isolated from *Frankenia laevis* and *Reaumuria mucronata* [76], also 3,3',4',5-tetrahydroxy-7-methoxyflavone;3'-O-*β*-D-glucuronopyranoside, 3,4',5-tri-O–sulfate (55) reported from *Tamarix aphylla* [77], 3,3',5,7-tetrahydroxy-4'-methoxyflavone;3-O–sulfate (Tamarixetin-3-sulfate) (56) from *Tamarix elongate* and *Tamarix laxa* [70], 3,5,7-trihydroxy-4'-methoxyflavone;3-O –sulfate (57) from *Tamarix spp.* [78], 3,5-dihydroxy-4',7-dimethoxyflavone; 3-O–sulfate from *Tamarix aphylla* [79], and 3,5-dihydroxy-4',7-dimethoxyflavone; 3,5-Di-O–sulfate (58) from *Tamarix amplexicaulis* [55].

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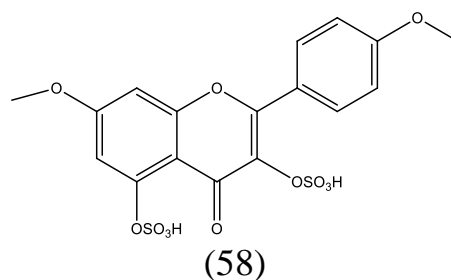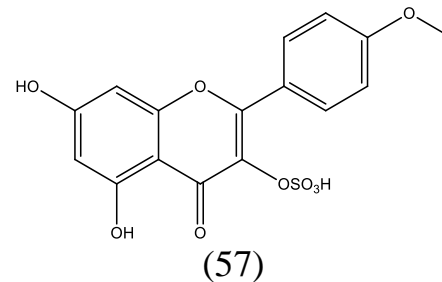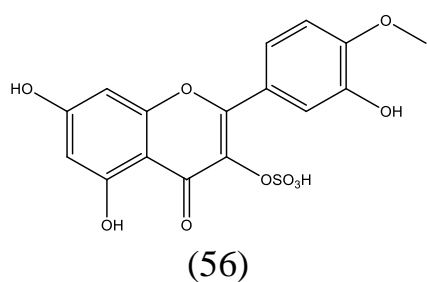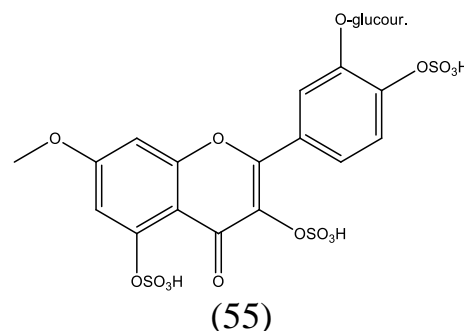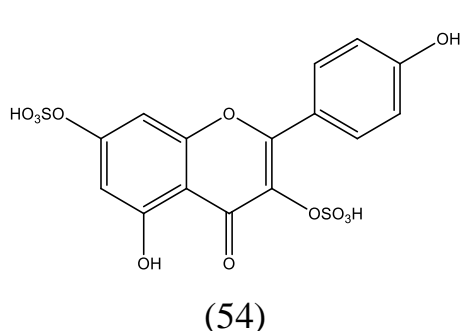
Isoflavonoids and aurones are another two groups identified in halophytes; as 2',6,7-trihydroxyisoflavone; 6,7-methylene ether (59) (2`-hydroxy-6,7 methylenedioxy isoflavone) and 2',7-dihydroxy-6 methoxy isoflavone (60) which isolated from *Salicornia europaea* [80], 3',4,4',6-tetrahydroxyaurone; 4-O-*β*-D-glucopyranoside (Cernuoside) (61) isolated from *Limonium bonduelli* [81], and 4,4',6-trihydroxyaurone (62) from *Limonium sp*.[82].
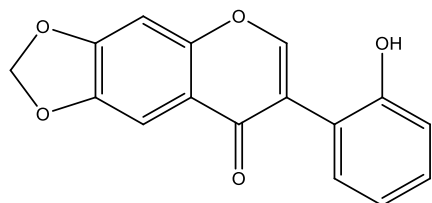
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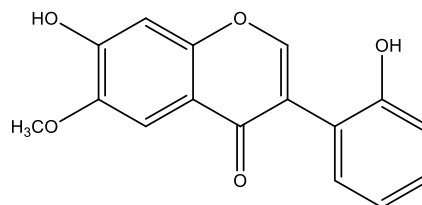
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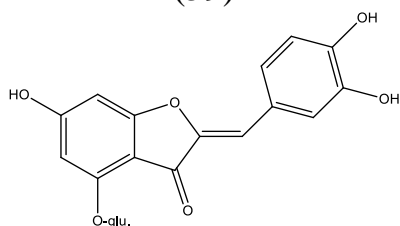
(61)
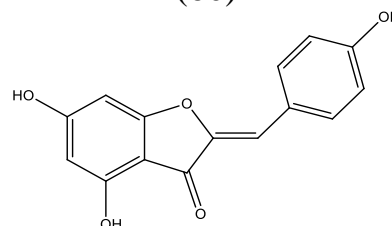
(62)

1.6.3 Isolation of essential oils from halophytes

Essential oils (also called volatile or ethereal oils, because they evaporate when exposed to heat in contrast to fixed oils) are odorous and volatile compounds found only in 10% of the plant kingdom and are stored in plants in special brittle secretory structures, such as glands, secretory hairs, secretory ducts, secretory cavities or resin ducts [83].

Essential oils are becoming more popular, because many synthetic drugs are connected with unpleasant side effects, such as nephrotoxicity or ototoxicity. Volatile oils also represent an interesting alternative due to an emerging resistance of microorganisms against synthetic agents. Essential oils can exert not only bacteriostatic and bactericidal effects, but also demonstrate activity against fungi and yeasts [84]. Essential oils are complex mixtures of volatile, lipophilic and odiferous substances from the secondary metabolism of plants. They are mainly composed of monoterpenes, sesquiterpenes and their oxygenated derivatives (alcohols, aldehydes, esters, ketones, phenols and oxides) [85].

The knowledge of composition of essential oils and their therapeutic properties have contributed to the development of their cultivation and markets. Although only 100 species are well known for their essential oils, there are over 2000 plant species distributed over 60 families such as *Lamiaceae*, *Umbelliferae* and *Compositae*, which can biosynthesize essential oils. They are about 3,000 essential oils, out of which approximately 300 are commercially important and are traded in the world market [83].

The plants having essential oils generally have the highest concentration at some particular time. Therefore better yield of essential oil plant material have to be

collected at this particular time. e.g. From jasmine at sunset. There are four methods of extractions of oils.
a) Expression method
b) Steam distillation method
c) Extraction by means of volatile solvents
d) Adsorption in purified fats

Steam distillation is most widely used method. In this method, macerated plant material is steam distilled to get essential oils into the distillate form these are extracted by using pure organic volatile solvents. If compound decomposes during steam distillation, it may be extracted with petrol at 50 °C. After extraction, solvent is removed under reduced pressure [86].

The volatile oils were also reported in a number of halophytic plants and revelaed the presence of valuable and useful components as in *Tamarix boveana;* The chemical composition of the volatile oils obtained from the whole aerial part, flowers, leaves and stems by steam distillation was analyzed using gas chromatograph (GC)-flame ionization detectors (FID) and GC–MS. Sixty-two components were identified. Hexadecanoic acid (18.14%), docosane (13.34%), germacrene D (7.68%) (63), fenchyl acetate (7.34%) (64) and Benzyl benzoate (4.11%) were found to be the major components in the whole aerial parts. This composition differed according to the tested part: 2.4 Nonadienal (65) was the main compound in the flowers (12.13%) while germacrene D was the major component in leaves (31.43%) and hexadecanoic acid in the stems (13.94%) [87].

(63)

(64)

(65)
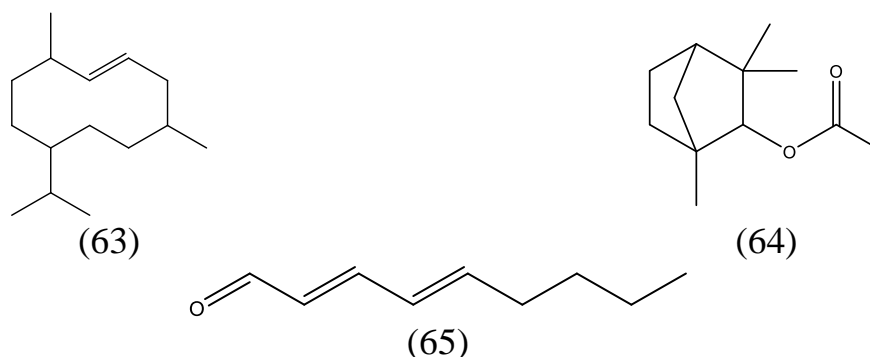
Also the volatile constituents of the essential oil of *Zygophyllum album* L., have been analyzed and a total of 100 volatile compounds were identified and (*E*)-β-damascenone (11.8%) was the major component of the oil [88].

1.7 Biological review of halophytes
At least 120 families of the Kazakhstan flora possessed more than 50 different kinds of biological activity. As an example, antibacterial activity was revealed in about 60% of families (95 of 161) and about 30% of genera (320 of 1118); fungicidal in about 50 and 23%, antiprotozoal in 9 and 33%, and insecticidal in 9 and 27% of the families and genera, respectively. In about 21% of the families and 5% of the genera there were species with antiviral activity and in about 16% and 5%, respectively with antitumor activities [89].

In Central Asia and the Caucasus, tinctures, decoctions of *Camphorosma lessingii* used externally for treatment of fungal diseases of the skin; whereas internally used as diuretic, diaphoretic and for treatment of rheumatism [14].

Moreover the plant *Zygophyllum fabago* is used for treatment of some diseases of the bladder, rheumatism, skin diseases, as an anthelmintic, anti-cephalic and laxative. The roots in Central Asia used as broth for carbuncles, wound healing, while leaves used fresh as blister. In the Caucasus also used as an anthelmintic, wound healing and detoxification. Ointment in clinical trials proved to be effective for boils, carbuncles, dermatitis, and chronic eczema. Also noticed that the feeding of dry and fresh plants in the experiments resulted in the death of guinea pigs and rabbits [90].

1.7.1 Economic potentials of halophytes

1) Medicines: Ethno-botanical studies on coastal halophytes have been conducted, which revealed about 50 plant species with different medicinal properties. Different plant parts of these plants are used to treat 12 disease conditions however, the use of leaves was highest followed by whole plants. Proper identification of bioactive compounds responsible to cure different diseases may not only help in uplift of local pharma-industry but also the socio-economic status of the people [91]. Halophytes may be potentially useful for economic applications as new sources of natural antioxidants in dietary food. Halophytes are known for their ability to resist and quench toxic reactive oxygen species (ROS) because they are equipped with powerful antioxidant enzyme systems. Crude methanolic extracts of halophytes have been investigated for their anticarcinogenic and chemopreventive activities by evaluating the levels of hepatic antioxidant defense [92]. Non-enzymatic antioxidants such as ascorbic acid, α-tocopherol, flavonoids and phenolics acids are present in the halophytes plants. For example, the halophyte plant *Crithmum maritimum* or sea fennel (family: *Apiacea*) contains vitamin C, carotenoids. *Limonium axillare* (family: *Limoniacea*, order: *Plumbaginales*) contain flavonoids [93]. Another report demonstrate the medicinal importance of coastal halophytic species along the Arabian Sea coast which are reported for their use in treating a number of ailments in local communities (table 1) [94].

2) Edible oil: Seeds of many halophytes contain appreciable amount of oil and may serve as a source of edible oil. Recent Research results revealed that the seeds of *Suaeda fruticosa* and *Arthrocnemum macrostachyum* contain about 25% oil, while those of *Halopyrum mucronatum, Cressa cretica, Haloxylon stocksii* and *Alhaji maurorum* contain 22.7%, 23.3%, 23.2% and 21.9% oil respectively. Shoots of *Salicornia bigellovi, Sesuvium portulacastrum, Chenopodium album, Portulaca oleracea,* and *Suaeda maritima* are used for vegetables, salads and pickles. Contents of unsaturated fatty acids were high (65–74%) in oils derived from halophytes due to the presence of 12 important unsaturated fatty acids [91, 94].

Table 1 – Traditional uses of halophytes reported as medicine

Name of species	Family	Preparation	Medicinal use
1	2	2	4
Acacia nilotica L.	*Mimosaceae*	Decoction	Asthma, diarrhea, demulcent
Achyranthes aspera L.	*Amaranthaceae*	Powder	Asthma, cough, pneumonia, joint pain, labor discomfort
Aerva javanica (Burm. f.) Juss.	*Amranthaceae*	Paste/ Decoction	Wounds, Jaundice, Diabetes, cough, headaches
Alhagi maurorum Medic.	*Fabaceae*	Decoction	Laxative, diuretic, eye infections
Amaranthus virdis L.	*Amaranthaceae*	Potherb	Constipation, gall bladder/kidney stones
Arthrocnemum indicum (Willd.)	*Chenopodiaceae*	Ashes	Alexipharmic
Atriplex stocksii Boiss	*Chenopodiaceae*	Infusion	Fever, jaundice, dropsy, liver disease
Calotropis procera (Ait.) Ait. f.	*Asclepidaceae*	Paste	Tooth & stomach aches
Capparis decidua Forssk.	*Capparidaceae*	Decoction	Carminative, aphrodisiac, ulcer, cough, asthma, stomach aches
Chenopodium album L.	*Chenopodiaceae*	Potherb	Constipation
Citrullus colocynthis L.	*Cucurbitaceae*	Toothache, Pills,	Toothache, Constipation, bleeding, piles, diabetes, leucorrhea, Asthma
Cleome brachycarpa Vahl.	*Capparidaceae*	With sesame oil	Itching, Joint pain, skin diseases, inflammation
Cleome viscosa L.	*Capparidaceae*	Juice	Ear infection, pain, deafness
Corchorus depressus (L.) Stocks	*Tiliaceae*	Infusion	Dysuria
Cressa cretica L.	*Convolvulaceae*	Paste	Sores
Cymbopogon jwarancusa Boiss.	*Poaceae*	Decoction	Fever, catarrh, joint pain, inflammation
Cynodon dactylon (L.) Pers.	*Poaceae*	Paste	Cuts, wounds
Cyperus rotundus L.	*Cyeraceae*	Poultice	Acne, Carbuncle
Desmostachya bipinnata L.	*Poaceae*	Potherb	Carbuncle
Digera muricata (L.) Mart	*Amranthaceae*	Powder	Constipation
Euphorbia caducifolia Haines	*Euphorbiaceae*	Raw	Increase male sexual vigor
Fagonia indica Burm. f.	*Zygophyllaceae*	Decoction	Skin eruption, malarial fever
Glinus lotoides L.		Powder	Blood purifier
Gynandropsis gynandra (L.) Briq.	*Capparidaceae*	Decoction	Stomach ache and skin inflammation
Haloxylon stocksii (Boiss.) Benth. & Hook.	*Chenopodiaceae*	Decoction	Tooth and stomach aches
Heliotropium curassavicum L.	*Boraginaceae*	Paste	Boils

Table 1 continue

1	2	3	4
Heliotropium strigossum Willd.	*Boraginaceae*	Paste	Boils, Ulcer, wounds
Heliotropium. eichwaldi Steud.	*Boraginaceae*	Raw	Earache
Ipomoea pes-caprae (L.) Swt.	*Convolvulaceae*	Decoction	Diarrhea, pains, vomiting, inflammation of legs, piles
Leucas urticifolia (Vahl) Rr. B.	*Labiatae*	Decoction	Cold, fever, gastrointestinal problems
Peganum harmala L.	*Zygophyllaceae*	Decoction	Anti-microbial, colic, lumbago/Rheumatisms, back pains
Portulaca oleracea L.	*Portulacaceae*	Infusion	Stomach ache, gastric trouble
Rhizophora mangle L.	*Rhizophoraceae*	Maceration, tea	Diarrhea, diabetes
Suaeda monoica Forsk. ex Gmel.	*Chenopodiaceae*	Paste	Ointment for wounds
Salvadora oleoides Decne.	*Salvadoraceae*	Decoction	Purgative, aphrodisiac, diarrhea
Salvadora persica L.	*Salvadoraceae*	Decoction	Asthma, cough
Salsola imbricata Forssk.	*Chenopodiaceae*	Infusion	Insecticidal, Vascular hypertension
Solanum surattense Burm.f.	*Solanaceae*	Pills	Bronchial, asthma, headache, cough, joint pain And chest pain, vomiting, burning feet
Suaeda fruticosa Forssk.	*Chenopodiaceae*	Water and Alcoholic extracts	Antibacterial
Trianthema portulacastrum L.	*Aizoaceae*	Decoction	Asthma
Tribulus terrestris L.	*Zygophyllaceae*	Infusion	Urinary calculi, spermatorrhoea, general debility, Asthma, Kidney stones
Withania somnifera (L.) Dunal	*Solanaceae*	Decoction	Anthelmintic, leucorrhoea, tuberculosis
Zaleya pentandra (L.) Jeffrey in Kew Bull	*Aizoaceae*	Powder	Influenza, phlegmatic cough
Ziziphus nummularia (Burm. f.) Wight & Arn.	*Rhamnaceae*	Decoction	Digestive, blood purifier, sores, skin diseases
Zygophyllum simplex L.	*Zygophyllaceae*	Decoction	Helminthiases

1.7.2 Antidiabetic activity

The aqueous extract of *Suaeda fruticosa (Chenopodiaceae)* at a dose of 192 mg/kg produces a significant decrease in blood glucose levels in normal rats, and even more in diabetic rats. This hypoglycemic effect might be due to an extra-pancreatic action of the aqueous extract of SF, since that the levels of plasma insulin

unchanged between the values before and after treatment [95]. In addition, the ability of *Zygophyllum gaetulum* leaves to lower the blood glucose was studied in 13 patients with non-insulin-dependent diabetes mellitus. The reaction time of the *Z. gaetulum* aqueous extract at a single oral dose (440 mg/kg) producing a significant hypoglycemic effect is 6 h after administration, the same dose ingested twice daily resulted in a significant reduction of blood glucose during the first week, and maintained the patient in normoglycaemia throughout the 2 week course of treatment, with no change in body weight [96], in a different study the anti-diabetic activity of the same plant was carried out to evaluate its hypoglycemic activity, Oral and intraperitoneal administration of the plants (0.7 g/kg) produced a significant hypoglycemic effect in normal as well as in hyperglycemic rats. The infusions increased significantly plasma insulin levels in normal rats. It is suggested that the hypoglycemic activity of these plants may be mediated through enhancement of peripheral metabolism of glucose and an increase in insulin release [97]. Another study demonstrated that the treatment of diabetic rats with *Zygophyllum coccineum* L have exerted a considerable hypoglycemic effect. In addition, this herb could ameliorate the impaired renal function and inhibit liver damage associated with alloxan diabetes [98]. Also *Z. album* aqueous extract exerts anti-diabetic activity on streptozotocin (STZ)-induced diabetic mice [99]. Furthermore, the effects of oral administration of ethanolic *Zygophyllum album* extract during 15 days in Streptozotocin-induced diabetic mice were investigated. The extract shows a significant decrease of the activity of blood glucose, lipid peroxidation in the liver and the pancreas. Furthermore, the treated diabetic mice showed significant increase in enzymatic and nonenzymatic antioxidants of defense systems such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), vitamin C, glutathione reductase (GSH) and significant decrease in vitamin E. These suggest that the extract from *Zygophyllum album* could exert their antidiabetic activities through their antioxidant properties [100]. Another study of extraction of a total triterpene glycosides preparation from *Zygophyllum oxianum* and its influence on blood sugar levels in rats with alloxan hyperglycemia and alloxan diabetes are addressed. This preparation had marked hypoglycemic actions on prophylactic and therapeutic administration [19].

1.7.3 Antipyretic activity

The antipyretic activity of different extracts from *Calotropis procera, Cotula cinerea* and *Zygophyllum gaetulum* was investigated experimentally in rats. The antipyretic effect was retained in all extracts tested and was comparable to that of acetylsalicylic acid used as the standard drug [101].

1.7.4 Antimicrobial activity

The extract of *Zygophyllum fabago* was found to be highly effective against *Candida albicans* and *Escherichia coli [102],* while Chloroformic and methanolic extracts of the halophytes *Eryngium maritimum* L., *Crithmum maritimum* L. and *Cakile maritime* Scop. were tested for their antimicrobial activities against 12

bacterial and yeast strains; only one bacterial strain (*Listeria monocytogenes*) was not inhibited by plants extracts, and a polar (chloroformic) fractions were generally more active than polar (methanolic) ones [103]. Another study of the edible halophyte *Mesembryanthemum edule (Aizoaceae)* revealed that organ extracts showed activity against food borne bacteria and fungi. The inhibitory percentages ranged from 0 to 94%. Stems showed the strongest antibacterial activity (inhibition of the growth in 6 of the 7 strains tested) especially against *Micrococcus luteus* (82%) followed by leaves and roots. Regarding fraction polarity, 60% and 20% aqueous methanolic fractions were the most and the less active fractions, respectively. All the organ fractions showed a high antifungal activity, notably against *Kloeckera apiculata* (85% for leaf 40%) and *Candida albicans* (77% for stem 40%). Even more, leaf, stem and root fractions were very potent in inhibiting growth of filamentous fungi, with inhibition percentages varying between 23% and 99% [104]. Furthermore, *Tamarix gallica* L. extracts showed appreciable antibacterial properties against human pathogen strains. The mean inhibition zone was from 0 to 6.5mm when the concentration increased from 2 to 100 mg/l. The strongest activity was recorded against *Micrococcus luteus* and the lowest activity was observed against *Escherichia coli*. Moreover, organ extracts show a weakly to moderate activity against *Candida* [105]. In addition to the previous, the study of antimicrobial activity of different extracts of *Zygophyllum oxianum* against several bacterial strains and fungi showed that the *n*-butanol extract from all aerial parts exhibits significant activity against Candida sp. but only weak antibacterial activity [106]. Seven extracts of *Zygophyllum geatulum* endemic plant of Morocco, were tested against various pathogenic fungi using three different techniques, an agar diffusion method used to determine the active extracts, microbroth dilution to assess and determine the minimal inhibitory concentration (MICs) and a macro dilution method to evaluate effect on mold species growth. The results showed that methanolic, ethanolic, chloroformic and aqueous extracts are most efficient on the growth of yeasts. Methanolic extract exhibited a high inhibitory activity on all tested yeasts. MICs are ranged between 200 and 600 mug/ml. On the other hand. ethanolic extract is only active on *Candida albicans* strains with the MICs located between 0.8 and 1 mg/ml. However, chloroformic and aqueous extracts showed moderate effects, their MICs were ranged between 0.3 and 0.6 mg/ml. Effects of the different extracts on the growth of moulds showed that methanolic extract gave the best antifungal effect at 4 mg/ml. At 1 mg/ml it reduced growth of *Aspergillus flavus* and *Aspergillus parasiticus* respectively of 17% and 67%. Chloroformic extract decreased weights of *Aspergillus flavus, Aspergillus parasiticus, Aspergillus niger* and *Penicillium cyclopium*, respectively, of 80%, 65%, 85% and 89%. The ethanolic extract showed only a partial effect on the fungal growth [107].

1.7.5 Antioxidants

Zygophyllum album exhibited a significant antioxidant activity in experimentally diabetic mice [99] and exhibited high in vitro and ex vivo antioxidant activities [108], while the three halophytic species *Eryngium maritimum* L.,

Crithmum maritimum L. and *Cakile maritime* Scop. had relatively strong total antioxidant activities [103]. In addition, study of different organs extracts of *Mesembryanthemum edule* L. (sourfig, *Aizoaceae*) showed a high antioxidant activity as compared to positive control BHT, with maximal efficiency for stems followed by leaves and roots. The highest polyphenolic levels were found in stems and leaves (86.5 and 68.7 mg GAE g^{-1} DW, respectively), suggesting that their strong antioxidant activity could be attributed to these phytochemicals. The HPLC analysis revealed that the main phenolic compounds were quercitrin and avicularin (1.4 and 1.15 mg g^{-1} DW, respectively) in the leaves, while catechin and procyanidin B2 (1.66 and 1.54 mg g^{-1} DW, respectively) were the most abundant phenolics in the stems. Overall, the strong antioxidant activity and richness of *M. edule* aerial tissues suggest that it could be advantageously used as a functional or nutraceutical food, to prevent or moderate oxidative stress-related diseases [109]. Another halophytic plant *Nitraria tangutorun* Bobr. with two variations (purple fruit and red fruit) were investigated for antioxidant activity; the extract of the two variations showed significantly different antioxidant activity ($p<0.01$) according to DPPH, ABTS and FRAP assay. Purple fruit possessed higher antioxidant activity than red fruit. There were significant correlations between antioxidant activity and both the total polyphenol content and anthocyanins content [110]. In addition to the previous; extracts and fractions of *Limoniastrum guyonianum* Boiss. showed variable and powerful antioxidant and antiradical-scavenging activities. Therefore, *L. guyonianum* extracts could serve as valuable sources of natural antioxidants in both nutraceutical and food industries. This great antioxidant capacity of this halophytic species is related to the presence of potent flavonoids, with strong antioxidant ability [111]. Furthermore the flavonoids in ethyl acetate and butanolic fractions of *Atriplex halimus* possess potential antioxidant activity which explains the relation structure-activity [112]. Also the triterpene Norhederagenin 3-O-β-D-glucuronopyranosyl-28-O-β-D-glucopyranoside isolated from the halophyte *Salicornia herbacea* exerted significant antioxidant activity on both authentic peroxynitrite and peroxynitrite generated from morpholinosydnonimine (SIN-1) [113]. Also the methanol extract of *Salicornia herbacea* evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and β-carotene-linoleic acid bleaching test. The extract was an efficient radical scavenger (IC_{50} = 55.3 ± 2.7 μg/mL) and exhibited a moderate inhibition of lipid peroxidation of linoleic acid emulsion [114]. In addition, *Limonium axillare* extract showed a higher antiradical activity in comparison with BHA, Antioxidant activity was determined using NBT/Riboflavin method [93].

1.7.6 Cytotoxicity and Anticancer activity

The edible specie *Zygophyllum album* had appreciable anticancer activity, especially against lung carcinoma cell lines, and displayed an important anti-inflammatory activity in RAW 264.7 macrophages [108]. Another investigation of cytotoxicity assays of different extracts of *Zygophyllum oxianum* against 5637 human urinary bladder cells the *n*-butanol extract showed only weak activity. Otherwise the chloroform-methanol (2:1) extract of stems and leaves has a remarkable cytotoxicity

against 5637 cells with an IC$_{50}$ value of 6.2µg/ml [106]. From another species of *Zygophyllum*; 3β-(3,4-Dihydroxycinnamoyl)-erythrodiol is a cytotoxic compound isolated from the roots of *Zygophyllum geslini* which exhibits cytotoxicity towards KB cells [115]. 27-nor-triterpenoid glycoside, 3-O-β-D-glucopyranosyl-pyrocincholate, together with 3-O-6-deoxy-β-D-glucopyranosyl-pyrocincholate and quinovic acid are a number of compounds isolated from *Zygophyllum fabago* L. showed some antitumor activities by MTT assay [31].

1.7.7 Anti-inflammatory activity

The anti-inflammatory effect of ethanolic extract with aqueous extract of *Zygophyllum gaetulum* was estimated by measuring the oedema induced by carragenin according to the method of Winter and al. ethanolic extract of *Zygophyllum gaetulum* reduced the increase of the paw volume with a percentage of inhibition of 46% ($p<0.01$), this percentage was 47.48% ($p<0.01$) with aqueous extract. The inhibition decrease in time, it arrived to 39% ($p<0.01$) at the sixth hour while the activity of aqueous extract decrease a lot, [116]. *Zygophyllum simplex* Linn. is another species of *Zygophyllum* also showed anti-inflammatory activity; the ethyl acetate extract was screened for anti-inflammatory activity by carrageenan induced rat paw oedema in rats, a significant anti-inflammatory activities were observed at a dose of 100, 200 and 400 mg/kg body weight. Dose dependent anti-inflammatory activities were also observed [117].

1.7.8 Phytotoxicity and other activities

The effects of aqueous extracts from *Zygophyllum fabago* L. on two phytopathogenic fungal species (namely, *Fusarium oxyosporum* f. sp. *melonis* and *Pythium aphanidermatum*) were studied under laboratory conditions. The plant extracts inhibited the growth of *F. oxyosporum* and *P. aphanidermatum* (the maximum mean inhibition that was recorded with the 10% w/v extracts was 42.9% and 85.3%, respectively) suggesting the potential of *Zygophyllum fabago* as a growth inhibitor of *F. oxyosporum* and *P. aphanidermatum* [118]. Furthermore, extracts of roots and aerial shoots of *Zygophyllum coccineum* L., *Z. album* L. and *Z. dumosum* Boiss were tested against spore germination of *Verticellium albo-atrum* and *F. oxysporum* f.sp. lycopersici. the methanol extracts possessed a high inhibitory effect toward spore germination of both fungi, while the aqueous methanolic root extracts were inhibitory to the test fungi and that of aerial shoot showed specific suppression [119].

Isorhamnetin 3 - β-D-glucopyranoside isolated from *Salicornia herbacea* reported as potent candidate of antiobesity agent via alleviation of lipid accumulation [120]. In addition, extracts of *Zygophyllum coccineum* L. displayed significant antihypertensive properties in normotensive as well as in spontaneously hypertensive rats [121].

2 MATERIALS AND METHODS

2.1 Materials
2.1.1 Plant material

1) *Zygophyllum fabago:* was collected at Almaty region in May 2012 and identified by botanist N. G. Gemedzhieva, head of laboratory of plant resources, Sc.D. of biology, institute of botany and phytointroduction. The air-dried aerial parts of *Zygophyllum fabago* was cut into small pieces and stored at room temperature.

2) *Camphorosma lessingii:* The aerial parts of *Camphorosma lessingii* collected in August 2013 from Almaty region. The identification of the plant materials was confirmed by botanist K. T. Abdikulova, biodiversity and bioresource department, faculty of biology and biotechnology, Al-Farabi Kazakh National University. The green leaves were air-dried for one week, stored at room temperature. The dried samples were ground using a mill to obtain coarse powder.

2.1.2 General laboratory chemicals

Anisaldehyde (4-methoxybenzaldehyde)	fisher scientific
2-Butanol	fisher scientific
Dimethylsulfoxide	fisher scientific
Hydrochloric acid	fisher scientific
Potassium hydroxide	fisher scientific
Pyridine	fisher scientific
Concentrated sulphuric acid	fisher scientific
Concentrated ammonia solution	fisher scientific
Acetic anhydride	fisher scientific
Ortho-phosphoric acid 85% (p.a.)	fisher scientific
Trifluroacetic acid	fisher scientific

2.1.3 Chromatographic and UV materials
1) Stationary phases

Pre-coated TLC plates, Silica Gel $G_{60}F_{254}$, layer thickness 0.2 mm	Merck
Silica Gel 60, 0.04 - 0.063 mm mesh size	Merck
Pre-coated TLC plates , RP-18, F_{254} S, layer thickness 0.25 mm	Merck
RP-18, 0.04 - 0.063 mm mesh size	Merck
Sephadex LH-20, 0.25 - 0.1 mm mesh size	Merck
Diaion HP20	S6+upelco

2) Spray reagents

Vanillin/H_2SO_4, anisaldehyde/H_2SO_4, Ceric sulphate in 60%H_2SO_4, ammonia vapor, *o*-toluidine, ninhydrin, diazotized *p*-nitroaniline (DzPNA), 1% aluminum chloride solution, Ferric ammonium alum, Lieberman-Burchard reagent.

3) UV-Shift reagents

Sodium methoxide 2.5% in methanol (w/v), aluminum chloride 5% in methanol (w/v), hydrochloric acid 18% aqueous solution (v/v), sodium acetate anhydrous coarsely powdered.

The reagents were stored in amber-colored bottles and kept refrigerated until use. TLC (thin layer chromatography) and PC (paper chromatography) was used to monitor the identity of each of the fractions and the qualitative purity of the isolated compounds. It was also utilized to optimize the solvent system that would be applied for column chromatography. Tests of the major active constituents: the air dried powdered aerial parts of the plants subjected to quantitative phytochemical tests for determination of the percentage of different groups of naturally occurring compounds according to the reported methods [122-125].

2.1.4. Solvents
1) General solvents
Acetone, *n*-butanol, dichloromethane, ethyl acetate, *n*-hexane and methanol were used. The solvents were certified by ACS (American Chemical Society) and supplied from fisher scientific.
2) Solvents for NMR
Chloroform-*d*   Cambridge Isotope Laboratories, Inc.
DMSO-d_6 Cambridge Isotope Laboratories, Inc.
Pyridine-d_5 Cambridge Isotope Laboratories, Inc.

2.2 Methods
2.2.1 Chromatographic methods
Chromatography refers to any separation method in which the components are distributed between stationary phase and mobile phase. The separation occurs because sample components have different affinities for the stationary and mobile phases and therefore move at different rates along the TLC plates and the column.

2.2.1.1 Thin layer chromatography and paper chromatography
Paper chromatography – PC (paper brand Watman S2, Germany), with the following solvent systems:
I   *n*-Butanol-acetic acid-water (BAW) 40:12.5:29
II   6 % acetic acid
III   Benzene-acetic acid-water 60:70:30
IV   Na-formate-formic acid- water 10:1:20

TLC was performed on pre-coated TLC plates with silica gel $G_{60}F_{254}$ (layer thickness 0.2 mm, E. Merck, Germany) with the following solvent systems:
V   *n*-Hexane-ethyl acetate 90:10
VI   *n*-Hexane-ethyl acetate 80:20
VII   Chloroform-methanol 90:10
VIII   Chloroform-methanol 80:20
IX   Chloroform-methanol 1:1
X   Ethyl acetate-methanol 9:1
XI   *n*-Hexane-acetone 80:20
XII   *n*-Hexane-acetone 60:40
XIII   Ethyl acetate-chloroform-methanol-water 80:40:11:2

XIV   Ethyl acetate-chloroform-methanol-water 15:8:4:1
XV   Ethyl acetate-chloroform-methanol-water 6:4:4:1

TLC on reversed phase RP18 F254 (layer thickness 0.25 mm, Merck, Germany) was used for polar substances and using the different solvent systems of MeOH:H_2O (90:10, 80:20, 70:30 and 60:40). The band separation on TLC was detected under UV lamp at 254 and 366 nm, followed by spraying the TLC plates with anisaldehyde/H_2SO_4 or vanillin/H_2SO_4 reagent and subsequent heating at 100 °C.

2.2.1.2 Vacuum liquid chromatography (VLC)

Vacuum liquid chromatography is a useful method as an initial isolation procedure for large amounts of sample. The apparatus consists of a 50 cm sintered glass filter funnel with an inner diameter of 5 cm. Silica gel 60 was packed to a hard cake at a height of 30-40 cm under applied vacuum. The sample used was adsorbed onto a small amount of silica gel using volatile solvents. The resulting sample mixture was then packed onto the top of the column. Using step gradient elution with non-polar solvent (e.g. *n*-Hexane or DCM) and increasing amounts of polar solvent (e.g. EtOAc or MeOH) successive fractions were collected. The flow was produced by vacuum and the column was allowed to run dry after each fraction collected.

2.2.1.3 Column chromatography

Fractions derived from VLC were subjected to repeated separation through column chromatography using appropriate stationary and mobile phase solvent systems previously determined by TLC.
The following separation systems were used:

1) Normal phase chromatography using a polar stationary phase, typically silica gel, in conjunction with a non-polar mobile phase (e.g. *n*-Hexane, DCM) with gradually increasing amounts of a polar solvent (e.g. EtOAc or MeOH). Thus hydrophobic compounds elute more quickly than do hydrophilic compounds.

2) Reversed phase (RP) chromatography using a non polar stationary phase and a polar mobile phase (e.g. H_2O, MeOH). The stationary phase consists of silica packed with n-alkyl chains covalently bound. For instance, C-8 signifies an octanyl chain and C-18 an octadecyl ligand in the matrix. The more hydrophobic the matrix on each ligand, the greater the tendency of the column to retain hydrophobic moieties. Thus hydrophilic compounds elute more quickly than do hydrophobic compounds. Elution was performed using H_2O with gradually increasing amounts of MeOH.

3) Size exclusion chromatography involves separations based on molecular size of compounds being analyzed. The stationary phase consists of porous beads (Sephadex LH-20). The larger compounds will be excluded from the interior of the bead and thus will elute first. The smaller compounds will be allowed to enter the beads and elute according to their ability to exit from the small sized pores they were internalized through. Elution was performed using MeOH or MeOH:DCM (1:1).

4) Ion exclusion chromatography uses ion exchange resin beds (Diaion HP-20) that act as a charged solid separation medium. The components of the processed sample have different electrical affinities to this medium and are differently retained by the resins due to these different affinities. Therefore, by elution, these components can be recovered separately at the outlet of the resins bed. Elution was performed using H_2O with gradually increasing amounts of MeOH and finally acetone.

2.2.1.4 Flash chromatography (Biotage)

Flash chromatography is a preparative column chromatography based on optimized prepacked columns and an air pressure driven eluent at a high flow rate. It is a simple and quick technique widely used to separate a variety of organic compounds. Normally, the columns are dry Silica Gel 60 GF254 pre-packed, of 18 cm height, vertically clamped and assembled in the system. The column is filled and saturated with the desired mobile phase just prior to sample loading. Samples are dissolved in a small volume of the initial solvent used and the resulting mixture was then packed onto the top of the column using special syringe. The mobile phase (isocratic or gradient elution) is then pumped through the column with the help of air pressure resulting in sample separation. This technique is considered as a low to medium pressure technique and is applied to samples from few milligrams to some gram of sample.

2.2.1.5 Solid phase extraction (SPE)

SPE is a separation process by which compounds that are dissolved or suspended in a liquid mixture are separated from other compounds in the mixture according to their physical and chemical properties. The stationary phase comes in the form of a packed syringe-shaped cartridge, a 96 well plate, a 47- or 90-mm flat disk, which can be mounted on its specific type of extraction manifold. The manifold allows multiple samples to be processed by holding several SPE media in place and allowing for an equal number of samples to pass through them simultaneously. A typical cartridge SPE manifold can accommodate up to 24 cartridges. Application of vacuum speeds up the extraction process by pulling the liquid sample through the stationary phase. The analytes are collected in sample tubes inside or below the manifold after they pass through the stationary phase. Solid phase extraction cartridges are available with a variety of stationary phases, each of which can separate analytes according to different chemical properties. Most stationary phases are based on silica that has been bonded to a specific functional group. Some of these functional groups include hydrocarbon chains of variable length (for reversed phase SPE), quaternary ammonium or amino groups (for anion exchange), and sulfonic acid or carboxyl groups (for cation exchange).

2.2.2 Structure elucidation
2.2.2.1 Mass spectrometry (MS)

1) Liquid chromatography mass spectrometry (LC/MS): High pressure liquid chromatography is a powerful method for the separation of complex mixtures,

especially when many of the components may have similar polarities. If a mass spectrum of each component can be recorded as it elutes from the LC column, quick characterization of the components is greatly facilitated. Usually, ESI-MS is interfaced with LC to make an effective on-line LC/MS. HPLC/ESI-MS was carried out using a Finnigan LCQ-DECA mass spectrometer connected to a UV detector. The samples were dissolved in water/MeOH mixtures and injected to HPLC/ESI-MS set-up. For standard MS/MS measurements, a solvent gradient that started with acetonitrile:nanopure H_2O (10:90), adjusted with 0.1 % HCOOH, and reached to 100 % acetonitrile in 35 minutes was used.

2) High resolution mass spectrometry (HR-MS): High resolution is achieved by passing the ion beam through an electrostatic analyzer before it enters the magnetic sector. In such a double focusing mass spectrometer, ion masses can be measured with an accuracy of about 1 ppm. With measurement of this accuracy, the atomic composition of the molecular ions can be determined. HRESI-MS was measured on a Micromass Qt of two mass spectrometer at Helmholtz Centre for Infection Research, Braunschweig. The time-of-flight analyzer separates ions according to their mass-to-charge ratios (*m/z*) by measuring the time it takes for ions to travel through a field free region known as the flight (National Center for Natural Products Research, School of Pharmacy, the University of Mississippi, MS, USA).

2) Field desorption mass − spectroscopy ((FD) recorded on a Varian - MAT 112 S, Finnigan MAT 112, PP, , ACU on March 12 MAT Jeol - JMS HX-110 (International Center for Chemical Sciences, HEJ Research Institute of Chemistry and Dr. Panjwani Center for Molecular Medicine and Drug Research University of Karachi, Karachi, Pakistan).

2.2.2.2 Nuclear magnetic resonance spectroscopy (NMR)

NMR and 2D-NMR spectra were recorded on a Varian Mercury 400 MHz, Brucker AM 200, 300, 500, 600 FT NMR and Ultrashield Bruker 500 MHz Plus spectrometer The HR-ESI-MS spectra were measured using a Bruker Bioapex-FTMS with electrospray ionization (ESI). The GC/Ms was interfaced to a HP 5973 quadruple mass selective detector. The injector temperature was 250°C, and 1 μL injections were performed in the split (1:10) mode using helium as carrier gas (National Center for Natural Products Research, School of Pharmacy, the University of Mississippi, MS, USA, International Center for Chemical Sciences, H.E.J. Research Institute of Chemistry and Dr. Panjwani Center for Molecular Medicine and Drug Research, University of Karachi, Karachi, Pakistan and School of Chemistry, Bangor University, LL57 2UW, UK).

2.2.2.3 Infra red spectroscopy (IR)

IR spectra were recorded on a JASCO IR A-1 (Japan) and Shimadru IR-460, Japan, tablets with KBr (International Center for Chemical Sciences, H.E.J. Research Institute of Chemistry and Dr. Panjwani Center for Molecular Medicine and Drug Research University of Karachi, Karachi, Pakistan).

2.2.2.4 Ultra violet spectroscopy (UV)

UV spectra were recorded on spectrophotometer Shimadru UV-240, Japan and the SF-26"LOMO» (International Center for Chemical Sciences, H.E.J. Research Institute of Chemistry and Dr. Panjwani Center for Molecular Medicine and Drug Research University of Karachi, Karachi, Pakistan; spectroscopic laboratory at department of organic chemistry and chemistry of natural products and polymer, Al-Farabi Kazakh National University).

2.2.2.5 Melting point Determination

The melting point is determined on a Kofler bench spectroscopic laboratory at department of organic chemistry and chemistry of natural products and polymer, Al-Farabi Kazakh National University).

2.2.3 Fatty and amino acids analysis

The composition of the saturated and unsaturated carboxylic acids (fatty acids) in plants is determined by gas-liquid chromatography apparatus «CARLO-ERBA-420" using helium as a carrier gas, flame ionization detector, carrier gas velocity 30 ml/min, detector temperature 188 ˚C, oven temperature 230 ˚C, adsorbent Cellite 545 on Chromosorb WAW. The chloroform extract of plant species is added to 10 ml of methanol and 2-3 drops of acetyl chloride and then carried out methylation at 60-70 ˚C in a special system for 30 minutes. Methanol was removed using a rotary evaporator, and the samples are extracted with 5 mL of *n*-hexane and analyzed by gas chromatography for 1 hour.

Analysis of amino acids was carried out chromatographically using helium as carrier gas, flame ionization detector 300 ˚C and condenser temperature 250 ˚C on Chromosorb WAW. Aqueous extract of the plant was hydrolyzed in HCl for 24 hours. The resulting hydrolyzate was evaporated to dryness in a rotary evaporator at 40 ˚C, after centrifugation at 2.5 thousand revolutions per minute the resulting precipitate was dissolved in sulfosalicylic acid and amino acids are eluted through an ion exchange column Dausk-50. On freshly obtained elutes 2, 2-dimethoxypropane and propanol saturated with HCl were added. The resulting mixture is heated at 110 ˚C for 20 minutes, then addition of a freshly prepared acylating reagent (1 volume of acetic anhydride and 2 volumes of triethylamine and 5 volumes of acetone), evaporation of the sample to dryness, addition of ethyl acetate and saturated aqueous solution of NaCl. Finally, the ethyl acetate layer is analyzed on the amino acid analyzer (Carlo-Erba) (ASE "Center of Physico-chemical methods of research and analysis," Republican State Enterprise "Al-Farabi Kazakh National University, MON RK).

2.2.4 Analysis of the lipophilic constituents

The air-dried aerial parts of plants were pulverized separately. The dried powder (100 g) was extracted three times with 70% ethanol (3 × 500 mL) at room temperature and mixed the extracts, and then it was evaporated on a vacuum rotary evaporator. The ethanol extract was suspended in water and extracted successively

with hexane (3 × 300 mL, 25 °C) to give *n*-hexane residue which is then ready for gas chromatography.

GC-MS analysis of the *n*-hexane extract was performed using Electron Impact Ionization (EI) (Gas Chromatography coupled to Mass Spectrometer), with silica capillary column coated with PES, He carrier gas, flow rate, 1mL/min, gradient column temperature (60- 290 ºC), injector temperature 310 ºC and ion source detector (EI-70eV). Identification of the components was calculated from the peak areas without using correction factors. Components were identified by comparing retention times and full mass spectra with NIST and Wiley electronic libraries (Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumqi, China).

2.2.5 Essential oil analysis

According to the standard methods for essential oils preparation, it is needed air-dried plant materials such as leaves, flowers, fruit, berries and branches. The oils contained within plant cells are liberated through heat and pressure from these parts of the plant matter, and the color may vary from a pale to deep yellow depending of the plant part used. The extraction of essential oils from plant material can be achieved by various methods, of which the most commonly used methods include hydro-distillation (with a collecting solvent which is then removed under vacuum), steam and steam/water distillation.

The plant material was pulverized, passed through 24-mesh sieve, and then placed in a steam distillation vessel. The sample was soaked for 4 hrs. and subjected to hydro-distillation using Ginsberg's apparatus for 8 hrs. NaCl was added until saturation was reached. Chloroform was then used as the solvent to extract the oil for three times. The extraction was enriched to 2 ml. The sample was dried by anhydrous Na_2SO_4, filtered by a microporous membrane, stored in a sealed container, and refrigerated prior to analysis.

Extracted volatile compounds were analyzed using gas chromatography (GC) equipped with mass spectroscopic detector 7890A/5975C (Agilent, USA) and auto sampler Combi-PAL (CTC Analytics, Switzerland). For sample preparation, we used the solid-phase micro-extraction SPME, which allows to efficiently concentrating the volatile organic compounds contained in the sample from micro-polymer coating, the samples were placed in pre-conditioned 20 ml vials (Agilent, USA) and sealed with lids conditioned silicone gaskets / Teflon (PTFE). Samples then extracted with an auto-sampler at temperature 30 ºC for 30 seconds. Extracted volatile compounds were desorbed by inserting the SPME fiber into the injector port (splitless mode, 240 °C) of a GC. The desorption time was 1 min. The desorbed volatile compounds were separated on a capillary column, DB-5MS (60-m length, internal diameter 0.32-mm, film thickness 0.2-μm). Helium was used as the carrier gas at a flow rate of 1 ml/min. The oven temperature was initially set at 40 °C for 10 min, increased to 240 °C at a rate of 10 °C /min, and held at 240 °C for 20 min. MSD interface temperature was 280 ° C. Data is collected in the selected-ion-monitoring (SIM) mode; in the range of

mass numbers 35-350. Response factor of the detector is adjusted to 1.0 and delay of solvent was 5.00 minutes.

Volatile compounds were identified using gas chromatography-mass spectrometry [Agilent MSD ChemStation (Ver. E.02.02 SP1)]; Separated compounds were tentatively identified by comparing the mass spectral data with the reference spectra in a mass spectral library (Natl. Inst. for Standard Technology, Manchester, U.K.) Wiley 8th edition, containing 782 thousand spectra as well as the retention indices with the reported values [126], (ASE "Center of Physico-chemical methods of research and analysis," Republican State Enterprise "Al-Farabi Kazakh National University, MON RK).

2.2.6 Preliminary phytochemical screening

I. Determination of moisture content: Record to the nearest 0.01g the mass of a high-silica or porcelain evaporating dish fitted with a heavy-duty aluminum foil cover. The dish shall have a capacity of not less than 100 ml. Mix thoroughly the representative sample and place a test specimen of (l0 g) in the container. Crush soft lumps with a spoon or spatula. The thickness of peat in the container should not exceed 3 cm. Cover immediately with the aluminum foil cover and record the mass to the nearest 0.01g. Dry uncovered for at least 16hr at105 °C or until there is no change in mass of the sample after further drying periods in excess of 1h. Remove from the oven, cover tightly, cool in a desiccator, and record the mass. Calculate the moisture content as follows:

$$Moisture\ Content\ (\%) = \frac{A - B}{A} x\ 100$$

Where:

A = mass of the as-received test specimen, g, and

B = mass of the oven-dried specimen, g [127].

II. Determination of ash value: Two grams of dried and powdered plant material was taken in the pre-weighed clean sintered silica crucibles. Then, they were incinerated by gradual increasing of the temperature (400-550 °C) in the muffle furnace till white ash obtained until constant weight of ash obtained. The crucible was cooled to room temperature in a desecrator and weighed the ash and calculated the % of total ash with reference to air dried sample of the crude drug using following formula:

$$Total\ Value\ (\%) = \frac{Z - X}{Y} x\ 100$$

Where:

Z= Weight of the crucible;

X = Weight of the crucible with ash; and

Y = Weight of the powder taken (g) [123, 128].

III) Mineral analysis: ashing 2.0 g dried and ground sample in a muffle furnace at 550°C. The ash was analyzed for macro and micro elements by atomic absorption spectrophotometer "ASSIN firm Karl Zeiss" (Laboratory of analytical chemistry and chemistry of rare elements Al-Farabi Kazakh National University, laboratory microanalysis - Institute of Chemistry of NAH RK named. A.B. Bekturova; potassium content found on the flame photometer PFM-80-2 (Laboratory of the Department of Macromolecular Compounds and Chemical Physics, Al-Farabi Kazakh National University).

VI) Acid hydrolysis: Acid hydrolysis of the compounds carried by 5% HCl on a boiling water bath, while mild acid hydrolysis was sampled at a certain time interval. The hydrolysis products were identified by m.p., PC and TLC in appropriate solvent systems with authentic samples.

The content of amino acids and flavonoids determined photometrically using the complexation reaction of flavonoids with aluminum chloride, and amino acids with ninhydrin reagent [122, 129]. The color intensity of the resulting complexes are measured at photo colorimeter FEC-72 with a light filter number 2 (425 nm) for flavonoids and amino acids used filter number 9 (450 nm). The calculation was performed on a sample calibration curve for flavonoids quercetin and a mixture of four amino acids to the amino acids, built in similar conditions. The content of free carbohydrates determined by spectrophotometric method [130], and the calculation of the calibration curve constructed under similar conditions for glucose.

2.2.7 Biological screening
2.2.7.1 *In vitro* phytotoxic bioassay

Phytotoxic activity was determined by using the modified protocol of *Lemna minor*[131] at (International Center for Chemical Sciences, H.E.J. Research Institute of Chemistry and Dr. Panjwani Center for Molecular Medicine and Drug Research, University of Karachi, Karachi, Pakistan). The medium was prepared by mixing various constituents in 100 ml distilled water and the pH was adjusted (5.5-6.5) by adding KOH solution. The medium was then autoclaved at 121°C for 15 minutes. The extracts dissolved in ethanol (20mg/ml) served as stock solution. Nine sterilized flasks, three for each concentration, were inoculated with 1000 μl, 100 μl and 10 μl of the stock solution for 500, 50 and 5 ppm respectively. The solvent was allowed to evaporate overnight under sterile conditions. To each flask, medium (20 ml) and plants (10), each containing a rosette of three fronds of *Lemna minor* L., was added. One other flask supplemented with solvent and reference growth inhibitor (paraquat), served as positive control. All flasks were plugged with cotton and kept in the growth cabinet at (28°± 1C °) for 7 days. The number of fronds per flask were counted and recorded on day seven. The results were interpreted by analyzing the growth regulation in percentage calculated with reference to the negative control by the following formula:

$$\text{Growth regulation (\%)} = \frac{100 - \text{No. of fronds in test sample}}{\text{No of fronds in negative control}} X\ 100$$

2.2.7.2 Evaluation of protein tyrosine phosphates1B activity

The in vitro PTP1B activity assay was conducted based on a protocol previously described by Taghibiglou et al. [132]. During the assay, p-NPP was used as substrate. p-NPP was diluted in the assay buffer (50 mM HEPES, pH 7.3, 100 mM NaCl, 0.1% BSA, and 1 mM DTT). In the evaluation of PTP1B inhibitor, different concentrations of the tested extracts were incubated with GST-PTP1B1-321, and the enzyme activities were detected in a 96-well microplate spectrophotometer (MD) at 30 °C. The enzyme activities were determined by measuring the absorbance at 405 nm generated by the formation of product p-NPP. The PTP1B inhibition was expressed as percentage of inhibition and calculated by the following equations:

$$\text{Reaction rate(\%)} = \frac{PTP1B_{test}}{PTP1B_{control}} X\ 100 \tag{1}$$

$$\text{Inhibition rate (\%)} = 100 - reaction\ rate\ (\%) \tag{2}$$

All assays were undertaken in triplicate and all data were expressed as mean ± standard deviation for the number of experiments. One-way ANOVA was used to assess significant differences among the treatment groups. The concentration of tested samples required to inhibit 50% of the activity under the assay conditions was determined from dose-response curves and defined as the IC_{50} value (Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumqi, China).

2.2.7.3 Anti-tumor activity

Cell Culture: Human hepatocarcinoma cell line (Hep G2), Colon carcinoma cells (HCT-116), breast adenocarcinoma cells (MCF-7) was purchased from ATCC, USA, were used to evaluate the cytotoxic effect of the tested extract. Cells were routinely cultured in DMEM (Dulbeco's Modified Eagle's Medium), except colon cells that were cultured in McCoy's media. Media was supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 units/ml penicillin G sodium, 100 units/ml streptomycin sulphate, and 250 ng/ml amphotericin B. Cells were maintained at sub-confluency at 37ºC in humidified air containing 5% CO_2. For sub-culturing, monolayer cells were harvested after trypsin/EDTA treatment at 37°C. Cells were used when confluence had reached 75%. Tested sample was dissolved in dimethyl sulphoxide (DMSO), and then diluted thousand times in the assay. All cell culture material was obtained from Cambrex BioScience (Copenhagen, Denmark). All chemicals were from Sigma/Aldrich, USA, except mentioned. All experiments were repeated three times, unless mentioned.

Cytotoxicity of tested samples was measured against different tumor cells using the MTT Cell Viability Assay. MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay is based on the ability of active mitochondrial dehydrogenase enzyme of living cells to cleave the tetrazolium rings of the yellow MTT and form a dark blue insoluble formazan crystals which is largely impermeable

to cell membranes, resulting in its accumulation within healthy cells. Solubilization of the cells results in the liberation of crystals, which are then solubilized. The number of viable cells is directly proportional to the level of soluble formazan dark blue color. The extent of the reduction of MTT was quantified by measuring the absorbance at 570 nm (Hansen et al, 1989).

Reagents preparation:

MTT solution: 5mg/ml of MTT in 0.9% NaCl.

Acidified isopropanol: 0.04 N HCl in absolute isopropanol.

Cells (0.5X105 cells/ well), in serum-free media, were plated in a flat bottom 96-well microplate, and treated with 20µl of different concentrations of each tested sample for 48 h at 37º C, in a humidified 5% CO_2 atmosphere. After incubation, media were removed and 40 µl MTT solution / well were added and Incubated for an additional 4 h. MTT crystals were solubilized by adding 180 µl of acidified isopropanol / well and plate was shacked at room temperature, followed by photometric determination of the absorbance at 570 nm using microplate ELISA reader. Triplicate repeats were performed for each concentration and the average was calculated. The relative cell viability was expressed as the mean percentage of viable cells as compared to the respective untreated cells (control), with cytotoxicity indicated by <100% relative viability.

Percentage of relative viability was calculated using the following equation:

$$\text{Percentage of relative viability (\%)} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100$$

Then the half-maximal growth inhibitory concentration (IC_{50}) was calculated from the line equation of the dose-dependent curve. The results were compared with the cytotoxic activity of paclitaxel, a known anti-cancer drug (Cancer Biology Laboratory, Excellence for Advanced Science, Department of Biochemistry, National Research Centre, Dokki, Giza, Egypt).

2.2.7.4 Antimicrobial activity

Three type strains, *Staphyloccocus aureus* ATCC 6538, *Escherichia coli* ATCC 11229, *Candida albicans* ATCC 10231 were obtained from the National Center for Medical Culture Collections (CMCC), China. All strains were stored at −80 °C in the appropriate medium. Antimicrobial activity was tested using the agar well diffusion method [133]. Luria-Bertani (LB) and Sabaurauds agar (SDA) were sterilised in an autoclave and cooled to 45–50 °C before be poured into 100 mm Petri dishes. The agar plates were stored at 4 °C before used.

Staphyloccocus aureus ATCC 6538 and *Escherichia coli* ATCC 11229 were cultured overnight at 37 °C in LB. *Candida albicans* ATCC 10231 was cultured overnight at 37 °C in SDA. Petri dishes with 20 mL of medium were prepared, previously inoculated with 200 µL of the culture suspension. The wells (6 mm) were made and the sample diluted in DMSO to test concentration (100 mg/mL) was added

(20 μL/well) and the same volume (20 μL) of DMSO was used as a control. The inoculated plates were incubated for 24/48 h. After incubation, the diameter of the inhibition zone was measured with calipers (Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumqi, China).

3 Results and Discussion

3.1 Qualitative and quantitative analysis of *Zygophyllum fabago* and *Camphorosma lessingii*

The plant materials of *Z. fabago* and *C. lessingii* which belonging to the obligate halophytes were collected from saline soils at Almaty region (Kazakhstan). The air-dried aerial parts was cut into small pieces and stored at room temperature 25 °C for one week. The dried samples were ground using a mill to obtain coarse powder.

The moisture content, total ash, Qualitative and quantitative contents of biologically active constituents of both *Z. fabago* and *C. lessingii* were determined according to methods reported in the State Pharmacopoeia XI edition techniques [134].

The amount and composition of ash remaining after combustion of plant material varies considerably according to the part of the plant, age, environment etc. The constituents of the ash also vary with time and from organ to organ. Ash usually represents the inorganic part of the plant and is useful in determining authenticity and purity of sample and these values are important qualitative standards. The ash content is a measure of the total amount of minerals present within a plant, whereas the mineral contents are a measure of the amount of specific inorganic components present within it.

Moisture content is an important factor because appearance and stability of dried plants depends on the amount of water they contain and the propensity of microorganisms to grow depends on their water content. The results in table 2 showed low moisture content of the aerial parts (8.26%) in *Z. fabago* and (7.71%) in *C. lessingii*. The ash content can be affected by many factors including type of species and variety, choice of soil type and location.

The ash content significantly increased as the salinity increased and there were significant differences in the comparisons between ash content in *Z. fabago* 5.43% and in *C. lessingii* 25.53% indicating that there was a difference in soil salinity between the two species [135, 136].

Furthermore plants respond to salinity stress through a set of changes in growth and basic biological functions; including photosynthesis, photorespiration, synthesis of life-saving molecules [137], so by means of titration, spectrophotometric methods gas-liquid (GLC), paper (PC) and thin-layer chromatography (TLC) in different solvent systems using specific developers; the groups of biologically active substance of the aerial parts of the tested species were screened and examined which revealed that the major groups in both plants are saponins, organic acids amino acids flavonoids and carbohydrates and chromones. In *Z. fabago* the chromones not detected but determined by considerable amount in *C. lessingii* [135, 136]. The presence of high contents of saponins, polyphenolic and amino acids in both plants are in agreement with the fact that these constituents work as protectants of halophytes from salinity [138-140]. The results are presented in table 2.

Table 2 – Qualitative and quantitative screening of the powdered aerial parts of *Zygophyllum fabago* and *Camphorosma lessingii*

Species	Contents, %									
	Moisture content	Ash	Carbohydrates	Flavonoids	Amino acids	Saponins	Alkaloids	Vitamin C	Organic acids	Chromones
Z. fabago	8.26	5.43	0.90	1.10	0.95	10.31	0.44	– *	7.96	–*
C. lessingii	7.71	25.53	0.70	1.90	1.54	5.20	– *	0.57	2.60	2.80

* – : Not detected

Some of heavy metals (Fe^{+++}, Cu^{++} and Zn^{++}) are essential for plants. The availability of heavy metals in medium varies, and metals such as Cu^{++}, Zn^{++}, Fe^{+++}, Mn^{++}, Mo^{++}, Ni^{++} and Co^{++} are essential micronutrients, whose uptake in excess to the plant requirements result in toxic effects. They are also called as trace elements due to their presence in trace (10 mg kg^{-1}, or mg L^{-1}) or in ultra-trace (1 μ kg^{-1}, or mg L^{-1}) quantities in the environmental matrices. The essential heavy metals (Cu^{++}, Zn^{++}, Fe^{++}, Mn^{++} and Mo^{++}) play biochemical and physiological functions in plants. Two major functions of essential heavy metals are the following: (a) Participation in redox reaction, and (b) Direct participation, being an integral part of several enzymes [141], in addition potassium is essential in the maintenance of cellular water balance, pH regulation and it is associated with protein and carbohydrate metabolism [142]. Halophytes tolerate salinity through the uptake or repulsion of ions, increasing of organic solutes, change of stomata, water content and other physiological changes [143]. The amount of inorganic ions in plant tissue increased with an increase in salinity to sustain the osmotic potential and maintain a flow of water into the plant 144][. Many plants develop mechanisms either to exclude salt from their cells or to tolerate its presence within the cells. During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis, and energy and lipid metabolism are affected [145]. High salinity also results in increased cytosolic Ca^{++} that is transported from the apoplast and intracellular compartments. The resultant transient Ca^{++} increase potentiates stress signal transduction and leads to salt adaptation [145].

The elemental analysis of the two species showed the presence of eleven macro and microelements as shown in table 3 and figs 3 and 4 with different levels and percent with the highest percent of macroelements K^+, Na^+, Mg^{++}, Ca^{++} and Fe. Na^+ was found with the highest level (58%) in *Z. fabago* followed by K^+ (24%), Ca^{++} (11%) and Mg^{++} (7%), while in *C. lessingii* Ca^{++} represented the high level by (33%) followed by Na^+ (30%), Mg^{++} (21%) and K^+ (15%) [135, 136].

Table 3 – Micro and macro elemental contents of the aerial parts of *Zygophyllum fabago* and *Camphorosma lessingii*

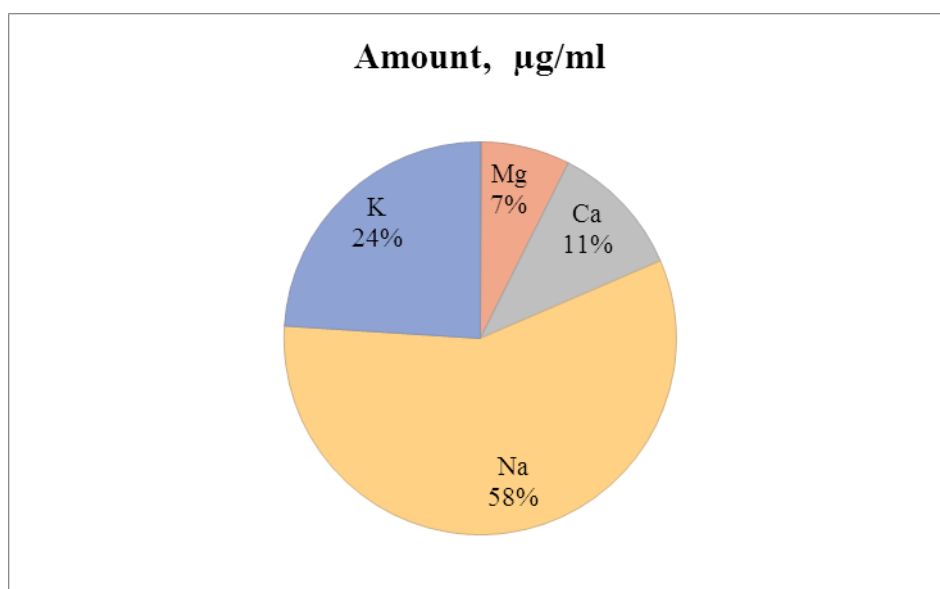| Species	Macro and Micro-elements *µg/ml*										
	Ni^{++}	Mn^{++}	Pb^{++}	Cd^{++}	Cu^{++}	Zn^{++}	Fe^{+++}	Mg^{++}	Ca^{++}	Na^+	K^+
Z. fabago	0.007	0.073	0.014	0.002	0.029	0.040	0.255	22.058	33.702	174.174	72.725
C. lessingii	0.598	4.309	1.398	0.082	1.049	3.429	42.350	1237.960	2018.070	1791	932.450

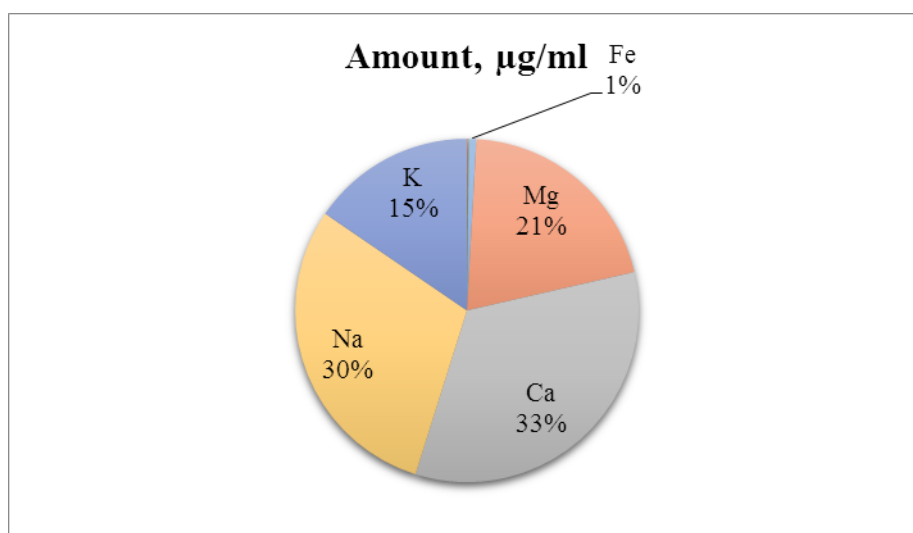Figure 3 – Diagram of macro-and micronutrient contents of *Zygophyllum fabago*

Figure 4 – Diagram of macro-and micronutrient contents of *C. lessingii*

It is clear from the previous results that the two species were grown in different environment which is clear from the high percent of salts in *C lessingii* compared to *Z. fabago* and this also confirmed from the ash content which was found with high percent in *C. lessingii*, but in the two species the results confirmed that the content of Na^+ is more than K^+ and also the content of Ca^{++} is more than Mg^{++}. The importance of high concentrations of both of Na^+ and Ca^{++} comes from that both two elements play an important role for halophytic plants that Ca^{++} gives protection against the effects of heavy metals and conveys some resistance to excessively saline conditions and low pH, whereas Na^+ can function as an osmotic stabilizer in halophytic plants; these have become adapted so that, in saline soils with low water potential, they can accumulate abnormally high concentrations of Na^+ ion in vacuoles, and thereby maintain sufficient turgor for growth [146]. The investigation of the elemental analysis reported here for the first time for the two species.

3.2 Extraction and solvent partitioning of *Zygophyllum fabago*

The air dried ground plant (2.7 Kg) was exhaustively percolated with 70% ethanol for 72 hrs. at room temperature. The solvent was evaporated under reduced pressure using rotary vacuum evaporator leaving 320 g residue. The alcoholic extract was dissolved in 1000 ml of distilled water/alcohol mixture (9:1) and successively extracted with *n*-hexane (5 X 500 ml), chloroform (4 X 500 ml), ethyl acetate (3 X 500 ml) and butanol (3 X 500 ml). Each extract was separately evaporated to dryness using rotary vacuum evaporator under reduced pressure at a temperature not exceeding 45 ℃. The resulted extracts were (4.23 g, 4.93 g, 5.72 g and 70.0 g respectively). Each of the obtained fractions was subjected to thin layer (TLC) and paper (PC) chromatographic techniques and then column chromatography for isolation of its major constituents (fig 5).
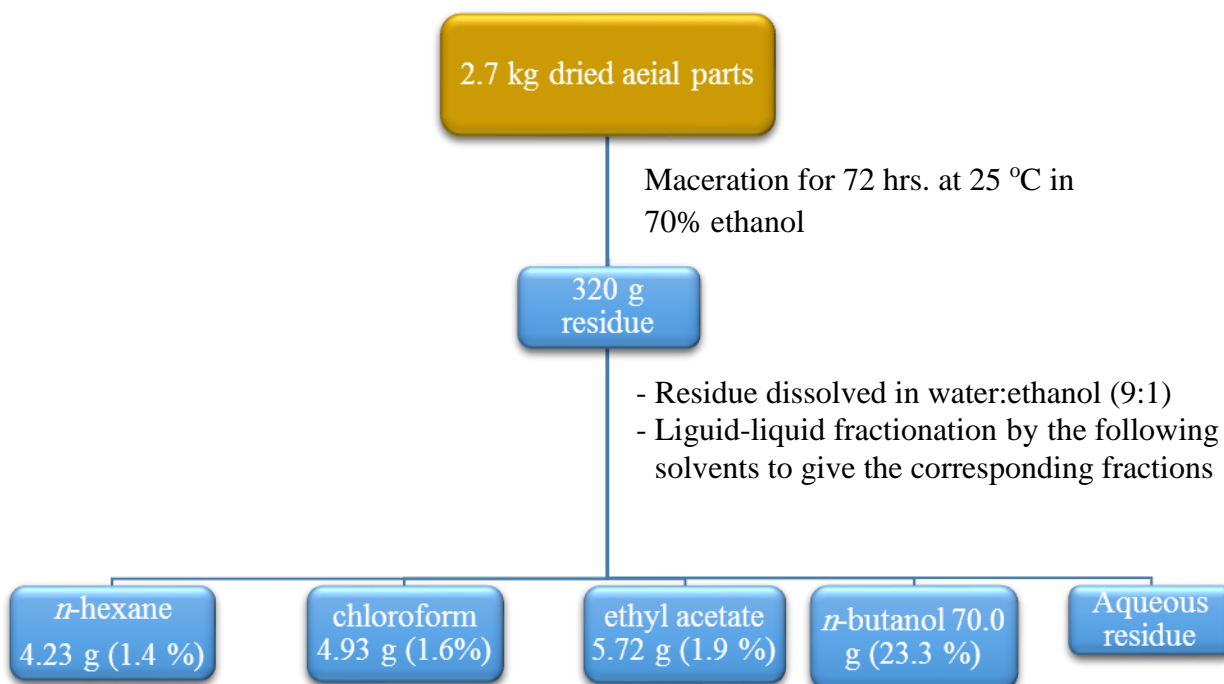
2.7 kg dried aeial parts

Maceration for 72 hrs. at 25 °C in 70% ethanol

320 g residue

- Residue dissolved in water:ethanol (9:1)
- Liquid-liquid fractionation by the following solvents to give the corresponding fractions

| *n*-hexane 4.23 g (1.4 %) | chloroform 4.93 g (1.6%) | ethyl acetate 5.72 g (1.9 %) | *n*-butanol 70.0 g (23.3 %) | Aqueous residue |

Figure 5 – Scheme of extraction and fractionation of the ethanolic extract of the aerial parts of *Zygophyllum fabago*

3.2.1 Chromatography of the *n*-hexane fraction of *Zygophyllum fabago*

The *n*-hexane fraction obtained above (4.2 g) was found to contain large amounts of chlorophyll which hindered the isolation of its contents, together with fatty acids sterols and lipophilic contents, it was fractionated on a silica gel column. Elution was begun with *n*-hexane and polarity was gradually increased in a gradient elution technique and finally end with ethyl acetate. The elute was collected in fractions, each fraction was concentrated under reduced pressure and monitored by silica gel plates using systems V and VI as developers. The plates were dried and sprayed with anisaldehyde/H_2SO_4 followed by heating at 100 °C for 10 minutes for location of the spots on TLC. Similar fractions were combined together and concentrated under reduced pressure. The preliminary hexane eluent was submitted to GC analysis for determination of the fatty acids and lipophilic contents, while the main fraction (Hex-A) subjected to recolumn on silica gel using system VII to afford the compound **1.1** in pure form. The results were illustrated in fig. 6.

3.2.2 Chromatography of the chloroform fraction of *Zygophyllum fabago*

The chloroform fraction obtained above (4.93g) was fractionated on a silica gel column using chloroform - methanol. Elution was begun with chloroform and polarity was gradually increased in a gradient elution technique and finally ends with methanol. The elute was collected in fractions, each fraction was concentrated under reduced pressure and monitored by silica gel plates using systems VII and VIII as developers. The plates were dried and sprayed with anisaldehyde/H_2SO_4 followed by

heating at 100 °C for 10 minutes for location of the spots on TLC. Similar fractions were combined together and concentrated under reduced pressure. The results were illustrated in (Fig. 22). two main fractions were collected (Ch-A and Ch-B), sub-fraction Ch-B was subjected to recolumn on sephadex LH-20 using systems IX afforded two sub-fraction Ch-B1 and Ch-B2, sub-fraction Ch-B1 subjected to recolumn on sp column using system IX to give compound **1.2**, while CH-B2 was subjected to recolumn again using sephadex LH-20 and system IX led to compounds **1.3** and **1.4** in pure states, The results were illustrated in fig 6a.

3.2.3 Chromatography of the ethyl acetate fraction of *Zygophyllum fabago*

The ethyl acetate fraction (5.7 g) was found rich in polyphenolics and terpenoids, was chromatographed in silica gel column built in ethyl acetate. The column was eluted with different ratio of ethyl acetate and methanol in gradient elution manner. Fractions 20 ml each, were collected and the similar fractions were pooled together after screening by TLC to six main fractions (fig 6). Sub-fraction Eth-A subjected to repeated chromatography; first on silica gel using ethyl acetate followed by purification on sephadex LH-20 column using system IX to afford compound **1.5**. While sub-fraction Eth-B subjected to recolumn on sephadex LH-20 using system IX giving three compounds **1.6**, **1.7** and **1.8** in pure forms. The sun-fraction Eth-C was subjected therefore to recolumn in silica gel column built in system X and eluted with the same system afford three sub-fractions (Eth-C1, 2, 3), which were subjected to repeated column chromatography on sephadex LH-20 column using system IX to afford compounds (**1.9-1.14**). Furthermore, the sub-fractions (Eth-D, F and G) were subjected to repeated column on sephadex LH-20 using methanol as eluent to yield compounds **1.15-1.21**. Compound **1.18**, **1.19** and **1.21** identified as phenolic acids by method of PC by direct comparison with authentic samples using systems I, III, IV and (Diazotized *P*-nitroaniline + Na_2CO_3) as detecting reagent. The results were illustrated in fig 6b.

3.2.4 Chromatography of the *n*-butanol and aqueous fractions of *Zygophyllum fabago*

The residue from *n*-butanol fraction (51.5 g) was dissolved in minimum quantity of methanol and made into slurry with minimum amount of silica gel. It was subjected to chromatographic separation over a column of silica gel (VLC column) built in ethyl acetate and eluted with increasing amounts of methanol. Elutes of 500 mL were collected each time and the solvent was distilled on a water bath. The homogeneity of the fractions was examined by TLC on silica gel plates. The spots developed were visualized under UV light and then by exposure to anisaldehyde/H_2SO_4 followed by heating at 100 °C for 10 minutes. Similar fractions were combined and purified. The VLC afforded three sub-fractions (But-A, B, C); the three sub-fractions were subjected to repeated recolumn on sephadex LH-20 using methanol as eluent as to separate the compounds (**1.22-1.29**) in pure state. The results represented in fig 6(a).

In the aqueous fraction of *Z. fabago* by method of PC using systems (I-IV) and specific detecting methods and reagents (UV, NH$_3$, Ferric ammonium alum, *o*-toluidine and ninhydrin) detected carbohydrates and amino acids. Identification of monosaccharide was carried out by PC using system I and *o*-toluidine reagent. This method led to the identification of galactose, glucose, and arabinose in the aqueous fraction of *Z. fabago*. Qualitative and quantitative amino acid analysis conducted on the amino acid analyzer (table 6 and fig 10).
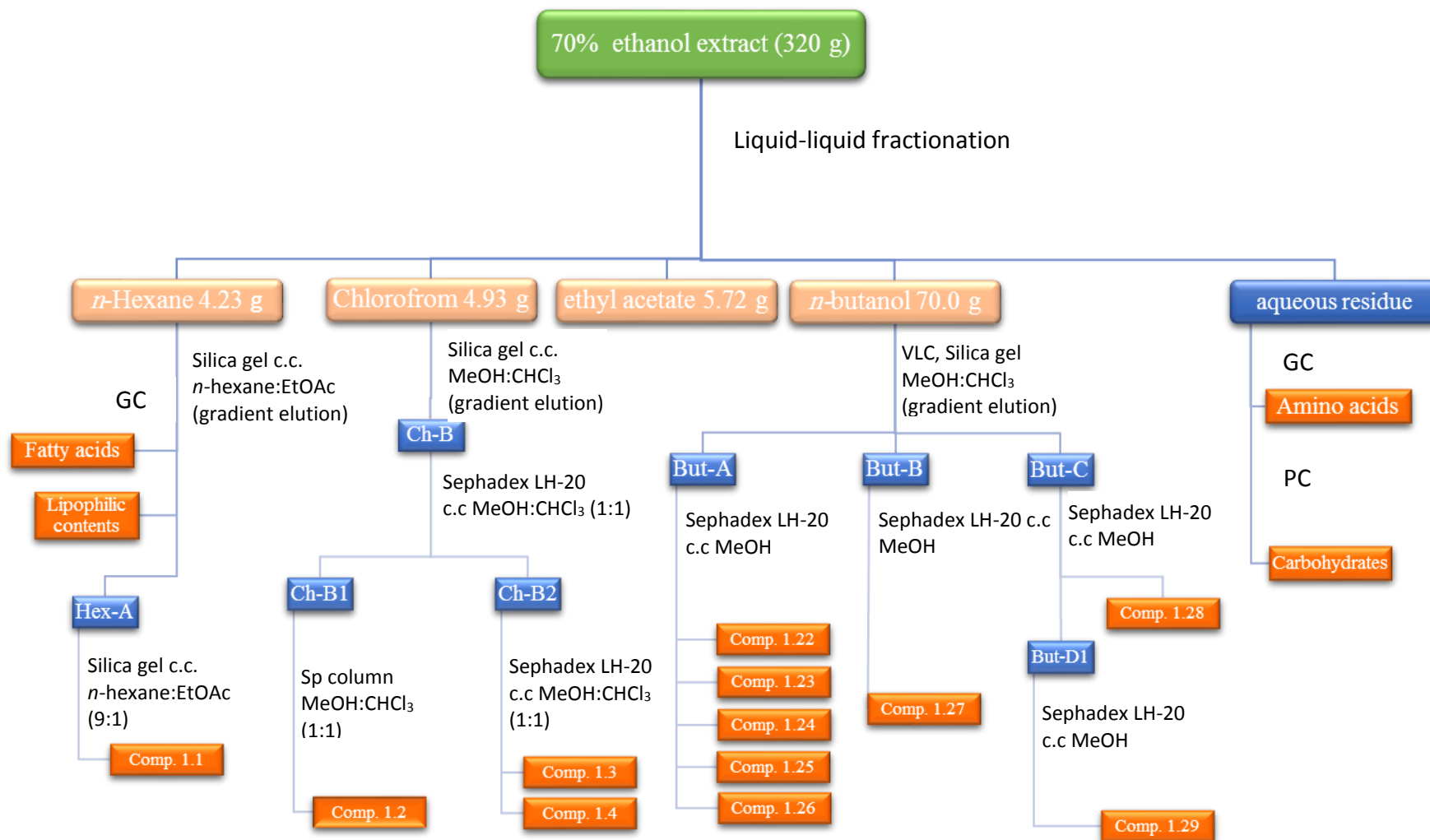
Figure 6a – Scheme of isolation of compounds from ethanolic extract of *Zygophyllum fabago*
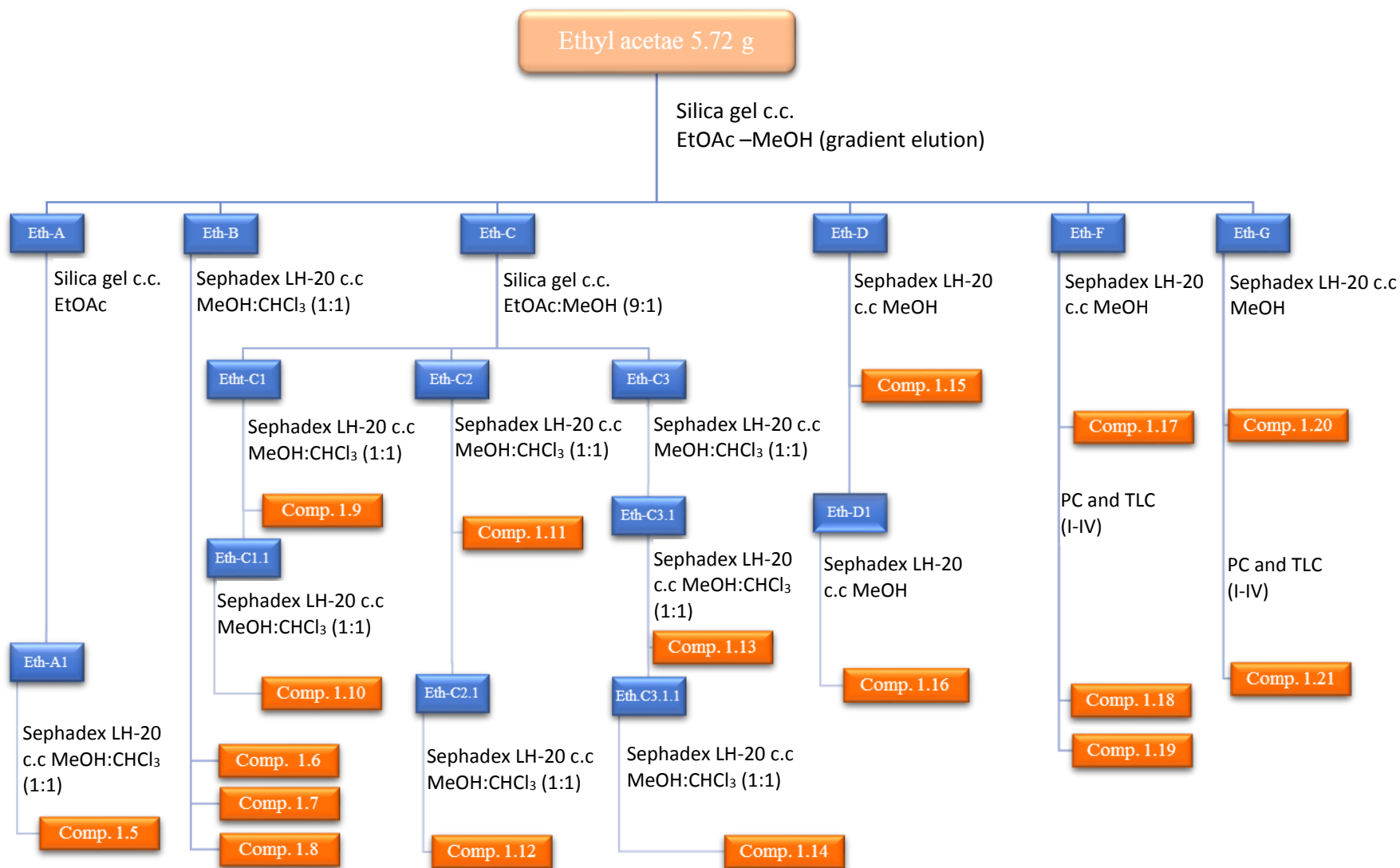
Figure 6b – Scheme of isolation of compounds from ethanolic extract of *Zygophyllum fabago*

3.3 Extraction and solvent partitioning of *Camphorosma lessingii*

The air-dried powdered aerial parts (3.9 kg) of *Camphorosma lessingii* were exhaustively extracted by maceration for 72 hrs. at room temperature with ethanol (70%) till complete exhaustion. The ethanolic extract was concentrated under reduced pressure using rotary vacuum evaporator at a temperature not exceeding 45 ℃ giving 400 g residue. The combined dried ethanolic extract (400 g) was digested in a least amount of distilled water/ethanol mixture (9:1), transferred to a separating funnel and successively partitioned with *n*-hexane (5 X 500 ml), chloroform (4 X 500 ml), ethyl acetate (3 X 500 ml) and *n*-butanol (3 X 500 ml) respectively. Each fraction was concentrated under reduced pressure to give the corresponding soluble fractions, *n*-hexane (8.3 g), chloroform (55.5 g), ethyl acetate (7.5 g) and *n*-butanol (51.5 g) respectively. Each of the obtained fractions was subjected to thin layer (TLC) and paper chromatography (PC) chromatographic studies and then column chromatography for isolation of its major constituents (fig 7).

3.3.1 Chromatography of the *n*-hexane and chloroform fractions of *Camphorosma lessingii*

The *n*-hexane fraction obtained above (8.3 g) was found to contain large amounts of chlorophyll which hindered the isolation of its contents, together with fatty acids sterols and lipophilic contents, so the *n*-hexane was submitted to GC analysis for determination of the fatty acids and lipophilic contents. The results presented in table 5 and 9.

The chloroform fraction was concentrated to a thick state. TLC in solvent systems (VI, VII) using specific reagents (UV light, NH_3, $CeSO_4$, conc. H_2SO_4), extracts of C. lessingii detected terpenoids, chromones. Further separation of substances from the chloroform extracts carried out according to the scheme in fig 7.

The extract was dissolved in the minimum amount of solvent and mixed with silica gel and dried in a vacuum desiccator contain $CaCl_2$. The resulting mass was powdered and transferred to a column packed with silica gel slurred in *n*-hexane. The column was eluted initially with *n*-hexane followed by gradient systems of *n*-hexane – acetone. Fractions were collected, concentrated under reduced pressure and monitored by precoated silica gel plates and systems VI, VII as developers. Location of spots under UV and after spraying the chromatograms with NH_3, $CeSO_4$, or conc. H_2SO_4, followed by heating at 110℃ for 10 minutes were determined. Similar fractions were compiled together and concentrated under reduced pressure.

Further increasing the concentration of acetone to obtain sub-fraction (Ch-A) which was further eluted with systems (VI) in gradient elution manner to get compound **2.1** in an individual state. Repeated chromatography on sub-fraction (Ch-A) by means of TLC identified two spots as terpenoids by their pink colour spots after spraying with $CeSO_4$. Repeated chromatography of this fraction using silica gel and (*n*-hexane - acetone, 6:4) led to isolation of compounds **2.2** and **2.3** in pure forms. Further investigation of sub-fraction (Ch-B) by column chromatography using

hexane-acetone (9:1, 8:2, 7:3), led to the isolation of substances **2.4** (*n*-hexane - acetone 7:3) and **2.5** (*n*-hexane - acetone, 6:4).

3.3.2 Chromatography of the ethyl acetate and aqueous fractions of *Camphorosma lessingii*

The ethyl acetate fraction of *Camphorosma lessingii* was studied by PC using specific developer (I-IV) and spraying reagents (UV light, NH_3, Diazotized *P*-nitroaniline + 15% Na_2CO_3, Ferric ammonium alum) Substance **2.6** attributed to flavonoids while **2.7**, **2.8, 2.9** and **2.10** attributed to phenolic substances. Isolation offlavonoids was done by adsorption chromatography on polyamide column. Fractions are monitored in the above solvent systems with corresponding developers. As a result received 2 sub-fractions (fig 7), after that, each of the fractions investigated separately. Fraction (Eth-A) was therefore subjected to recolumn on silica gel led to the isolation of an individual substances **2.6** in pure state, while compound (**2.7-2.10**) were identified by means of paper chromatography (I-V) by direct comparison with authentic samples and Diazotized *P*-nitroaniline + 15% Na_2CO_3 revealing reagent. While fraction (Eth-B) subjected to recolumn on polyamide using CH_3OH - H_2O as eluent by gradient elution method. As a result substance **2.11**are eluted in individual state.

In the aqueous fraction of *C. lessingii* by method of PC using systems (I-IV) and specific detecting methods and reagents (UV, NH_3, Ferric ammonium alum, *o*-toluidine and ninhydrin) detected carbohydrates and amino acids. Identification of monosaccharaides was carried out by PC using system I and *o*-toluidine reagent. This method led to the identification of galactose, glucose, and rhamnose in the aqueous fraction of *C. lessingii*. Qualitative and quantitative amino acid analysis conducted on the amino acid analyzer (table 7 and fig 11).
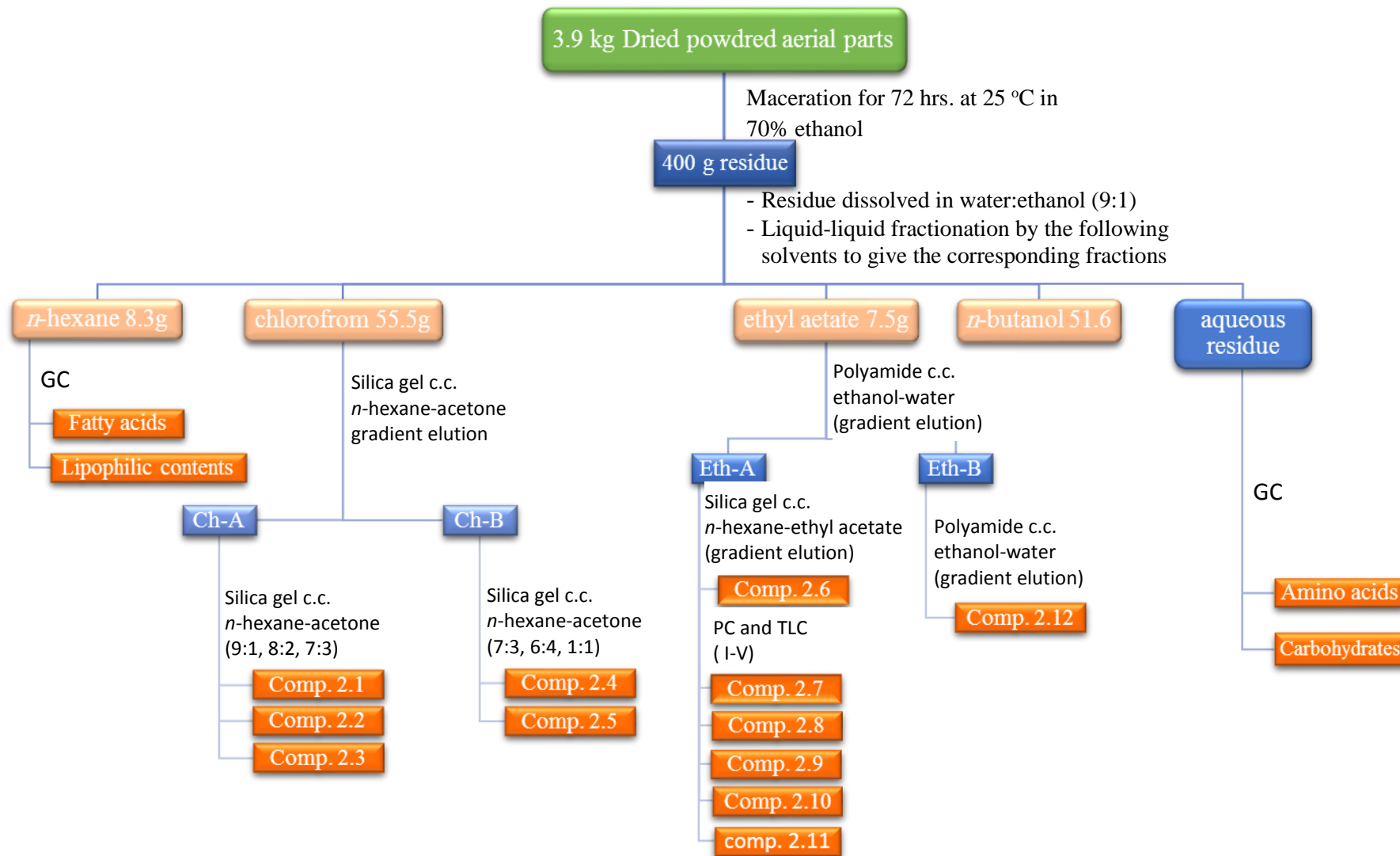
Figure 7 – Scheme of extraction, fractionation and isolation of compounds from ethanolic extract of *Camphorosma lessingii*

3.4 Analysis of fatty and amino acid contents of *Zygophyllum fabago* and *Camphorosma lessingii*

3.4 1 Analysis of fatty acid contents

Fatty acids are the constituents of all plant cells, where they function as membrane components, storage products, metabolites, and as a source of energy [147]. They are also important nutrient substances and metabolites in living organisms [148]. Fatty acids, especially essential fatty acids (EFAs), are of vital significance for human beings. The role of EFAs such as linoleic (18:2ω6) and γ-linolenic (18:3ω6) acids is crucial [28].

Salinity is one of the key environmental factors that modifies fatty acid composition, and is hence important to salt tolerance of oil seed plants. Changes in the ratio of fatty acid saturation/unsaturation in response to salt stress, and a reduction in the concentrations of triacylglycerols containing primarily unsaturated fatty acids has been reported in seed oil from plants under salt stress. There is evidence that unsaturated fatty acids in membrane lipids could protect the photosynthetic machinery against salt stress. As such, linoleic and linolenic acids and their long-chain derivatives are important components of plant cell membranes [149].

The results of analysis of the fatty acids constituents of the aerial parts of the *Zygophyllum fabago* and *Camphorosma lessingii* as shown in table 4 and 5 and figs. 8 and 9 revealed the presence of eight saturated (8.9%), monounsaturated (78.0%) and polyunsaturated (13.2%) known fatty acids in *Z. fabago* while in *C. lessingii* the percent of saturated (18.2%), monounsaturated (33.5%) and polyunsaturated (48.3%) was found different.

Table 4 – Composition of the saturated and unsaturated carboxylic acids (fatty acids) in *Zygophyllum fabago*

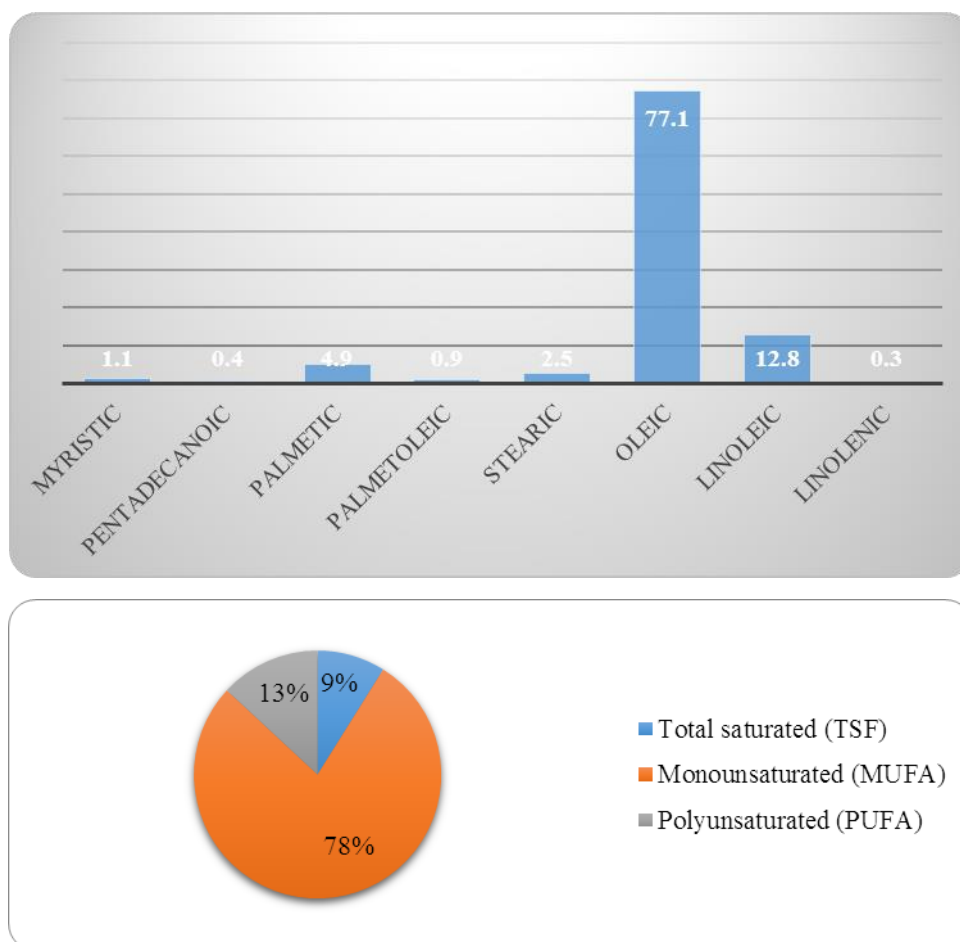| Fatty acids	No. of carbon atoms	Relative percentage %	Peak No
Myristic	$C_{14:0}$	1.10	1
Pentadecanoic	$C_{15:0}$	0.42	2
Palmetic	$C_{16:0}$	4.90	3
Palmetoleic	$C_{16:1}$	0.91	4
Stearic	$C_{18:0}$	2.50	5
Oleic	$C_{18:1}$	77.13	6
Linoleic	$C_{18:2}$	12.81	7
Linolenic	$C_{18:3}$	0.30	8
Total saturated (TSF)		8.92	
Monounsaturated (MUFA)		78.04	
Polyunsaturated (PUFA)		13.21	

Figure 8 – Composition of the fatty acid contents in *Zygophyllum fabago*

The major fatty acids in *Z. fabago* were oleic (77.1 %), linoleic (12.8 %), palmitic (4.9 %), stearic (2.5 %) and myristic (1.1 %) acid, while in *C. lessingii* were Linoleic (46.1 %), Oleic (30.6%), palmitic (8.5 %), stearic (5.1 %) and pentadecanoic (2.8 %) acid.

Table 5 – Composition of the saturated and unsaturated carboxylic acids (fatty acids) in *Camphorosma lessingii*

Fatty acids	No. of carbon atoms	Relative percentage %	Peak No
1	2	3	4
Myristic	$C_{14:0}$	1.82	1
Pentadecanoic	$C_{15:0}$	2.81	2
Palmitic	$C_{16:0}$	8.50	3
Palmetoleic	$C_{16:1}$	2.92	4
Stearic	$C_{18:0}$	5.13	5
Oleic	$C_{18:1}$	30.61	6
Linoleic	$C_{18:2}$	46.12	7
Linolenic	$C_{18:3}$	2.20	8

Table 5 continue

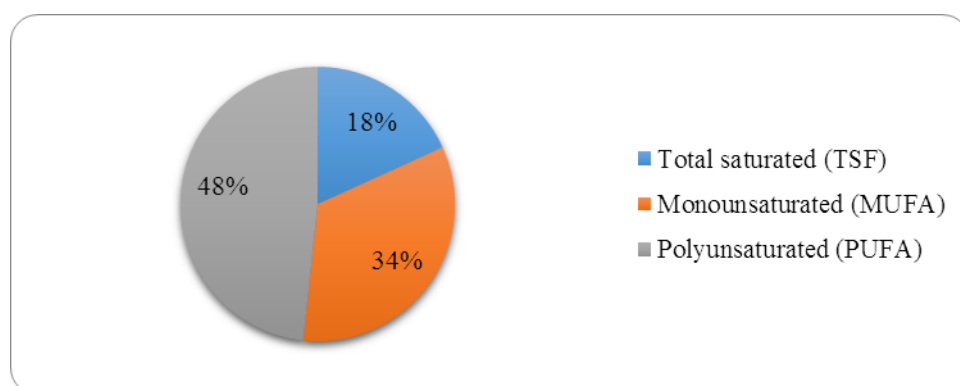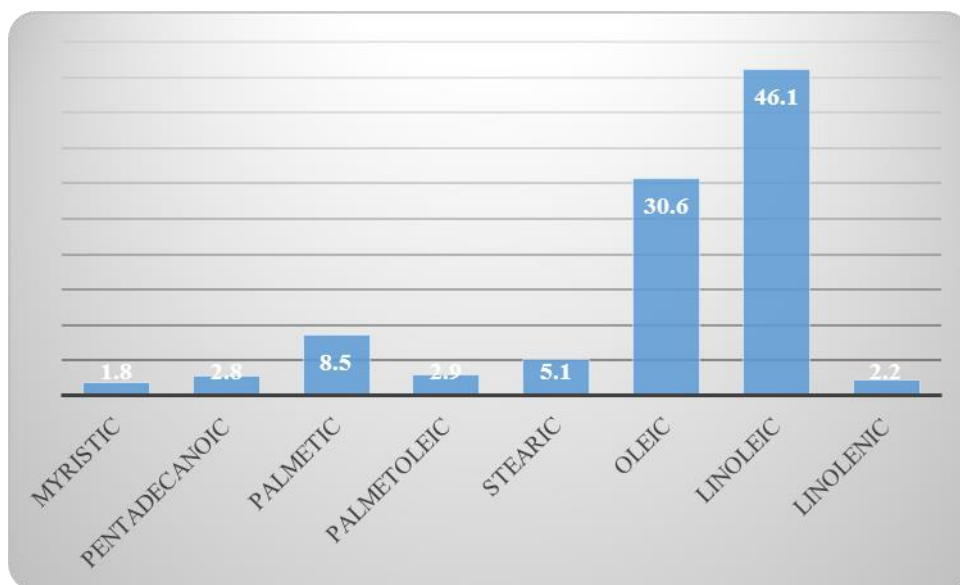| 1	2	3	4
Total saturated (TSF)		18.26	
Monounsaturated (MUFA)		33.53	
Polyunsaturated (PUFA)		48.32	

Figure 9 – Composition of the fatty acid contents in *Camphorosma lessingii*

The results also revealed that there were a significant difference between the two species whereas the content of TSF and PUFA in *C. lessingii* two and three times more than in *Z. fabago;* whereas the MUFA was found also two times more than in *Z. fabago* and the percent of total unsaturated fatty acids in both plants 91.1% and 81.8% was found very great compared to the percent of saturated ones 8.9% and 18.2%. It is of interest to note that these results reported here for the first time for the two species [135, 150]. And this result was found in agreement with the results which mentioned the presence of relationship between the ratio of fatty acid saturation/unsaturation in response to salt stress [149] and the high content of unsaturation of fatty acids also counteracts water or salt stress [145].

3.4.2 Analysis of amino acid contents

A number of nitrogen-containing compounds (NCC) accumulate in plants exposed to saline stress. The most frequently accumulating NCC includes amino acids, amides, imino acids, proteins, quaternary ammonium compounds, and polyamines. NCC accumulation is usually correlated with plant salt tolerance. Amino acids have been reported to accumulate in higher plants under salinity stress. The important amino acids include alanine, arginine, glycine, betaine serine, leucine, and valine, together with the imino acid, proline, and the non-protein amino acids, citrulline and ornithine. Amides such as glutamine and asparagine have also been reported to accumulate in plants subject to salt stress [139, 145].

GC analysis of the amino acids constituents of the aerial parts of the *C. lessingii* and *Z. fabago* (table 6 and 7) and (figs 11 and 12) revealed the presence of twenty amino acids but differs in their percentages, the major amino acids in *C. lessingii* were glutamic acid (27.1%), aspartic acid (9.4 %), proline (7.8 %), alanine (6.5 %), serine (6.0 %), tyrosine (5.2%) and leucine (4.9%), whereas in *Z. fabago* glutamic acid (23.4 *µg/g*), aspartic acid (12.8 *µg/g*), alanine (10.5 *µg/g*), proline (7.6 *µg/g*), arginine (5.1 *µg/g*), leucine (4.7 *µg/g*) and serine (4.2 *µg/g*).

Table 6 – Composition of the amino acid contents in *Zygophyllum fabago*

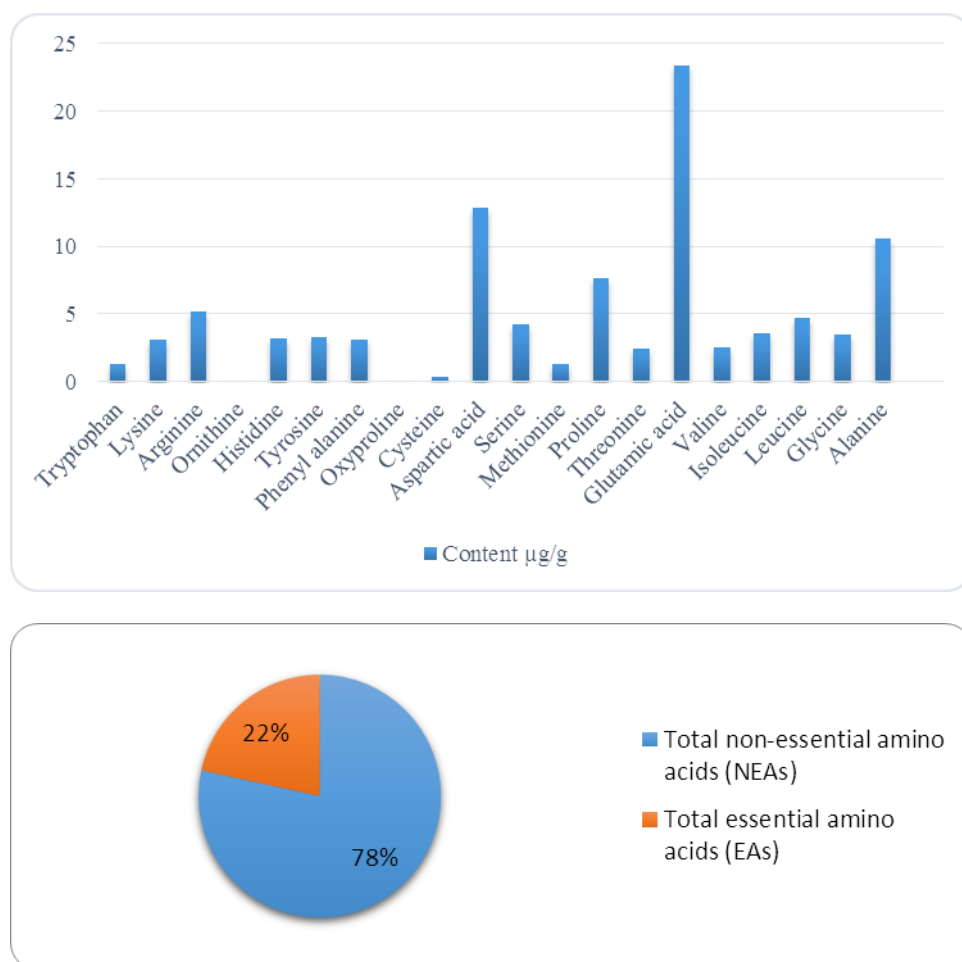| Peak no.	Amino acids	Content *µg/g*	Peak no.	Amino acids	Content *µg/g*
1	Tryptophan	1.27	11	Serine	4.28
2	Lysine	3.12	12	Methionine	1.32
3	Arginine	5.18	13	Proline	7.68
4	Ornithine	0.04	14	Threonine	2.45
5	Histidine	3.20	15	Glutamic acid	23.42
6	Tyrosine	3.32	16	Valine	2.56
7	Phenyl alanine	3.05	17	Isoleucine	3.58
8	Oxyproline	0.03	18	Leucine	4.75
9	Cysteine	0.38	19	Glycine	3.48
10	Aspartic acid	12.86	20	Alanine	10.54
Total non-essential amino acids (NEAs)			75.68		
Total essential amino acids (EAs)			20.83		
Total amino acids			96.51		

Figure 10 – Composition of the amino acid contents in *Zygophyllum fabago*

Table 7 – Composition of the amino acid contents in *Camphorosma lessingii*

| Peak no.	Amino acids	Content *µg/g*	Peak no.	Amino acids	Content *µg/g*
1	Tryptophan	1.92	11	Serine	6.07
2	Lysine	2.46	12	Methionine	0.67
3	Arginine	3.87	13	Proline	8.88
4	Ornithine	0.04	14	Threonine	2.78
5	Histidine	2.30	15	Glutamic acid	27.15
6	Tyrosine	5.26	16	Valine	3.80
7	Phenyl alanine	3.17	17	Isoleucine	2.15
8	Oxyproline	0.03	18	Leucine	4.98
9	Cysteine	0.46	19	Glycine	4.45
10	Aspartic acid	9.46	20	Alanine	8.15
Total non-essential amino acids (NEAs)			73.82		
Total essential amino acids (EAs)			24.23		
Total amino acids			98.05		
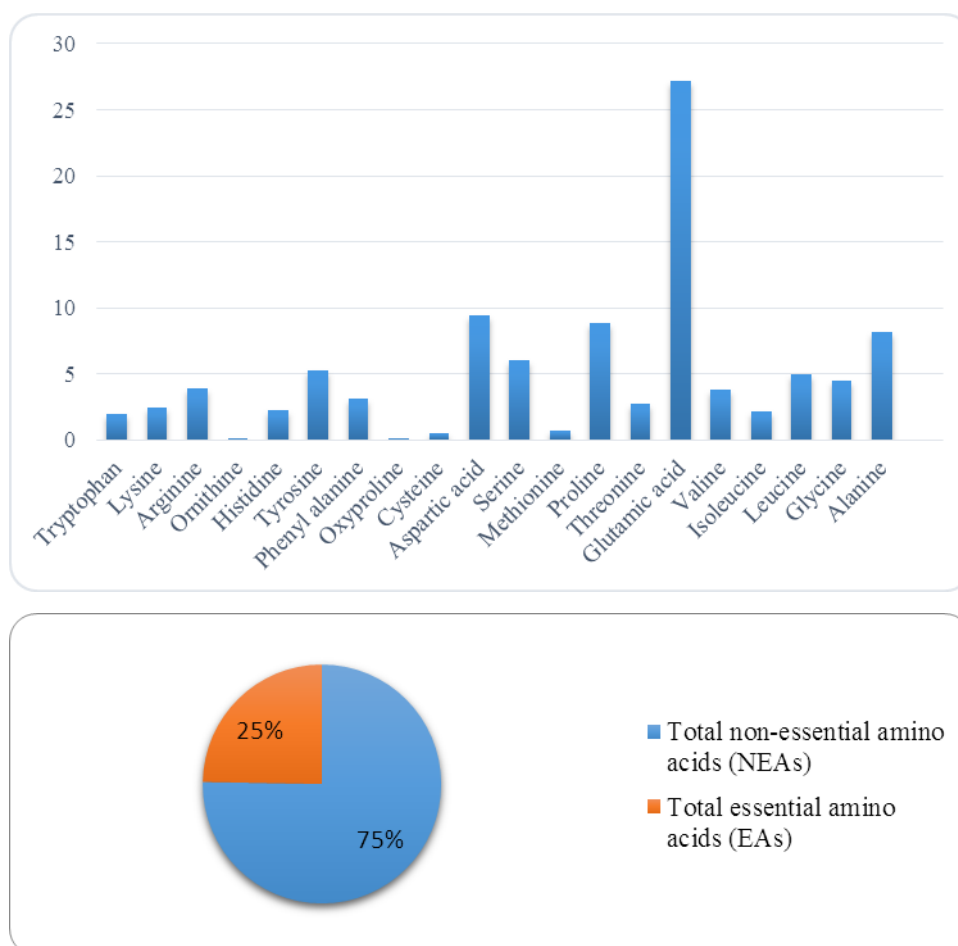
Figure 11 – Composition of the amino acid contents in *Camphorosma lessingii*

the results showed similarity of the amino acid contents of the two species whereas the most abundant amino acid were glutamic acid by approximately the same percent followed by aspartic acid, also the results showed that the amino acids which were found with high percent were the same in the two species with little difference in the sequence, in addition the percent of the total amino acids, total essential and total non-essential amino acids were approximately the same. Also the percent of proline was found approximately close to each other (7.6%) and (8.8%) in *Z. fabago* and *C. lessingii* respectively and this another significant observation because proline, which occurs widely in higher plants, accumulates in larger amounts than other amino acids in salt stressed plants. and is one of the common characteristics in many plants under saline conditions [139], because tolerance of halophytes to ionic and osmotic components of salt stress are linked to their ability to synthesize osmoprotectants in order to maintain a favorable water potential gradient and to protect cellular structures. Proline is one of these osmoprotectants which is also found to have significant beneficial functions under metal stress by three major actions, namely metal binding, antioxidant defense and signaling [151]. The results of the investigation of amino acids reported here for the first time for the two species [135, 150].

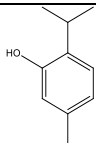### 3.5 Analysis of lipophilic contents of *Zygophyllum fabago* and *Camphorosma lessingii*
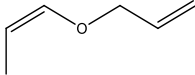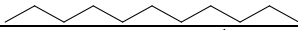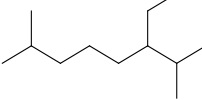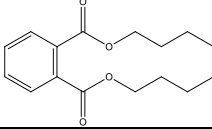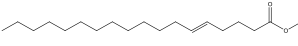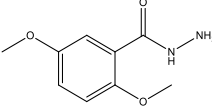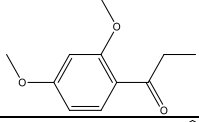
Lipids are vital structural components of cell membranes and serve as storage products. Lipid composition of plasma membranes modifies as the environment changes. These modifications are vital in sustaining membrane fluidity, integrity and functionality when confronting external perturbations [149].

GC-MS analysis of the lipophilic constituents of *C. lessingii* and *Z. fabago* as shown in table 8 and 9 revealed the presence of twelve known compounds in *C. lessingii* which comprising (32.31%) of the total lipophilic constituents, the major compounds are 2-(4-methoxyphenyl)-5-phenyl-1,3,4-thiadiazole (11.71%), 2,5-dimethoxy benzohydrazide (6.07%), heneicosane (5.08%), eicosane (3.62%), 1-(2,4-dimethoxyphenyl)propan-1-one (1.98%) and dimethylchrysin (1.14%). In addition to a number of unknown constituents which represent about (67.69%) while in *Z. fabago* identified seven components which cla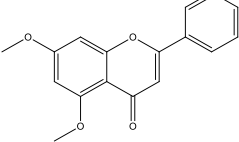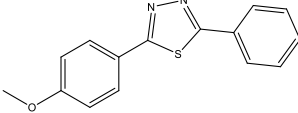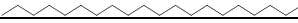rified about 51.76 % of the total fraction while the 48.24 % contain a number of unknown compounds which can`t identified by GC-MS. 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester by 18 % represent the major percent of the identified components, followed by 2-pentadecanone 6,10,14-trimethyl by 8.91%, hexadecanoic acid ethyl ester by 8.77 % and decane by 5.60 %.

Table 8 – Component composition of lipophilic constituents of *Zygophyllum fabago*

Compounds	R_t	M. formula	Content %	Structure
Decane	12.928	$C_{10}H_{22}$	5.60	
Tetradecane	15.703	$C_{14}H_{30}$	2.30	
2-Pentadecanone, 6,10,14-trimethyl-	25.898	$C_{18}H_{36}O$	8.91	
n-Hexadecanoic acid	27.031	$C_{16}H_{32}O_2$	4.03	
Phthalic acid, butyl hexyl ester	27.128	$C_{18}H_{26}O_4$	4.04	
Hexadecanoic acid, ethyl ester	27.359	$C_{18}H_{36}O_2$	8.77	
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	32.161	$C_{16}H_{22}O_4$	18.11	
Total known				51.76
Total unknown				48.24

Table 9 – Component composition of lipophilic constituents of *Camphorosma lessingii*

Compounds	R_t	M. formula	Content %	Structures
Thymol	29.37	$C_{10}H_{14}O$	0.05	
(Z)-1-(2-propenyloxy) prop-1-ene	32.81	$C_6H_{10}O$	0.09	
Undecane	42.70	$C_{11}H_{24}$	0.27	
3-ethyl-2,7-dimethyloctane	46.93	$C_{12}H_{26}$	0.39	
Dibutyl phthalate	50.33	$C_{16}H_{22}O_4$	1.59	
5-octadecenoic acid, methyl ester	53.65	$C_{19}H_{36}O_2$	0.32	
2,5-dimethoxybenzohydrazide	59.58	$C_9H_{12}O_3N_2$	6.07	
1-(2,4-dimethoxyphenyl)propan-1-one	60.03	$C_{11}H_{14}O_3$	1.98	
Dimethylchrysin	76.95	$C_{17}H_{14}O_4$	1.14	
2-(4-methoxyphenyl)-5-phenyl-1,3,4-thiadiazole	64.03	$C_{15}H_{12}ON_2S$	11.71	
Eicosane	71.00	$C_{20}H_{42}$	3.62	
Heneicosane	81.91	$C_{21}H_{44}$	5.08	
Total known			32.31	
Total unknown			67.69	

The identified components in both species contain hydrocarbons, esters, ketones, acids, phenols and other nitrogenous compounds. The hydrocarbons, esters and ketones identified in the two species with differences in their percent where hydrocarbons reported by relatively the same percent 7.9% in *Z. fabago* and 9.36% in *C. lessingii* while the ester and ketone contents showed a large difference where esters and ketones represented by 30.92 and 8.91% in *Z. fabago* and 3.14 and 1.98% in *C. lessingii* respectively. Moreover the acids 4.03% reported only in *Z. fabago* and nitrogenous 17.78% and phenols 0.05% in *C. lessingii*. the results which obtained here were reported for the first time for both species [152]. The results in the present study are consistent with the previous observations which demonstrated that

lipophilic constituents have vital roles in the tolerance to several physiological stressors [145].

3.6 Investigation of the essential oil constituents of *Camphorosma lessingii*

Essential oils are defined as any volatile oil(s) that have strong aromatic components and that give distinctive odour, flavor or scent to a plant. These are the by-products of plant metabolism and are commonly referred to as volatile plant secondary metabolites [153] Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices nutrition and insecticides [154]. It is also known for their therapeutic properties hence, used in the treatment of various infections [155, 156]. In addition strong in vitro evidence indicates that essential oil can act as antibacterial agent against a wide spectrum of pathogenic bacteria strains [157-161]. Besides their antibacterial and antifungal activities, essential oils have also been reported to possess interesting antiviral activities [162]. Essential oils can also be used for the treatment of non-pathogenic diseases [163]. Some essential oils also exert hypotensive activity when applied in vivo and they are used for treating hypertension [164]. Moreover essential oils and their individual aroma components showed cancer suppressive inactivity when tested on a number of human cancer cells lines including glioma, tumors, breast cancer, leukaemia and others [165].Essential oils also are reported to have insecticidal properties essentially as ovicidal, larvicidal, growth inhibitor, repellence and antifeedant [166].

Essential oils are multicomponent mixtures of organic compounds found naturally in various parts of aromatic plants. They have very complex chemical properties and various efficacy modes. The chemical composition of the volatile oil of *Camphorosma lessingii* is presented in table 10. A total of eighty one compounds were detected from which 37 were identified, which constitute about 60.79 % of the volatile oil. Table 9 showed the main component of volatile oil from the aerial parts of *Camphorosma lessingii* were terpenoids (mono, sesqui and diterpenes), hydrocarbons, hydroxy compounds, esters, acids, nitrogenous and other miscellaneous compounds. The main components are; hexanedioic acid, bis (2-ethylhexyl) ester (38.26%), *n*-hexadecanoic acid (3.74%), octadecanoic acid (3.61%), benzenamine, N,N-diethyl (1.85%) while monoterpenes; α-pinene (1.13%) was the main components, sesquiterpene; spathulenol (0.54%) was the main one and for diterpenes found kaur-16-ene, (8*β*,13*β*) (0.32%). The results of the investigation of the essential oils reported here for the first time for the genus *Camphorosma* [167].

Table 10 – Percent composition of *Camphorosma lessingii* essential oils
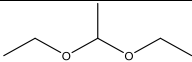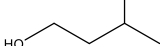
Compounds	R_t	Content%	M. formula	Structures
1	2	3	4	5
1,1-diethoxy ethane	10.59	0.45	$C_6H_{14}O_2$	
1-Butanol, 3-methyl	11.01	0.19	$C_5H_{12}O$	

Table 10 continue
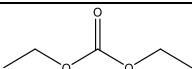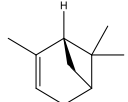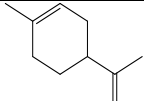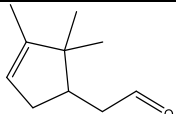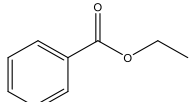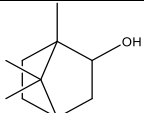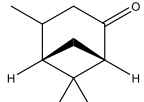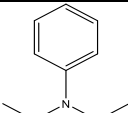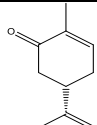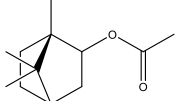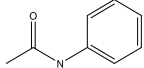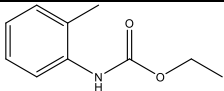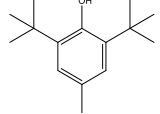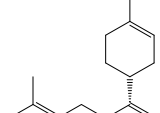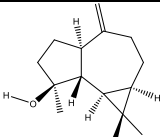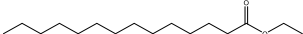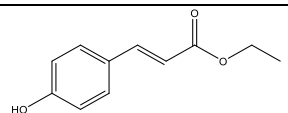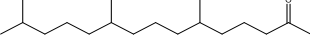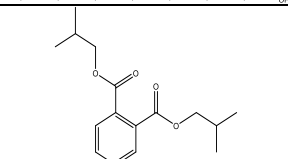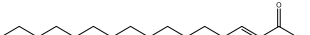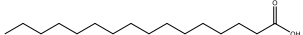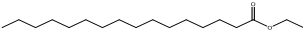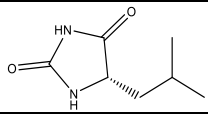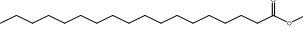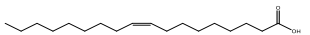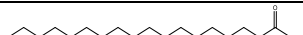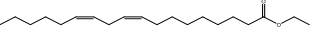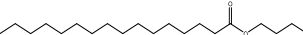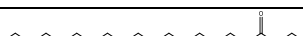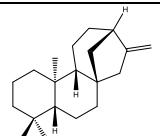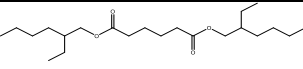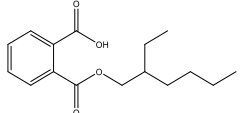
1	2	3	4	5
Carbonic acid, diethyl ester	13.37	0.27	$C_5H_{10}O_3$	
α-Pinene	18.38	1.13	$C_{10}H_{16}$	
Camphene	18.84	0.08	$C_{10}H_{16}$	
D-Limonene	20.62	0.33	$C_{10}H_{16}$	
Campholenic aldehyde	22.53	0.11	$C_{10}H_{16}O$	
Benzoic acid, ethyl ester	23.23	0.14	$C_9H_{10}O_2$	
Borneol	23.39	0.17	$C_{10}H_{18}O$	
2-Pinen-4-one (1S,5S)	23.96	0.52	$C_{10}H_{14}O$	
Benzenamine, N,N-diethyl	24.05	1.85	$C_{10}H_{15}N$	
d-Carvone (+)	24.48	0.16	$C_{10}H_{14}O$	
Bornyl acetate	25.08	0.20	$C_{12}H_{20}O_2$	
Acetamide, N-phenyl	26.26	1.19	C_8H_9NO	
Ethyl N-(2-methylphenyl) carbamate	27.59	0.16	$C_{10}H_{13}NO_2$	
Butylated Hydroxytoluene	28.01	0.11	$C_{15}H_{24}O$	
β-Bisabolene	28.09	0.22	$C_{15}H_{24}$	

Table 10 continue

1	2	3	4	5
(+) Spathulenol	29.18	0.54	$C_{15}H_{24}O$	
Tetradecanoic acid, ethyl ester	31.24	0.14	$C_{16}H_{32}O_2$	
p-Hydroxycinnamic acid, ethyl ester	31.70	0.14	$C_{11}H_{12}O_3$	
2-Pentadecanone,6,10,14-trimethyl	31.80	0.09	$C_{18}H_{36}O$	
Pentadecanoic acid	31.91	0.11	$C_{15}H_{30}O_2$	
1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	32.08	0.62	$C_{16}H_{22}O_4$	
trans-2-Hexadecenoic acid	32.77	0.37	$C_{16}H_{30}O_2$	
n-Hexadecanoic acid	32.95	3.74	$C_{16}H_{32}O_2$	
Hexadecanoic acid, ethyl ester	33.30	1.13	$C_{18}H_{36}O_2$	
2,4-Imidazolidinedione, 5-(2-methylpropyl)-, (S)	34.41	0.16	$C_7H_{12}N_2O_2$	
Octadecanoic acid, methyl ester	34.58	0.14	$C_{19}H_{38}O_2$	
Oleic Acid	34.69	1.34	$C_{18}H_{34}O_2$	
Octadecanoic acid	34.89	3.61	$C_{18}H_{36}O_2$	
Linoleic acid ethyl ester	34.93	1.34	$C_{20}H_{36}O_2$	
Hexadecanoic acid, butyl ester	35.12	0.70	$C_{20}H_{40}O_2$	
Octadecanoic acid, ethyl ester	35.18	0.33	$C_{20}H_{40}O_2$	
Acetic acid, octadecyl ester	35.31	0.17	$C_{20}H_{40}O_2$	
Kaur-16-ene, (8*β*,13*β*)	36.24	0.32	$C_{20}H_{32}$	
Hexanedioic acid, bis(2-ethylhexyl)ester	36.93	38.26	$C_{22}H_{42}O_4$	
1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	38.36	0.26	$C_{16}H_{22}O_4$	
Total Known		60.79		
Total Unkown		39.21		

3.7 Identification of triterpenes and sterols

Compound **1.5** was obtained as white powder, m.p.282-284 °C (MeOH) it gave positive results to the libermann-Burchard test.with $R_f = 0.65$ (ethyl actate).

The ^{1}H-NMR (table 11 and fig 12) of compound **1.5** exhibited four tertiary and two secondary methyls characterized by the singlets at δ_H 1.05 (3H-24), 1.18 (3H-26), 1.25 (3H-25), 1.25 (3H-23) and the doublets at δ_H 1.19 (3H, d, J= 6.2 Hz, H-29) and 0.98 (3H, d, J= 6.3 Hz, H-30), one doublet at δ_H 3.33 (1H, d, J= 8.6 Hz, H-3), the ^{1}H-NMR spectrum also displayed one olefinic proton at δ_H 5.85 (1H, br.s, H-12).

The ^{13}C-NMR and DEPT (table 11 and fig 13-14) spectra of the compound **1.5** showed 30 carbon resonances confirmed the presence of triterpenoidal aglycone moiety, the thirty carbons were resolved as six methyls at δ_C 16.3 (C-25), 16.3 (C-24), 17.8 (C-29), 18.4 (C-26), 21.4 (C-30) and 28.6 (C-23), nine methylenes at δ_C 18.3 (C-6), 22.7 (C-11), 24.3 (C-16), 25.3 (C-15), 27.2 (C-2), 30.1 (C-21), 36.19 (C-22), 36.6 (C-1) and 38.8 (C-8), six methines of which one oxygenated at δ_C 77.3 (C-3), one olefinic at δ_C 128.2 (C-12) and other four methines at δ_C 36.8 (C-20), 38.7 (C-19), 46.4 (C-9) and 55.2 (C-5), eight quaternary carbons, of which two carboxylic at δ_C 176.4 (C-27) and 178.6 (C-28), one olefinc at 132.9 (C-13) and other five at δ_C 39.3 (C-4), 39.3 (C-8), 47.6 (C-17), 55.6 (C-14) and 38.8 (C-10).

HMBC cross-peaks (fig 15) between δ_H 5.85 (H-12) and δ_C 22.7 (C-11), 46.4 (C-9) and 55.6 (C-14) and COSY cross-peaks (fig 17) between δ_H 5.85 (H-12) and δ_H 1.96 (H-11) revealed the position of the C=C bond.

The above structural elucidation of **1.5** was further supported by its ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY), heteronuclear multiple quantum correlation (HMQC) fig 16 and HMBC data, respectively.

Based on the previous ^{1}H-NMR, ^{13}C-NMR analysis and HMBC, HMQC and COSY correlations and comparing with the literature data [168], the structure of compound **1.5** was recognized as 3β-3-hydroxyurs-12-ene-27, 28-dioic acid (Quinovic acid). This compound previously isolated from the genus *Zygophyllum* [169].
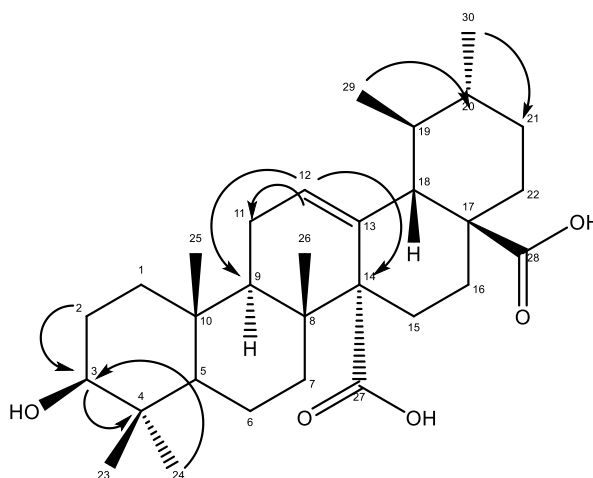
3β-3-hydroxyurs-12-ene-27, 28-dioic acid (Quinovic acid) (Compound **1.5**)

Table 11 – ^{1}H and ^{13}C-NMR spectral data and ^{13}C-^{1}H long –rang correlation of compound **1.5** in *DMSO- d$_6$*

Carbon	δc	Dept	δ$_H$(J_{HH} in Hz)	Cross peaks (δ$_C$) in HMBC spectrum
1	36.64	CH$_2$		
2	27.29	CH$_2$	1.85 (m, 2H)	77.3 (C-3)
3	77.31	CH	3.33 (d, J = 8.6 Hz, 1H)	16.3 (C-24), 28.6 (C-23), 39.3 (C-4)
4	39.34	C		
5	55.29	CH	0.98 (s, 1H)	
6	18.32	CH$_2$		
7	38.80	CH$_2$	2.05 – 1.88 (m, 2H),	
8	39.34	C		
9	46.47	CH	2.51 (m, 1H)	
10	38.8	C		
11	22.71	CH$_2$	1.96 (m, 2H)	46.4 (C-9)
12	128.26	CH	5.85 (br.s, 1H)	22.7 (C-11), 46.4 (C-9), 55.6 (C-14)
13	132.91	C		
14	55.62	C		
15	25.31	CH$_2$		
16	24.33	CH$_2$		
17	47.61	C		
18	53.97	CH	2.51 (m, 1H)	
19	38.67	CH		
20	36.83	CH		
21	30.11	CH$_2$		
22	36.19	CH$_2$		
23	28.63	CH$_3$	1.25 (s, 3H)	16.3 (C-24), 39.3 (C-4), 77.3 (C-3), 55.2 (C-5)
24	16.38	CH$_3$	1.05 (s, 3H)	28.6 (C-23), 39.3 (C-4), 55.2 (C-5), 77.3 (C-3)
25	16.30	CH$_3$		
26	18.47	CH$_3$	1.18 (s, 3H)	39.3 (C-8), 46.4 (C-9), 55.6 (C-14)
27	176.45	C		
28	178.67	C		
29	17.81	CH$_3$	1.19 (d, J = 6.2 Hz, 3H)	
30	21.42	CH$_3$	0.98 (d, J = 6.3 Hz, 3H)	

Figure 12 – ^{1}H-NMR spectrum of compound **1.5** in *DMSO- d$_6$*

Figure 13 – ^{13}C-NMR spectrum of compound **1.5** in *DMSO- d$_6$*

Figure 14 – DEPT spectrum of compound **1.5** in *DMSO- d$_6$*

Figure 15 – HMBC spectrum of compound **1.5** in *DMSO- d$_6$*

Figure 16 – HMQC spectrum of compound **1.5** in *DMSO- d₆*

Figure 17 – COSY spectrum of compound **1.5** in *DMSO- d₆*

Compound **1.29** was obtained as white powder, m.p.247-249 °C (MeOH), with $R_f = 0.19$ (system XIV) it gave positive results to the libermann-Burchard and Molish tests. Furthermore, the HR-ESI-MS $[M-1]^+$ of this compound showed m/z = 647.417, corresponding to the molecular formula $C_{36}H_{56}O_{10}$.

The ^{1}H-NMR and ^{13}C-NMR spectra (table 12 and fig 18-20) of compound **1.29** showed the presence of quinovic acid as aglycone ^{1}H-NMR spectrum displayed one anomeric proton at δ_H 4.56 (1H, d, J=7.6 Hz, H-1) indicating the presence of sugar moiety of β-configuration.

The ^{13}C-NMR and DEPT spectra of the compound **1.29** showed 36 carbon resonances confirmed the presence of one hexose sugar moiety with quinovic acid. The ^{13}C-NMR spectrum also showed anomeric carbon at δ_C 105.3 and other sugar carbons at δ_C 76.6, 76.9, 70.2, 74.0 and 61.3 which showed that the sugar was a β-glucose [170]. The sugar was also confirmed by acid hydrolysis which gave glucose and aglycone moieties. The HMQC (fig 21) used to correlate the carbon to its corresponding proton while the HMBC correlation (fig 24) between H-1' of glucose at δ_H 4.56 and C-3 of the aglycone at δ_C 87.9 revealed that glucose was attached at C-3 of the aglycone.

On the basis of all the above evidences and comparing with the literature data [171], the structure of compound **1.29** was assigned as 3-O-β-D-glucopyranosyl-quinovic acid. This compound also isolated from some members of the genus *Zygophyllum* [171]. The above structure was further supported by its ^{1}H-^{1}H COSY, HMQC and HMBC data (fig 23-25).

3-O-β-D-glucopyranosyl-quinovic acid (Compound **1.29**)

Table 12 – ¹H and ¹³C-NMR spectral data and ¹³C-¹H long –rang correlation of compound **1.29** in *DMSO- d₆*

Carbon	δc	Dept	δ_H(J_{HH} in Hz)	Cross peaks (δ_C) in HMBC spectrum
1	35.91	CH₂		
2	25.66	CH₂		
3	87.92	CH	2.92 (br.s, 1H)	
4	39.05	C		
5	55.29	CH	1.02 (d, J = 11.1 Hz, 1H)	18.2 (C-24), 17.8(C-6), 36.2(C-10), 39.0(C-4)
6	17.83	CH₂		
7	38.63	CH₂		
8	38.71	C		
9	46.22	CH	2.56 (d, J = 8.5 Hz, 3H)	
10	36.26	C		
11	22.44	CH₂		
12	127.96	CH	5.90 (s, 1H)	55.3(C-14)
13	132.65	C		
14	55.37	C		
15	24.08	CH₂		
16	25.03	CH₂		
17	47.34	C		
18	53.71	CH	2.56 (d, J = 8.5 Hz, 3H)	
19	36.56	CH		
20	38.50	CH		
21	29.85	CH₂		
22	36.34	CH₂		
23	27.77	CH₃	1.35 (s, 3H)	87.9(C-3), 74.0(C-5`), 55.2(C-5), 39.0(C-4)
24	18.20	CH₃	1.15 (s, 3H)	87.9(C-3), 39.0(C-4), 27.7(C-23)
25	16.15	CH₃	1.30 (s, 3H)	36.2(C-10), 46.2(C-9), 55.2(C-5)
26	17.55	CH₃	1.20 (s, 3H)	38.7(C-8), 46.2(C-9), 55.3
27	176.18	C		
28	178.38	C		
29	16.57	CH₃	1.23 (br.s, 3H)	
30	21.17	CH₃	1.28 (br.s, 3H)	
1`	105.35	CH	4.56 (d, J = 7.6 Hz, 1H)	87.9(C-3), 105.3(C-1`)
2`	76.62	CH	3.46 (m, 1H)	70.2(C-4`), 74.0(C-5`)
3`	76.95	CH		
4`	70.25	CH	3.43 (m, 1H)	61.3 (C-6`), 76.9(C-3`)
5`	74.03	CH	3.38 (m, 1H)	
6`	61.30	CH₂	4.06 (d, J = 10.7 Hz, 2H)	
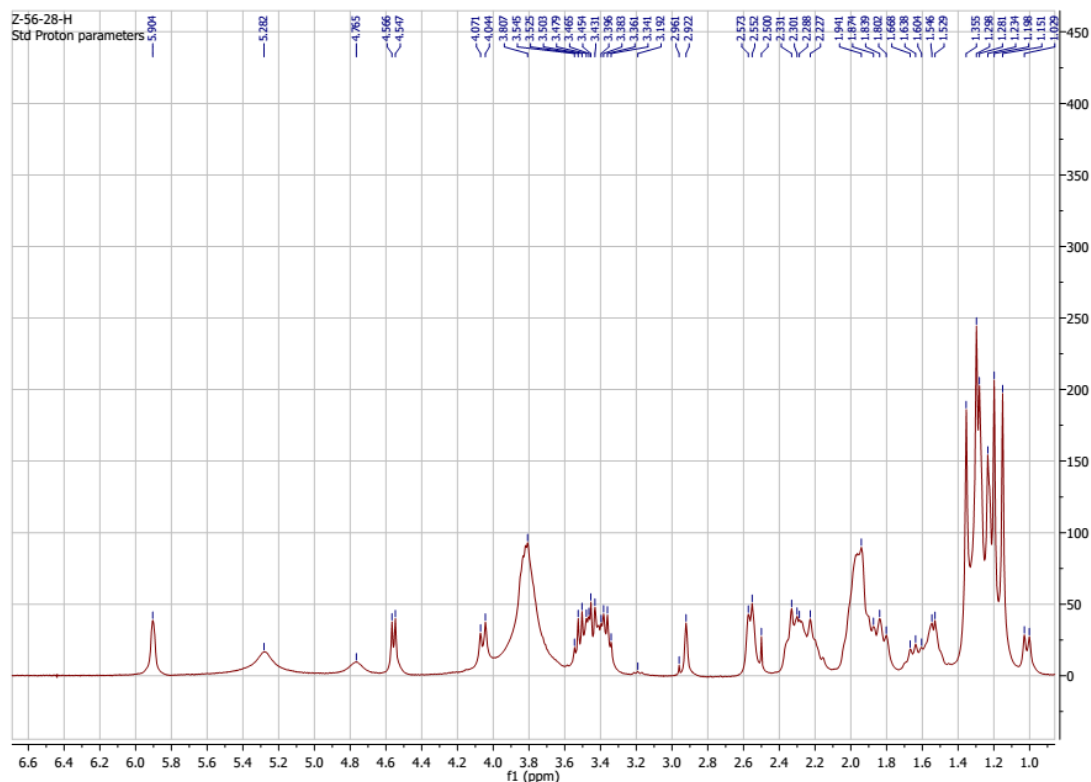
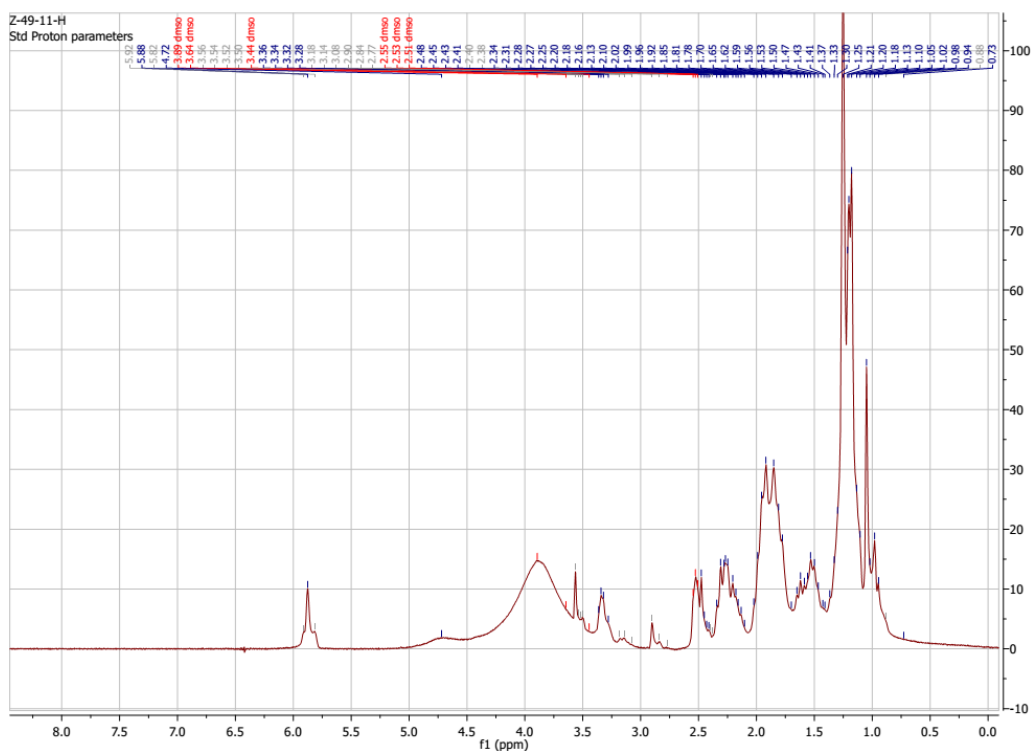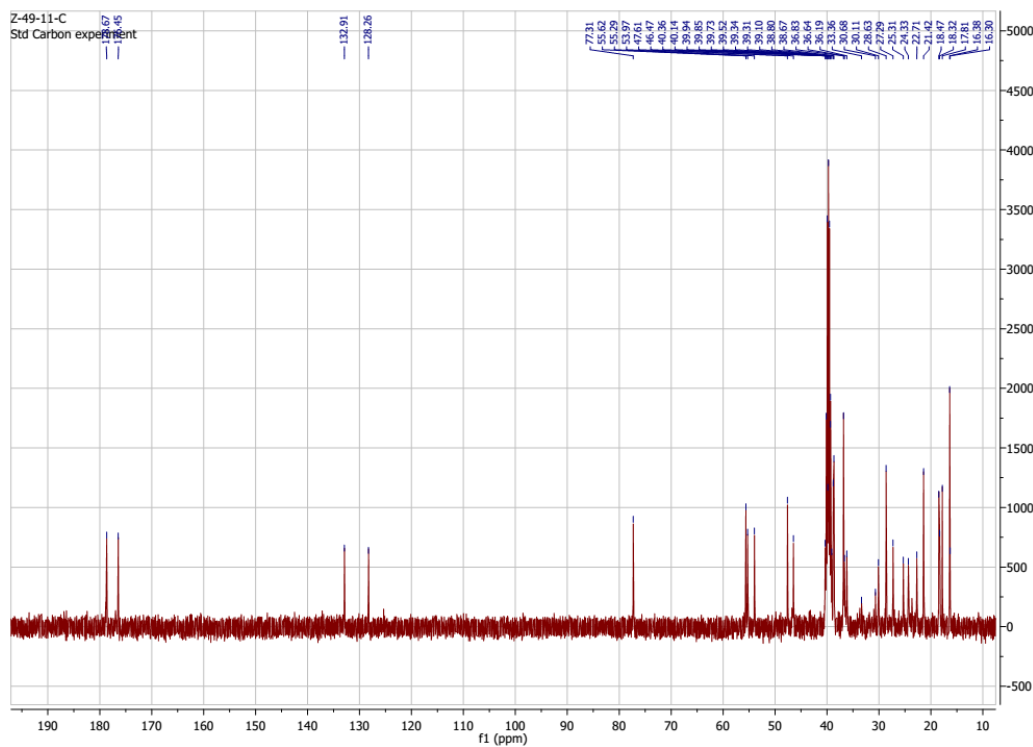Figure 18 – ^{1}H-NMR spectrum of compound **1.29** in *DMSO- d$_6$*
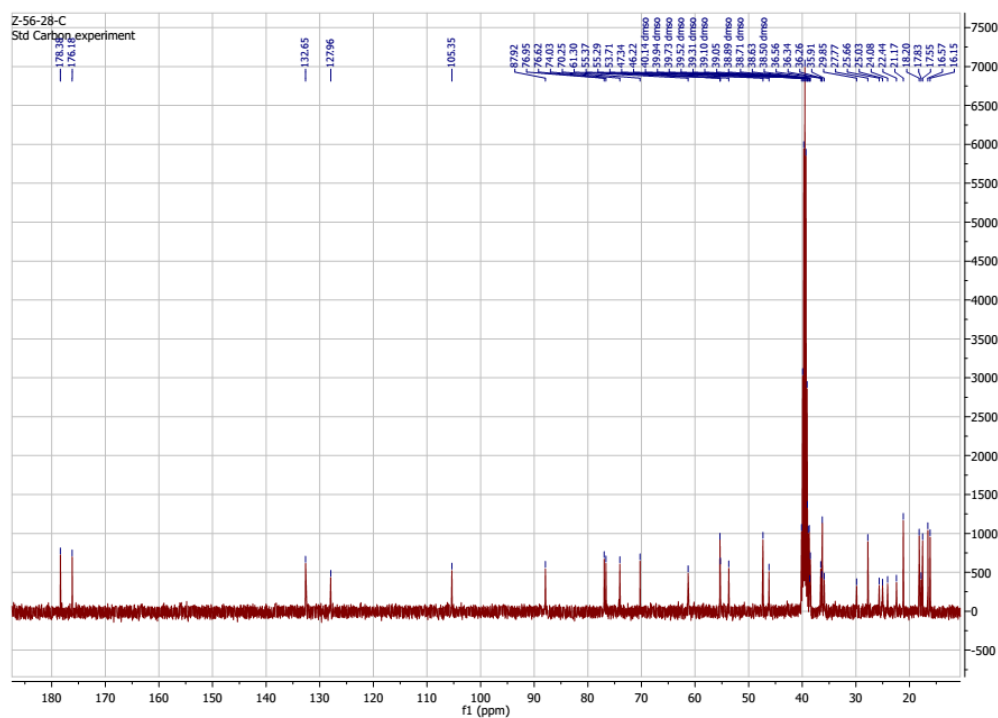
Figure 19 – ^{13}C-NMR spectrum of compound **1.29** in *DMSO- d$_6$*
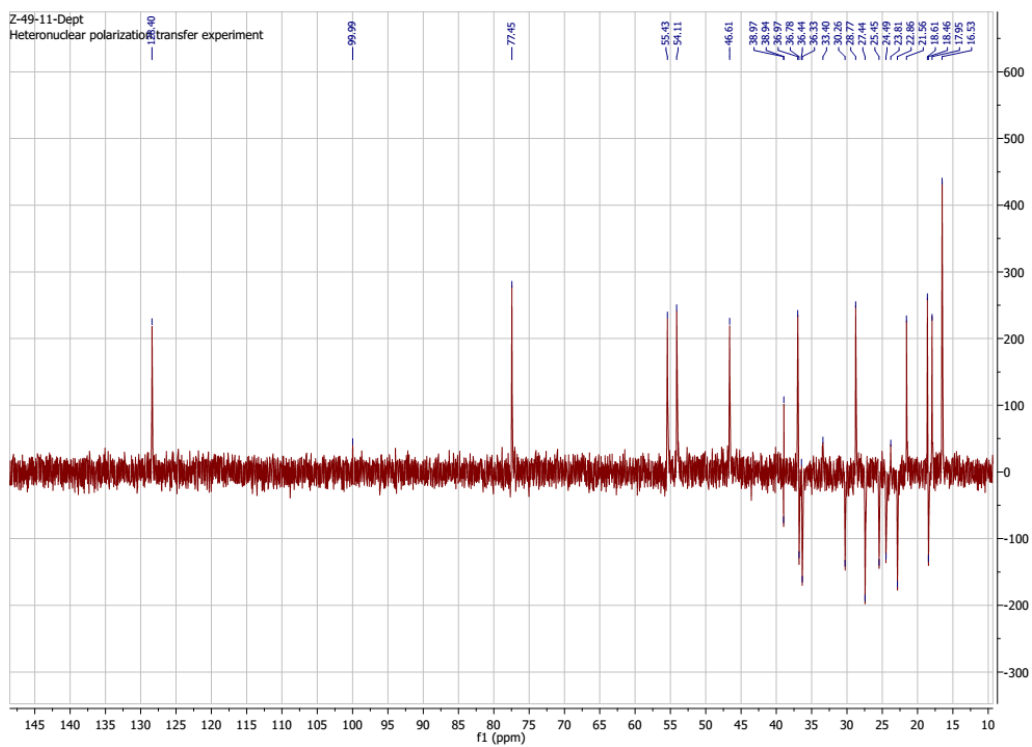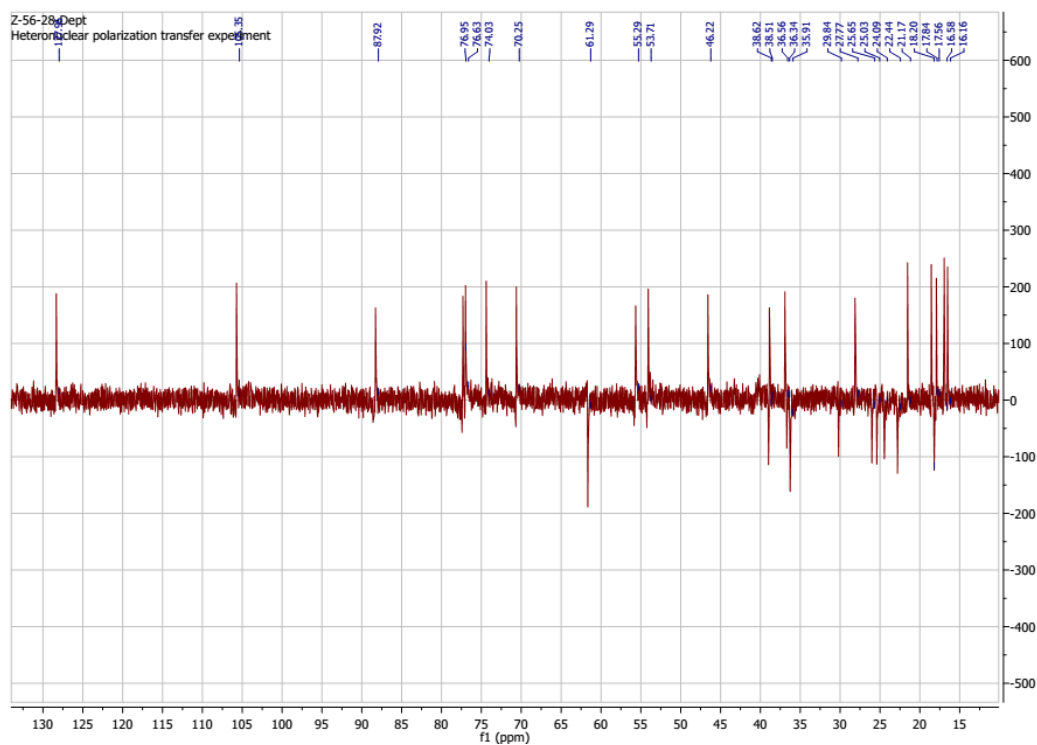
Figure 20 – DEPT spectrum of compound **1.29** in *DMSO- d₆*
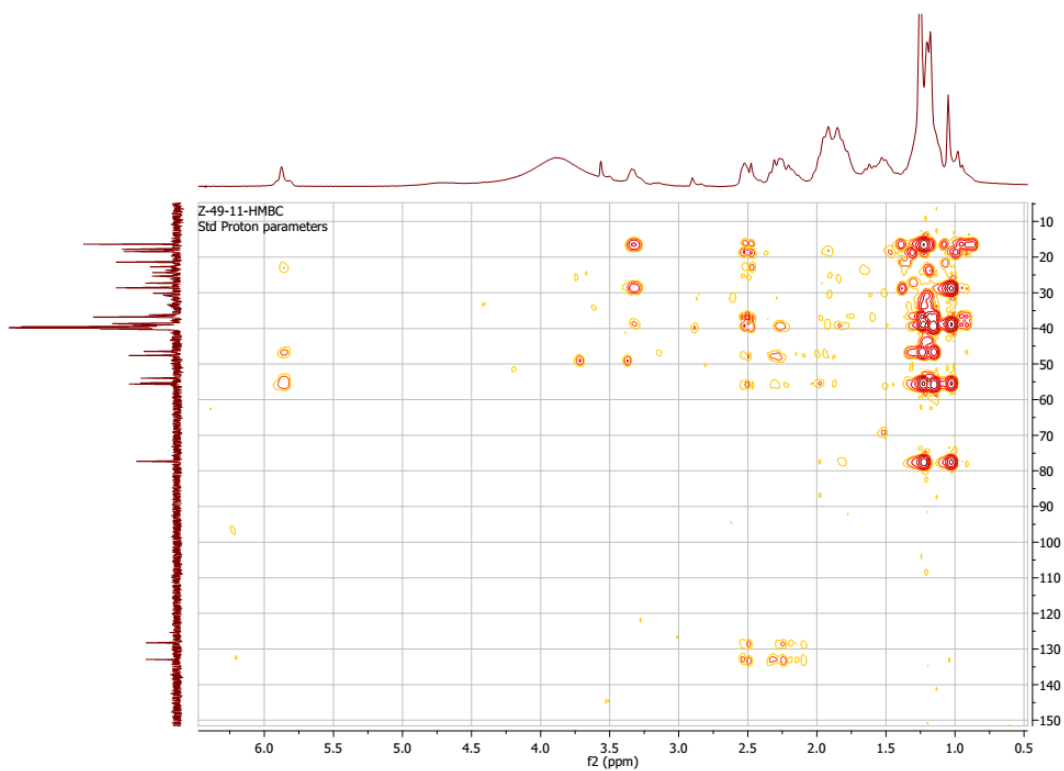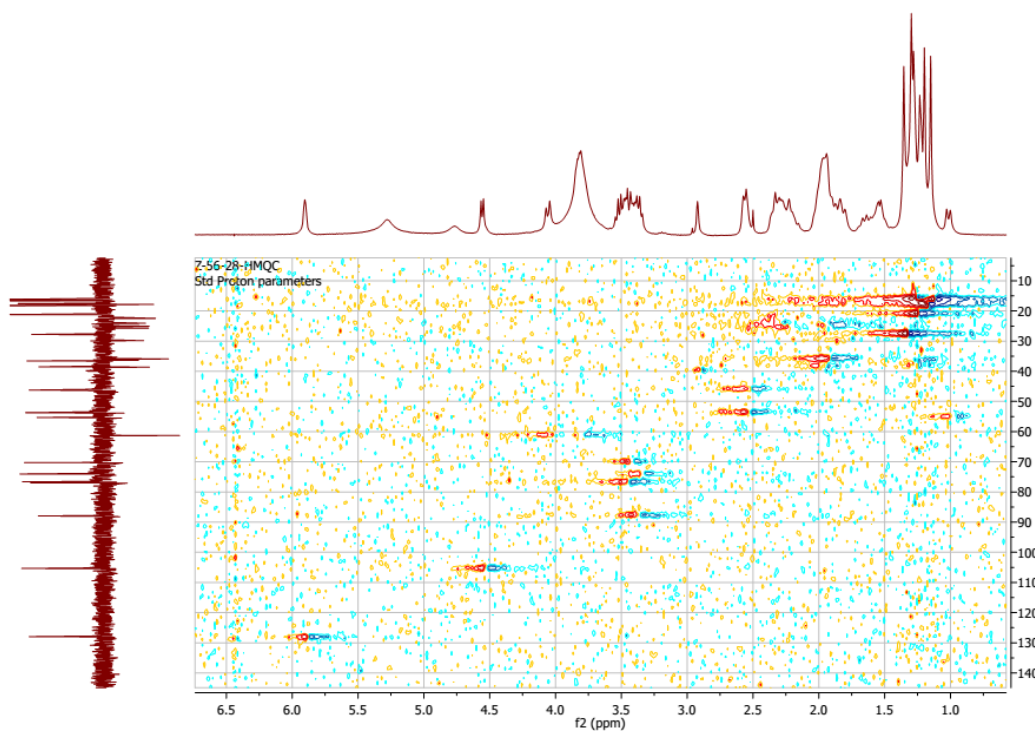

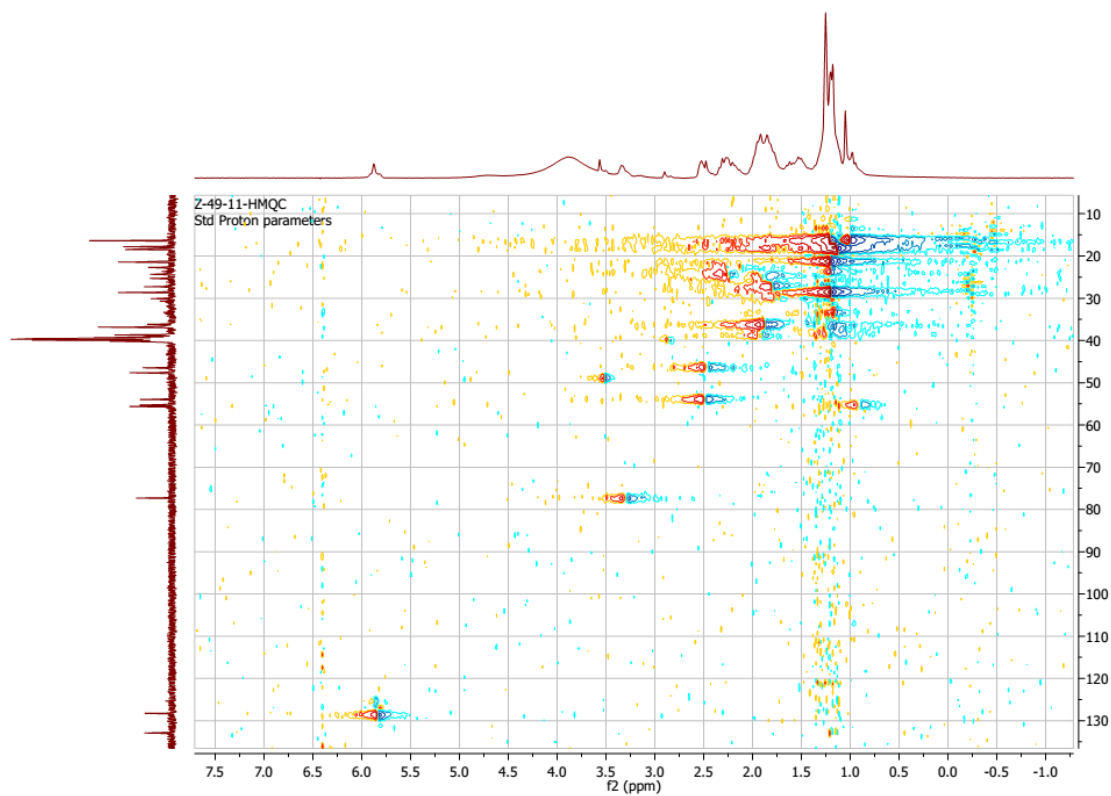Figure 21 – HMQC spectrum of compound **1.29** in *DMSO- d₆*
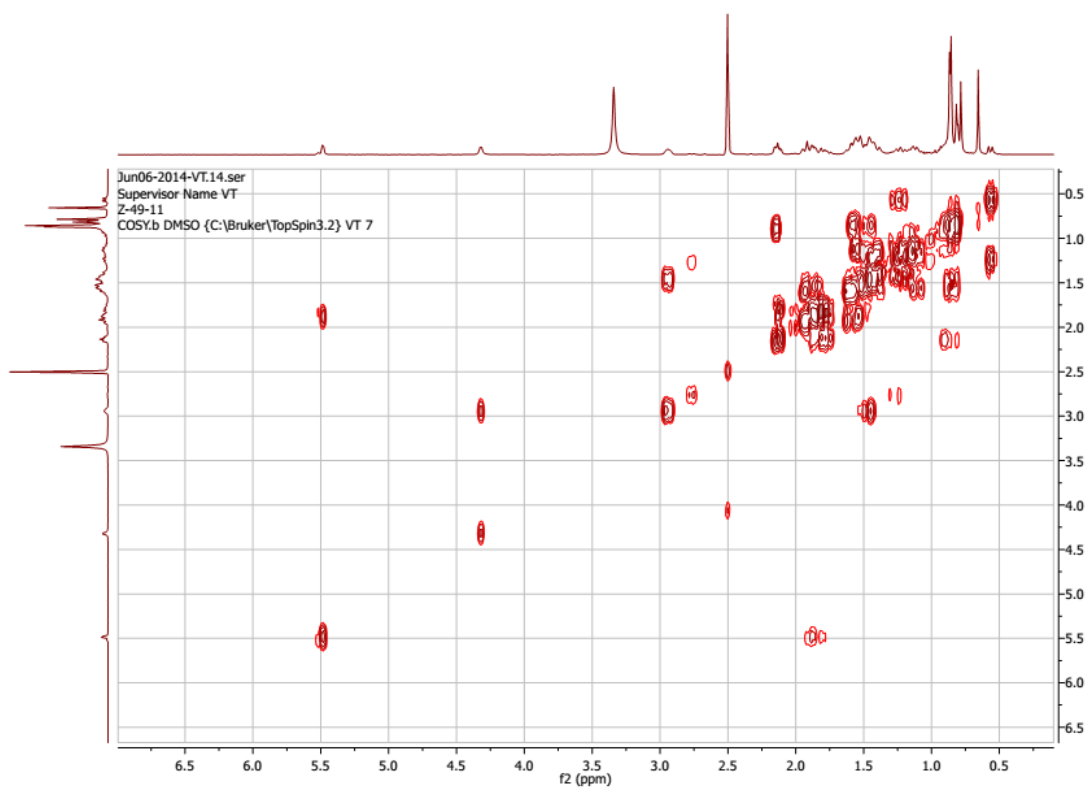
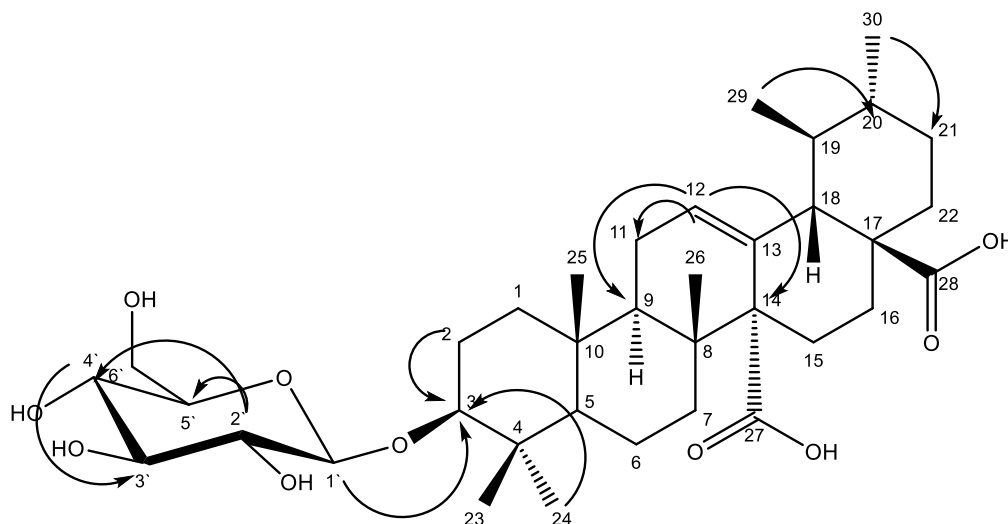Figure 22 – HMBC spectrum of compound **1.29** in *DMSO- d₆*
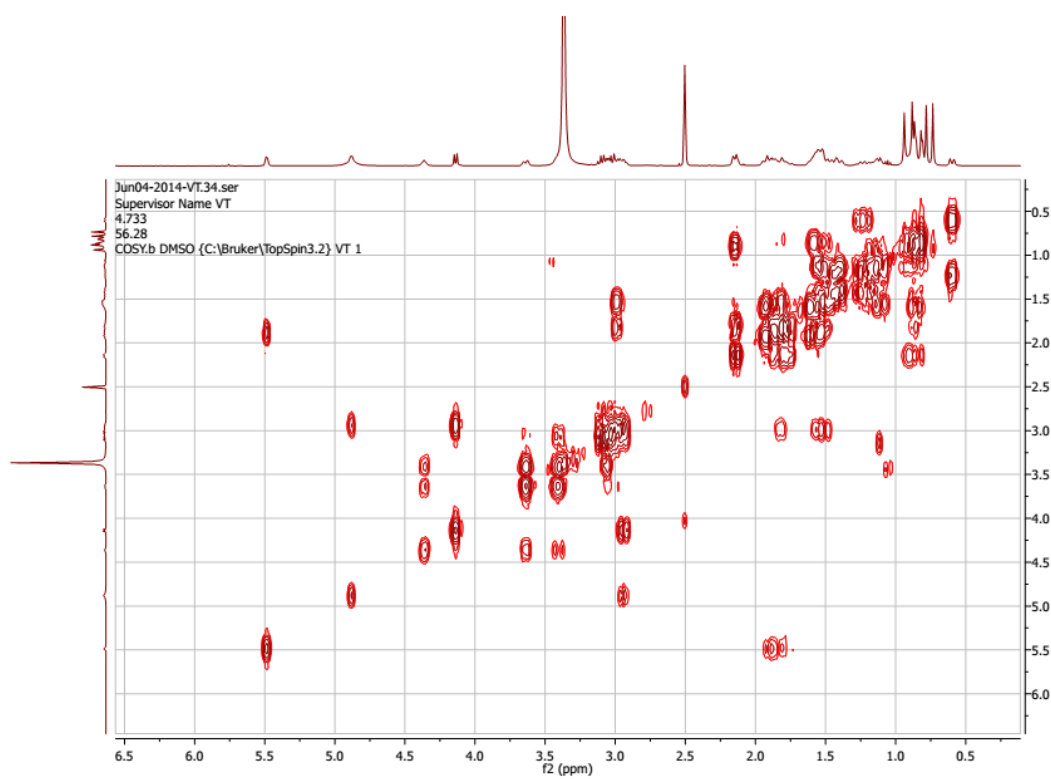
Figure 23 – COSY spectrum of compound **1.29** in *DMSO- d₆*

Compound **1.12** was obtained as white powder, m.p. 208-209 $^\circ$C, with R_f =0.44 (system X), it gave positive results to the libermann-Burchard and Molish tests. Furthermore, the HR-ESI-MS [M-1]$^+$ of this compound showed m/z = 617.381, corresponding to the molecular formula $C_{35}H_{54}O_9$.

The ^{1}H-NMR and ^{13}C-NMR spectra (table 13 and fig 24-26) of compound **1.12** showed 35 carbon resonances, indicating the presence of a single sugar moiety with a triterpenoid aglycone, the olefinic resonances of the aglycone at δ_C 134.63and 129.46, corresponding to quaternary and methine carbons suggested the urs-l2-ene skeleton with a carboxylic group at C-27. The carbonyl carbons at δ_C 180.8 and 178.59 showed the presence of two unsubstituted carboxylic groups at C-17 and C-14, respectively, in the aglycone moiety [172, 173]. The ^{13}C-NMR spectral data of **1.12** were consistent with quinovic acid as the aglycone [172-174].

The methine and methyl resonances at δ_C 108.0, 76.0, 79.11, 71.74, and 67.57 were due to the sugar moiety suggested that the sugar may be pentose sugar. The large coupling constants of the anomeric proton in the ^{1}H-NMR spectrum at δ_H 4.70 (1H, d, J=7.5 Hz, H-1) (table 13) indicated their β-nature and the glycosylation site was assigned to C-3 of quinovic acid.

The ^{13}C-NMR spectrum also showed anomeric carbon at δ_C 108.0 and other sugar carbons at δ_C 76.0, 79.11, 71.74, and 67.57, together with the ^{1}H-NMR spectrum which showed six clear signals at δ_H 4.70 (1H, d, J=7.5 Hz, H-1`), 4.00 (1H, d, $J$ = 8.0 Hz,H-2`), 4.15 (1H, d, J = 8.6 Hz, H-3`), 4.21 (1H, dd, $J$ = 9.6, 5.1 Hz, H-4`), 4.36 (1H, dd, J = 11.3, 5.2 Hz, H-5`) and 3.75 (1H, t, $J$ = 10.9 Hz, H-5`) which assigned to C-1` - C-5` of xylose [175]. The HMQC spectrum (fig 27) showed that the ^{13}C-NMR signals at 108.09, 76.0, 79.11, 71.74, and 67.57 can be correlated with ^{1}H-NMR signals at 4.70, 4.00, 4.15, 4.21, 4.36 and 3.75, respectively. The sugar was also confirmed by acid hydrolysis which gave D-xylose and aglycone moieties. The downfield ^{13}C-NMR signal at δ_C 89.0 and the HMBC correlation (fig 28) between H-1' of xylose at δ_H 4.70 and C-3 of the aglycone at δ_C 89.0 revealed that xylose moiety was attached to the C-3 of the aglycone. Another confirmation for the site of attachment was the correlation of the H-3 of quinovic acid at δ_H 3.21 and the anomeric carbon of the sugar at δ_C 108.0. The above structural elucidation of **1.12** was further supported by its ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) (fig 29), heteronuclear multiple quantum correlation (HMQC) and HMBC data, respectively. On the basis of the above results, compound **1.12** was established as 3-O-β-D-xylopyranosyl quinovic acid and this is the first report of this compound from natural source.

Table 13 – ¹H and ¹³C-NMR spectral data and ¹³C-¹H long –rang correlation of compound **1.12** in pyridine- d_5

Carbon	δc	Dept	δ$_H$(J_{HH} in Hz)	Cross peaks (δ$_C$) in HMBC spectrum
1	39.58	CH$_2$		
2	26.05	CH$_2$		
3	89.00	CH	3.21 (dd, J = 11.8, 4.4 Hz, 1H)	17.4 (C-24), 28.6 (C-23), 40.0 (C-4), 108.0 (C-1`)
4	40.01	C		
5	56.55	CH	0.97 (s, 1H)	19.1 (C-6), 28.6 (C-23), 40.0 (C-4), 89.0 (C-3)
6	19.17	CH$_2$		
7	37.54	CH$_2$	1.98 (d, J = 13.3 Hz, 2H)	
8	40.55	C		
9	47.72	CH	2.74 (dd, J = 11.6, 4.8 Hz, 1H)	19.5 (C-26), 40.5 (C-8)
10	37.7	C		
11	23.87	CH$_2$		
12	129.46	CH	6.02 (s, 1H)	47.7 (C-9), 57.3 (C-14), 55.4 (C-18)
13	134.63	C		
14	57.31	C		
15	27.34	CH$_2$		
16	26.90	CH$_2$		
17	49.26	C		
18	55.44	CH	2.82 (d, J = 11.4 Hz, 1H)	26.9 (C-16), 38.2 (C-19), 49.2 (C-17), 57.3 (C-14), 129.4 (C-12), 134.6 (C-13), 180 (C-28)
19	38.29	CH		
20	39.80	CH		
21	31.10	CH$_2$		
22	38.03	CH$_2$		
23	28.62	CH$_3$	1.16 (s, 3H)	17.4 (C-24), 40.0 (C-4), 89.0 (C-3)
24	17.43	CH$_3$	0.97 (s, 3H)	28.6 (C-23), 40.0 (C-4), 89.0 (C-3)
25	16.96	CH$_3$	0.90 (s, 3H)	47.7 (C-9)
26	19.53	CH$_3$	1.12 (s, 3H)	37.5 (C-7), 40.5 (C-8), 47.7 (C-9), 57.3 (C-14)
27	178.59	C		
28	180.8	C		
29	18.76	CH$_3$	1.23 (d, J = 6.0 Hz, 3H)	39.8 (C-20), 55.4 (C-18)
30	21.95	CH$_3$	0.82 (d, J = 6.3 Hz, 3H)	18.7 (C-29), 31.1 (C-21), 39.8 (C-20)
1`	108.09	CH	4.70 (d, J = 7.5 Hz, 1H)	67.5 (C-6`), 89.0 (C-3), 108.0 (C-1`)
2`	76.01	CH	4.00 (d, J = 8.0 Hz, 1H)	79.1 (C-3`), 108.0 (C-1`)
3`	79.11	CH	4.15 (d, J = 8.6 Hz, 1H)	71.7 (C-4`), 76.0 (C-2`), 79.1 (C-3`)
4`	71.74	CH	4.21 (dd, J = 9.6, 5.1 Hz, 1H)	67.5 (C-6`)
5`	67.57	CH$_2$	4.36 (dd, J = 11.3, 5.2 Hz, 1H) 3.75 (t, J = 10.9 Hz, 1H)	71.7, 79.1, 108.0 (C-1`)
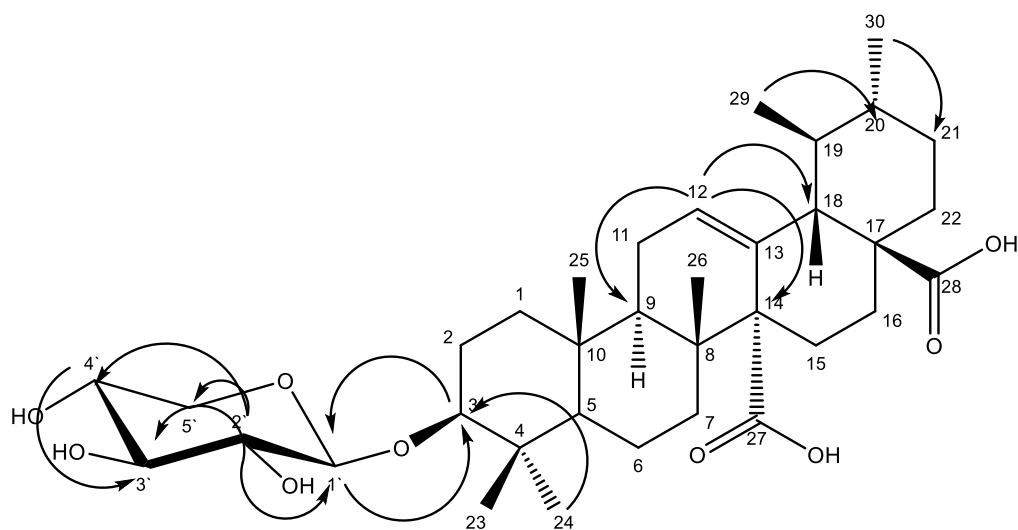
3-O-β-D-xylopyranosyl quinovic acid (Compound **1.12**)
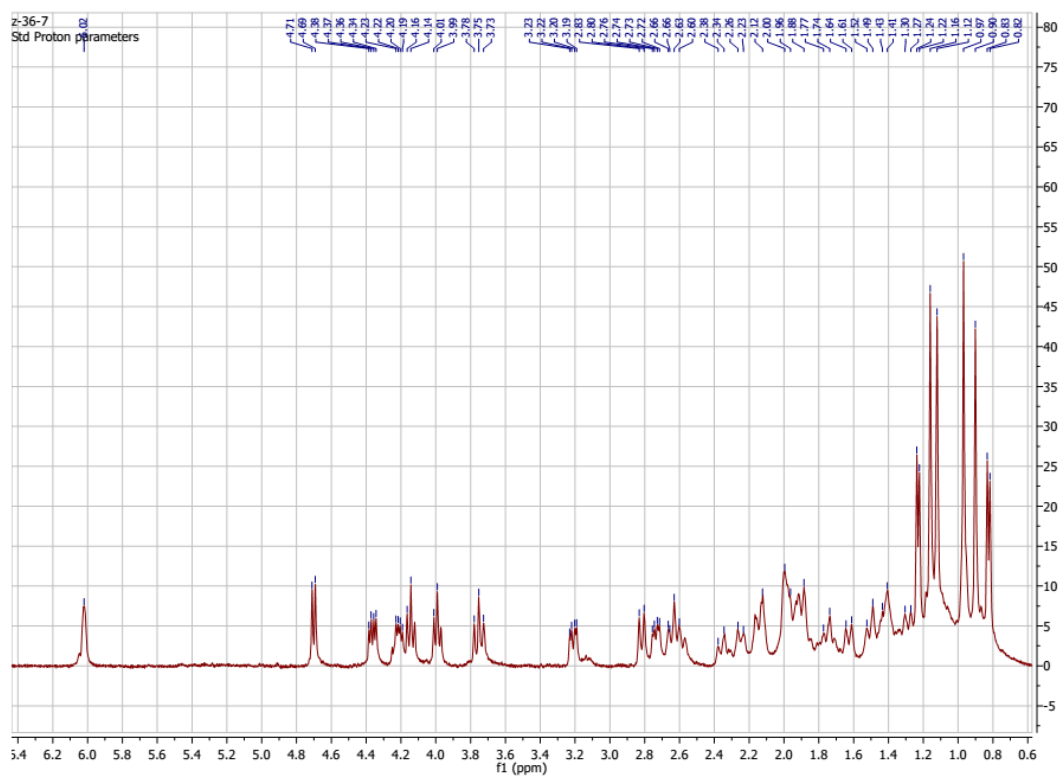
Figure 24 – ¹H-NMR spectrum of compound **1.12** in *Pyridine- d5*

Figure 25 – ^{13}C-NMR spectrum of compound **1.12** in *Pyridine- d$_5$*

Figure 26 – DEPT spectrum of compound **1.12** in *Pyridine- d$_5$*

Figure 27 – HMQC spectrum of compound **1.12** in *Pyridine- d₅*
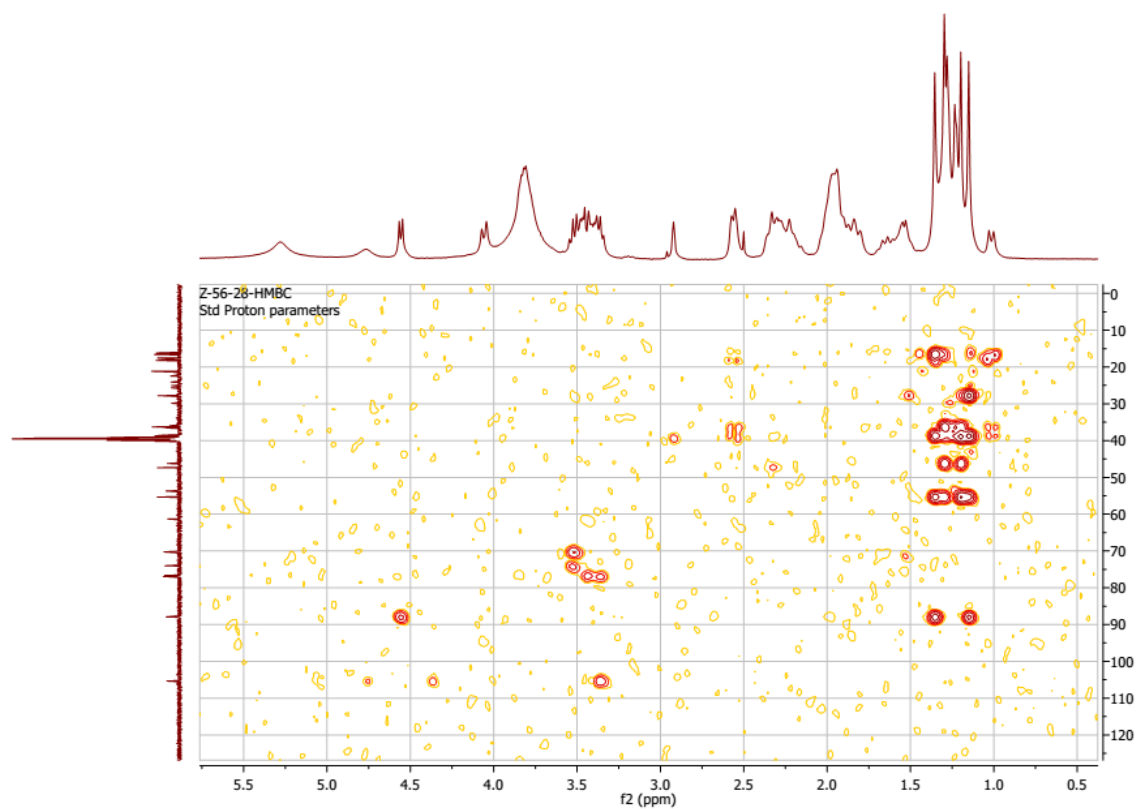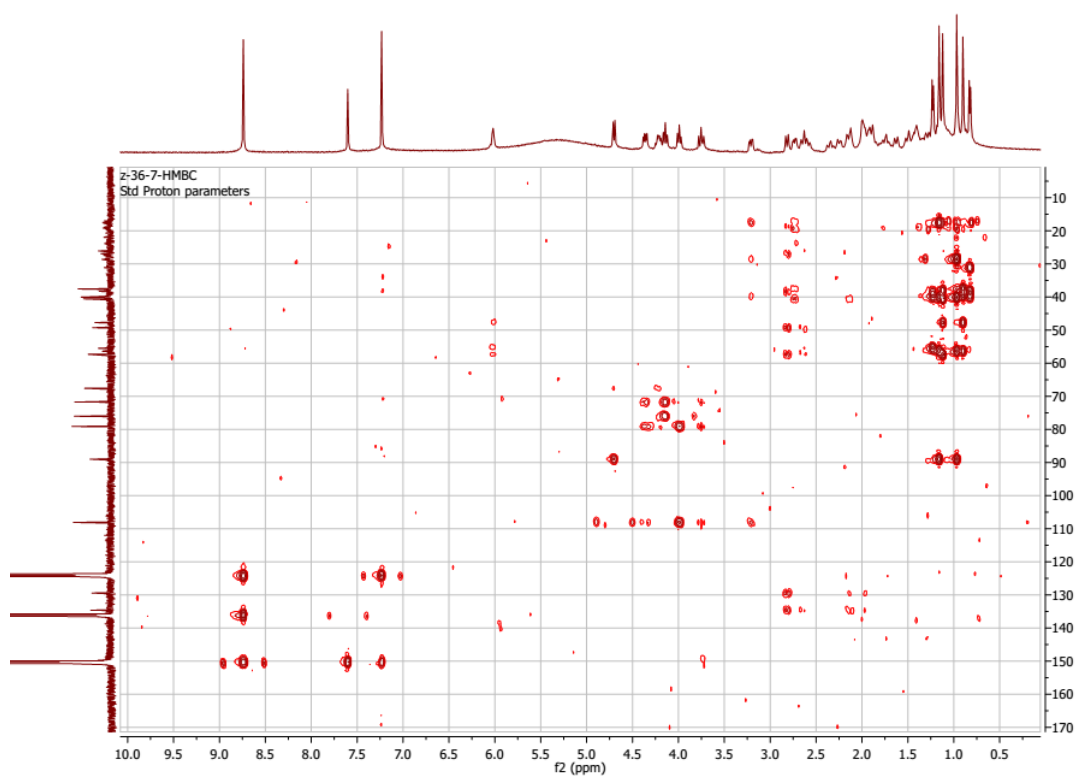

Figure 28 – HMBC spectrum of compound **1.12** in *Pyridine- d₅*
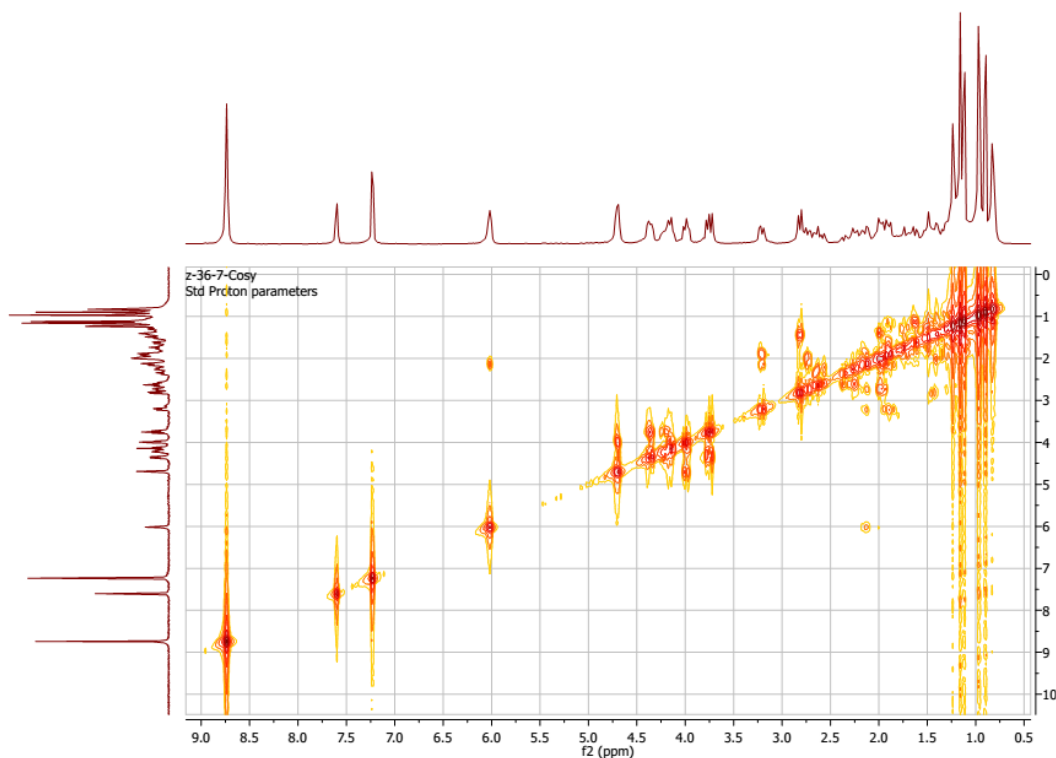
Figure 29 – COSY spectrum of compound **1.12** in *Pyridine- d_5*

Compound **1.22** was obtained as white needles, m.p. 193-195 °C, with R_f=0.40 (system XIII), it gave positive results to the libermann-Burchard and Molish tests. Furthermore, the HR-ESI-MS [M-1]+ of this compound showed m/z = 631.381, corresponding to the molecular formula $C_{36}H_{56}O_9$.

The ^{13}C-NMR spectra (fig 31-32), summarized in table 14, showed 36 carbon resonances, indicating the presence of a single sugar moiety with a triterpenoid aglycone. The methine and methyl resonances at δ_C 107.31, 78.98, 77.57, 76.52, 73.35 and 19.09 were due to the sugar moiety but they do not coincide with the ^{13}C-NMR data of any common sugar moiety [176, 177]. Similarly, the ^{1}H-NMR spectrum (fig 30) also indicated six clear signals at δ_H 1.63 (d, *J* = 6.04 Hz), 3.91 (m), 4.060 (m), 3.81-3.69 (m) and 4.64 (d, *J*=7.8 Hz) due to the sugar moiety. The sugar moiety was identified as D-quinovose by direct comparison of data with that reported for quinovose [168] The downfield ^{13}C-NMR signal at δ_C 89.48 showed that the sugar moiety was attached to the C-3 of the aglycone [172]. The olefinic resonances of the aglycone at δ_C 134.85 and 129.81, corresponding to quaternary and methine carbons suggested the urs-12-ene skeleton with a carboxylic group at C-27 [173]. The carbonyl carbons at δ_C 181.19 and 179.01 showed the presence of two unsubstituted carboxylic groups at C-17 and C-14, respectively, in the aglycone moiety [172, 173] The ^{13}C-NMR spectral data of compound **1.22** were consistent with quinovic acid as the aglycone [172-174]. The carbons were related to the corresponding protons by HMQC (fig 33) correlations. The site of attachment of the sugar was confirmed by COSY (fig 35) correlation which showed correlations between the anomeric proton of sugar at δ_H 4.64 and H-3 δ_H 3.16 of the quinovic acid and from the HMBC

correlation (fig 34) between H-1' of sugar at δ_H 4.64 and C-3 of the aglycone at δ_C 89.4 revealed that sugar was attached at C-3 of the aglycone.

Table 14 – ^{1}H and ^{13}C-NMR spectral data and ^{13}C-^{1}H long –rang correlation of compound **1.22** in *pyridine- d5*

Carbon	δc	Dept	$\delta_H(J_{HH}$ in Hz)	Cross peaks (δ_C) in HMBC spectrum
1	39.93	CH$_2$		
2	27.59	CH$_2$	2.14 (d, J = 15.4 Hz, 2H)	
3	89.48	CH	3.16 (s, 1H)	17.9 (C-24), 28.8 (C-23), 40.8 (C-4), 107.3 (C-1`)
4	40.84	C		
5	56.67	CH	0.95	40.8 (C-4)
6	19.45	CH$_2$	1.62 (dd, J = 12.4, 6.0 Hz, 2H)	
7	37.94	CH$_2$	1.96	
8	40.72	C		
9	48.02	CH	2.66	
10	37.83	C		
11	24.68	CH$_2$		
12	129.81	CH	6.02 (d, J = 17.4 Hz, 1H)	
13	134.85	C		
14	57.57	C		
15	26.31	CH$_2$	2.56	
16	27.16	CH$_2$	2.56	
17	49.57	C		
18	55.73	CH	2.75	
19	38.53	CH		
20	40.27	CH		
21	31.44	CH$_2$		
22	38.35	CH$_2$		
23	28.86	CH$_3$	1.14 (s, 3H),	17.9 (C-24), 40.8 (C-4), 56.6(C-5), 89.4(C-3)
24	17.90	CH$_3$	0.95 (s, 3H),	28.8 (C-23), 40.8 (C-4), 56.6(C-5), 89.4(C-3)
25	17.43	CH$_3$	0.91 (s, 3H)	37.8 (C-10), 48.0 (C-9)
26	19.74	CH$_3$	1.09 (s, 3H),	40.7 (C-8), 57.5 (C-14)
27	178.95	C		
28	181.19	C		
29	19.59	CH$_3$	1.22 (d, J = 5.7 Hz, 3H),	55.7 (C-18) , 38.5 (C-19)
30	22.22	CH$_3$	0.84 (d, J = 6.1 Hz, 3H)	31.4 (C-21), 40.2 (C-20)
1`	107.31	CH	4.64 (d, J = 7.8 Hz, 1H)	89.4 (C-3)
2`	76.52	CH	3.91 (m, 1H)	78.9 (C-3`), 107.3 (C-1`)
3`	78.98	CH	4.06 (m, 1H)	76.5 (C-2`)
4`	77.57	CH		
5`	73.35	CH	3.81 – 3.69 (m, 1H)	76.5 (C-2`), 107.3 (C-1`)
6`	19.09	CH$_3$	1.63 (3H)	73.3 (C-5`), 76.5 (C-2`)

Another confirmation for the site of attachment was the correlation of the H-3 of quinovic acid at δ_H 3.16 and the anomeric carbon of the sugar at δ_C 107.3. The above structural elucidation of **1.22** was further supported by its (^1H–^1H COSY), (HMQC) fig 33 and HMBC data respectively, which led us to confirm the structure as 3-O-[*β*-D-quinovopyranosyl]-quinovic acid, and reported for the first time from *Zygophyllum fabago*.
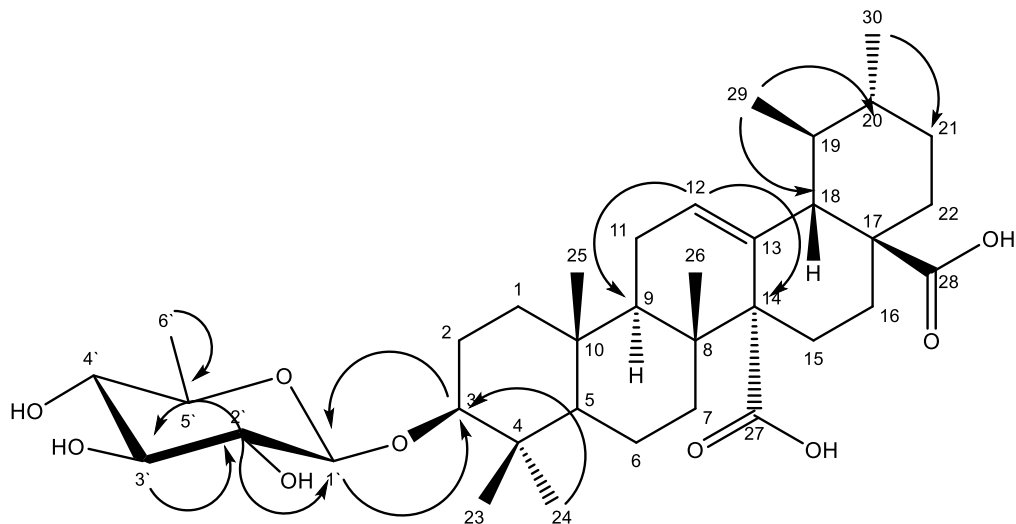
3-O-[*β*-D-quinovopyranosyl]-quinovic acid (Compound **1.22**)
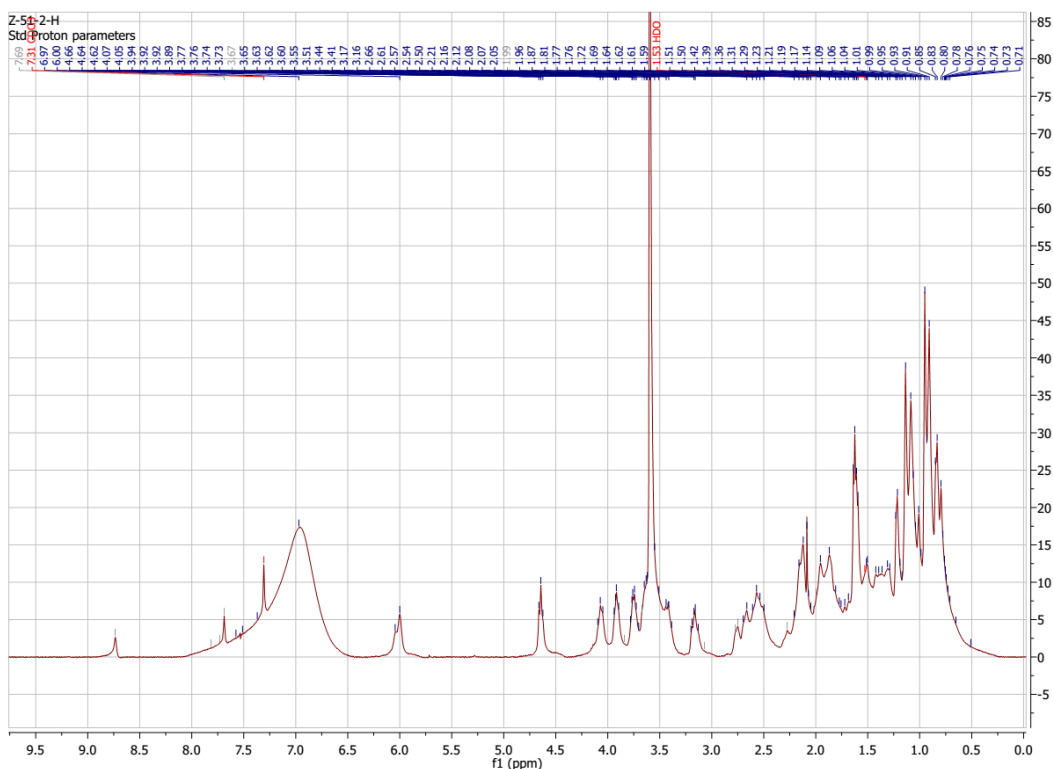
Figure 30 – ^{1}H-NMR spectrum of compound **1.22** in *Pyridine- d_5*
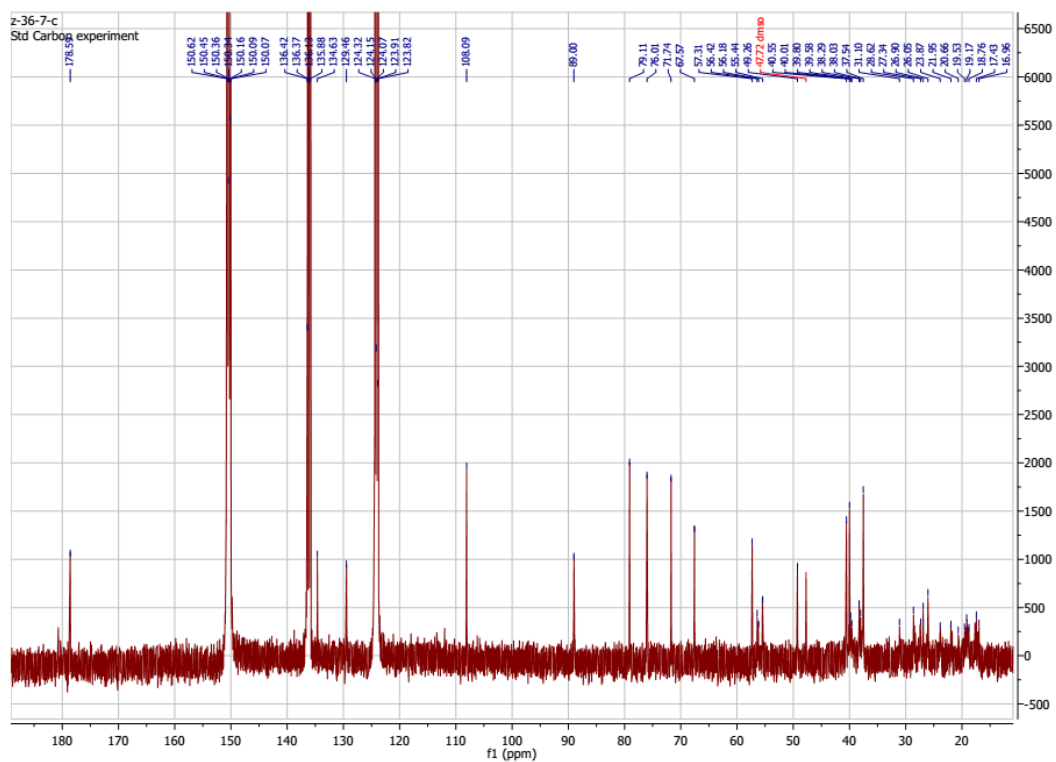
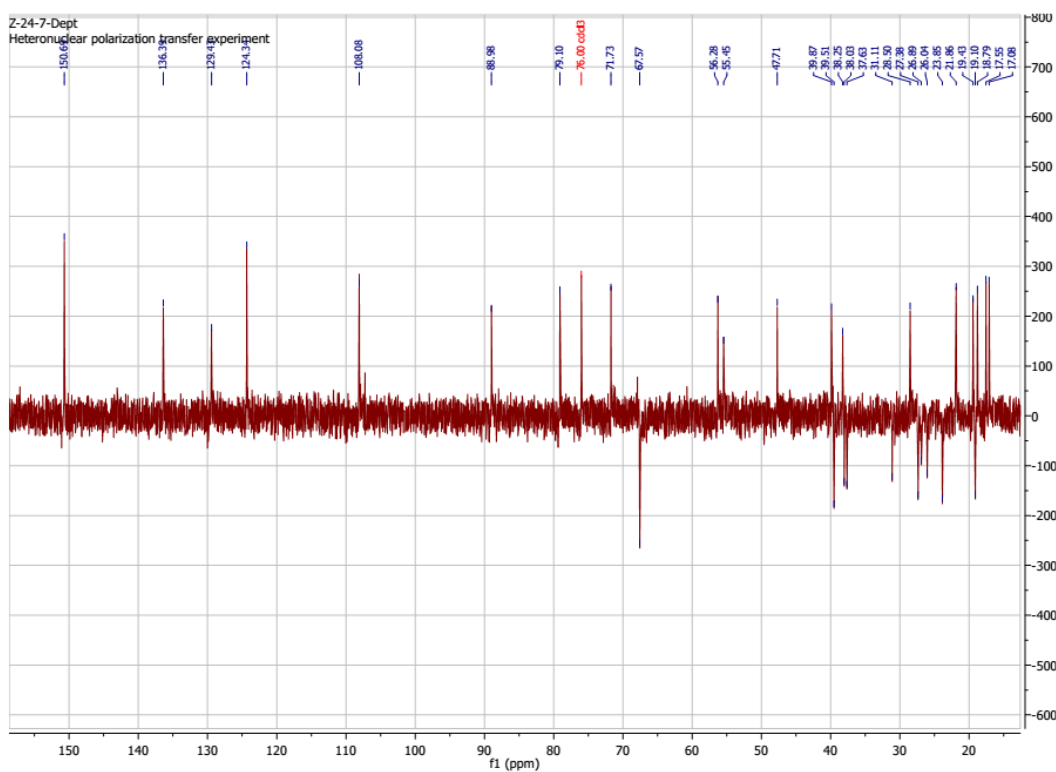Figure 31 – ^{13}C-NMR spectrum of compound **1.22** in *Pyridine- d₅*
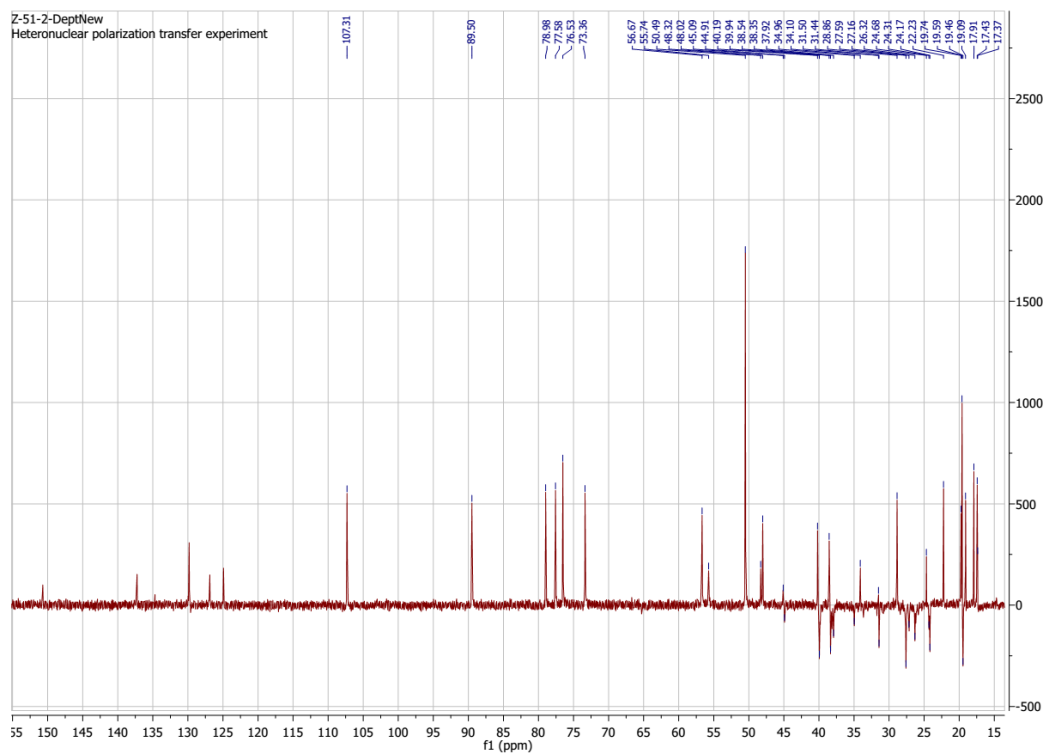
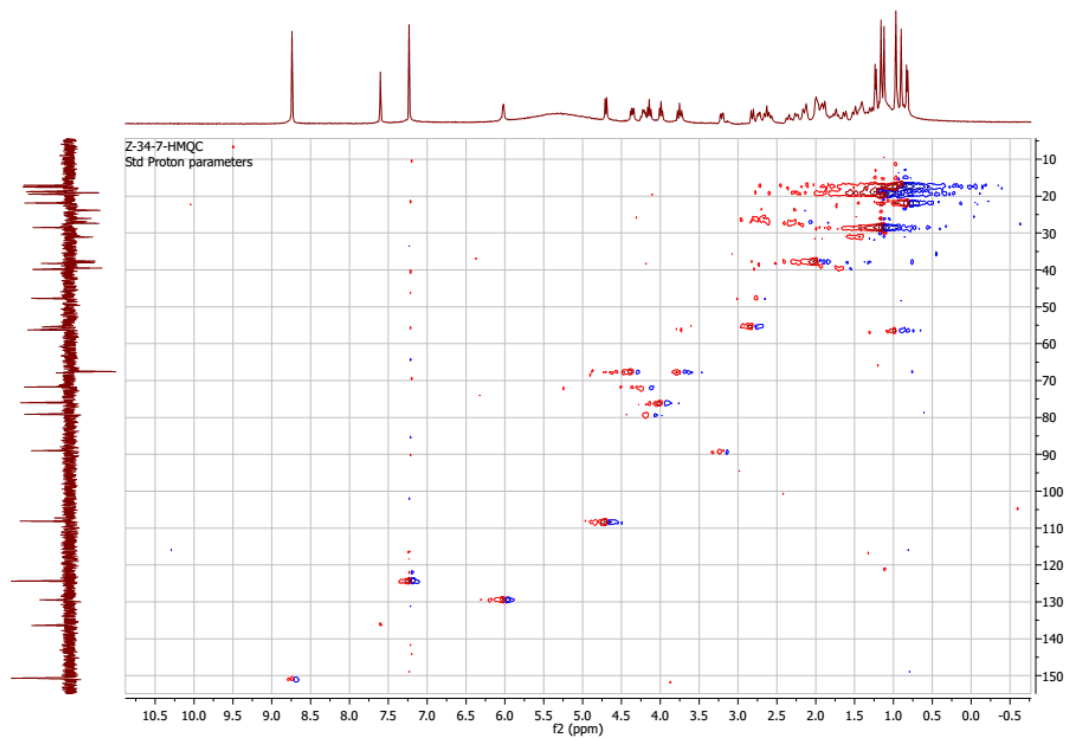Figure 32 – DEPT spectrum of compound **1.22** in *Pyridine- d₅*
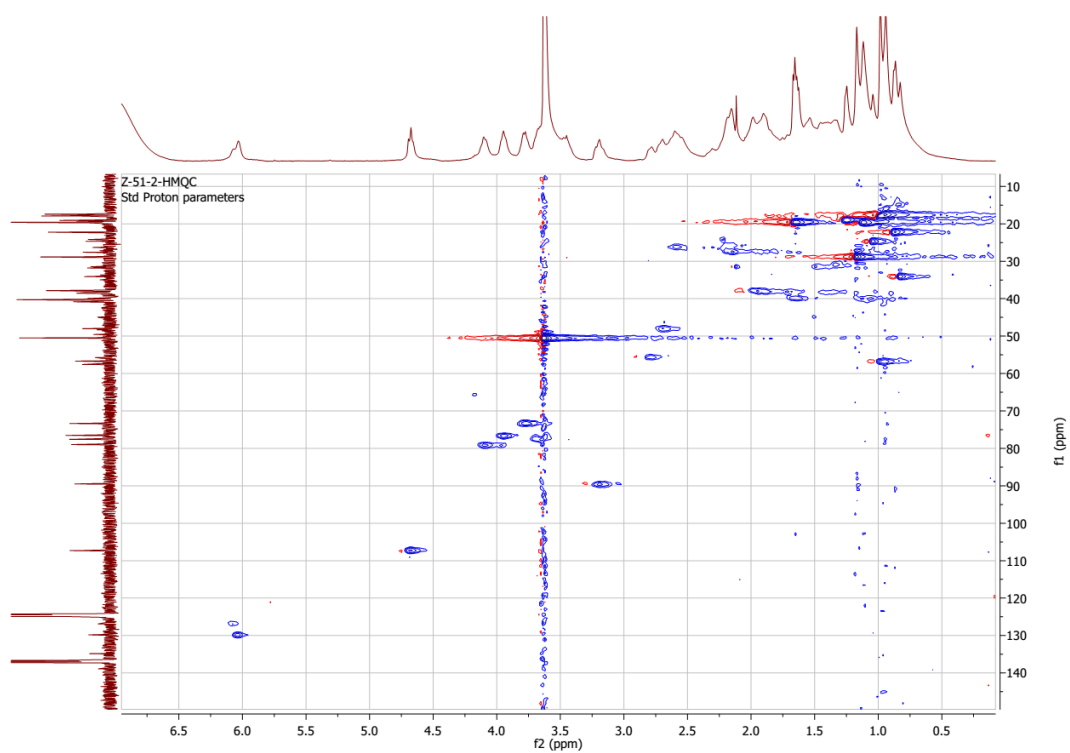
Figure 33 – HMQC spectrum of compound **1.22** in *Pyridine- d₅*
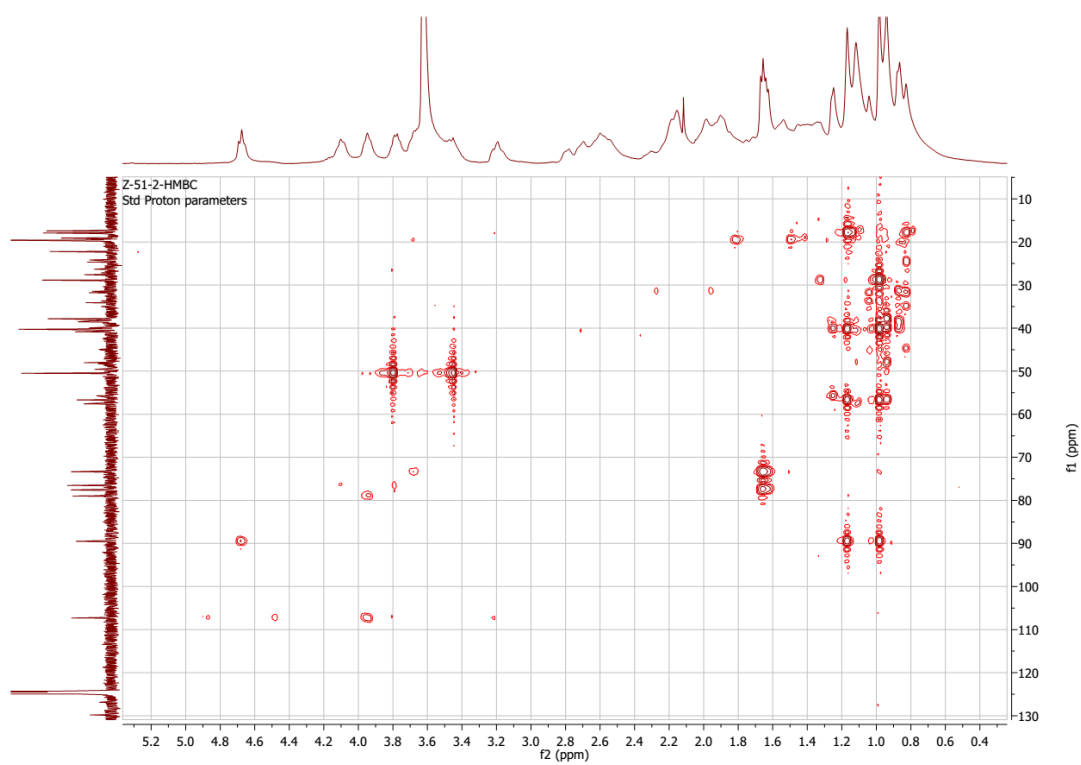
Figure 34 – HMBC spectrum of compound **1.22** in *Pyridine- d₅*
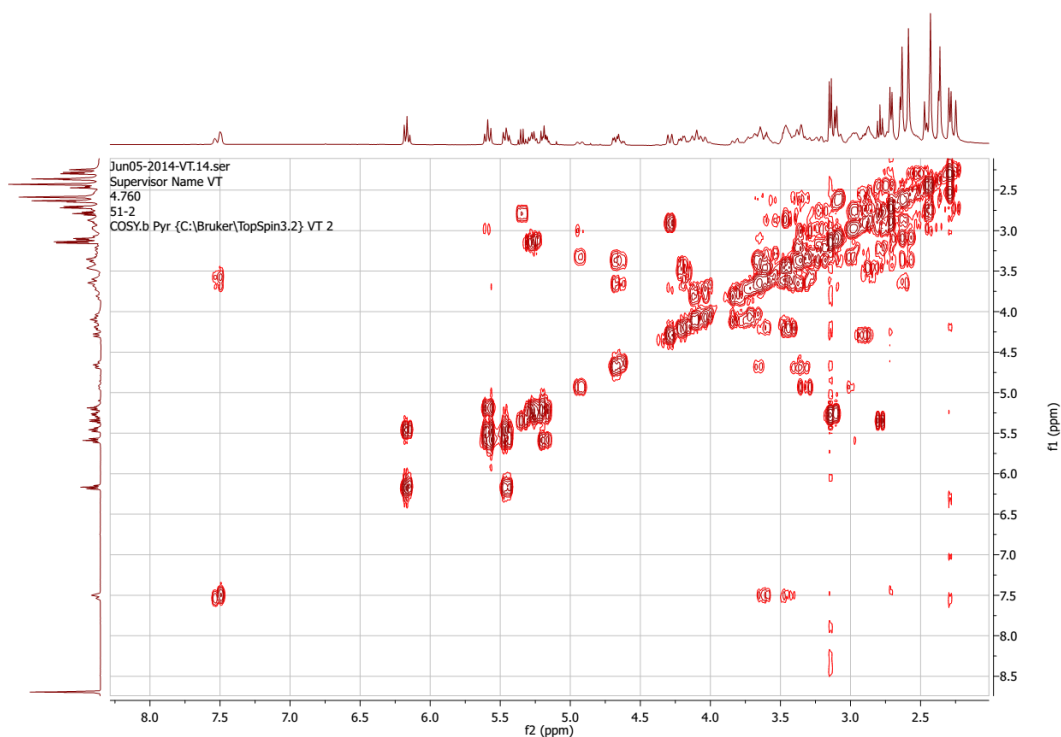
Figure 35 – COSY spectrum of compound **1.22** in *Pyridine- d₅*

Compound **1.28** was obtained as white needles, m.p. 215-216 °C, with R_f = 0.26 (system XIV) it gave positive results to the libermann-Burchard and Molish tests. HR-ESI-MS $[M-1]^+$ showed m/z = 711.362 which corresponding to the molecular formula ($C_{36}H_{56}O_{12}S$).

The ^{13}C-NMR spectrum (fig 37), summarized in table 15, showed 36 carbon resonances, indicating the presence of a single sugar moiety with a triterpenoid aglycone. The methine and methyl resonances at δ_C 105.36, 76.96, 76.81, 76.62, 71.21 and 18.20 were due to the sugar moiety. The sugar moiety was identified as D-quinovose by direct comparison of data with that reported for quinovose [168]. The downfield ^{13}C-NMR signal at δ_C 87.92 showed that the sugar moiety was attached to the C-3 of the aglycone [172]. The olefinic resonances of the aglycone at δ_C 132.66 and 127.95, corresponding to quaternary and methine carbons suggested the urs-12-ene skeleton with a carboxylic group at C-27 [173]. The carbonyl carbons at δ_C 178.41 and 176.20 showed the presence of two unsubstituted carboxylic groups a C-17 and C-14, respectively, in the aglycone moiety [172, 173].

The ^{13}C-NMR spectral data of **1.28** were consistent with quinovic acid as the aglycone [172-174]. The downfield signal at δ_C 76.9.0 assigned for C-2 of the sugar moiety compared to the unsubstituted one indicated the presence of a group attached to the C-2 oxygen of the sugar moiety. Acid hydrolysis followed by treatment with $BaCl_2$ and comparing the HR-ESI-MS established the presence of a sulfate moiety.

Table 15 – ^{1}H and ^{13}C-NMR spectral data and ^{13}C-^{1}H long –rang correlation of compound **1.28** in *pyridine- d5*

Carbon	δ_c	Dept	$\delta_H(J_{HH}$ in Hz)	cross peaks (δ_C) in HMBC spectrum
1	35.91	CH$_2$	1.95 (d, J = 28.2 Hz, 1H)	87.9(C-3), 25.7(C-2), 55.2(C-5)
2	25.78	CH$_2$		
3	87.92	CH	3.51 – 3.39 (m, 1H)	105.3(C-1`), 76.6(C-2`)
4	39.05	C		
5	55.28	CH	1.06 (d, J = 10.7 Hz, 1H)	17.8(C-6), 16.5(C-24), 36.2(C-10)
6	17.85	CH$_2$		
7	38.6	CH$_2$		
8	38.74	C		
9	46.22	CH	2.61 (d, J = 7.7 Hz, 1H)	
10	36.27	C		
11	22.44	CH$_2$		
12	127.95	CH	5.95 (s, 1H)	22.4(C-11), 46.2(C-9), 55.3(C-14)
13	132.66	C		
14	55.37	C		
15	24.09	CH$_2$		
16	25.04	CH$_2$		
17	47.34	C		
18	53.72	CH	2.61 (d, J = 7.7 Hz, 1H)	17.5(C-29), 36.5(C-19), 47.3(C-17), 132.6(C-13), 127.9(C-12)
19	36.57	CH		
20	38.51	CH		
21	29.86	CH$_2$		
22	36.34	CH$_2$		
23	27.73	CH$_3$	1.40 (s, 3H)	16.5(C-24), 39.0(C-4), 55.2(C-5), 87.9(C-3)
24	16.57	CH$_3$		
25	16.16	CH$_3$	1.19 (s, 3H)	
26	18.12	CH$_3$		
27	176.20	C		
28	178.41	C		
29	17.55	CH$_3$	1.28 (s, 6H)	
30	21.17	CH$_3$	1.34 (s, 12H)	
1`	105.36	CH	4.60 (d, J = 7.6 Hz, 1H)	76.6(C-4`), 87.9(C-3), 105.3(C-1`)
2`	76.96	CH		
3`	76.81	CH	3.54 (m, J = 8.7 Hz, 1H)	71.2(C-5`)
4`	76.62			
5`	71.21	CH		
6`	18.20		1.67 – 1.52 (m, 3H)	71.2(C-5`)

The ^{1}H-NMR spectrum (fig 36) showed anomeric proton signal of the sugar moiety at δ_H 4.60 (d, J = 7.6 Hz). From the coupling constant data the anomeric configuration of the sugar unit was found to be *ß*-quinovose. The point of attachment of the quinovopyranose moiety to the aglycone was determined from the chemical shift data of the glycosylated position as well as from the HMBC interactions (fig

38). The C-3 of the aglycone was shifted downfield to δ87.92 due to glycosylation. The point of attachment of the sugar unit at this position was also supported by the HMBC interactions of the anomeric proton of the sugar unit to the C-3 of aglycone. Thus, the proton signal at δ_H 4.60 (H-1′) showed HMBC correlations with the carbon signal at δ_C 87.92 (C-3). The H-3 of aglycone (δ_H 3.51), in turn, also showed HMBC interactions with the anomeric carbon signal at δ_C 105.3.

On the basis of above observations, the structure of compound **1.28** was established as 3-O-[β-D-2-O-sulphonyl-quinovopyranosyl]-quinovic acid. This compound isolated here for the first time from *Z. fabago*.
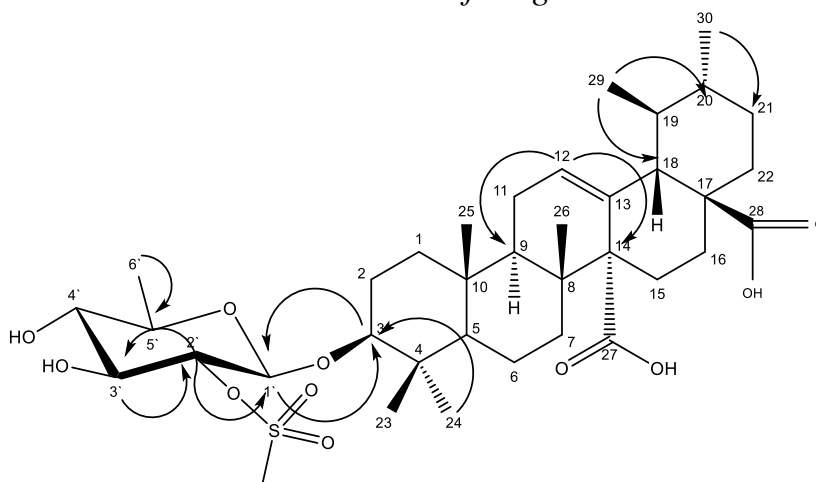
3-O-[β-D-2-O-sulphonyl-quinovopyranosyl]-quinovic acid (Compound **1.28**)
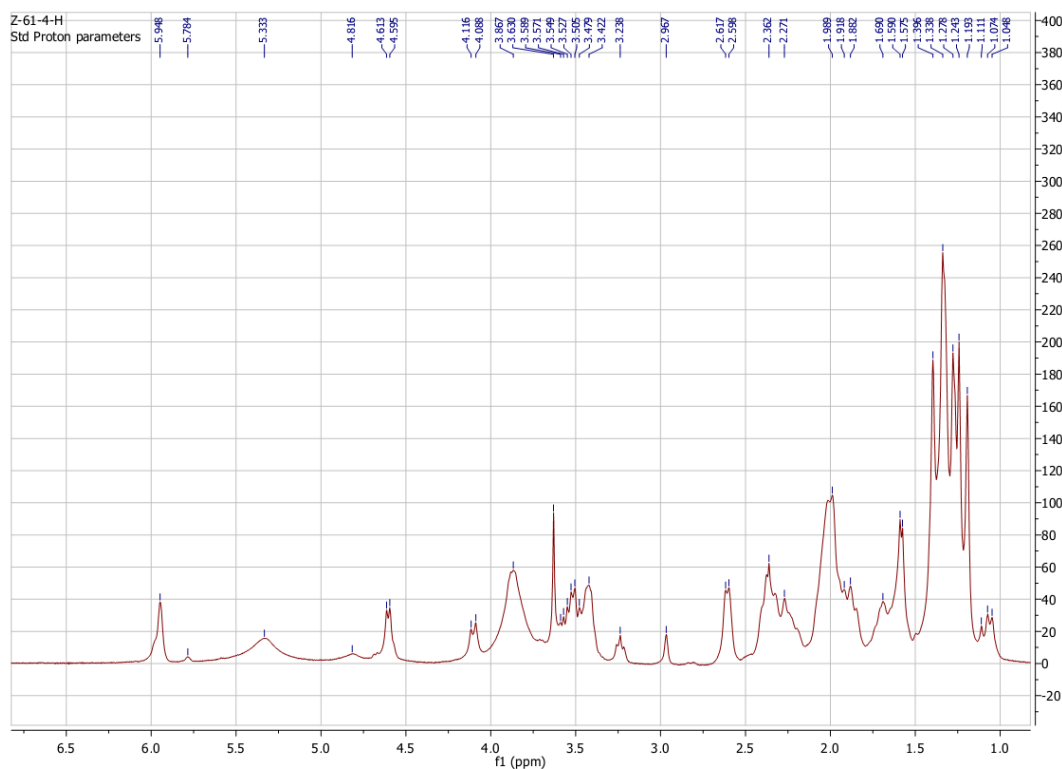
Figure 36 – ¹H-NMR spectrum of compound **1.28** in *Pyridine- d₅*
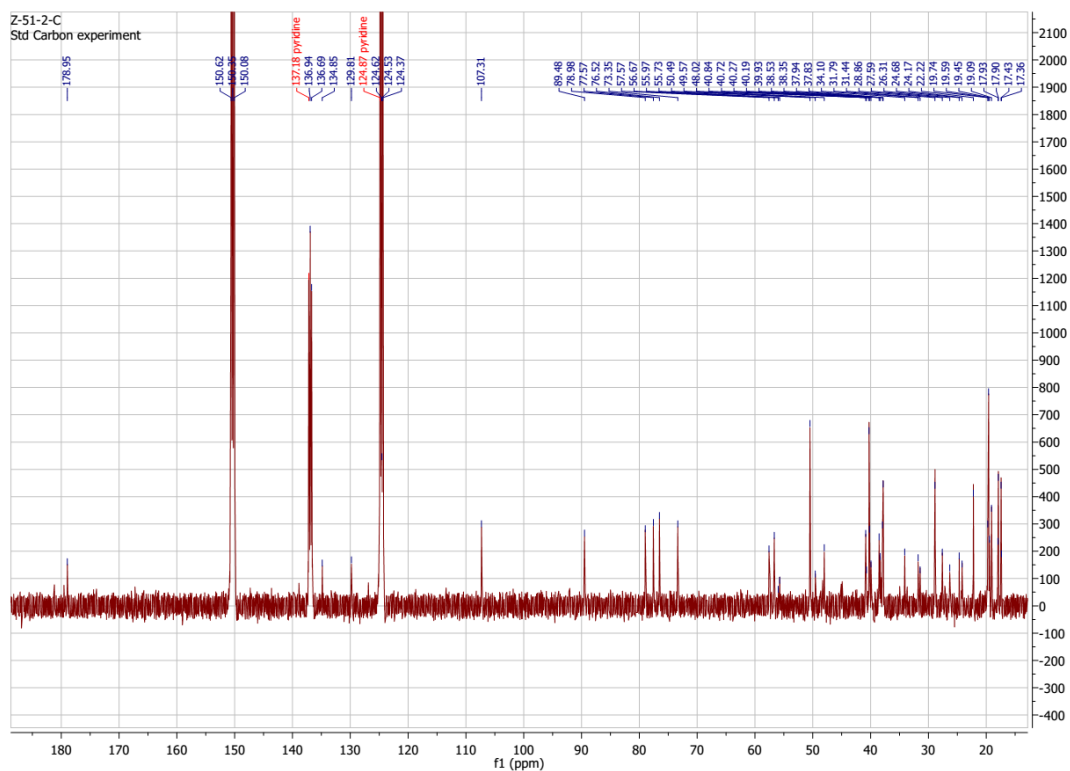
Figure 37 – ^{13}C-NMR spectrum of compound **1.28** in *Pyridine- d_5*

Figure 38 – HMBC spectrum of compound **1.28** in *Pyridine- d_5*

Compound **1.24** was isolated as white powder, with R_f= 0.32 (system XV) and gave positive results to Liebermann–Burchard and Molish tests. HR-ESI-MS [M-1]$^+$ showed m/z =587.397 corresponding to the molecular formula $C_{35}H_{56}O_7$.

The[1]-HNMR spectrum showed four tertiary at δ_H 0.90, 0.96, 1.11 and 1.16, two secondary δ_H 0.83 and 1.24 methyl groups and one olefinic proton at δ_H 6.07 – 5.96 (m, 1H). The[13]C-NMR spectrum (fig 40-41) of the aglycone table 16 displayed 29 carbon signals including one oxygenated CH group signal at δ_C 88.8, one olefinic carbons δ_C 129.2, one quaternary at δ_C 134.3 and one carboxyl carbon at δ_C 178.31, six methyl groups at δ_C 28.30, 17.36, 16.87, 19.20, 18.54 and 21.65 which showed a nor-triterpenoid skeleton. The above data of the aglycone part of compound **1.24** were quite similar to those of quinovic acid [169]. The distinct difference being the absence of 28-carboxylic group. The methine and methyl resonances at δ_C 106.9, 78.55, 77.11, 76.11, 72.9 and 19.0 were due to the sugar moiety. Similarly, the ^{1}H-NMR spectrum (fig 39) also indicated six clear signals at δ_H 1.66 (d, J = 5.9 Hz), 3.84 (m), 4.11 (m), 3.74-3.66(m), 3.97 (m) and 4.70 (d, J=7.8 Hz) due to the sugar moiety. The sugar moiety was identified as D-quinovose by direct comparison of data with that reported for quinovose [168]. The OH group at C-3 of aglycone was disposed β and equatorial on the basis of coupling constant of H-3 (dd, J = 11.6, 4.5 Hz). The anomeric proton signal of the sugar moiety was observed at δ_H 4.70 (d, J = 7.8). The spin constitution of the sugar moiety was observed from the ^{1}H–^{1}H-COSY (fig xxx) correlations between the sugar protons. From the coupling constant data, the anomeric configuration of the sugar unit was found to be β-quinovose.

The point of attachment of the quinovopyranoside moiety to the aglycone was determined from the chemical shift data of the glycosylated position as well as from the HMBC interactions (fig 42). The C-3 of the aglycone was shifted downfield to δ_C 88.8 due to glycosylation. The point of attachment of the sugar unit at this position was also supported by the HMBC interactions of the anomeric proton of the sugar unit to the C-3 of aglycone. Thus, the proton signal at δ_H 4.70 (H-1′) showed HMBC correlations with the carbon signal at δ_C 88.8 (C-3). The H-3 of aglycone (δ_H 3.21), in turn, also showed HMBC interactions with the anomeric carbon signal at δ_C 106.9.

The HMBC spectrum showed also correlations for H-12/C-9, C-11, H-18/C-19. The above structural elucidation was further supported by its ^{1}H–^{1}H COSY, HMQC (fig 43) and HMBC data. From these results, the structure of compound **1.24** was established as 28-Nor-quinovic acid-3-O-β-quinovopyranoside. And this is the first report for isolation of this compound from natural source.

Table 16 – ^{1}H and ^{13}C-NMR spectral data and ^{13}C-^{1}H long –rang correlation of compound **1.24** in *pyridine- d5*

Carbon	δc	Dept	δ$_H$(J_{HH} in Hz)	cross peaks (δ$_C$) in HMBC spectrum
1	37.82	CH$_2$		
2	25.80	CH$_2$	2.27 – 2.19 (m, 2H)	
3	88.80	CH	3.21 (dd, J = 11.6, 4.5 Hz, 1H),	106.9 (C-1′), 77.1 (C-4′), 28.3 (C-23), 17.3 (C-24)
4	39.78	C		
5	56.12	CH		
6	18.90	CH$_2$	1.67 (d, J = 5.9 Hz, 3H)	
7	39.38	CH$_2$	1.98 (td, J = 9.5, 5.2 Hz, 3H)	56.1(C-5), 19.2 (C-26)
8	40.33	C		47.5 (C-9), 19.2 (C-26)
9	47.51	CH	2.73 (dd, J = 11.4, 5.1 Hz, 1H)	129.2 (C-12), 19.2 (C-26), 16.8 (C-25)
10	37.31	C		
11	23.64	CH$_2$		
12	129.29	CH	6.07 – 5.96 (m, 1H)	47.5 (C-9), 23.6 (C-11)
13	134.36	C		
14	57.05	C		
15	27.13	CH$_2$	2.67 – 2.53 (m, 2H)	
16	26.66	CH$_2$	2.67 – 2.53 (m, 2H)	27.1 (C-15)
17	38.04	CH		
18	55.23	CH	2.81 (d, J = 11.6 Hz, 1H)	39.6 (C-19)
19	39.66	CH		
20	38.04	CH		
21	30.88	CH$_2$	1.43 (d, J = 12.2 Hz, 4H)	21.6 (C-30)
22	37.38	CH$_2$		
23	28.30	CH$_3$	1.16 (s, 3H)	88.8 (C-3), 56.1 (C-5), 17.3 (C-24)
24	17.36	CH$_3$	0.96 (s, 3H)	88.8 (C-3)
25	16.87	CH$_3$	0.90 (s, 3H)	56.1 (C-5), 37.8 (C-1)
26	19.20	CH$_3$	1.11 (s, 3H)	
27	178.31	C		
28	-	-		
29	18.54	CH$_3$	1.24 (d, J = 5.9 Hz, 3H)	38.0 (C-20), 55.2 (C-18)
30	21.65	CH$_3$	0.84 (d, J = 6.1 Hz, 3H)	38.0 (C-20), 37.3 (C-22)
1′	106.95	CH	4.70 (d, J = 7.8, 1H)	76.1 (C-2′), 77.1 (C-4′), 88.8 (C-3)
2′	76.11	CH	3.97 (m, 1H)	78.5 (C-3′), 72.9 (C-5′)
3′	78.55	CH	4.11 (td, J = 9.0, 3.3 Hz, 1H),	76.1 (C-2′), 77.1 (C-4′)
4′	77.11	CH	3.74 – 3.66 (m, 1H)	19.0 (C-6`)
5′	72.94	CH	3.84 – 3.76 (m, 1H),	77.1 (C-4′), 19.0 (C-6′), 106.9 (C-1`)
6′	19.07	CH$_3$	1.66 ((d, J = 5.9 Hz, 3H)	77.1 (C-4′)
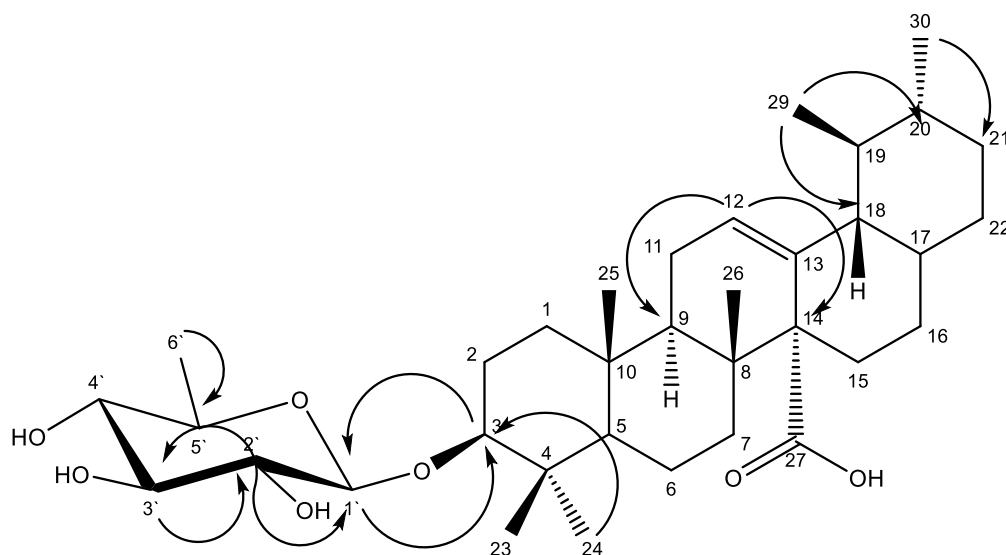
28-Nor-quinovic acid-3-O-β-quinovopyranoside (Compound **1.24**)
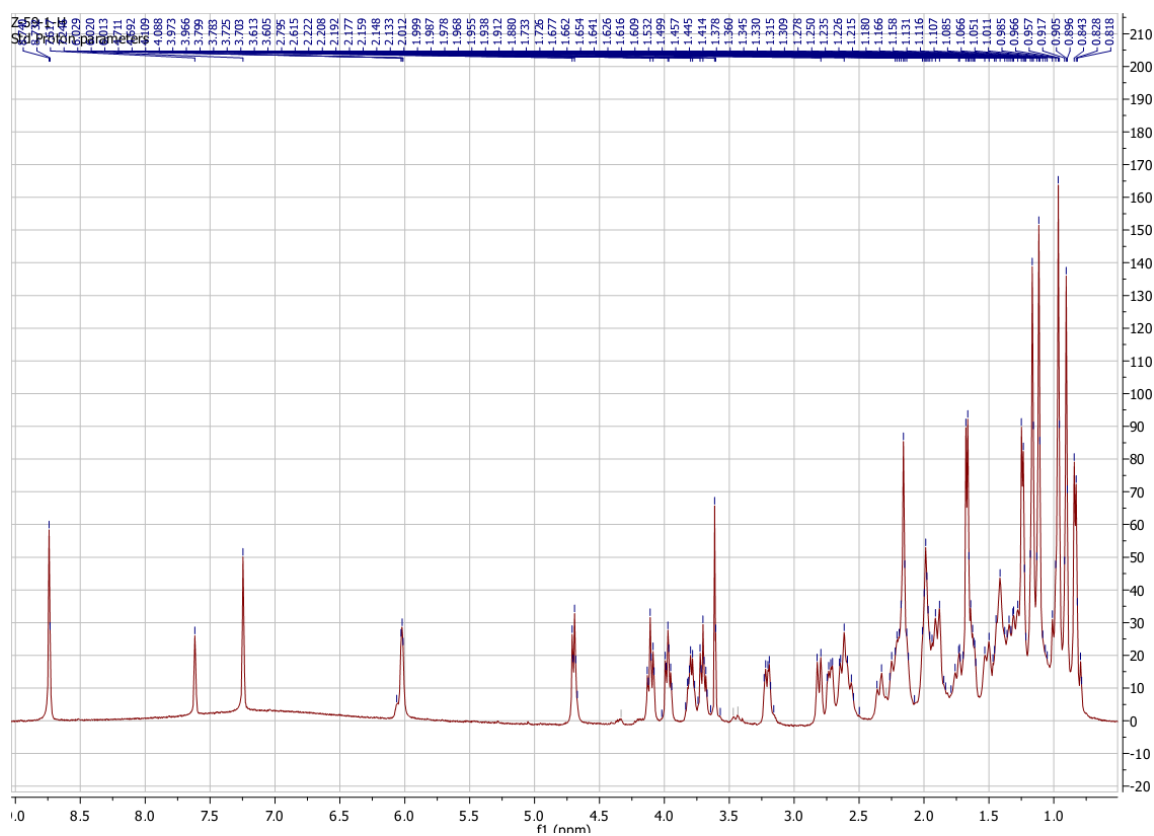
Figure 39 – ¹H-NMR spectrum of compound **1.24** in *Pyridine- d₅*
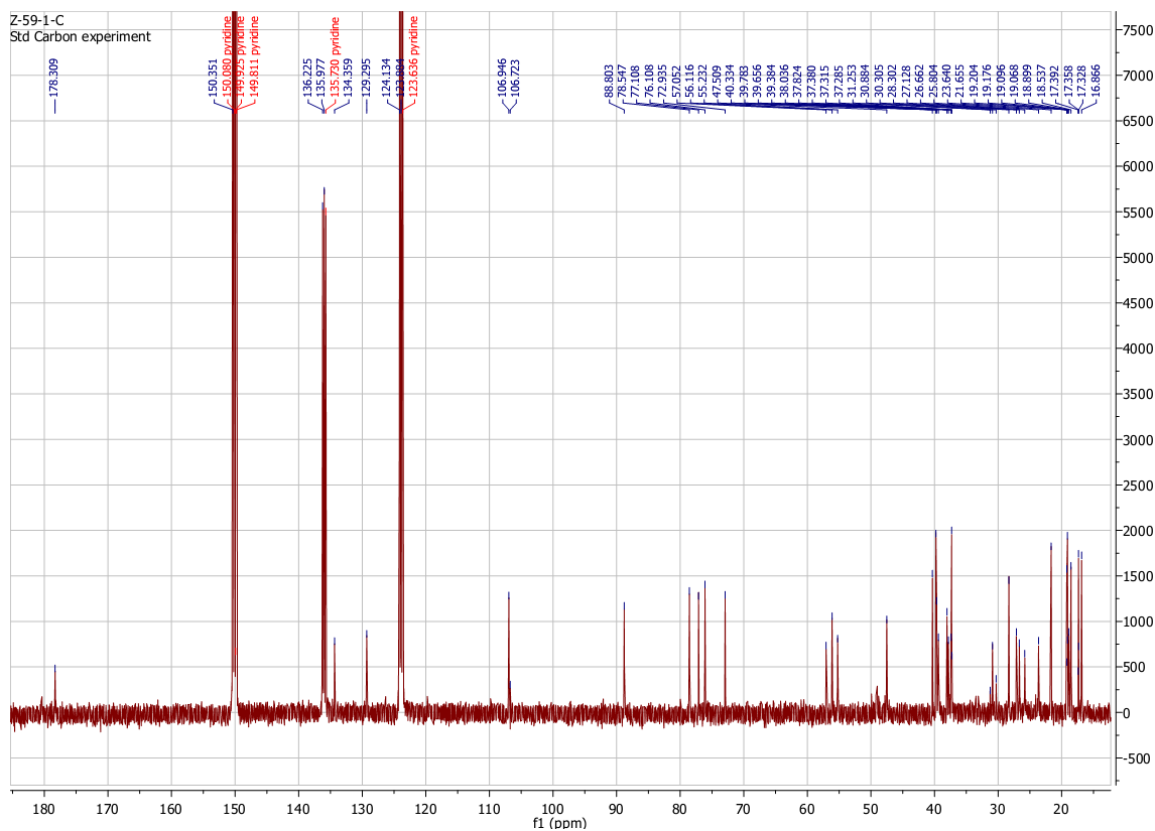
Figure 40 – ^{13}C-NMR spectrum of compound **1.24** in *Pyridine- d$_5$*

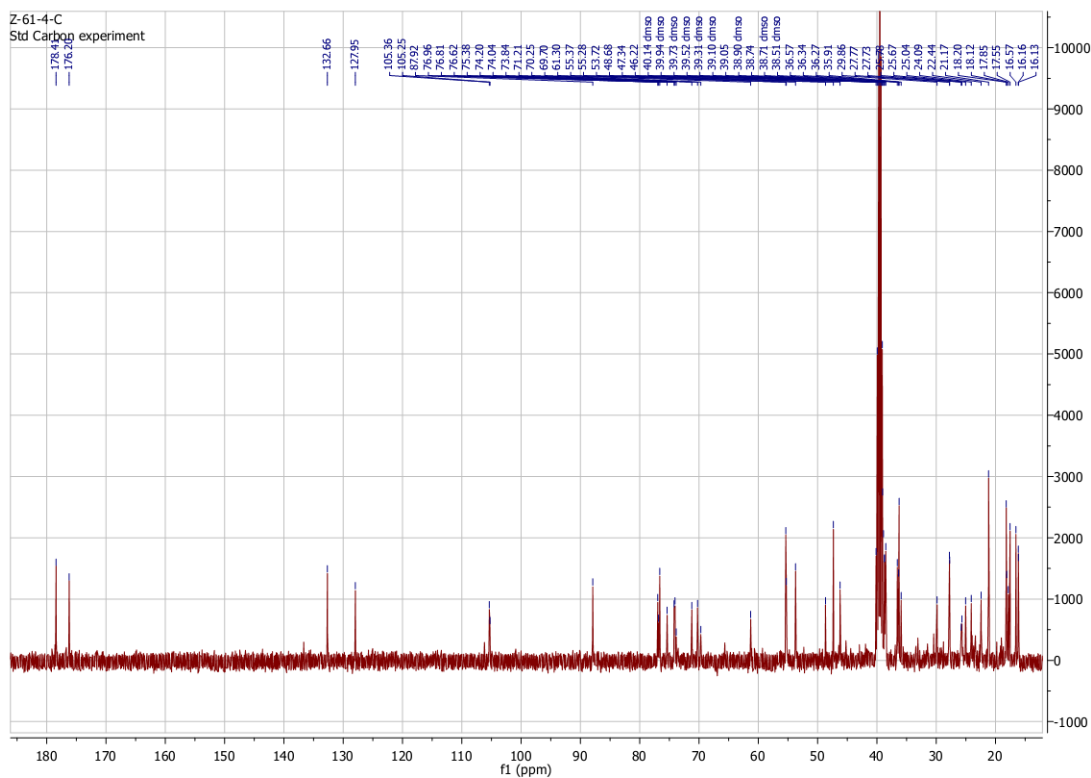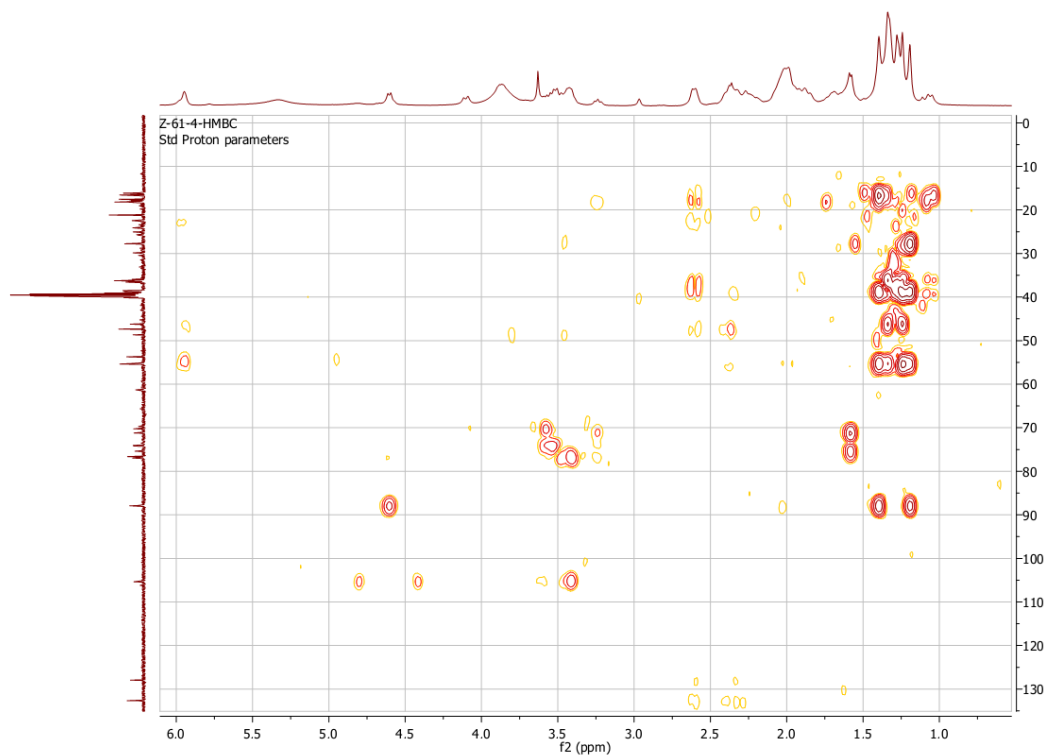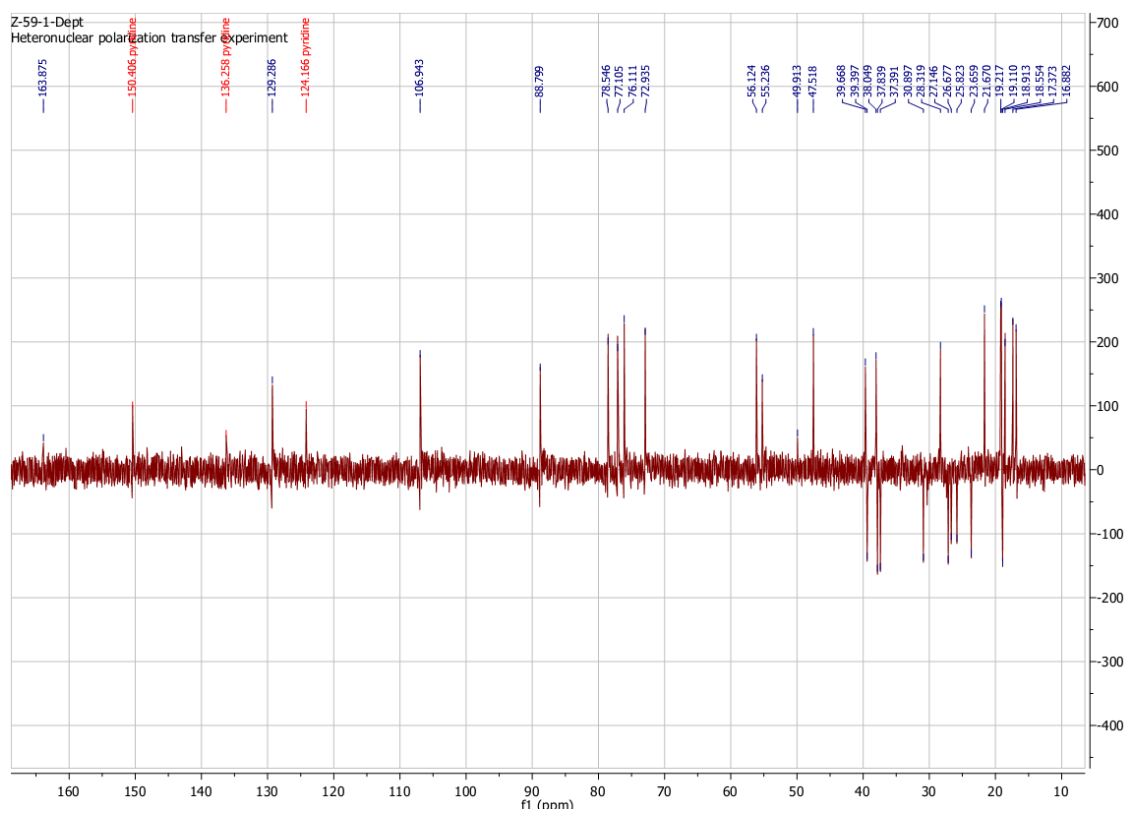Figure 41 – DEPT spectrum of compound **1.24** in *Pyridine- d$_5$*
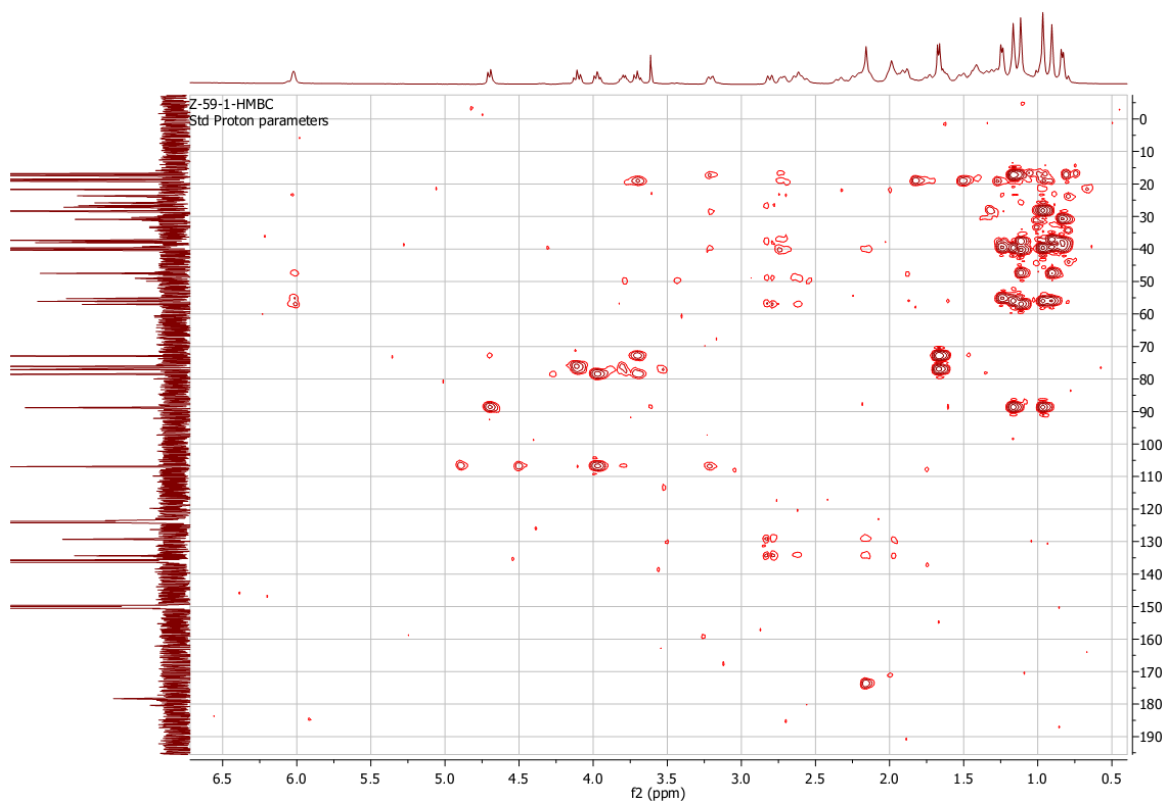
Figure 42 – HMBC spectrum of compound **1.24** in *Pyridine- d₅*
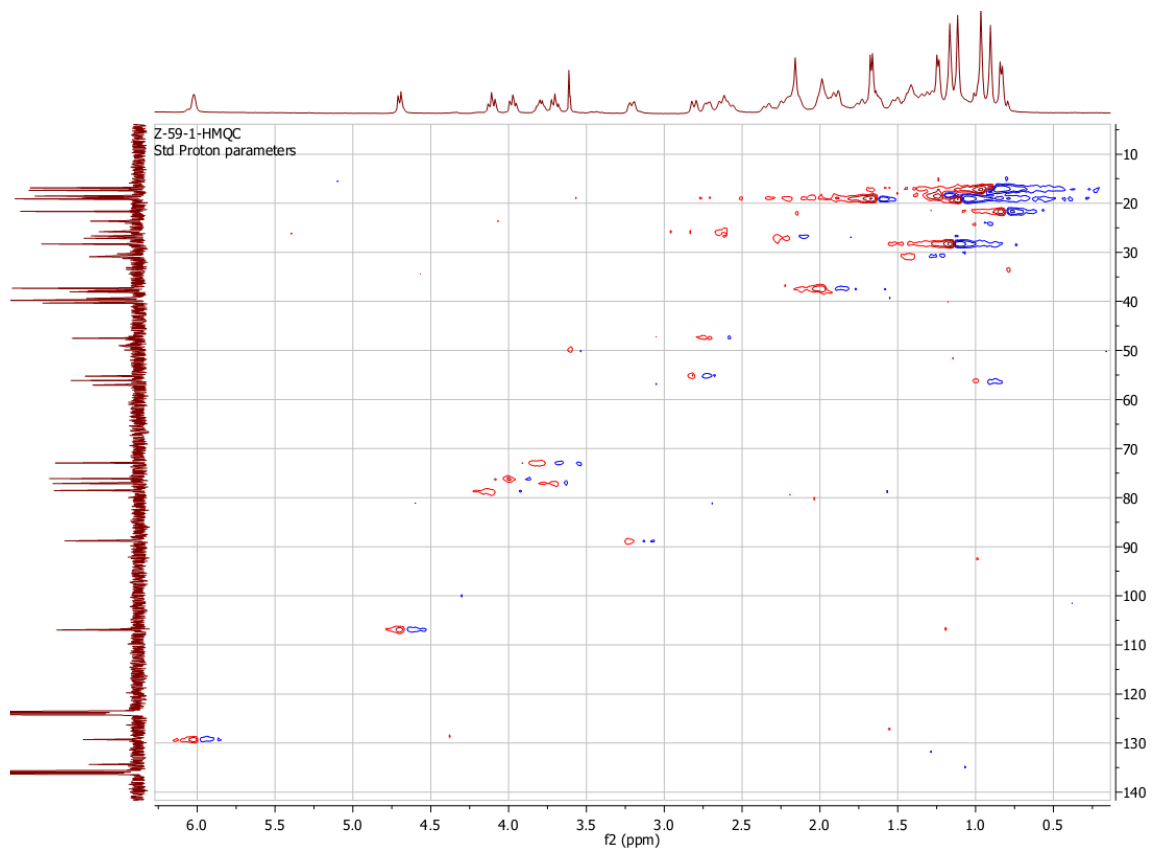
Figure 43 – HMQC spectrum of compound **1.24** in *Pyridine- d₅*

Compounds **2.2** and **2.3** were obtained as white crystals with m.p. 198-200°C and 220-221°C and R_f = 0.36 and 0.34 (system XI) respectively. It gave positive results to the libermann-Burchard and Molish tests. The mass - spectra (FAB method) of both substances 2.2 and 2.3 revealed a molecular ion peak with m/z 618, which corresponds to the molecular formula $C_{36}H_{58}O_8$. The IR spectrum of the substance, showed bands of stretching vibrations for (C = O) group at 1720-1724 cm^{-1}, in the region 3400-3412 cm^{-1} stretching vibrations of OH groups and three intense bands at1026-1070 cm^{-1} and 890 cm^{-1} indicate the presence of pyranose form of sugar with β-configuration of the glycosidic bond. By means of acid hydrolysis and using PC and TLC (System I, VI) identified D-glucose and the aglycone moiety of the triterpene. In the ^{1}H-NMR spectrum of compounds 2.2 and 2.3 (table 15), observed signals of the anomeric proton of glucose at δ_H 4.57 (1H, d, J = 7.0-7.5 Hz) confirmed the β-configuration of the anomeric center of D-glucopyranose. The remaining protons of glucose appear at δ_H 4.44-3.92. The mass spectrum also showed a characteristic peak at m/z 455 method (EC/mass), due to the loss of glucose [M-162]$^+$ in both compounds, this molecular ion peak with m/z 455 corresponds to the molecular formula of oleanolic acid $C_{30}H_{46}O_2$. Other proton of the aglycone moiety were observed in the ^{1}H-NMR spectrum in the form of seven singlet signals of quaternary methyl groups, at δ_H 0.91-0.83 (each 3H, H-23-27,29,30). Doublet at δ_H 3.58 (**2.2**) and 3.81 (**2.3**) belong to the methine proton at H-3. The magnitude of spin-spin coupling J = 2.0 Hz indicates the α-orientation of the proton at C-3. In addition the oleifinc proton at δ_H 5.33 (1H, br.s) in both compounds confirmed the presence of double bond.

For substances **2.2** the structure was confirmed by ^{13}C-NMR spectra. Which showed signals at δ_C 122.5 (C-12) and 143.6 (C-13) which serves as a diagnostic feature for $\Delta^{12,13}$ – oleanolic acid triterpene (table 17) [178]. The spectrum showed also signal δ_C 183.0 (C-28) corresponding to the COOH group, signal at δ_C 79.0 of the methine (C-3) and signal at δ_C 105.6 (C-1') of the anomeric carbon of glucose, other sugar carbons observed at δ_C 62.8 (C-6'), 71.3 (C-4'), 72.8 (C-2'), 75.6 (C-3'), 77.4 (C-5'). The site of attachment of sugar with the aglycone moiety was determined from HMBC correlations. Thus, in the HMBC spectrum of compound (**2.3**), the anomeric proton (H-1') of glucose at δ_H 4.57 showed correlation with C-3 of terpenoid at δ_C 79.0 ppm, and for compound (**2.3**), the correlation were found at C-28 δ_C 183.0. Consequently, the sugar moiety confirmed for 2.2 at C-3, and for **2.3** at C-28 positions. On the basis of chemical and physico-chemical methods of analysis **2.2** was identified as oleanolic acid -3-O-β-D-glucopyranoside, and **2.3** as Oleanolic acid 28-O-β-D-glucopyranoside ester. These compounds have previously been isolated from *Camphorosma monspeliacum*, but this is the first repot for isolation from *Camphorosma lessingii* [149].

Table 17 – ^{1}H and ^{13}C-NMR spectral data of **2.2** and **2.3** in CDCl$_3$

Carbon	Compound **2.2**			Compound **2.3**
	δc	Dept	δ$_H$ (J_{HH} in Hz)	δ$_H$(J_{HH} in Hz)
1	38.3	CH$_2$		
2	26.9	CH$_2$		
3	79.0	CH	3.58 (d, J = 2.0, 1H)	3.81 (d, J = 2.0, 1H)
4	38.8	C		
5	55.2	CH		
6	18.1	CH$_2$		
7	32.5	CH$_2$		
8	39.3	C		
9	47.6	CH		
10	37.1	C		
11	22.8	CH$_2$		
12	122.5	CH	5.33 (br.s, 1H)	5.33 (1H, br.s, H-12)
13	143.6	C		
14	41.6	C		
15	27.6	CH$_2$		
16	23.4	CH$_2$		
17	45.8	C		
18	41.0	CH		
19	46.5	CH$_2$		
20	30.7	CH		
21	33.8	CH$_2$		
22	32.6	CH$_2$		
23	28.1	CH$_3$	0.88 (s, 3H)	0.89 (s, 3H)
24	15.3	CH$_3$	0.84 (s, 3H)	0.85 (s, 3H)
25	15.5	CH$_3$	, 0.87 (s, 3H)	0.88 (s, 3H)
26	17.2	CH$_3$	0.83 (s, 3H)	0.84 (s, 3H)
27	25.9	CH$_3$	0.91 (s, 3H)	0.91 (s, 3H)
28	183.0	C		
29	23.6	CH$_3$	0.85 (s, 3H)	0.86 (s, 3H)
30	33.1	CH$_3$	0.86 (s, 3H)	0.87 (s, 3H)
1'	105.6	CH	4.57 (d, J = 7.5, 1H)	4.57 (d, J = 7.0, 1H)
2'	72.8	CH	4.07 (t, J=9.5, 1H)	4.07 (t, J=9.5, 1H)
3'	75.6	CH	4.29 (t, J=9.5, 1H)	4.31 (t, J=9.5, 1H)
4'	71.3	CH		3.98-3.92 (m, 1H)
5'	77.4	CH	3.98-3.94 (m, 1H)	3.98-3.92 (m, 1H)
6'	62.8	CH2	4.42 (d, J = 12.0, 3.0, 2H)	4.44 (dd, J=12.0, 3.0, 2H)
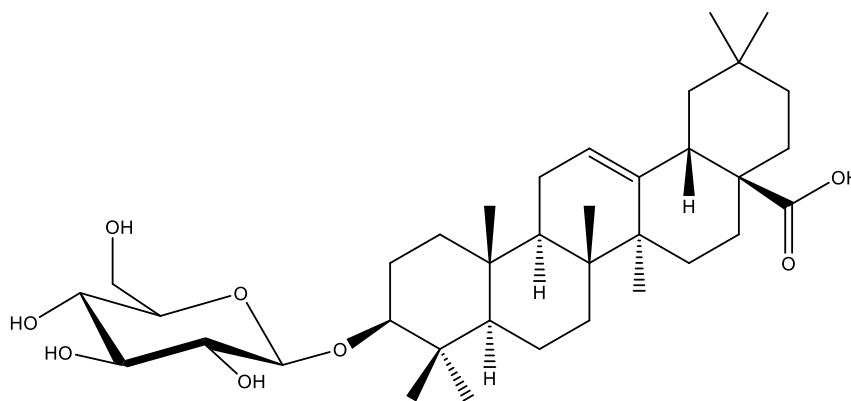
Oleanolic acid -3-O-*β*-D-glucopyranoside (Compound **2.2**)
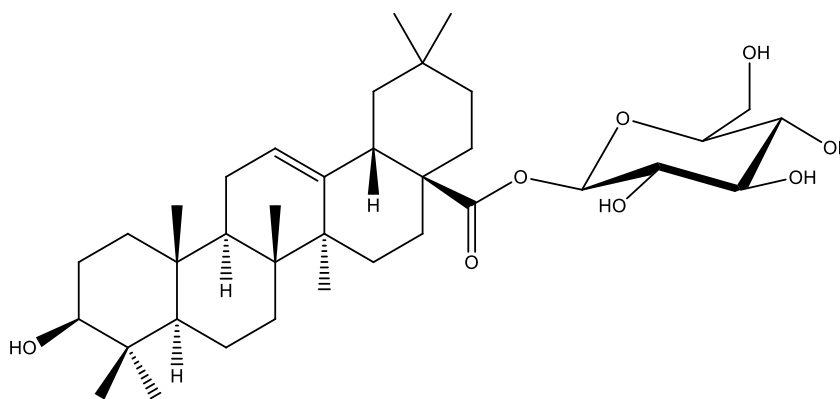
Oleanolic acid 28-O-*β*-D-glucopyranoside ester (Compound **2.3**)

Compound **1.1** and **2.1** were isolated from *Zygophyllum fabago* and *Camphorosma lessingii,* respectively. They isolated as a white powder m.p. 136-140 °C, R_f = 0.71 (system XI) and showed positive Liebermann-Burchard reaction and Salkowski's test indicated its sterol nature [179].

The ^{1}H-NMR spectral data (400 MHz, CDCL$_3$) together with the ^{13}C-NMR and DEPT experiment (table 18) showed 29 signals which indicating its steroidal nature. The spectral features and the other physical properties are in close agreement to those observed for *β*-sitosterol in literature [180, 181].
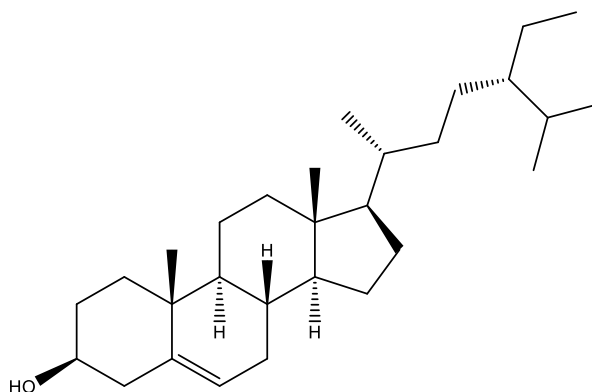
β-Sitosterol (Compound **1.1**, **2.1**)

Table 18 – ^{1}H and ^{13}C-NMR spectral data of compounds **1.1**, **2.1** and **1.15** in chloroform-*d*

Carbon	Compounds **1.1** and **2.1**			Compound **1.15**		
	δ_C	Dept	$\delta_H(J_{HH}$ in Hz)	δ_C	Dept	$\delta_H(J_{HH}$ in Hz)
1	37.3	CH$_2$		38.0	CH$_2$	
2	31.6	CH$_2$		30.7	CH$_2$	1.74 (dt, J = 12.4, 5.4 Hz, 2H),
3	71.7	CH	3.49 (tt, J = 10.7, 4.7 Hz, 1H)	79.1	CH	3.62 (m, 1H),
4	42.3	CH$_2$	2.30 – 2.17 (m, 2H)	39.8	CH$_2$	2.50 (t, J = 12.6 Hz, 2H)
5	140.8	C		141.4	C	
6	121.7	CH	5.32 (d, J = 5.0 Hz, 1H)	122.4	CH	5.38 (d, J = 5.0 Hz, 1H)
7	31.9	CH$_2$	2.48 (m, 2H)	32.7	CH$_2$	2.44 (m, 2H)
8	31.9	CH		32.6	CH	2.16 (d, J = 12.0 Hz, 1H)
9	50.2	CH		50.8	CH	
10	36.5	C		37.4	C	
11	21.1	CH$_2$	1.50 – 1.43 (m, 2H)	21.8	CH$_2$	2.04 – 1.98 (m, 2H)
12	39.8	CH$_2$	2.04 – 1.89 (m, 2H)	40.5	CH$_2$	1.89 (d, J = 5.5 Hz, 2H)
13	42.3	C		43.0	C	
14	56.1	CH		56.7	CH	
15	24.4	CH$_2$		25.0	CH$_2$	1.41 (dd, J = 10.9, 5.3 Hz, 2H)
16	28.3	CH$_2$		29.0	CH$_2$	1.41 (dd, J = 10.9, 5.3 Hz, 2H)
17	56.8	CH	1.17 – 1.10 (m, 1H)	57.3	CH	
18	12.0	CH$_3$	0.66 (s, 3H).	12.7	CH$_3$	0.69 (s, 3H)
19	19.5	CH$_3$	0.98 (s, 3H)	19.9	CH$_3$	0.90 (s, 3H)
20	36.2	CH	1.27 – 1.19 (m, 1H)	36.9	CH	1.16 – 1.07 (m, 1H)
21	18.8	CH$_3$		19.5	CH$_3$	1.02 (d, J = 6.7 Hz, 3H)
22	34.0	CH$_2$		34.7	CH$_2$	1.57 (t, J = 13.9 Hz, 2H)
23	26.1	CH$_2$		26.9	CH$_2$	
24	45.8	CH		46.5	CH	
25	29.2	CH	1.70 – 1.58 (m, 1H)	30.0	CH	
26	19.9	CH$_3$	0.83 (d, 3H)	20.5	CH$_3$	0.88 (s, 3H)
27	19.1	CH$_3$	0.81 (d, J = 1.8 Hz, 3H)	19.7	CH$_3$	0.96 (br s, 3H)
28	23.1	CH$_2$	0.90 (d, J = 6.3 Hz, 2H),	23.9	CH$_2$	
29	11.9	CH$_3$	0.84 (s, 3H),	12.5	CH$_3$	0.92 (br s, 3H)
1'				103.9	CH	5.07 (d, J = 7.6 Hz, 1H)
2'				75.8	CH	4.04 – 3.93 (m, 1H)
3'				78.9	CH	4.58 (m, 1H)
4'				72.2	CH	4.30 (m, 1H)
5'				78.6	CH	4.08 (d, J = 7.9 Hz, 1H)
6'				63.3	CH$_2$	4.64 – 4.50 (m, 1H) 4.43 (dd, J = 11.9, 5.3 Hz, 1H)

Compound **1.15** was obtained as white granular powder (methanol), m.p. 276 – 278 °C. R_f = 0.43 (system XII) and showed positive Liebermann-Burchard reaction, Salkowski's and Molish tests indicated its steroidal glycoside nature [179].

The ^{1}H-NMR spectrum together with ^{13}C-NMR (table 18) showed the presence of 35 carbons which confirmed our suggestion. The β-configuration of the methyl group was confirmed by the comparison of chemical shifts of carbons and protons of the side chain in the ^{1}H and ^{13}C-NMR spectra with β-sitosterol [182]. Alkaline hydrolysis of compound yielded D-glucose and an aglycone that was identified as β-sitosterol by direct comparison with an authentic sample (Co-TLC, mmp). Based on these findings, the structure of compound **1.15** has been established as β-sitosterol 3-O-β-D-glucopyranoside.
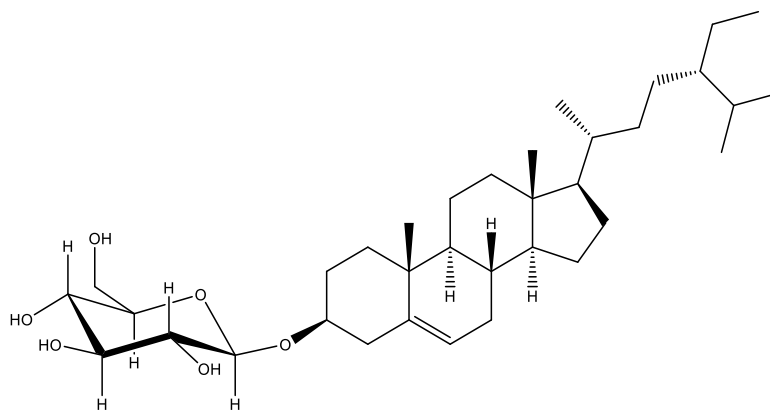
β-sitosterol 3-O-β-D-glucopyranoside (Compound **1.15**)

The study of the triterpenoid and sterols contents of *Z. fabago* revealed the presence of ursane type triterpenes; 3β-3-hydroxyurs-12-ene-27, 28-dioic acid (Quinovic acid), 3-O-β-D-glucopyranosyl-quinovic acid, these compounds were isolated before from some members of the family *Zygophyllaceae* [31, 169, 171], while 3-O-β-D-xylopyranosyl quinovic acid and 28-Nor-quivovic acid-3-O-β-D-quinovopyranosyl are reported here for the first from natural source (new compounds), while 3-O-[β-D-quinovopyranosyl]-quinovic acid and 3-O-[β-D-2-O-sulphonyl-quinovopyranosyl]-quinovic acid are reported for the first time from *Z. fabago*.

Furthermore from *Camphorosma lessingii* isolated oleanane type triterpenes; oleanolic acid-3-O-β-D-glucopyranoside, and oleanolic acid 28-O-β-D-glucopyranoside ester. These compounds have previously isolated from *C. monspeliacum*, but this is the first repot for isolation from *C. lessingii* [149]. The sterols also (β-sitosterol and β-sitosterol 3-O-β-D-glucopyranoside) isolated from *Z. fabago* and *C. lessingii* but for the *C. lessingii,* this is the first report of its isolation. The presence of triterpenoids in both species is in accordance with the fact that the triterpenoids play an important role in halophytic plants for protection from salinity in both salt-secretors and non-secretors. This particularly holds true for root tissues that directly encounter salt, and are the primary sites of perception and injury for salinity [138].

3.8 Identification of polyphenolic compounds

Compound **1.6** was isolated as a yellow amorphous powder, m.p. 276 °C (MeOH) and R_f =0.47 (system VII). It gave a yellow orange color with $AlCl_3$ and green color with $FeCl_3$ suggested that the compound maybe flavonoid. Furthermore, the HR-ESI-MS [M-1]$^+$ of this compound showed m/z = 285.053, corresponding to the molecular formula $C_{15}H_{10}O_6$. UV spectra were taken in MeOH. Absorption maximas were 270 and 374 nm identical to that of the standard kaempferol [183]. Its ^{1}H- and ^{13}C-NMR spectra (table 19) showed the presence of aromatic system with 13 carbon signals.

These findings together with other 2D NMR analyses and comparing data with those of literature [184] confirmed that the compound was determined as 3,4`,5,7-tetrahydroxyflavone, namely Kaempferol, and this is the first report about isolation of this compound from *Zygophyllum fabago*.
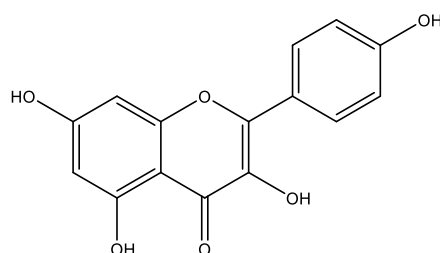
Kaempferol (Compound **1.6**)

Compound **1.7, 2.5** were obtained as a crystalline yellow solid (m.p. 315 °C) and R_f =0.45 (system VII). It gave a yellow orange color with $AlCl_3$ and green color with $FeCl_3$ suggested that the compound maybe flavonoid. Furthermore, the HR-ESI-MS [M-1]$^+$ of this compound showed m/z = 301.040, corresponding to the molecular formula $C_{15}H_{10}O_7$. UV spectra were taken in MeOH. Absorption maximas were 266 and 375 nm.

The ^{1}H-NMR, ^{13}C-NMR spectra together with DEPT experiment (table 19) revealed the presence of 15 carbon signals.

From the previous findings and by comparison of ^{1}H- and ^{13}C-NMR data with those of literature [185] the compound **1.7, 2.5** were identified as 3`4`5, 7-tetrahydroxy flavonol (Quercetin), and this is the first report also about isolation of this compound from *Zygophyllum fabago* and *Camphorosma lessingii*.
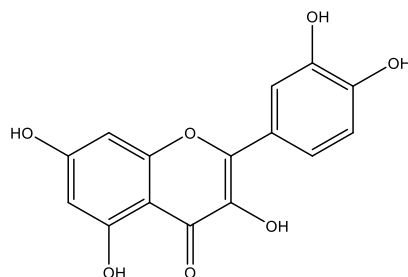
Quercetin (Compound **1.7, 2.5**)

Table 19 – ^{1}H and ^{13}C-NMR spectral data of compounds **1.6** and **1.7**

Carbon	Compound **1.6** (chloroform-*d*)			Compounds **1.7, 2.5** (*DMSO- d$_6$*)		
	δc	Dept	δ$_H$(*J$_{HH}$* in Hz)	δc	Dept	δ$_H$(*J$_{HH}$* in Hz)
2	146.9	C		147.7	C	
3	135.8	C		135.7	C	
4	176.0	C		175.8	C	
5	160.8	C		160.7	C	
6	98.3	CH	6.19 (br.s, 1H)	98.2	CH	6.19 (s, 1H)
7	164.0	C		163.9	C	
8	93.6	CH	6.44 (br.s, 1H)	93.4	CH	6.42 (s, 1H)
9	156.3	C		156.1	C	
10	103.1	C		103.0	C	
1′	121.8	C		122.0	C	
2′	129.6	CH	8.04 (d, *J* = 6.4 Hz, 1H)	115.6	CH	7.67 (s, 1H)
3′	115.5	CH	6.92 (d, *J* = 7.9 Hz, 2H)	145.1	C	
4′	159.3	C		146.8	C	
5′	115.5	CH	6.92 (d, *J* = 7.9 Hz, 2H)	115.1	CH	6.89 (d, *J* = 8.5 Hz, 1H)
6′	129.6	CH	8.04 (d, *J* = 6.4 Hz, 1H)	120.0	CH	7.54 (d, *J* = 8.7 Hz, 1H)

Compounds **1.8, 2.6** were obtained as a crystalline yellow solid (m.p. 306-307 ºC) and R$_f$ =0.0.38 (system VIII). It gave a yellow orange color with AlCl$_3$ and green color with FeCl$_3$ suggested that the compound maybe flavonoid. Furthermore, the HR-ESI-MS [M-1]$^+$ of this compound showed m/z = 315.056, corresponding to the molecular formula C$_{16}$H$_{12}$O$_7$. UV spectra were taken in MeOH. Absorption maximas were 254 and 370 nm.

The ^{1}H-NMR, ^{13}C-NMR spectra together with DEPT experiment (table 20) of compound 1.8 revealed 16 carbon signals also indicated a flavonol moiety.

The presence and position of the methoxy group was confirmed by ^{13}C-NMR and HMBC analyses and by comparing with that reported for Isorhamnetin [186], and this is the first report about isolation of this compound from *Zygophyllum fabago* and *Camphorosma lessingii*.
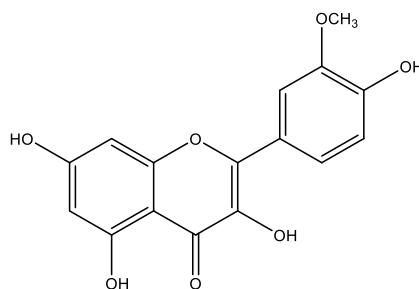
Isorhamnetin (Compound **1.8, 2.6**)

Compounds **1.20, 2.12** were isolated as yellow crystals (m.p. 175-179 ºC) from ethyl acetate and R$_f$ =0.34 (system II). It extracted by eluting the sephadex LH-20 column with MeOH. The compound gave a Purple colored spot on TLC when

examined under UV light, which can be characterized as flavonoid. The compound had positive results on test for sugar and flavonoid moiety which suggested that the compound maybe a flavonoid glycoside. Compound gave a yellow orange color with $AlCl_3$ and green color with $FeCl_3$. Furthermore, the HR-ESI-MS [M-1]$^+$ of this compound showed m/z = 477.119, corresponding to the molecular formula $C_{22}H_{22}O_{12}$.

The ^{1}H-NMR spectral data (table 20) displayed signals typical for flavonoid glycoside. The presence of the methoxy group at C-3' was supported by δ_H 3.82 (3H, s). Furthermore the ^{1}H-NMR spectra of the compound exhibited signal at δ_H 5.54 (1H, d, J= 7.0 Hz) applicable for sugar anomeric proton suggesting the presence of β-glyosidic linkage. Acid hydrolysis of this compound under reflux condition provided isorhamnetin and glucose when compared with authentic sample by Co-PC. This structure was further confirmed by ^{13}C-NMR spectral studies.

The ^{13}C NMR spectral data and DEPT experiment (table 20) of the compound showed a total of twenty one signals for the carbon atoms. The glycosylation site at C-3 hydroxyl was confirmed through the downfield resonance of C-2 at δ_C 156.5 and the upfield signal of C-3 at δ_C 133.0 and from the HMQC and HMBC correlation of the anomeric proton of sugar δ_H 5.5 to C-3 at δ_C 133.0 also the position of the methoxy group at C-3′ also confirmed by the its correlation to C-4′ at ring B. On the basis of these spectral data and comparing with that reported [187], the compound **1.20, 2.12** were identified as Isorhamnetin-3-O-β-glycoside, and this is the first report about isolation of this compound from *Zygophyllum fabago*.
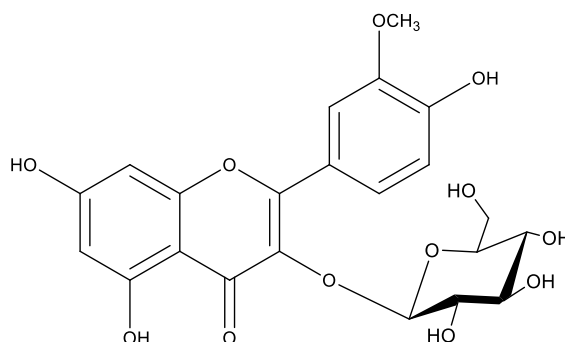
Isorhamnetin-3-O-β-glycoside (Compound **1.20, 2.12**)

Table 20 – ^{1}H and ^{13}C-NMR spectral data and of Compounds **1.8, 2.6** and **1.20, 2.12** in *DMSO- d$_6$*

Carbon	Compounds **1.20, 2.12**			Compound **1.8, 2.6**		
	δc	Dept	δ$_H$(J_{HH} in Hz)	δc	Dept	δ$_H$(J_{HH} in Hz)
2	156.55	C		146.6	C	
3	133.05	C		135.8	C	
4	177.49	C		175.8	C	
5	161.33	C		160.6	C	
6	98.94	CH	6.20 (d, J = 2.1 Hz, 1H),	98.2	CH	6.14 (s, 1H)
7	164.2	C		164.1	C	
8	93.91	CH	6.44 (d, J = 2.1 Hz, 1H)	93.6	CH	6.42 (s, 1H)
9	156.40	C		156.2	C	
10	104.06	C		103.0	C	
1′	121.20	C		122.0	C	
2′	113.56	CH	7.92 (s, 1H),	111.7	CH	7.70 (s, 1H)
3′	149.52	C		148.8	C	
4′	147.01	C		147.3	C	
5′	115.32	CH	6.91 (d, J = 8.5 Hz, 1H)	115.4	CH	6.87 (d, J = 7.6 Hz, 1H)
OCH$_3$	55.79	CH$_3$	3.82 (s, 3H)	55.7	CH$_3$	3.78 (s,3H)
6′	122.16	CH	7.49 (dd, J = 8.35, 2.0 Hz 1H),	121.7	CH	7.63 (d, J = 8.7 Hz, 1H),
1″	100.89	CH	5.54 (d, J = 7.0 Hz, 1H)			
2″	74.46	CH	3.23 (d, J = 3.2 Hz, 1H)			
3″	76.52	CH	3.23 (d, J = 3.2 Hz, 1H)			
4″	69.92	CH	3.16 (s, 1H), 3.10 (s, 2H)			
5″	77.56	CH	3.16 (s, 1H), 3.10 (s, 2H)			
6″	60.70	CH$_2$	3.3 (m, 2H)			

Compounds **1.27** were isolated as yellow crystals, m.p. 175-179 ºC with R$_f$ =0.35 (system XIV). The compound had positive results on test for sugar and flavonoid moiety which suggested that the compound maybe a flavonoid glycoside. Compound gave a yellow orange color with AlCl$_3$ and green color with FeCl$_3$. Furthermore, the HR-ESI-MS [M-1]$^+$ of this compound showed m/z = 447.088, corresponding to the molecular formula $C_{21}H_{20}O_{11}$.

The ^{1}H-NMR spectral data (table 21) displayed signals typical for flavonoid glycoside. the proton signals at δ$_H$ 8.48 (2H, d, J = 8.4 Hz, H-2′, H-6′) and 7.33 (2H, d, J = 8.5 Hz, H-3′, 5′), together with those at δ$_H$ 6.78 (1H, s, H-6) and 6.81 (1H, s, H-8) confirmed the 1,4-disubstituted and 1,2,3,5-tetrasubstituted aromatic rings, respectively. Furthermore the anomeric proton at δ$_H$ 6.57 (1H, d, J = 7.5 Hz, H-1``) and sugar protons at δ$_H$ 4.08-4.48 (5H, m, H-2″-H-5″) confirmed a glycosylated flavonoid structure having a glucose unit.

The ^{13}C-NMR exhibited upfield shift for C-3 at δ$_C$ 136.6 ppm, compared to unsubstituted kaempferol [188], revealing glycosylation at C-3. The position of sugar at C-3 was also confirmed by HMQC and HMBC correlations. The above findings together with comparing the data with that reported [189], the compound **1.27** is

identified as Kaempferol-3-O-β-D-glucopyranoside. This compound reported here for the first time from *Zygophyllum fabago*.
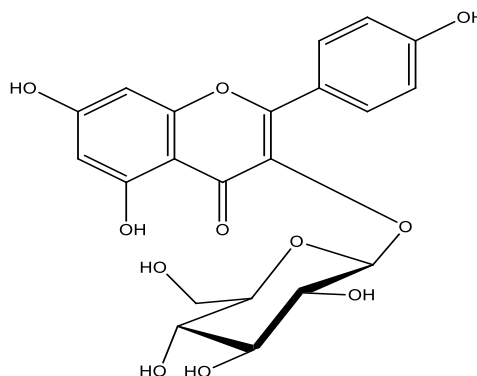
Kaempferol-3-O-β-D-glucopyranoside (Compound **1.27**)

Compound **2.4** was obtained as yellow crystalline powder with m.p. 110-112 °C (methanol) it has blue fluorescence under UV light and give a yellow color with ammonia vapor and cerium sulphate with R_f =0.40 (system XI). It showed two absorption bands in the UV spectrum observed at λ_{max} (MeOH) = 207-288 and 305-368 nm and absorption band of the carbonyl group in IR spectra at 1654 cm^{-1} which is characteristic for chromones due to the low negative inductive effect of the γ-pyrone hetero cycle [190]. The HR-ESI-MS [M-1]$^+$ of this compound showed m/z = 268, corresponding to the molecular formula $C_{16}H_{12}O_4$. Furthermore, in the ^{13}C-NMR spectrum and DEPT experiment, detected 16 carbon signals (table 21). The mass spectrum has a molecular fragmentation characterized for chromone nucleus with m/z 148 and phenoxyl with m/z 92. The presence of the characteristic fragment at m/z 120 corresponds to the decay mechanism retrodiene γ-pyrone cycle of chromone nucleus. The ^{13}C-NMR spectrum showed signals at δ_C 166.5 (C-2), 148.0 (C-1') and 116.2 (C-3) (table 21) characteristic for chromones according to the literature data [191]. In the ^{1}H-NMR spectrum the signals of the proton at C-3 appeared at δ_H 6.84 (1H, s), Two multiplet signals at δ_H 8.03 (2H, s, H-2', H-6'), and 8.04-8.06 (3H, H-3', H-4', H-5 ') indicate phenoxyl unsubstituted ring. The spectrum also showed proton at δ_H 7.58 (1H, d, J = 7.0 Hz, H-5), 7.09 (1H, dd, J = 8.8, 2.2 Hz, H-6), and 7.23 (1H, d, J = 2.1 Hz, H-8).

Moreover, ^{1}H-NMR and ^{13}C-NMR signals observed belonging to OCH$_3$ group. The mass spectrum (EI method, negative-ion FAB) observed the characteristic fragments at m/z 252 which confirmed the presence of one methoxy group. The structure was also confirmed by 2D NMR (HMBC). Which showed that the proton at C-3 of the pyrone cycle correlates with the carbon atoms C-2, C-3 and C-4, respectively. The position of OCH$_3$ group is also confirmed by the HMBC correlations. On the basis of physico-chemical methods of analysis of the structure of substance **2.4** set as 7-methoxy-2-phenoxychromone; this compound previously isolated from *Camphorosma monspeliacum* but reported her for the first time from *C. lessingii*.
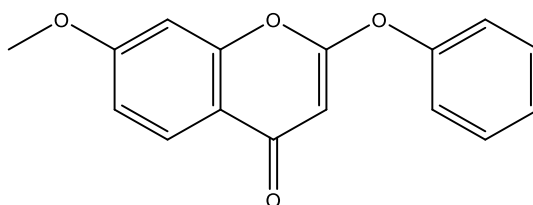
7-methoxy-2-phenoxy chromone (Compound **2.4**)

Table 21 – ^{1}H and ^{13}C-NMR spectral data of compound **2.4 and 1.27**

Carbon	Compound **2.4** in CDCl$_3$			Compound **1.27** in Pyridine		
	δc	Dept	δ$_H$(J_{HH} in Hz)	δc	Dept	δ$_H$(J_{HH} in Hz)
2	166.5	C		158.5	C	
3	116.2	CH	6.84 (s, 1H, c, H-3)	136.6	C	
4	180.0	C		179.7	C	
5	160.0	C	7.58 (d, J=8.0, 1H)	163.7	C	
6	107.5	CH	7.09 (dd, J=2.2, 8.8, 1H)	100.8	CH	
7	165.0	C		166.9	C	
8	101.6	CH	7.23 (d, J=2.1, 1H)	95.6	CH	
9	159.0	C		158.3	C	
10	118.0	C		106.2	C	
1′	148.0	C		122.9	C	
2′	132.8	CH	8.03 (m, 1H)	132.8	CH	8.48 (d, J = 8.4 Hz, 2H),
3′	127.5	CH	8.04-8.06 (m, 1H)	117.1	CH	7.33 (d, J = 8.5 Hz, 2H)
4′	159.0	C	8.04-8.06 (m, 1H)	162.6	C	
5′	127.4	CH	8.04-8.06 (m, 1H)	117.1	CH	7.33 (d, J = 8.5 Hz, 2H)
6′	130.2	CH	8.03 (m, 1H)	132.8	CH	8.48 (d, J = 8.4 Hz, 2H),
1″				104.2	CH	6.57 (d, J = 7.5 Hz, 1H),
2″				77.2	CH	4.42 (d, J = 7.0 Hz, 1H)
3″				79.4	CH	4.45 (m, 1H)
4″				72.3	CH	
5″				79.4	CH	4.08 (m, 1H)
6″				63.5	CH$_2$	4.48 (m, 1H)
OCH$_3$	56.60	CH$_3$	3.96 (s, 3H)			

Compound **1.25** was obtained as white crystals, melting point 210-213 °C with R$_f$ =0.23 (system XIV). HR-ESI-MS [M-1]$^+$ of compound showed m/z=, 163.058 which corresponding to the molecular formula C$_9$H$_8$O$_3$.

^{1}H-NMR (400 MHz, pyridine) data of compound **1.25** in table 22. Showed protons at δ$_H$ 8.15 (1H, d, J = 16 Hz, H-7), 7.67(2H, m, H-2, H-6), 7.31 (2H, d, J = 8.3 Hz H-3, H-5), 6.86 (1H, d, J = 16 Hz, H-8). ^{13}C-NMR (400 MHz, pyridine) data of compound XXX showed signals at δ$_C$ 170.88 (–COOH), 145.47 (C-7), 127.25 (C-1), 163.97 (C-4), 116.93 (C-3, C-5), 131.37 (C-2, C-6), 117.86 (C-8). According to ^{1}H-NMR and ^{13}C-NMR data, and comparing with that reported [192], compound **1.25** was identified as *P*-coumaric acid. The structure was further confirmed by 2D-NMR.
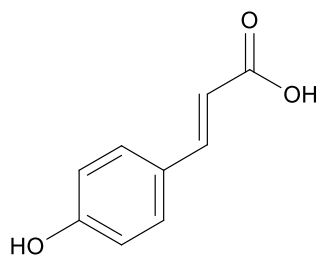
P-coumaric acid (Compound **1.25)**

Compound **1.26** was isolated as white crystal, m.p. 169°C with R_f =0.24 (system XIV). HR-ESI-MS [M-1]⁺ of compound showed m/z=, 177.029 which corresponding to the molecular formula $C_{10}H_{10}O_3$.

^{1}H-NMR data of compound **1.26** in table 22 showed similarity to compound **1.25** with protons at δ_H 3.81 (3H, s), 8.15 (d, J = 15.9 Hz, 1H), 6.86 (d, J = 15.9 Hz, 1H, H-8), 8.52 (d, *J* = 8.2 Hz, 2H), 8.11 (d, *J* = 8.1 Hz, 2H, H-3, H-5). ^{13}C-NMR (400 MHz, pyridine) showed signals at δ_C 56.59 (C4 -OCH₃), 114.58 (C-3, C-5), 117.62 (C-8), 127.25 (C-1), 133.51 (C-3, C-5), 145.47 (C-7), 161.95 (C-4), 170.14 (C-9). According to ^{1}H-NMR and[13]C-NMR data and comparing with that reported [193, 194], compound **1.26** was identified as *p*-methoxycinnamic acid. The structure was further confirmed by 2D-NMR.
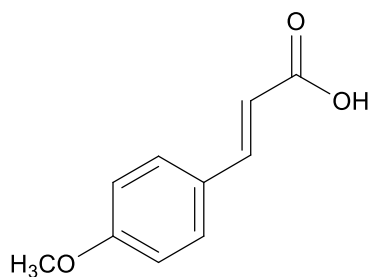
p-methoxycinnamic acid (Compound **1.26**)

Table 22 – ^{1}H and ^{13}C-NMR spectral data of compound **1.25 and 1.26** in Pyridine

Carbon	Compound **1.25**			Compound **1.26**		
	δ_c	Dept	$\delta_H(J_{HH}$ in Hz)	δ_c	Dept	$\delta_H(J_{HH}$ in Hz)
1	127.25	C		127.25	C	
2,6	131.37	CH	7.67 (d, J = 8.2 Hz, 2H)	133.51	CH	8.52 (d, J = 8.2 Hz, 2H)
3,5	116.93	CH	7.31 (d, J = 8.3 Hz, 2H)	114.58	CH	8.11 (d, J = 8.1 Hz, 1H)
4	163.97	C		161.95	C	
7	145.47	CH	8.15 (d, J = 16.0 Hz, 1H)	145.47	C	8.15 (d, J = 15.9 Hz, 1H)
8	117.86	CH	6.86 (d, J = 16.0 Hz, 1H)	117.62	CH	6.86 (d, J = 15.9 Hz, 1H)
9	170.88	C		170.14	C	
				56.59	OCH₃	3.81 (s, 3H)

Compound **1.2** was isolated as white crystalline crystals, m.p.63-64°C with Rf= 0.43 (ethyl acetate). ^{1}H-NMR (400 MHz, Pyridine-d_5) table 23 showed protons at δ_H 0.89 (t, J = 6.7 Hz, 3H C-16) indicating terminal methyl protons, 1.22-1.38 (m, 10×CH$_2$, 20H, C-4 to C-13), 2.55 (m, 2H, C-2) and 1.87 − 1.78 (m, 2H, C-3) indicates CH$_2$ protons adjacent to carboxylic group, respectively.

^{13}C-NMR (400 MHz, Pyridine-d_5) table 23 showed signals at δ_C 15.16 (CH$_3$, C-16) for the terminal methyl, 176.98 (COO, C-1) identical to the carboxylic group, the other signals at δ_C 30.47-31.40 (CH$_2$, C-4 to C-13), 23.81 (CH$_2$, C-15), 32.99 (CH$_2$, C-14), together with HR-ESI-MS [M-1]$^+$ of this compound which showed m/z = 255.231, corresponding to the molecular formula $C_{16}H_{32}O_2$ according the previous findings and comparing with that reported [195] confirmed that the compound **1.2** is Palmitic acid.
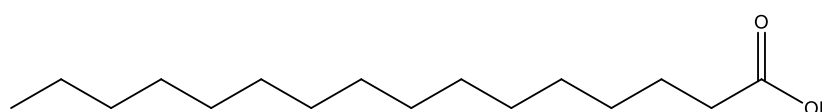
Palmitic acid (Compound **1.2**)

Table 23 – ^{1}H and ^{13}C-NMR spectral data of compound **1.2** in Pyridine

Carbon	δ_c	Dept	δ_H(J_{HH} in Hz)
1	176.98	C	
2	35.77	CH$_2$	2.55 (s, 2H)
3	26.52	CH$_2$	1.87 – 1.78 (m, 2H),
4-13	31.40-30.47	CH$_2$	1.22-1.38 (20H, m)
14	32.99	CH$_2$	1.28 (m, 2H),
15	23.81	CH$_2$	1.28 (s, 18H),
16	15.16	CH$_3$	0.89 (t, J = 6.7 Hz, 3H)

Substances (**1.19, 1.21** and **2.7-2.11**) on the basis of physico-chemical properties (table 24) and comparison with authentic samples using method of paper chromatography PC (I-V) and Diazotized *P*-nitroaniline as detecting reagent identified as vanillic, isovanillic, *p*-hydroxybenzoic, ferulic and protocatechuic acids.

Table 24 – Qualitative analysis of phenolic acids (**1.19, 1.21** and **2.7-2.11**)

Compound	R$_f$ values		Diazotized *P*-nitroaniline + 15% Na$_2$CO$_3$	m.p. °C
	III	IV		
1.19, **2.7**	0.65	0.45	Lilac	210
1.21, **2.8**	0.97	0.67	Violet	250-252
2.9	0.65	0.46	Light Blue	213-214
2.10	0.75	0.40	Rose red	174
2.11	0.62	0.49	Brown	200
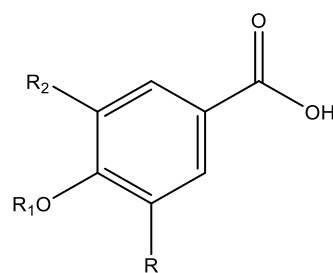
P-Hydroxybenzoic acid   (Compound **2.9**) R = H, R$_1$ = H, R$_2$ = H
Vanillic acid   (Compound **1.19** and **2.7**) R = OCH$_3$, R$_1$ = H, R$_2$ = H
Isovanillic acid   (Compound **1.21** and **2.8**) R = OH, R$_1$ = CH$_3$, R$_2$ = H
Protocatechuic acid   (Compound **2.11**) R = OH, R$_1$ = H, R$_2$ = H
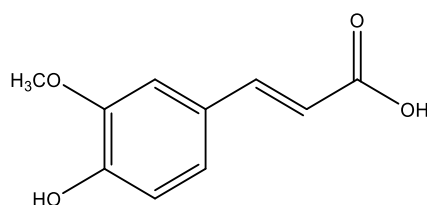
Ferulic acid   (Compound **2.10**) R= OCH$_3$

The polyphenolic compounds identified and reported in both plants as flavonoids, phenolic acids and chromones, but the later was reported only in *Camphorosma lessingii* and not detected in *Zygophyllum fabago.* The study declared the presence of Quercetin, Isorhamnetin, Isorhamnetin-3-O-*β*-glycoside form both plants, while Kaempferol and Kaempferol-3-O- O-*β*-glycoside isolated only from *Z. fabago* and this is the first report of isolation of these flavonoids from *Z. fabago.* The chromone 7-methoxy-2-phenoxychromone isolated here for the first time from *C. lessingii* but previously isolated from *C. monspeliacum.* Concerning the phenolic acids also highlighted similarity to greater extent that in both plants recognized vanillic, and isovanillic, while *C. lessingii* showed additionally, *p*-Hydroxybenzoic procatechoic acid and ferulic acids. *Z. fabago* showed *p*-coumaric acid, *P*-methoxycinnamic acid in addition to Palmitic acid. The identification of these phenolic acids was done for the first time in the two examined plants. Natural antioxidants occur in all plant parts, and the typical compounds that exhibit antioxidant activities include phenolics, carotenoids and vitamins. Among the various kinds of natural antioxidants, polyphenols constitute the main powerful compounds. Structurally, phenolics comprise an aromatic ring, bearing one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymerized compounds. In plants, polyphenol synthesis and accumulation is generally stimulated in response to biotic/abiotic stresses, such as salinity [140].

3.9 Biological screening

Natural products have served as an important source of drugs since ancient times and a significant part of today's drugs are somehow derived from natural sources. In the recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [196]. Several salt marsh plants have traditionally been used for medical, nutritional, and even artisanal purposes. Currently, an increasing interest is granted to these species because of their high content in bioactive compounds (primary and secondary metabolites) such as polyunsaturated fatty acids, carotenoids, vitamins, sterols, essential oils (terpenes), polysaccharides, glycosides, and phenolic compounds. These bioactive substances display potent antioxidant, antimicrobial, anti-inflammatory, and anti-tumoral activities, and therefore represent key-compounds in preventing various diseases (e.g. cancer, chronic inflammation, atherosclerosis and cardiovascular disorder) and ageing processes [197].

3.9.1 Antimicrobial activity of *Zygophyllum fabago*

The extracts of *Z. fabago* and *C. lessingii* obtained with different solvents were checked for both antibacterial and antifungal activity. However, the results of only *n*-butanol and *n*-hexane fractions of *Z. fabago* are mentioned here because other extracts did not show considerable antimicrobial activity. All fractions were screened against *Staphyloccocus aureus*, and *Candida albicans*. The results obtained as shown in table 25 revealed that the *n*-hexane and *n*-butanol fractions of *Zygophyllum fabago* exhibited a significant antifungal and antibacterial activity against *Candida albicans* with inhibition zones of 9.5 mm and against *Staphylococcus aureus* with inhibition zones of 10 mm compared to positive controls Ampicillin sodium and Amphotericin B with inhibition zones of 14 mm and 15 mm respectively. Moreover, the results obtained give a scientific support and confirm the traditional medicinal use of *Z. fabago* in skin diseases as wound healing, for carbuncles and as blister [90].

Table 25 – Antimicrobial test result of total extract and *n*-hexane fraction of *Zygophyllum fabago*

Sample	Sample con. (*mM*)	Sample amount (*ul*)	Inhibitory zone diameter (*mm*)	
			CA*	SA*
Ampicillin sodium salt	10 mg/ml	5	14	—
Ampicillin sodium salt	1 mg/ml	5	—	—
Amphotericin B	5 mg/ml	20	—	15
n-Butanol fraction	100	20	—	10
n-Hexane fraction	100	20	9.5	—

*CA : *Candida albicans*   EC :   SA : *Staphylococcus aureus*

3.9.2 Anti-leishmanial activity of *Zygophyllum fabago*

Leishmania, a trypanosometed protozoan that is transmitted by the female *Phlebotomus* sand fly causing leishmaniasis is prevalent in four continents. It is considered to be endemic in 88 countries, 72 of which are developing countries [198]. Leishmania patients are highly susceptible to HIV infection. It is now considered a genuine AIDs related opportunistic disease largely due to latent infection by immunosuppression [199]. Many novel compounds isolated from various medicinal plants have been reported for their leishmanicidal activity [200-207]. The current therapy against leishmaniasis is unsatisfactory [208]. The recommended drugs for leishmania are the pentavalent antimonials sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime) drugs that have been used for over 50 years, but they require long courses of parenteral administration for treating leishmania. The use of these recommended drugs also exhibit therapeutic failure, side effects and long duration for healing of lesions [209, 210].

The anti-leishmanial screen tests samples for their ability to inhibit *Leishmania donovani*, a fly-borne protozoan that causes visceral leishmaniasis. Crude extracts are initially tested in a primary screen at 80µg/mL in duplicate and percent inhibitions (% inh.) are calculated relative to negative and positive controls.

The results obtained as shown in table 26 for the evaluation of the anti-leishmanial activity of the different fractions of *Zygophyllum fabago* showed that the *n*-hexane and chloroform fractions showed a significant activity against *Leishmania donovani* with percent of inhibition about 93% of the *n*-hexane fraction and 86% of the chloroform fraction compared to the standard drug Amphotericin B that shows 99% inhibition. Moreover, the results obtained give a scientific support and confirm the traditional medicinal use of *Z. fabago* in skin diseases as wound healing, dermatitis, and chronic eczema [90].

Table 26 – Anti-leishmanial test results of different fractions of *Zygophyllum fabago*

Samples	Test con. (µg/mL)	*L. donovanii* % Inh.
n-Hexane fraction	80	93
Chloroform fraction	80	86
Amphotericin B*	100	99

*The drug control amphotericin B is used as positive control.

3.9.3 *In vitro* phytotoxic activity of *Camphorosma lessingii*

Weeds have always been thought as one of the most serious agricultural and environmental problems. In agriculture, the control of weeds and their harmful effects is usually achieved by using herbicides belonging to different classes of organic synthetic chemicals [211]. The synthetic chemical pesticides are being used for prevention of crop production. However, some serious flaws are associated with the use of these synthetic pesticides including problems to human health and produces heavy environmental pollution. In Kazakhstan, approximately 2.5 million hectares of grain are heavily infested with weeds. Government subsidies for herbicide purchase

of US$2-3million/year resulted in herbicide use on 1.4million hectares [134]. These problems have resulted in the renewed interest in the development and use of botanical pesticides, which could be an appropriate and non-hazardous alternative to the currently used synthetic agrochemicals as the natural products [212]. Phytotoxic natural products may be utilized either directly or as lead compounds for the development of herbicides. [213]. Therefore, those phytotoxic plants have received special attention due to their agricultural potential to develop natural herbicides for eco-friendly weed management strategies [214].

Therefore, the ethanolic extract of *Camphorosma lessingii* was checked for phytotoxic activity and exhibited significant phytotoxic activity against growth of *Lemna minor* with 100 % growth regulation at 1000 µg/ml, 82% at 100 µg/ml and 47% at 10 µg/ml (table 27). The inhibitory effect was enhanced with increased concentrations, indicating that the phytotoxic activity of the ethanolic extract is dosage-dependent.

Table 27 – Phytotoxic test result of ethanolic extract of *Camphorosma lessingii*

Name of plant	Test con. (µg/mL)	No of fronds		Growth regulation %	Con. of std. drug (µg/mL)
		sample	control		
Lemna minor	1000	0	17	100	0.05
	100	3		82	
	10	9		47	

3.9.4 Antidiabetic activity of *Zygophyllum fabago* and *Camphorosma lessingii*

Type II diabetes is increasing at an alarming rate worldwide with a fundamental feature of resistance to insulin. Over the last decade, more and more evidences have shown that protein tyrosine phosphatase 1B (PTP1B) is involved in the down regulation of insulin and leptin signaling. The most convincing evidence is that PTP1B is involved in the insulin-signaling pathway deriving from the phenotype of the PTP1B knockout (PTP1B KO) mouse. The knockout mice (PTP1B-/-) completely deficient in the enzyme show increasing IR phosphorylation, increasing sensitivity to insulin and resistant to diet-induced obesity. Interestingly, the PTP1B-/- mice grow and develop normally, which are fertile and show no macroscopic or histological difference compared with wild-type mice (PTP1B+/+) [215, 216]. Thus, PTP1B appears to be a very attractive drug target for type II diabetes and obesity.

Therefore, the ethanolic extracts of the two species together with some selected fractions were assayed for their inhibitory activity against PTP1B, and the results are presented in table 24. The known PTP1B inhibitors, 3-(3,5-dibromo-4-hydroxy-benzoyl)-2-ethyl-benzofuran-6-sulfonic acid-(4-(thiazol-2-ylsulfamyl)-phenyl)-amide [IC_{50} = (1.46 ±0.40) µg/mL] were used as positive controls in this assay.

The results showed in table 28 revealed that all tested fractions showed significant potent inhibitory activity on PTP1B; but the *n*-butanol fraction of *Z. fabago* is the most potent fraction with an (IC_{50} value of 3.94 µg/mL), also the *n*-hexane fraction of *Z. fabago* found significantly potent inhibitor on PTP1B with an

(IC_{50} value of 4.33 µg/mL), furthermore the ethanolic extract of *Z. fabago* and *C. lessingii* showed significant inhibitory effect but with moderately high IC_{50} (17.50 µg/mL) and (11.26 µg/mL) respectively.

Table 28 – Inhibitory effect of total extracts and different fractions of *Zygophyllum fabago* and *Camphorosma lessingii* on PTP1B

Sample	IC_{50} (µg/mL)
n-Butanol fraction of *Z. fabago*	3.94 ± 0.14
n-Hexane fraction of *Z. fabago*	4.33 ± 0.17
Ethanol extract *C. lessingii*	11.26 ± 0.51
Ethanol extract *Z. fabago*	17.50 ± 0.87
PTP1B Inhibitor*	1.46 ± 0.40

*The drug control 3-(3,5-dibromo-4-hydroxy-benzoyl)-2-ethyl-benzofuran-6-sulfonic acid-(4-(thiazol-2-ylsulfamyl)-phenyl)-amide is used as positive control.

3.9.5 Anti-tumor activity of *Zygophyllum fabago* and *Camphorosma lessingii*

The cytotoxic activity against human hepatocarcinoma (Hep-G2), colon carcinoma (HCT-116), and breast adenocarcinoma (MCF-7) cells was estimated by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay (Hansen et al., 1989) [217], which is based on the cleavage of the tetrazolium salt by mitochondrial dehydrogenases in viable cells. Cells were seeded in a 96-well plate at a concentration of 5×104 cells/well and incubated overnight. After treatment with various concentrations of the compounds (0, 12.5, 25, 50, and 100 µg/mL, respectively), cells were incubated for an additional 24 h at 37°C in serum-free medium before being submitted to MTT assay.

The samples were tested against different human cancer cell lines including Hep G2, HCT-116, and MCF-7 cells. In Hep-G2 cells the results indicated that the *n*-butanol fraction of *Z. fabago* (Z-T-T) was the most potent cytotoxic sample (fig 44) as concluded from the low IC_{50} (fig 47 and table 29), while samples the total extract of *C. lessingii* (C-T), and *Z. fabago* (Z-T) and compound (**1.12**) were moderate inhibitors (fig 36) of the cell viability as concluded from their relatively low IC_{50} (fig 47 and table 29). While In colon HCT-116 cells the results indicated that the *n*-butanol fraction of *Z. fabago* (Z-T-T) was the most potent cytotoxic sample (fig 37) as concluded from the low IC_{50} (fig 47 and table 29), while the total extract of *C. lessingii* (C-T), and *Z. fabago* (Z-T) and compound (**1.12**) were moderate inhibitors (fig 37) of the cell viability as concluded from their relatively low IC_{50} (fig 47 and table 29). However the total flavonoid fraction (Z-F) was inactive. In breast MCF-7 cells the results indicated that all of the tested samples were inactive as anticancer agents towards breast cells (fig 46), although the *n*-butanol fraction of *Z. fabago* (Z-T-T) revealed a relative viability inhibition with high IC_{50} (fig 46, 47). And this found in accordance with the traditional use of *Z. fabago* in treatment of cancer diseases [218].

Table 29 – Cytotoxicity (IC$_{50}$, μg/mL) of total extracts and different fractions of *Zygophyllum fabago* and *Camphorosma lessingii* against human malignant cell lines after 48 hr. of incubation

Cell lines	Samples/ IC$_{50}$, μg/mL					
	C-T	Z-T	Z-F	Z-T-T	Comp. **1.12**	Paclitaxel*
MCF-7	76.21±0.30	69.59±0.54	153.78±0.30	51.90±0.47	84.80±0.38	0.87±0.20
Hep-G2	48.15±0.36	46.09±0.68	169.28±0.27	6.86±0.34	54.47±0.44	0.47±0.10
HCT-116	71.06±0.43	57.17±0.80	192.42±0.32	30.09±0.41	75.01±0.52	0.38±0.13

*The drug control Paclitaxel is used as positive control.
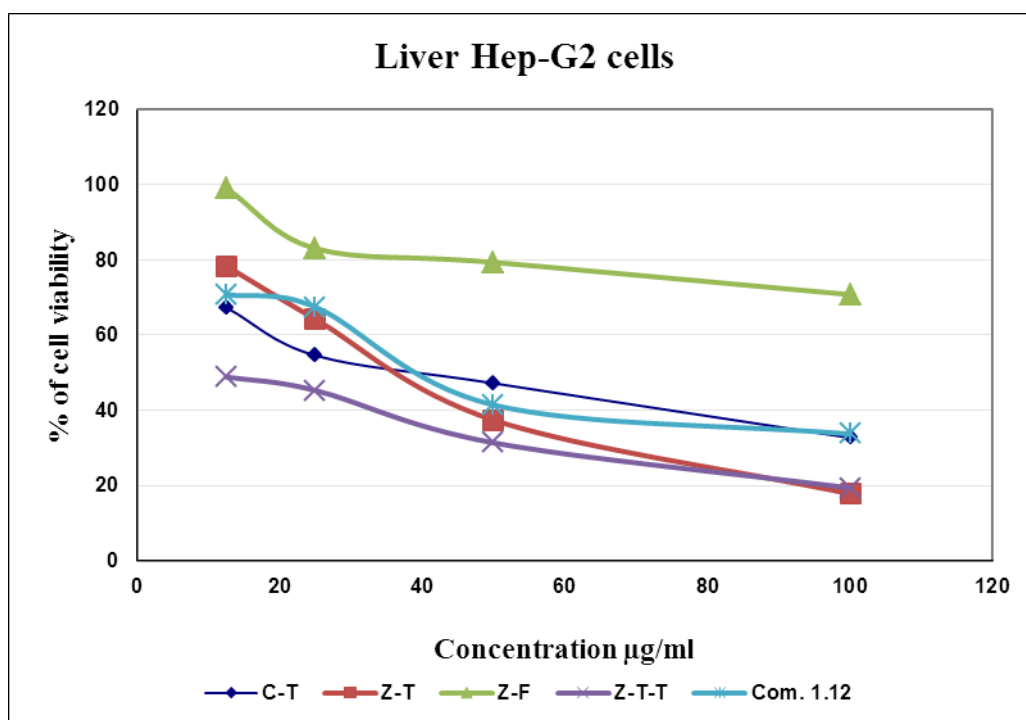
Figure 44 – Cell viability of liver Hep-G2 cells after the treatment with different concentrations of C-T, Z-F, Z-T-T, Z-T and comp. **1.12** for 48 hours, as measured by MTT assay. The data are presented as (Mean ± SE) of μg/mL.
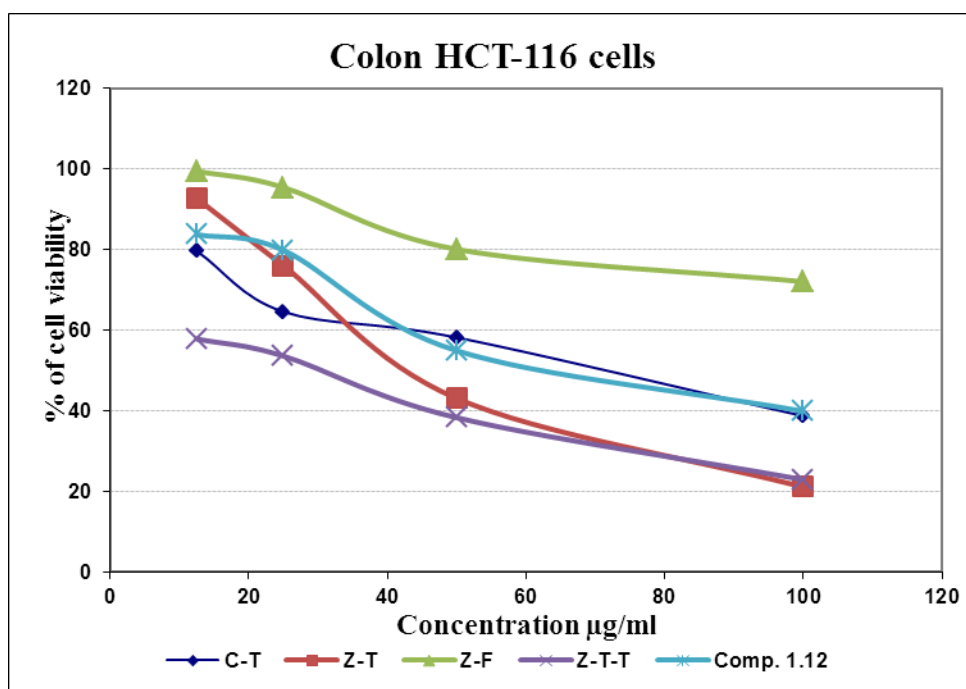
Figure 45 – Cell viability of colon HCT-116 cells after the treatment with different concentrations of C-T, Z-F, Z-T-T, Z-T and comp. **1.12** for 48 hours, as measured by MTT assay. The data are presented as (Mean ± SE) of µg/mL.
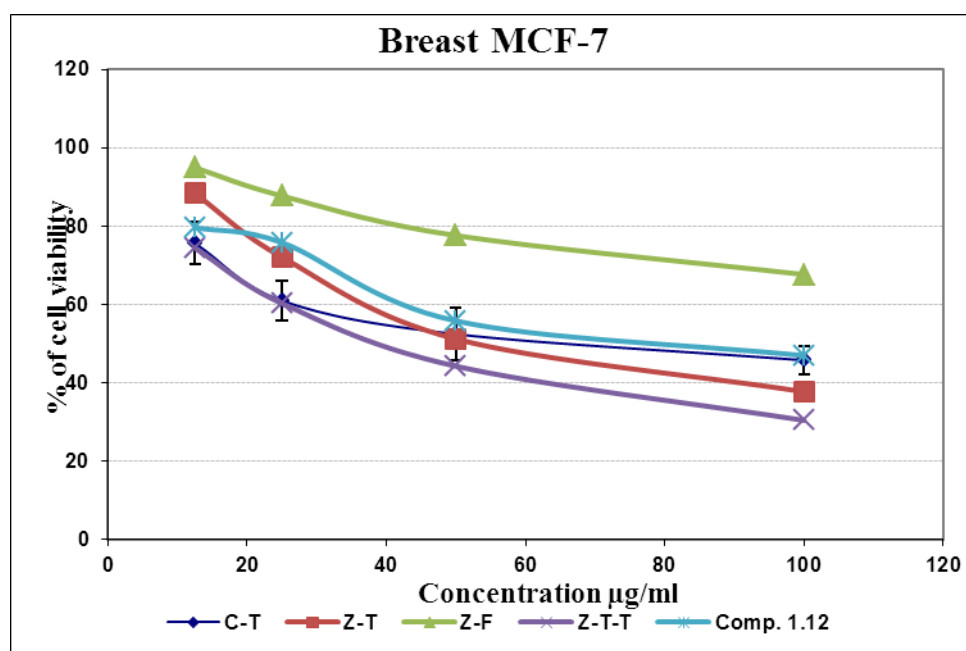
Figure 46 – Cell viability of breast MCF-7cells after the treatment with different concentrations of C-T, Z-F, Z-T-T, Z-T and comp. **1.12** for 48 hours, as measured by MTT assay. The data are presented as (Mean ± SE) of µg/mL.

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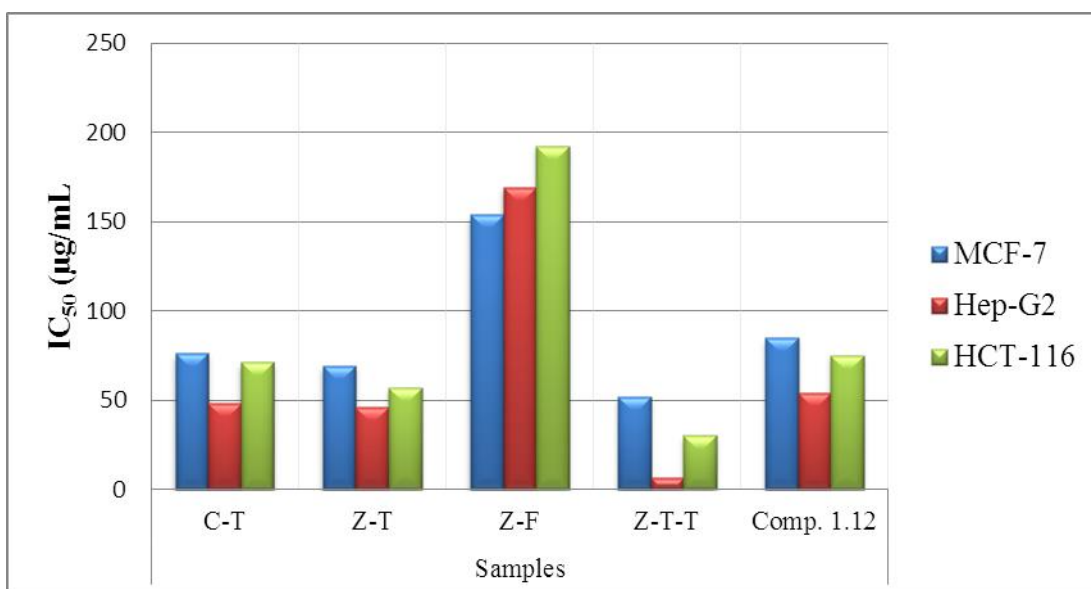
Figure 47 – Half maximum inhibitory concentration (IC$_{50}$, µg/mL) of tested samples in cell viability of colon HCT-116, liver Hep-G2 and breast MCF-7 cells after the treatment for 48 hours, as measured by MTT assay.

In view of the current biological screening results of the different extracts and fractions of *Zygophyllum fabago* and *Camphorosma lessingii* together with compound **1.12** which isolated from the ethyl acetate fraction of *Z. fabago*, ,it could be concluded that; the total ethanolic extract of *Z. fabago* and *C. lessingii* showed a moderate inhibitor cytotoxic activity against hepatocarcinoma (Hep-G2) and colon carcinoma (HCT-116) cells, while for the test of antidiabetic activity showed a significant inhibitory effect but with moderately high IC$_{50}$. The *n*-butanol fraction of *Z. fabago* were found the most potent fraction as cytotoxic agent against hepatic (Hep G2) and colon (HCT-116) cells and revealed a relative viability inhibition with high IC$_{50}$ against breast adenocarcinoma (MCF-7). It also showed potent inhibitory effect on PTP1B; and exhibited a significant antifungal and antibacterial activity against *Candida albicans* and *Staphylococcus aureus*. Moreover, the ethanolic extract of *C. lessingii* exhibited a dosage-dependent significant phytotoxic activity against growth of *Lemna minor*. In addition, the *n*-hexane and chloroform fractions of *Z. fabago* showed a significant anti-leishmanial activity against *Leishmania donovani* with percent of inhibition about 93% and 86% respectively. However, the *n*-hexane alone showed additional significantly potent inhibitory effect on PTP1B and exhibited a significant antifungal and antibacterial activity against *Candida albicans* and *Staphylococcus aureus*. These results reported here for the first time for the two species. Finally we can conclude that the results obtained give a scientific support and confirm the traditional medicinal use of *Z. fabago* in skin diseases as wound healing for carbuncles and as blister, dermatitis, chronic eczema and in treatment of cancer diseases [90, 218].

CONCLUSION

Based on the obtained results from the carried experiments the following could be concluded:

1. For the first time, a comparative study of the chemical composition of halophytes plants *Zygophyllum fabago* and *Camphorosma lessingii*. Revealed that both species contain high contents of saponins, organic and amino acids and flavonoids, while chromones reported only in *Camphorosma lessingii*. The obtained results and high elemental contents indicate that they were grown in different saline soils.

2. The Optimal conditions for isolation of biologically active substances and scheme of its separation using a flash, vacuum, solid-phase and other types of chromatography; allocated from *Zygophyllum fabago* 18 pure compounds (six triterpenoids, five flavonoids, two sterols, one aliphatic acid, four phenolic acids); while from *Camphorosma lessingii* isolated 12 substances (two triterpenoids, three flavonoids, one sterol, one chromone and five phenolic acids).

3. The method of gas-liquid chromatography of studied halophytic species identified 8 fatty acids, 20 amino acids. The lipophilic content revealed the presence of hydrocarbons, ketones, esters, acids and nitrogen-containing compounds. In *Zygophyllum fabago* identified 7 components while in *Camphorosma lessingii* -12 components were identified.

4. Establishment of the chemical composition of *Camphorosma lessingii* essential oils revealed the presence of 80 components, of which 37 were identified. The main components are Hexanedioic acid, bis (2-ethylhexyl) ester, *n*-hexadecanoic acid, octadecanoic acid, benzenamine, N,N-diethyl, α-pinene, spathulenol and kaur-16-ene, (8β,13β).

5. Structures of the isolated substances established by modern spectral analysis methods (IR, UV, ^{13}C-NMR, ^{1}H-NMR, 2D NMR: COSY - 45°, HMBC, HMQC; HRESI-MS, FAB, ECD, EI and FD mass spectrometry). From *Zygophyllum fabago* and *Camphorosma lessingii* 14 and 12 compounds respectively are reported for the first time.

6. Isolation and structure elucidation of two natural metabolites have not previously described in the literature: 3-O-β-D-xylopyranosyl-urs-12-ene-27, 28-dioic acid (3-O-β-D-xylopyranosyl-quinovic acid) and 28-Nor-quinovic acid-3-O-β-D-quinovopyranoside.

7. The biological screening (*in vitro*) of six extracts and one pure compound isolated from the studied halophytes. Revealed that ethanol, *n*-butanol and *n*-hexane extracts exhibit a sufficiently high cytotoxic, anti-diabetic, anti-microbial, anti-leishmanial and phytotoxic activity. The compound 3-O-β-D-xylopyranosyl quinovic acid showed pronounced cytotoxic activity against hepato and colon carcinoma cells.

Assessment of research tasks completeness: tasks fully implemented. Identified natural sources of biologically active substances from Kazakh halophytes species, studied their chemical composition, determined quantitative constituents of the major

groups of biologically active substances and established new structures not previously described in the literature of compounds.

Extracts and individual substances from the investigated species have phytotoxic, antidiabetic, anti-leishmanial and antitumor activity and could be used in the future for the development of new drugs in medicine and agriculture.

Recommendations for specific use of research results: the results could be used in research practice, as well as in medicine, agriculture and industry.

Assessment of technical and economic level of the work performed in comparison with the best field achievements:

- Develop optimal methods for the isolation and separation of new biologically active substances from Kazakh halophytic species of the genus *Camphorosma* (*C. lessingii*) and *Zygophyllum* (*Z. fabago*) families *Chenopodiaceae* and *Zygophyllaceae.*

- Complex of chemical, chromatographic and spectral methods (^{1}H-NMR, ^{13}C-NMR, 2D NMR - COSY- 45˚, HMQC, HMBC, and HRESI-MS, FAB, ECD, EI and FD mass spectrometry) established the structure of 30 individual compounds, of which two are new not previously described in the literature.

- Extracts and individual compounds isolated from the genus *Camphorosma* and *Zygophyllum* exhibited a significant activity as phytotoxic, antidiabetic, anti-leishmanial, antimicrobial and antitumor activity against specific types of cell lines. The results of investigation of halophytic species growing on the territory of Kazakhstan succeeded to recommend a new plant objects for use in medicine and agriculture.

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