AL-FARABI KAZAKH NATIONAL UNIVERSITY

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WORKING TOGETHER

AN EXAMPLE OF SUCCESSFUL SCIENTIFIC COLLABORATION BETWEEN PAKISTAN AND KAZAKHSTAN

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CONTENT

Chemical Research of Kazakhstan euhalophyte species *C. monspeliacum* of *Camphorosma genus* and *T. laxa, T. elongata* of *Tamarix genus* of *Chenopodiaceae, Tamaricaceae* families

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The objects of our investigation are the over-ground mass of *Camphorosma monspeliacum* (*Chenopodiaceae* family), *Tamarix laxa* and *Tamarix elongata* (*Tamaricaceae* family) plants. They were sampled in Almaty and Aral (dried bottom) areas in the blooming phase.

Isolation and Separation of Biologically Active Compounds

For extraction of bioactive substances from *C. monspeliacum, T. laxa* and *T. elongate,* comparative research of propyl, ethyl and methyl alcohols, acetone and their aqueous solutions was carried out. It was determined that the greatest BAS quantity is extracted by 70%-ethyl alcohol during 72 hours at room temperature. The obtained water-alcohol extracts were filtered then and concentrated in the vacuum of water jet pump until the full alcohol was removed. Prior the separation of bioactive substances, the fractional extraction of water-alcohol extracts of *C. monspeliacum, T. laxa* and *T. elongata* plants was carried out by means of chloroform and ethyl acetate. Chloroform, ethyl acetate and water solutions of investigated plant species were analyzed by means of GLC, PC and TLC methods. Chloroform extracts of *C. monspeliacum, T. laxa* and *T. elongata* plants contain chlorophylls, lipophilic substances, high saturated and unsaturated carboxylic acids (fatty acids) and terpenoids. However, the extracts of *C. monspeliacum* contain chromones. Ethyl acetate extracts *of C. monspeliacum, T. laxa* and *T. elongata* plants contain phenolic acids, flavonoids and their glycoside forms. Water solutions contain aminoacids and carbohydrates. Additionally, sulphate forms of flavonoids and hydrolysable tanning agents were identified in the water solutions of *T. laxa* and *T. elongata* plants. Hence, the 20 substances were shown in the water-alcohol

extracts of *C. monspeliacum* plants and 30 substances were obtained in the water-alcohol extracts of *T. laxa* and *T. elongata* plants.

Extraction of individual plants was carried out by means of adsorption distributing chromatography (polyamide, silica gel), gel chromatography (LH-20), preparative HPLC, PC and TLC. **10** individual compounds from *C. monspeliacum* plants: 3 terpenoids (substances **2.1, 2.4** and **2.5**), 3 chromones (substances **2.6, 2.7** and **2.8**), 3 flavonoids (substances **2.14, 2.20** and **2.22**) and 1 phenolic acid (substance **2.17**).

26 individual compounds were extracted from *T. laxa* and *T. elongata* plants: 3 terpenoids (substances **2.1 – 2.3**). 17 Flavonoids (substances **2.9-2.16, 2.19-2.25, 2.26-2.28**), 4 phenolic acids (substances **2.17, 2.18, 2.29, 2.30**) and 2 hydrolyzable tannins (substances **2.31, 2.32**) were also extracted.

Determination of Terpenoids Structure

Ain accordance to the results of TLC (the developer is sulfate of cerium) and positive reaction with the Liberman's reagent it is stated that the main components of chloroform extract are terpenoids: substances **2.4** and **2.5** from the plants of *C.monspeliacum*, substances **2.1-2.3** are from the plants of *T. laxa* and *T. elongata*. Five substances (**2.1 – 2.5**) were evolved from the chloroform extracts of *T. laxa, T. elongata* and *C. monspeliacum* by employing adsorption distributing chromatography (silica gel) and preparative TLC techniques.

Substance **2.1** was obtained from three investigated species of plants; substances **2.2** and **2.3** were obtained from the plants of *T. laxa* and *T. elongata* and substances **2.4** and **2.5** were obtained from the plants of *C. monspeliacum*.

On the basis of physical-chemical data and in comparison with the literature data substance **2.1** is β-sitosterol; substance **2.2** is methyl ether of the 3-β-al-D-fridoolean-14-en-28 carboxylic acid; substance **2.3** is 3-α-[3″,4″-Dihydroxy-trance-cinnamyl-oxy-D-fridoolean-14-en-28-carboxylic acid (izotamarixen); substance **2.4** is 3-O-β-D-glucopiranoside of the oleanolic acid; substance **2.5** is 28-O-β-D-glucopiranoside of the oleanolic acid.

Earlier β-sitosterol was identified by the others foreign and home scientists for the plants of *Chenopodiaceae* and *Tamaricaceae* families. It

should be noted that β-sitosterol was found in all the investigated plants. Substance **2.3** was earlier obtained from Kazakhstan species *Tamarix hispida*. Substances **2.4** and **2.5** were described in literature, but for the first time they were obtained from the plants of *Camphorosma* genus.

Substance **2.2** (0.34%) was related to pentacycle triterpenoids because of claret coloring by sulfate of cerium and Liberman-Burhard reaction. In IR spectrum there are absorption bands which are typical for methyl, methyne, methylene and ketonic groups in the range of 2864-2933 cm^{-1} and 1689 cm^{-1}, respectively. In the mass spectrum the peak of molecular ion (*m/z* 482) corresponding to $C_{32}H_{50}O_3$ molecular formula was registered by means of EI-MS and FAB-MS (-ve) methods. Fragments with m/z 248, 204, 189 and 133 correspond to retro-dien disintegration on Diels-Alder, which is typical for pentacycle triterpenoids (Fig. 3).

Also, 32 signals of the carbon atoms were observed in a spectrum of NMR ^{13}C. By means of DEPT in a normal phase seven methyl (C-23-27, C-29-30), four methyne (C-5, C-9, C-15,C-18), and in a return phase ten methylene carbon atoms (C-1, C-2, C-6, C-7, C-11, C-12, C-16, C-19, C-21, C-22) were registered. Proton signals of seven methyl groups in a spectrum NMR ^{1}H were registered at δ 0.82-0.98 as 3H singlets, and twenty protons of methylene groups were registered as multiple signals at δ 1.08-1.98.

Besides, in a spectrum NMR ^{1}H at δ 5.54 olefinic proton resounds at double bond H-15 (1H, dd, J_1 11.0 and J_2 =3.4 Hz,), indicating that substance **2.2** refers to taraxeran-14-en type or to D-fridoolean derivatives, and at δ 3.30 to the β-form.

In ^{13}C NMR spectrum, ^{13}C signals of carbon atoms with double bond (C-14 and C-15) resound at δ 161.0 and δ 117.0; signals C-28 and C-3 resound at δ 179.0 and δ 76.0, respectively. Singlet at δ 3.80 in a spectrum of NMR of ^{1}H and the area δ 56.0 in a spectrum of NMR ^{13}C is typical for the methyl ether of carbonyl group at C-28, but in a spectrum NMR ^{13}C of the substances **2.2** an additional signal of aldehydic carbon atom was found out at δ 238.0 The presence of carbonyl groups of aldehydic and methyl ether was also proved by means of mass-spectrometry, fragment with m/z 438 (Fig. 1).

COSY-45° and HMBC spectroscopy were used to prove the double bond position of $COOCH_3$, CHO and CH_3 groups. Carbon atom C-15 in

the HMBC spectrum interacts with the protons H-16, H-18, and C-14 with the protons at C-26, C-18. The COSY-45° spectrum also confirms the interaction of H-15 and H-16 protons. Hence, according to 2 D spectra the double bond is located between C-14 and C-15 atoms.

Position of $COOCH_3$ group was proved by the HMBC spectrum: signals of protons H-18, H-16 and H-22 interact with carbon atom at C-28 and C-17 positions. Carbon atom of aldehydic group interacts with a methyl proton at C-23 (δ 0.98), that confirms addition of the aldehydic groups at C-3 position. Besides, in the NMR ^1H spectrum the doublet of doublets signal with $J_1 = 9.0$, $J_2 = 4.7$ Hz, indicates the fact that the methyn proton at C-3 is in an axial, and aldehydic group is in an equatorial position. The methyl groups' positions were completely proved to be true by the HMBC spectrum. On the basis of physical and chemical analysis methods the structure for substance **2.2** was stated as methyl ether of the 3-β-al-D-fridoolean-14-en-28 carboxylic acid. **The substance 2.2 is a new chemical compound not described in the literature earlier.**

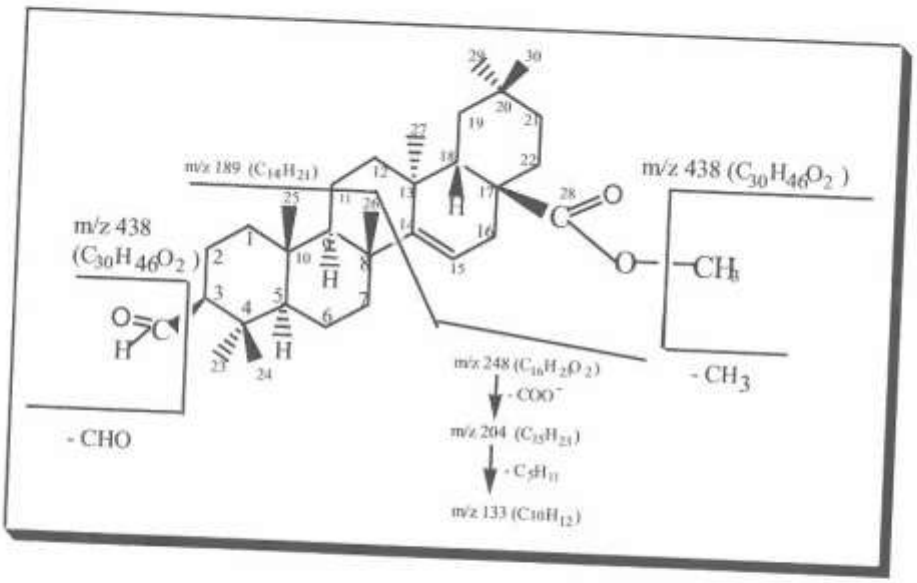

Figure 1 – Fragmentation scheme of methyl ether 3-β-al-D-fridoolean-14-en-28 carboxylic acid (**2.2**)

Chemical study of the genus *Camphorosma* plants *(C. monspeliacum)* and the genus *Tamarix* plants (*T. laxa, T. elongata*) indicates the prescence of pentacylic tri terpernoid. Such conclusion was made on the basis of study of the individual substances, by means of chroma-

tographic and spectral analysis methods. Thus, olean triterpenoids are typical for the plants of *Camphorosma* genus (*C. monspeliacum*) and fridoolean triterpenoids are typical and for the plants of *Tamarix* genus (*T. laxa* and *T. elongata*).

Determination of Structure Chromones

This class of natural chemical compounds was evolved from the chloroform extract of *C. monspeliacum* on silica gel and elucated, eluating with hexane and hexane-acetone mixture. Substances **2.6** (0.44%) and **2.7** (0.02%) gave bluish-dark blue fluorescence in UV-light and yellow coloring in ammonia vapor and with sulfate of cerium. In the UV spectrum of substances **2.6** and **2.7** in area λ_{max} = 207-283 and 306-368 nm two absorption bands were observed and the absorption band of carbonyl group in the range of 1654 cm^{-1} (**2.6**) and 1641 cm^{-1} (**2.7**) in the IR spectra, are typical for chromones, that is accounted for low negative inductive influence of the γ-pyrone cycle hetero atom. For substance **2.7** in the range of 3456 cm^{-1} the absorption band of hydroxyl group was additionally developed.

Molecular masses of substances **2.6** and **2.7** (by means of HREIMS method) are 268 (for substance **2.6**) and 314 (for substance **2.7**), that correspond to formulae $C_{16}H_{12}O_4$ (for substance **2.6**) and $C_{17}H_{14}O_6$ (for substance **2.7**). Besides, in the NMR ^{13}C spectrum (methods BB, DEPT) sixteen carbon atoms were registered for substance **2.6** and seventeen carbon atoms were registered for substance **2.7**.

Substances **2.6** and **2.7** are primarily related to the derivatives of 2-phenoxychromone. In mass-spectrum the formation of the chromone nucleus with m/z 148 was ac companied with the formation of the phenoxyl with m/z 92. The presence of the characteristic fragment with m/z 120 corresponds to retrodiene disintegration mechanism of γ-pyronic cycle of chromone nucleus. In ^{13}C NMR spectrum the the C-2 and C-1′ atom signals within the limits at δ 166.0-169.0 and δ 144.0-148.0, accordingly were typical for 2- phenoxychromones′ system. In the spectrum NMR ^{13}C of substances 2.6 and 2.7the C-2 signals resound at δ 166.5 (**2.6**) and δ 166.6 (**2.7**); and the C-1′ signals resound at δ 148.0 (**2.6**) and δ 147.5 (**2.7**), that corresponds to the literature data.

In the spectrum of NMR ^{1}H, substances **2.6** and **2.7** the proton signals at C-3 are at δ 6.84 (substance **2.6**) and δ 6.71 (substance **2.7**)

intervals as one-proton singlet, and carbon atoms at C-3 in the spectrum of NMR ^{13}C are shown at δ 116.2 (substance **2.6**) and δ 108.9 (substance **2.7**), respectively. Two multiple signals in spectrum NMR ^{13}C of substance **2.6** at δ 8.03 (2H, H-2 ′, H-6 ′) and δ 8.04-8.06 (3H, H-3 ′, H-4 ′, H-5 ′) specify on the unreplaced phenoxyl ring. For substance **2.7** signals of the phenoxyl protons were shown at δ 7.56 (2H, d, J =7.9 Hz, H-3 ′, H-5 ′) and δ 7.97 (2H, d, J =8.0 Hz, H-2 ′, H-6 ′), that confirms 4 ′-replacement.

In the spectrum of NMR ^{1}H, of substance **2.6** the proton of the chromone benzol ring at C-5 was exposed to disscreening influence of carbonyl group at C-4 and as distinct from the other protons exposed in weaker area at δ 7.58 (1H, d, J = 7.0 Hz). The signals H-6 (1H, dd, J_1 which were 8.8, J_2 = 2.2 Hz) and H-8 (1H, d, J 2.1 Hz) were shown at δ 7.09 and δ 7.23.

In substance **2.7** the benzene ring protons of chromone nucleus were shown at δ 6.54 and δ 6.81 areas as doublets with intensity in 1H with 1.9 and 2.0 Hz belonging to meta-interacting protons H-6, H-8; and H-6 signal resounds in a stronger field than H-8.

The location of chromone benzene ring signals in spectrum NMR ^{13}C of substance **2.7**, also, corresponds to 5,7- dire placement. The C-6 signals were found at δ 97.5, and the C-8 signals were found at δ 94.3.

Besides in spectra NMR ^{1}H and in NMR ^{13}C of substances **2.6** and **2.7**, signals belonging to $-OCH_3$ group were registered. In mass-spectrum (methods EI / MS, FAB-MS (-ve) the presence of one $-CH_3$ group in substance **2.6** and two $-CH_3$ groups in substance **2.7** were confirmed by observed characteristic fragments with m/z 252 (**2.6**) and 285 (**2.7**). The presence of one -OH group in substance **2.7** was proved to be true by formation of the fragment with m/z 251.

Determination of a fine structure of substances was proved to be true by means of 2D correlation HMBC spectra and hence proton at C-3 of pyrone cycle interacts with carbon atoms C-2, C-3 and C-4, respectively. The $-OCH_3$ groups' position was also proved to be true by means of HMBC spectrum. Three – proton singlet of the proton of $-OCH_3$ group of substance **2.6** interacts with the carbon atom in the C-7 position; and in substance **2.7** the interaction of two $-OCH_3$ groups' atoms with C-5 and C-7 carbon atoms was marked. On the basis of spectral and chemical analysis methods, the structure of the substances

2.25 was confirmed by the acid hydrolysis data and by the presence of optical rotation.

On the basis of physical and chemical characteristics, spectral analysis methods and according to the literature data, substance **2.12** was identified as 5,7,4 '-Trihydroxy-3'-methoxyflavon (chrysoeriol); substance 13 was identified as 7,3',4'- Trihydroxy-5-methoxyflavon.

For substances **2.9, 2.10, 2.11, 2.14, 2.15** and **2.16**, the following structures were identified: 3,7,3',4'-Tetrahydroxy-5-methoxyflavon (**2.9**); 3,5,7-trihydroxy-3',4'-dimethoxyflavon (**2.10**); 3,5,4'- trihydroxy-7,3'-dimethoxyflavon (ramnazine) (**2.11**); 3,5,7,3',4'-Pentahydroxyflavon (quercetin) (**2.16**); 3,5,7,4'-Tetrahydroxy-3'-methoxyflavon (isorhamnetin) (**2.14**); 3,5,7,3'-tetrahydroxy-4'-methoxyflavon (tamarixetine) (**2.15**).

Substance **2.19** was characterized as 3-O-β-D-glucopyranoside of kaempferide; **2.25** – 3-O-β-D-glucopyranoside of quercetin; **2.22** – 3-O-β-D-glucopyranoside of isorhamnetine; **2.20** – 3-O-β-D-galactopyranoside of isorhamnetine; **2.23** – 3-O-β-D-glucopyranoside of tamarixetine; **2.21** – 3-O-α-L-rhamnopyranoside of tamarixetine; **2.24** – 3-O-ά-L-arabopyranoside of tamarixetin.

Substances **2.14** and **2.22** were obtained from all the investigated species of plants (*C. monspeliacum, T. laxa* and *T. elongata*). For the plants of *Camphorosma* genus the substances **2.14** and **2.22** were new and for the plants of *Tamarix* genus substance **2.22** was also obtained for the first time. Substance **2.21** was new for the plants of *Camphorosma* genus, and substances **2.12, 2.13, 2.9-2.11, 2.19, 2.23** and **2.24** were new for the plants of *Tamarix* genus.

The chemical composition of the investigated species of euhalophyte plants of *Camphorosma* genus (*C. monspeliacum)* and *Tamarix* genus (*T. laxa, T. elongata)* showed, that flavonoids basically were presented in the form of methoxylazed flavonol (**2.9, 2.10, 2.11, 2.14** and **2.15**). The glycosidized forms were also found (**2.19-2.24**). The glycosidation in flavonols is carried out on OH-group at the C-3 aglycon position. The basic flavonol sugar component is glucose (**2.19, 2.22** and **2.23**). However, there were flavonols carbohydrate, part of which was presented by rhamnose (**2.21**), arabinose (**2.24**) and galactose (**2.20**). It should be noted, that flavonols (substances **2.12** and **2.13**) were obtained from the ethyl-acetate extract of the *T. laxa* and *T. elongata* plants, as distinct from the *C. monspeliacum* plants.

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Determination of the obtained Structure of Hydrolysable Tannins

The water extracts of the *T. laxa* and *T. elongata* plants containing several substances which are primarily related to hydrolyzable tannins were determined by means of the two-dimensional PC method with the specific developers' application. From the water extract two individual substances **2.31** and **2.32** were obtained by means of adsorption-distributive and gel-chromatography methods. On the basis of chemical (acid hydrolysis) and physical and chemical analysis methods in comparison with the literature data substance **2.31** had 2-galloyl-D-glucopyranose and compound **2.32** had 3-O-(1-dehydrodigalloyl)-4,6-hexahydroxydiphenoylglucopyranose structure. Compound **2.32** was obtained as a result of acid hydrolysis from *T. aphylla* and from Kazakhstan species *T. hispida.*

Thus, such individual substances as vanillic acid, isorhamnetin, 3-O-β-D-glucopyranoside of isorhamnetine, β-sitosterol and glucose were obtained from all the investigated euhalophyte plants of *Camphorosma* genus *(C. monspeliacum)* and *Tamarix* genus (*T. laxa, T. elongata*).

The genus *Camphorosma (C. monspeliacum)* are characterized with the triterpenoids of the oleanoic type and 2-phenoxychromomes; plants of *Tamarix* genus (*T. laxa* and *T. elongata)* are characterized with triterpenoids of the fridooleanoic type, tanning agents and sulphate form of flavonoids.

Methyl ether of 3-β-al-D-fridoolean-14-en-28 carboxylic acid obtined from *T. laxa* and *T .elongata* (substance **2.2**) and *C. monspeliacum* – 7-methoxy-2-phenoxychromone (**2.6**), 5,7-dimethoxy-2-(4′-hydroxyphenoxy) chromone (**2.7**) are new natural compounds.

Biological Activities of *Camphorosma* genus Plants *(C. monspeliacum)* and *Tamarix* genus plants (*T .laxa, T. elongata)*

For studying the biological activities of Kazakhstan euhalophyte species *C. monspeliacum* of *Chenopodiaceae* family and *T. laxa* and *T. elongata* of *Tamaricaceae* family the water-alcohol, chloroform, ethyl acetate, water extracts, flavonoid fractions and individual substances **2.3**, **2.6**, **2.7**, **2.11**, **2.25** and **2.26** were tested by means of standard informative tests (*in vitro*) in specialized laboratories of the H.E.J Research Institute of Chemistry and in the specialized laboratories of Dr. Panjwani Center of Molecular Medicine and Drug Research, Karachi University (Pakistan).

Water-alcohol, chloroform, ethyl acetate, water extracts from *C. monspeliacum, T. laxa* and *T elongata* possess antibacterial, antifungal and growth-regulating activities. The extracts from *T. laxa* and *T. elongata* have a high antioxidant activity. The extracts from *T. elongata* have a high antiamnezia activity.

Individual substances **2.3**, **2.11** and **2.26** also have potential antioxidant and antidiabetic – activities.

For the first time toxicity of plant extracts of *Camphorosma* genus *(C. monspeliacum)* and *Tamarix* genus (*T. laxa* and *T. elongata)* was investigated against larvae of *Artemia salina* shrimp (Brine Shrimp activity) and their immunomodulatory activity was also performed. At the concentration of 0.4 mg / mL, the chloroform extract of *C. monspeliacum* (CM-4), containing substances **2.6** (KM – 1) and **2.7** (KM – 2) as basic components shows a high immunomodulatory activity. Water-alcohol extracted TE-1 of *T. elongata* containing the substance **2.25** (GE-19) also showed a high immunomodulatory activity. Individual substances **2.6** (KM – 1) and **2.7** (KM – 2) at the 0.4 mg / mL concentration also possess immunomodulatory activity, and the substance **2.25** (GE-19) in similar concentration (0.4 mg / mL) enhances its action.

For the first time immunomodulatory and *Brine Shrimp* activities (investigation of toxicity of vegetative extracts and biologically active substances by means of larvae of *Artemia salina*) were established for these euhalophyte species such as *C. monspeliacum, T. laxa* and *T. elongata*. Besides, immunomodulatory activity for class of chromones (compounds **2.6** and **2.7**) was also investigated for the first time.

Conclusion:

The following conclusions were made from results of research:

[1]. New sources of bioactive substances of plants of *Chenopodiaceae* family of *Camphorosma* genus (*C. monspeliacum)* and *Tamaricaceae* family of *Tamarix* genus (*T. laxa and T. elongata*) were revealed: chromones and triterpenoids of olean series were revealed in *C. monspeliacum;* triterpenoids of friloolean series, hydrolysable tannins and sulphate form of flavonoids were revealed in *T. laxa* and *T. elongata;* 12 fatty acids and 18 amino acids were shown by GLC; the mineral composition of ash residues were also determined.

[2]. The scheme of separation of BAS was developed: 10 individual substances were deduced from *C. monspeliacum*, they are 3 terpenoids, 3 chromones, 3 flavonoids, 1 phenolic acid; 26 individual substances were deduced from *T. laxa* and *T. elongata*, they were 3 terpenoids, 17 flavonoids, 4 phenolic acids and 2 hydrolyzable tannins.

[3]. For the first time three new compounds were isolated from *T. laxa* and *T. elongata* methyl ether of 3β-al-D-fridoolean-14-en-28-carbonic acid; from *C. monspeliacum* 2-phenoxychromones – 7-methoxy-2-phenoxychromones and 5,7-dimethoxy-2-(4'-hydroxyphenoxy) chromone. 6 substances were new for the plants of *Camphorosma* genus and 11 substances were new for the *Tamarix* genus. Substances' structure was determined by means of chemical and spectral analysis methods (IR, UV, NMR ^{13}C -, NMR ^{1}H-, 2D – COSY 45°, HNQC, HMBC, mass spectrometry).

[4]. 15 extracts and 6 individual substances were produced. Antibacterial, antifungal, antioxidant, antiamnezia, antidiabetic and growth-regulating activities were shown. For the first time immunomodulatory and toxilogical activities (Brine Shrimp activity) were studied for the plants of *Camphorosma* genus (*C. monspeliacum)* and *Tamarix* genus (*T. laxa and T. elongata*). Immunomodulatory activity of chromones was also studied.