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TOP EDIBLE WILD PLANTS OF EASTERN MEDITERRANEAN REGION. PART V: ANTIOXIDANT ACTIVITY

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ABSTRACT

This is the fifth review in a series of articles that present the major medicinal activities and the phytochemistry of the most important wild edible plants of eastern Mediterranean region, which we named as the "Deca-plants" (D-P). The previous articles discussed the anticancer, anti-inflammatory, antidiabetic and antimicrobial and/or antiviral activities. The antioxidant activities of the D-P vary from moderate to very strong, and due to their nutritional importance, this medicinal, biological property, give them special health relevance and importance. With antioxidant property, eating these plants help their consumers avoid and ease oxidative stress that contributes and is involved in almost all known diseases. Since antioxidant activity is one of the most tested activities of plant materials, the literature reports about it are numerous and the number of methods and tests is very large, so we had to limit our review to the major publications. The major phytochemicals responsible for the antioxidant activities of the D-P will be indicated. Finally, for comparison and usefulness, in the last part of this article, five Non-Deca-Plants with notable, reported antioxidant activity will be shortly reviewed, when the criteria of selection are wild and edible.

KEYWORDS: ROS, oxidative stress, antioxidant, DPPH, ascorbic acid, SOD, FRAP, plant extracts, essential oils, BHT.

1. INTRODUCTION

Oxidation is a fundamental process in nature and it is the very major source of energy, resulting from metabolism of nutrition in living organisms, or fuel combustion outside of them. For examples, the metabolism of glucose $C_6H_{12}O_6$ and the combustion of natural gas used as fuel (methane, CH_4) are shown in the following reactions

$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ + Energy (ATP)

$CH_4 + 2\ O_2 \rightarrow CO_2 + 2\ H_2O$ + Energy (heat)

But undesired oxidation naturally occurs in living organisms and outside of then, and the most important example in the second group is the formation of rust, iron oxide, but also other metal oxide. It is estimated that the global formation of rust costs 3-4% of the gross domestic product (GDP, 2021), which translates to around 6 trillion USD.[1]

From human health perspective, the major cause of undesired oxidation within living organism are ROS, reactive oxygen species, that react molecules and living tissues, resulting health damages. Several factors are responsible for the formation of ROS, and the most important of them are shown in Figure 1.[2,3]

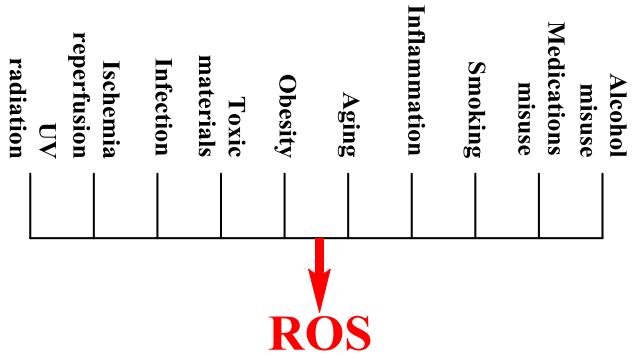


Figure 1: Major generators of ROS.[2,3]

ROS can be either radical, $O_2^{-\cdot}$, $HO_2^{-\cdot}$, $HO^{\cdot}$, $NO^{\cdot}$, $RO^{\cdot}$, $ROO^{\cdot}$, $RNO^{\cdot}$; or molecular, H_2O_2, $^1O_2$ (singlet) and HOC*l*.[4] The formation mechanisms of these ROS are very diverse, and it is out of the scope of this article to present all of them, but two selected reactions are shown below

$Fe^{+2} + H_2O_2 \rightarrow HO^{\cdot} + OH^- + Fe^{+3}$

$2\ O_2 + NADPH \rightarrow 2\ O_2^{-\cdot} + NADP^+ + H^+$

(NADPH, nicotinamide adenine dinucleotide phosphate).

The occurrence of undesired oxidation leads to oxidative stress, which is defined as imbalance between the formation of oxidative species and the ability of antioxidants to detoxify them.[5] Oxidative stress is one of the major causes of many health disorders as shown in Figure 2.[6,7]

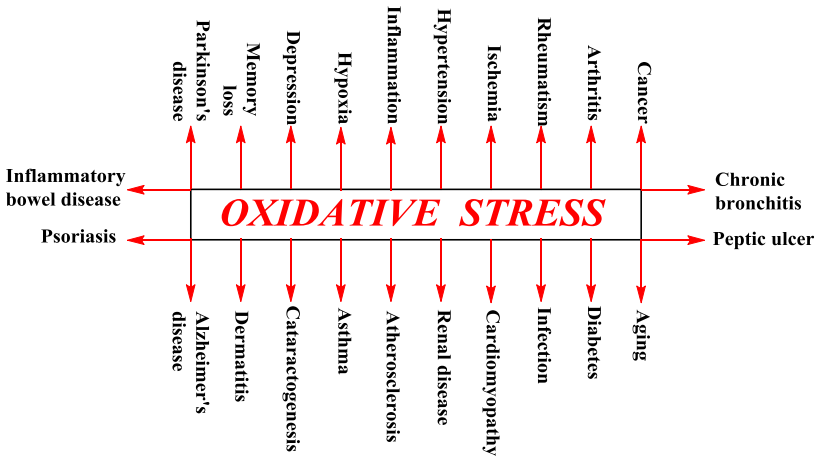


Figure 2: Damages Resulting from Oxidative Stress.[6,7]

The great majority of scientific literature that discuss oxidative stress relate only to ROS, reactive oxygen species, and to less extent, to RNS or reactive nitrogen species. But in recent decades, there is a growing awareness to the role of reactive sulfur species, RSS.[8] These include species such as H_2S_n , R_2S_n (n>1) and sulfur oxides.

Living organisms including our major interest, the human body, have natural antioxidant mechanisms that are supposed to detoxify oxidative stress. A notable example of these mechanisms can the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase.[9] But these mechanisms are not enough to balance oxidative reactive species generated inside the human body, due to the mentioned above reasons. So, human body needs dietary supplementation of antioxidants. The scientific literature about this topic is almost endless, with great numbers of research and review articles. Among the later, we chose to cite three publications, which to our humble understanding, summarize and represent most of the literature about this topic. Y-J. Zhang and colleagues reviewed antioxidants with focus on their activity in prevention and alleviating chronic diseases, but also present some other activities.[10] They presented the phytochemicals and the related diseases that they can treat, the bioavailability, and a very interesting section of the “adverse effects” of these natural products. Carefully reading of this section reveals that these “adverse effects” are in fact lack of antitumor activity, but not real damage that these compounds caused.

The review article of R. Guan and colleagues has close content to that of Y-J. Zhang and colleagues but has an additional value of presenting the structures and the sources of the natural antioxidants.[11] The article of E.D. Abeyrathne and colleagues has a clear advantage:

it presents plant and animal-based antioxidants.[12] Despite the great importance of the second group, it is often missing in such review articles. Almost all these antioxidants are peptides, and the structures of two of them (carnosine, anserine, Figure 3), that are found in meat.

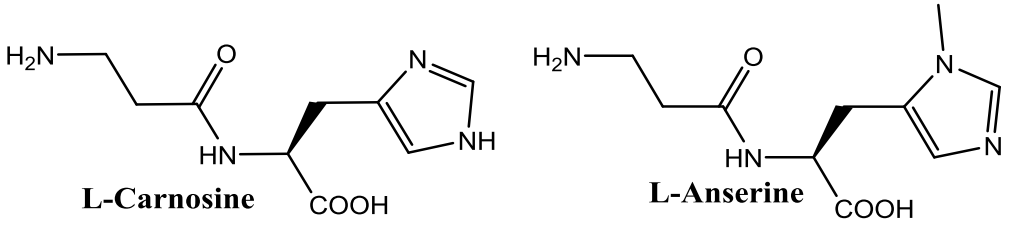


Figure 3: Antioxidant Peptides from Meat.[6,7]

Finally, the D-P are *Arum palaestinum* (Araceae), *Cichorium pumilum* (Syn. *Cichorium endivia*, Asteraceae), *Cyclamen persicum* (Primulaceae), *Foeniculum vulgare* (Apiaceae), *Gundelia tournefortii* (Asteraceae), *Majorana syriaca* (Syn. *Origanum syriacum*, Lamiaceae), *Malva sylvestris* (Malvaceae), *Micromeria fruticosa* (Lamiaceae), *Salvia fruticosa* (Syn. *S. triloba*, *S. libanotica*, *S. cypria*, *S. lobryana*, Lamiaceae), *Sinapis alba* (Brassicaceae).

2. Ethnomedicinal Antioxidant Activities of the Deca-Plants

Traditional healer and ethnomedicine literature do not mention terms such as oxidative stress and antioxidants. They were not aware of the connections between oxidative stress and health disorders, which they extensively knew and treated. But the term of “oxidation” was not known to them.

Although, they treated health disorders that emerge directly from oxidative stress, especially skin diseases, and vitiligo in particular. For example, traditional healer in Turkey used different parts of *Gundelia tournefortii* decoction to treat vitiligo.[13] But it is very accurate to say that antioxidant activity was not part of ethnomedicines.

3. Antioxidant Activities of the Deca-Plants and Their Natural Products

Antioxidant activities of all the D-P were reported, with clear differences between the ten species. Summary of published research findings is presented in Table 1.

Table 1: Published Antioxidant Activities of the D-P in Eastern Mediterranean region.

Testing Method, Results and Reference/s
Arum palaestinum
Leaves were successively extracted with water and ethyl acetate and the extract was tested with DPPH (1,1-diphenyl-2-picrylhydrazyl) method, showing strong activity.[14]
Leaves were separately extracted with water and 80% aqueous methanol. Extracts were tested with DPPH and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) methods resulting moderate activities.[15]
Leaves aqueous and ethanolic extracts were tested by DPPH method showing moderate and weak activities, respectively.[16]
Leaves ethanolic extract showed strong activities in DPPH and β-carotene bleaching methods.[17]
Leaves from two locations 96% aqueous ethanolic extracts had moderate and weak activities (DPPH, clear difference).[18]
Leaves methanolic extract had moderate activity in DPPH method.[19]
Leaves methanolic extract had high activity in in DPPH with Trolox as a reference.[20]
Four aqueous extracts were prepared from fresh, boiled, shade dried and oven dried leaves. Extracts were tested in DPPH methods with Trolox a reference. Oven dried had the strongest activity (IC_{50}).[21]
Leaves 70% aqueous ethanolic extract had moderate activity in DPPH test.[22]
Cichorium pumilum
Leaves 70% aqueous ethanolic extract had moderate activity in DPPH test.[22]
Aqueous and 70% aqueous methanolic extract were prepared (plant part/s not indicated) and tested with ABTS method with Trolox as a reference, showing good and moderate activities, respectively.[23]
Aqueous and 80% aqueous methanolic extract were prepared (plant part/s not indicated) and tested with ABTS method with Trolox as a reference, showing high and good activities, respectively.[24]
Seeds water extract had moderate activity based on total phenolic content (TPC) and four strong antioxidant compounds, compared with 19 other plants used in this study.[25]
Leaves 50% aqueous ethanolic extract had high activity compared with ascorbic acid (DPPH), and *in vivo* in rat model against acetaminophen liver toxicity (SOD concentrations).[26]
Fresh aerial parts were tested with saturated oxygen, compared with β-carotene, showing high activity.[27]
Leaves aqueous and ethanolic extracts showed high activity in ABTS and FRAP (ferric reducing antioxidant power) methods, as well as inhibition of hydrogen peroxide in an *in vitro* model of antioxidant enzymes.[28]
Leaves were extracted with 70% aqueous methanol, and extract was fractionized and analyzed with several solvents, yielding ten compounds (none of them new, **Figure 4**). Compounds 3, 7 and 8 showed significant activity in DPPH method.[29]
Leaves ethanolic and methanolic extracts had high activity, and higher than two other subspecies of *Cichorium*.[30]
Seed were separately extracted with 70% aqueous ethanol and chloroform, and tested with DPPH method, showing excellent and good activities, respectively.[31]
Whole plant 70% aqueous ethanol extract showed weak activity in DPPH and FRAP methods, but high activity in TAC method (total antioxidant capacity, phosphomolybdate complex reduction), with ascorbic acid as a reference in the three tests.[32]
Leaves 80% aqueous ethanolic extracts were prepared from three cultivars and tested with

DPPH, ABTS and hydrogen peroxide reducing methods, showing significant activities in all three tests. Detailed chemical composition is presented.[33]

Cyclamen persicum

Aqueous and 80% aqueous methanolic extract were prepared (plant part/s not indicated) and tested with ABTS method with Trolox as a reference, showing excellent activities.[24]

Tubers 70% aqueous methanol extract was analyzed affording saxifragifolin B and cyclamen, which had weak activity compared with ascorbic acid and catechin (**Figure 5**). The isolated compounds had also weak ferrous (Fe^{+2}) ion chelating activity.[34]

Leaves 80% aqueous methanolic extract had high activity in three tests, DPPH, FRAP and TEAC (Trolox equivalent antioxidant capacity). Trolox was standard in all tests.[35]

Leaves methanolic extract had moderated activity in DPPH test, with Trolox as a reference. Slight difference was indicated between wild and cultivated plants.[36]

Foeniculum vulgare

Leaves 70% aqueous ethanolic extract had moderate activity in DPPH test.[22]

Fresh aerial parts essential oil (EO) was obtained by hydrodistillation had moderate activity in TBRAS (thiobarbituric acid reactive substances) method, with butylated hydroxytoluene (BHT) and α-tocopherol as references (**Figure 6**).[37]

Seeds aqueous and ethanolic extracts had very strong activities in six methods, where ethanolic extract was more active. BHT, α-tocopherol and butylated hydroxyanisole (BHA) were references.[38]

EO from leaves and stems was produced by hydrodistillation and the remaining material was extracted with water. Both materials were analyzed and the isolated compounds were tested with three methods, with four reference compounds. Three of the isolated compounds (3-, 4- and 5-caffeoylquinic acids, **Figure 7**, caffeic acid reference) were most active.[39]

Seeds 70% ethanolic extract was separately prepared from wild and cultivated plants, and they were tested with three methods: DPPH, lipid peroxidation and β-carotene bleaching test. Both extracts had moderate activity, where extract of wild plants was way more active, especially in DPPH method (ascorbic acid was reference).[40]

Same research group and very similar work to ref. 39, from different locations.[41]

Seeds EO (hydrodistillation) and four extracts (MeOH, 80% MeOH/H_2O, EtOH, 80% EtOH/H_2O) were prepared and tested with two methods: DPPH and inhibition in linoleic acid system. Extracts were more potent than EO, and most active was 80% EtOH/Water. BHT was reference.[42]

Seeds EOs (hydrodistillation) were prepared from plants in three maturity stages and analyzed for chemical compositions. EOs and major component, *E*-anethole were tested with two methods: DPPH and inhibition in linoleic acid system. Mature seeds were most potent, but activities were almost the same. BHT was reference.[43]

EOs were separately obtained from aerial parts and seeds, using four different time of production (8 EOs). These were tested with three methods: TBARS (α-tocopherol as a reference), hydroxyl radical scavenging (mannitol) and 5-lipoxygenase inhibition (nordihydroguaiaretic acid). In all tests activity was weak to moderate.[44]

Seeds EO (steam distillation) and 50% aqueous methanolic extract were prepared and tested with DPPH and their effect on stability of sunflower oil, showing moderate activity.[45]

Seeds aqueous and 90% aqueous methanolic extracts were tested with DPPH and hydroxyl radical scavenging methods, showing high activity. Aqueous-methanolic extract was more active, and ascorbic acid was reference.[46]

Seeds EO (hydrodistillation) and four extracts (methanol, ethanol, diethyl ether and hexane) were prepared and tested with DPPH method. All materials showed moderate to high activity (alcoholic extracts) compared with four reference compounds.[47]

Seeds 96% aqueous ethanolic extract had stronger effect of oxidation prevention of olive oil

than BHT and BHA.[48]
Seeds 80% aqueous ethanolic extract was tested with four methods (DPPH, copper induced egg lecithin peroxidation, inhibition of copper induced protein oxidative modification, activity against oxidative damage to DNA). Results were moderate compared with BHA and BHT.[49]
Leaves were separately extracted with six solvents and tested with several methods, including DPPH, where quercetin was standard. Results were weak to moderate: ethanol > methanol > *n*-butanol > *n*-hexane > acetone > chloroform.[50]
A follow-up study of ref. 48 with soybean oil, instead of olive oil. Similar results.[51]
Aerial parts EO (hydrodistillation) and aqueous, ethyl acetate and diethyl ether extract were tested with DPPH method, compared with ascorbic acid. Activity was weak to moderate according to this order: ethyl acetate > ascorbic acid > EO > diethyl ether.[52]
Seeds 70% aqueous ethanolic extract was supplemented to female rats, resulted in increase of SOD and GPX concentrations in the animals blood.[53]
Aqueous extract of commercial samples (plant part/s not indicated) was tested with four methods, showing strong activity, with Trolox as a reference. The extract was added to cottage cheese which protected it against oxidation and microbial activity.[54]
Seeds EO (hydrodistillation) was effective in preventing steel corrosion in hydrochloric acid medium.[55]
Seeds ethanolic extract had weak activity in DPPH method compared with BHT.[56]
Seeds were extracted with chloroform, methanol, 80% aqueous methanol and water. These extracts were tested with DPPH, FRAP and phosphomolybdenum methods. In all tests, extracts had weak to moderate activities compared with BHT and ascorbic acid, especially in low concentrations. Activity order was chloroform < methanol < aqueous-methanol < water.[57]
Methanolic and 96% aqueous ethanolic extracts (plant part/s not indicated) were tested with DPPH, along with ground plant material (not extracted). Activity order was methanolic > aqueous-ethanolic > raw material. No reference is indicated.[58]
Seeds from China and Egypt were hydrodistilled to prepare EOs and separately extracted with 70% aqueous ethanol. EOs and extracts were tested with DPPH method showing significant differences (see section 4, **Discussion**). EOs were analyzed for chemical compositions and notable differences were indicated (see **Figure 8** and section 4).[59]
Seeds from three locations in Morocco and two locations in France were hydrodistilled, and their activities were tested with DPPH, FRAP and β-carotene bleaching methods. Moroccan EOs were active. Chemical compositions were also presented.[60]
Seeds 80% aqueous ethanolic extract was supplemented to pregnant female mice, resulting healthier offspring, indicated by several biomarkers, especially antioxidant activity (FRAP) of blood serum, compared with control.[61]
Seeds 50% aqueous methanolic extract had significant activity in Fenton reaction (Fe^{+2}/H_2O_2 system).[62]
Seeds ethanolic and acetone extracts had strong activities in DPPH test, where acetone extract had higher activity.[63]
Seeds EO had low activity in DPPH, ABTS and β-carotene bleaching methods.[64]
EOs of seeds from Germany, Iran and Turkey had significant activities in DPPH and ABTS methods, with BHT as a reference.[65]
Commercial EO had weak activity in DPPH, CUPRAC (cupric reducing antioxidant capacity) and ABTS methods, compared with activities of three other plats.[66]
Seeds were germinated for 9 days, then raw seeds and sprouts were separately extracted with 70% aqueous ethanol. Both extracts had significant activity in DPPH method, and *in vivo* after supplementation to rats with CCl_4-induced hepatotoxicity, where positive effects were

detected by several biomarkers, such as SOD concentrations.[67]
Seeds aqueous extract had weak activities in DPPH and F^{+2} chelating methods, with gallic acid and EDTA (ethylenediamine tetra acetic acid), respectively, as references.[68]
Seeds EO had significant activities in DPPH, TBARS and F^{+2} chelating methods, with ascorbic acid and BHT, as references.[69]
Seeds methanolic extract had high activity in DPPH method in 50-200 ppm.[70]
Liquid that resulted from seeds fermentation (14 days) in brine solution showed high activity in ABTS and DPPH methods.[71]
Commercial EO had moderate activities in DPPH compared with five other EOs.[72]
Female rats with bisphenol A toxicity showed higher antioxidant activity (FRAP) of blood serum after supplementation of 70% aqueous ethanol seeds extract.[73]

Gundelia tournefortii

Leaves and stems methanolic extract had moderate activity in DPPH method.[19]
Aqueous and 70% aqueous methanolic extract were prepared (plant part/s not indicated) and tested with ABTS method with Trolox as a reference, showing moderate activities.[23]
Aqueous and 80% aqueous methanolic extract were prepared (plant part/s not indicated) and tested with ABTS method with Trolox as a reference, showing moderate activities.[24]
Aerial parts and seeds were separately extracted with methanol and both extracts were tested with DPPH and TBRAS methods, showing weak to moderate activity in the first and strong in the second. Quercetin and α-tocopherol used as references.[74]
Aerial parts 80% aqueous ethanol extracts were prepared from plants harvested in four different locations in three growth stages (total of 12 extracts), and tested with DPPH method. No significant differences were observed between locations, but the highest activities were recorded for extracts of plants in flowering stage.[75]
Aerial parts were separately hydrodistilled for EO and extracted successively with chloroform, ethyl acetate, methanol and water. Extracts were tested with DPPH method showing weak to moderate activity, where methanolic extract was most active and BHT was reference.[76]
Seeds aqueous extract showed moderate hydrogen peroxide reduction, with Trolox as a reference.[77]
Methanolic extracts were prepared from leaves and stems (combined) and seeds, and they were tested with DPPH, FRAP and CUPRAC methods. Compared with other plants used in this study, both extracts had high activity, with Trolox as a reference.[78]
Aerial parts aqueous extract ameliorated oxidative stress caused by 0.05% H_2O_2 , as measured by several biomarkers in rats blood.[79]
Aerial parts aqueous extract supplemented to obese rats had positive effects on six antioxidant parameters such as SOD and GPX.[80]
Aqueous extract of flowering buds had significant activity in TBRAS method.[81]
Aerial parts methanolic extract had moderate activity in FRAP method.[82]

Majorana syriaca

Aerial ethanolic extract showed weak and moderate activities in DPPH and β-carotene bleaching methods, respectively.[17]
Aerial parts 70% aqueous ethanolic extract had moderate activity in DPPH test.[22]
Aqueous and 80% aqueous methanolic extract were prepared (plant part/s not indicated) and tested with ABTS method with Trolox as a reference, showing excellent and high activities, respectively.[24]
Leaves EO was obtained by steam distillation and found very potent in two methods: DPPH and FRAP, with BHT as a reference.[83]
Aerial parts were used to produce EO (hydrodistillation) and separately, extracts with water, methanol, *n*-hexane and dichloromethane (DCM). These materials were tested with DPPH

and β-carotene bleaching methods, where aqueous extract was most active, with BHT a reference.[84]
Aerial parts EO (hydrodistillation) was tested with six methods showing high activity, with BHT as a reference.[85]
Leaves were extracted successively with petroleum ether, diethyl ether, ethyl acetate and ethanol. Extracts were tested with DPPH method, resulting diethyl ether extract as most active. The identified major compounds of each fraction are shown in **Figure 9**.[86]
Ethyl acetate leaves extract had significant activity in TBRAS and hydrogen peroxide reduction methods.[87]
Flowering aerial parts EO (hydrodistillation) had strong activities in DPPH and β-carotene bleaching methods, especially in concentrations of 5 and 100 µg/mL, respectively.[88]
Leaves ethyl acetate extract stabilized corn oil in various temperatures and hydrostatic pressures, as measured by TBRAS method.[89]
Leaves EO (hydrodistillation) and 70% ethanolic extract had weak and moderate activities, respectively, in DPPH method. Ascorbic acid was reference.[90]
Aerial parts methanolic extract had moderate activity in DPPH, ABTS and FRAP methods. BHT and ascorbic acid were references.[91]
Commercial EO had significant effect of oxidation inhibition in chicken meat, with BHT and ascorbic acid as references.[92]
Leaves aqueous extract supplemented to humans and had positive effect that was measured by several biomarkers, such as SOD. Paracetamol was reference.[93]
Leaves methanolic extract was tested *in vitro* (blood) and *in vivo* (supplementation to humans), resulting improvement of several biomarkers (increase of SOD and GSH, glutathione; reduction of MDA, malondialdehyde).[94]
Leaves 80% ethanolic extract had high activity in DPPH test, reduced ROS in several cancer cells, and increased the concentration of N-acetyl cysteine (NAC, **Figure 10** with GSH and MDA). Analysis of extract composition indicated the presence of compounds shown in **Figure 11**.[95]
Leaves and flowers were extracted for antioxidant enzymes and phenolic compounds, resulting moderate content in both tests, compared with 15 other plants used in this study, all from the Lamiaceae family.[96]
Leaves aqueous extract fed to rabbits resulting improvement of several biomarkers, especially moderate decrease of myeloperoxidase concentrations.[97]

Malva sylvestris
Aerial parts 70% aqueous ethanolic extract had no activity in DPPH test.[22]
Leaves methanolic extract had moderated activity in DPPH test, with Trolox as a reference. Slight difference was indicated between wild and cultivated plants.[36]
Leaves aqueous extract had weak to moderate activity in DPPH method.[98]
Leaves, flowers, immature fruits and leafy flowered stems were separately extracted with methanol. Extracts were tested with DPPH, FRAP, β-carotene bleaching and TBRAS methods, resulting significant activities.[99]
Flowers aqueous extract had significant activity in DPPH, ABTS and β-carotene bleaching methods, with BHT as a reference.[100]
Seeds, stems, leaves and flowers were separately extracted with 96% aqueous ethanol. The four extracts were tested with DPPH, FRAP and TAC, resulting weak to moderate activities. Leaves extract had highest activity and seeds extract, lowest.[101]
Leaves and stems were extracted with methanol-ammonium citrate solution with pH=7.4 (6:40, v:v). Extract had high activity in DPPH method.[102]
Leaves ethanolic extract was prepared and fractionized with *n*-hexane, chloroform, ethyl acetate and water. All five extracts were tested with DPPH and ABTS methods, with BHT

and Trolox as references, respectively. Significant activities were measured.[103]
70% Aqueous ethanol was used for ultrasonic extraction of leaves and the products was tested with DPPH and FRAP methods, resulting high activities.[104]
Leaves 80% aqueous ethanolic extracts was tested with DPPH, FRAP and hydrogen peroxide reduction methods, resulting weak to significant activities, with α-tocopherol and gallic acid as references.[105]
Leaves methanolic extract had high and significant activities in ABTS and DPPH methods, respectively.[106]
Whole plants methanolic extract was partitioned with *n*-hexane, dichloromethane, *n*-butanol. All products were tested with DPPH and NO scavenging (with quercetin as a standard) methods, resulting highest activity for dichloromethane extract.[107]
Leaves were separately extracted with water, *n*-hexane, methanol and acetone, and all extracts were tested with DPPH and Fe^{+2} chelating tests. Compared with three other *Malva* subspecies, *M. sylvestris* had the strongest activity. Trolox was reference in these tests.[108]
Leaves aqueous extract was partitioned with *n*-butanol and both extracts were tested with DPPH, ABTS and FRAP methods, with ascorbic acid and Trolox as references. Compared with *M. pseudolavatera*, *M. sylvestris* had the stronger activity but moderate compared with references. A major component of each extract is shown in **Figure 12**.[109]
Leaves methanolic and dichloromethane extracts were tested with DPPH method showing moderate activity, but results for each extract are not presented.[110]

Micromeria fruticosa

Aerial parts EO (hydrodistillation) and methanolic extract were tested with DPPH and β-carotene bleaching methods, resulting weak to moderate activities, with BHT as a reference. The major components of EO were (%): isomenthone 3.92, pulegone 29.19, piperitone 3.12 and piperitenone 50.61.[111]
Aerial parts ethanolic extract was tested with DPPH method, resulting moderate activity, with BHT as a reference (IC_{50} = 50 and 91.4 µg/mL, respectively).[112]
Whole plant was separately extracted with methanol, ethanol, ethyl acetate and acetone. All extracts were tested with DPPH methods, showing excellent activities, with ascorbic acid as a reference. Ethanolic extract was used to treat mice with CCl_4-induced hepatotoxicity, showing moderate activity detected by antioxidant biomarkers.[113]
Aerial parts of plants from three locations were hydrodistilled to obtain EOs that were tested with DPPH method, resulting moderate activities, with Trolox as a reference.[114]
Aerial parts were separately extracted with chloroform and methanol and extracts were tested with PRAF, DPPH, ABTS and CUPRAC methods. With BHA, BHT and ascorbic acid as standards, extracts showed moderate to high activities, where methanolic extract was more potent.[115]
Pollen was extracted with 70% aqueous ethanol and extract was tested with PRAF and DPPH methods, showing high activities. BHT and ascorbic acid as references.[116]
Roots, stems, leaves and flowers were separately and successively extracted by *n*-hexane, ethanol and water, yielding 12 extracts that were tested with DPPH method, showing moderate activities.[117]
Leaves and stems were combinedly defatted with petroleum ether and separately extracted with methanol, *n*-butanol and water. Extracts were tested with DPPH method showing moderate activities.[118]
Leaves methanolic extract had moderate activities in DPPH and FRAP methods.[119]

Salvia fruticosa

Shoots were separately extracted with water and 80% aqueous methanol. Extracts were tested with DPPH and ABTS methods resulting moderate activities.[15]
Aerial parts 70% aqueous ethanolic extract had moderate activity in DPPH test.[22]

Aqueous and 80% aqueous methanolic extract were prepared (plant part/s not indicated) and tested with ABTS method with Trolox as a reference, showing excellent and moderate activities, respectively.[24]
Leaves aqueous extract supplemented to humans and had positive effect that was measured by several biomarkers, such as SOD. Paracetamol was reference.[93]
Leaves methanolic extract was tested *in vitro* (blood) and *in vivo* (supplementation to humans), resulting improvement of several biomarkers (increase of SOD and GSH, glutathione; reduction of MDA).[94]
Aerial parts aqueous extract was tested against lipid peroxidation (expressed as MDA equivalent) in cells, resulted from exposure to oxidizing ferric chloride or ozone. Compared with nine other plants and α-tocopherol as a reference, extract had moderate activity.[120]
Leaves aqueous extract had moderate activity in FRAP method.[121]
EO and methanolic extract were separately produced from aerial parts. EO was tested with DPPH method while methanolic extract was tested with DPPH and ABTS methods. Results showed moderate (EO) to significant (methanolic extract) activities. In this study, seasonal variations in chemical composition and antioxidant activity were investigated.[122]
Flowers and leaves were separately extracted with water, and extracts tested with hydroxyl radical scavenging, DPPH, FRAP and TAC methods. With BHA as control, extracts had weak activity, and leaves extract more active than flowers extract.[123]
Flowers and leaves from one location and leaves from nine other locations were separately extracted (microwave-assisted) with methanol, ethanol and ethyl acetate. Compared with six references, extracts had weak to moderate activities.[124]
Leaves EO was obtained by hydrodistillation, and separately, were successively extracted with dichloromethane, ethyl acetate and 75% aqueous ethanol. EO was tested with DPPH method and extracts with DPPH, FRAP and Fe^{+2} chelating methods. With gallic acid, BHA and chlorogenic acid, respectively, as references, all four plant products had weak to moderate activities. In this study wild and cultivated plants were compared.[125]
Dry leaves of wild and cultivated plants from different locations were used to obtain EO (hydrodistillation) and for extraction with 80% aqueous methanol. The process was repeated with dry leaves that were stored for up to six months. Extracts (8) were tested with DPPH method resulting activity decrease proportional to storage time. Compared with BHT as reference, extracts activities were significant.[126]
Aerial parts EO (hydrodistillation) had moderate activity in DPPH method.[127]
Leaves of plants harvested in 20 locations were extracted with methanol and tested with DPPH method. Compared with Trolox as a standard, extracts had moderate activities.[128]
Aerial parts ultrasonic-assisted extracts were prepared using nine different combinations of water, acetone, methanol and acetic acid (0.5%). Extracts were tested with DPPH and TAC methods. With ascorbic acid as a reference, extracts had significant activities.[129]
Aerial parts and roots from four different localities were separately and successively extracted with chloroform, ethyl acetate, methanol and *n*-butanol. All extracts (8) were tested with DPPH method resulting higher activity of aerial parts, in average.[130]
Aerial parts were extracted with methanol and partitioned with *n*-hexane, and both extracts were tested with β-carotene bleaching, DPPH and FRAP methods. With ascorbic acid, *n*-propyl gallate and BHT (respectively) as references, extracts had weak to moderate activities.[131]
Aerial parts were hydrodistilled to obtain EO that was tested with β-carotene bleaching, DPPH and FRAP methods, resulting moderate activities.[132]
Flowering buds EO (hydrodistillation) and water extract were separately prepared and tested with DPPH, ABTS and FRAP methods, showing moderate activities, with extract higher than EO. Chemical compositions of both materials are presented.[133]

Leaves were extracted with 20, 50, and 80% aqueous methanol, and all extracts were tested with DPPH and FRAP methods, resulting significant activities.[134]
Commercial aerial parts were used to prepare teas in two brewing variables: temperature (75-95 °C) and time (2-10 min), and all brews (10) were tested with DPPH and FRAP methods. Tea resulted from 2 minutes and 85 °C brewing conditions was most active.[135]
Fresh aerial parts were hydrodistilled to obtain EO, and dried stems and leaves were separately extracted with water and methanol. The three materials were tested with DPPH, β-carotene bleaching (quercetin), FRAP, superoxide scavenging (ascorbic acid) and Fe^{+2} chelating (EDTA) methods. EO had weakest activities, methanolic extract the highest except for chelating activity where aqueous extract was highest. All three materials were analyzed for chemical composition and the major component of each material is shown in **Figure 13**.[136]
Leaves were extracted with cyclohexane, dichloromethane, ethyl acetate and methanol, and all extracts were tested with DPPH method, with ascorbic acid as a reference. Cyclohexane extract was inactive, dichloromethane and ethyl acetate extracts had weak activities, but methanolic extract had significant activity. Very detailed chemical compositions are presented.[137]
Aerial parts ethanolic extract was tested with DPPH method, with ascorbic acid as a reference. Eight concentrations from 1.075 to $2x10^5$ µg/mL were used, and extract had significant activities in low and high concentrations.[138]
Leaves 96% aqueous ethanolic extract and EO (hydrodistillation) were tested with DPPH, FRAP (quercetin) and Fe^{+2} chelating (EDTA) methods. EO was inactive but extract had very strong activity.[139]
Aerial parts EO (hydrodistillation) was tested *in vitro* with myeloperoxidase inhibition, nitric oxide (NO) radical scavenging and TBRAS methods, resulting low to moderate activities.[140]

Sinapis alba

Aqueous and 70% aqueous methanolic extract were prepared (plant part/s not indicated) and tested with ABTS method with Trolox as a reference, showing moderate and weak activities, respectively.[23]
Aqueous extracts of regenerated plant parts had moderate and different activities in DPPH method: shoots > seed derived plantlets > plantlets > callus.[141]
Seeds *n*-hexane extract had moderate to significant activities in several concentrations, with no reported reference. The major components of the extract are shown in **Figure 14**.[142]
Aqueous extracts of three seeds:water ratios had moderate activities in FRAP method.[143]
Seeds EO (hydrodistillation) was tested with DPPH, FRAP, TBRAS (*n*-propyl gallate) and Fe^{+2} chelating (EDTA) methods, resulting significant activities.[144]
Fresh leaves were separately extracted with 80% and 96% aqueous ethanol. Fresh seeds were separately extracted with water and 1.5% aqueous cyclodextrin. All extracts were tested with DPPH method, with Trolox as reference, resulting moderate (80% EtOH) to weak (aqueous) activities.[145]
Leaves 80% aqueous ethanol extract was tested with TAC and DPPH methods. With Trolox as a reference, results showed moderate activities.[146]
Seeds were extracted with methanol/ acetone/ water mix (7:7:6, v/v/v) and extract was tested with $O_2^{\cdot -}$ scavenging, DPPH, FRAP (BHT, Trolox), $HO^{\cdot}$ scavenging (Trolox) and Fe^{+2} chelating methods. Results showed very strong activities, except for the chelating activity that was strong. Major components of the extract were 4-vinylsyringol, sinapic acid and *p*-hydroxybenzoic acid (**Figure 15**).[147]
Leaves, flowers and fruits were separately extracted with chloroform and 70% aqueous methanol, and the six extracts were tested with DPPH method. With ascorbic acid as a reference, activities ranged from weak (chloroform) to moderate (methanolic).[148]

Aqueous extracts were separately prepared using autoclaved and unautoclaved seeds, and both extracts were tested using FRAP, Fe^{+2} chelating and hydrogen peroxide inhibition methods. Autoclaved seeds extract was more active.[149]
Seeds from plant in three different maturation stages, grown in three different varieties, were extracted with 70% aqueous methanol (with 0.1% formic acid). All nine extracts were tested with ABTS and FRAP methods with Trolox as a reference, resulting moderate activities. In average, rutin, epicatechin and carnosolic acid were the major component (**Figure 16**).[150]

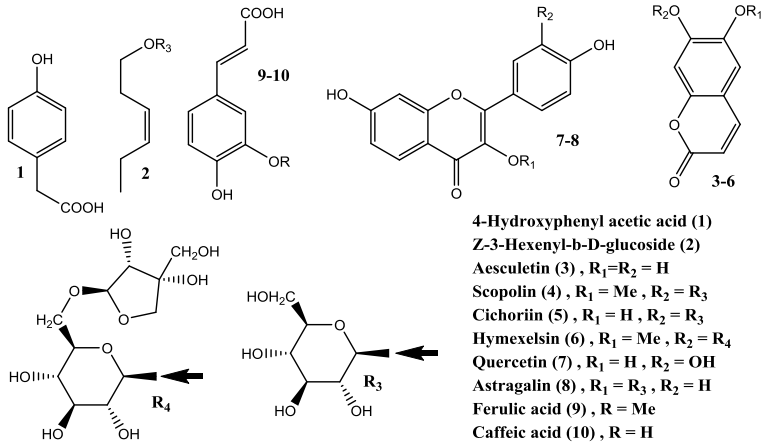


Figure 4: Antioxidant compounds from leaves of *C. pumilum*.[29]

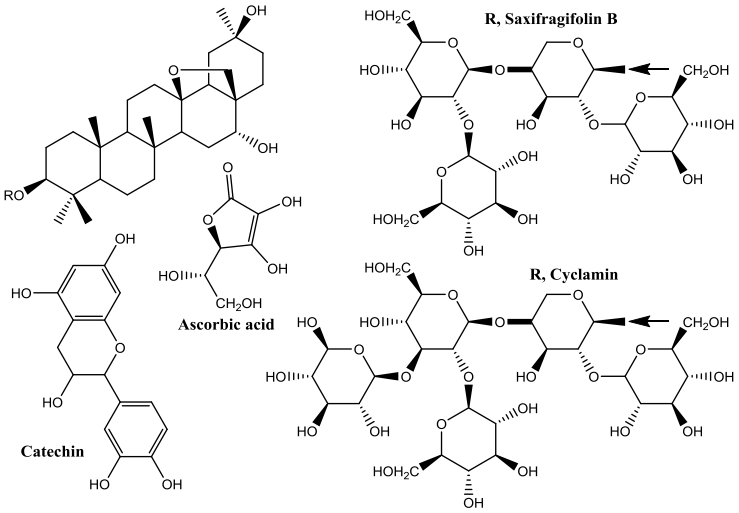


Figure 5: Antioxidant compounds from leaves of *C. persicum*.[34]

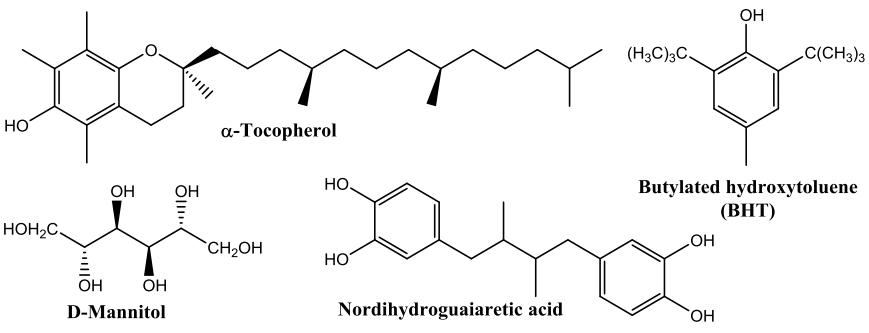


Figure 6: Antioxidant reference compounds.[37,44]

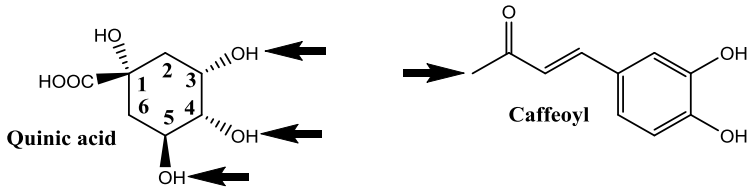


Figure 7: Three antioxidant compounds from *F. vulgare*.[39]

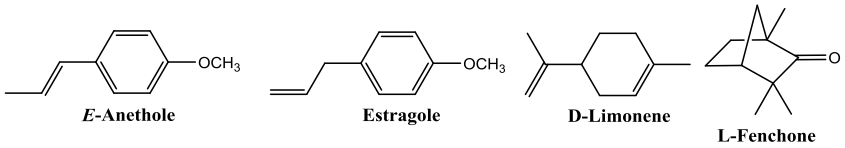


Figure 8: Major compounds of Chinese and Egyptian *F. vulgare* Eos.[59]

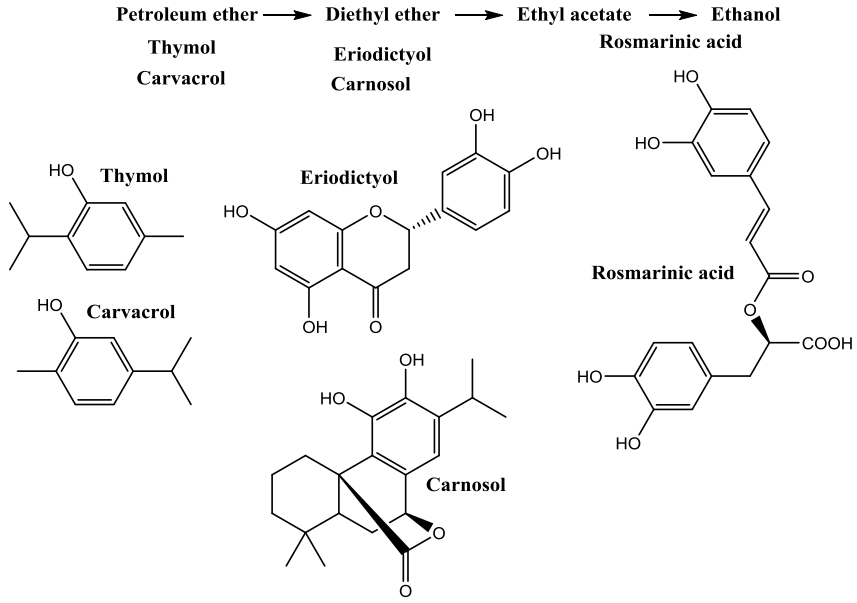


Figure 9: Major identified components of successive extraction of *M. syriaca* leaves.[86]

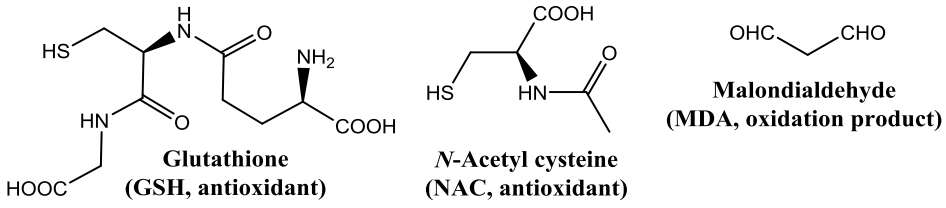


Figure 10: Oxidation and antioxidation biomarkers.

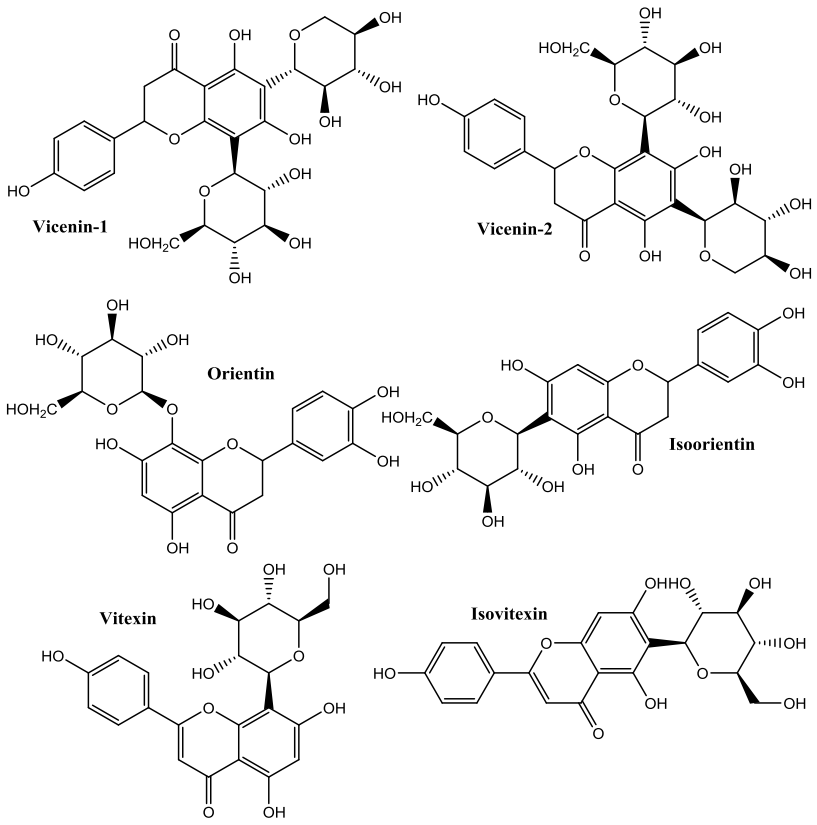
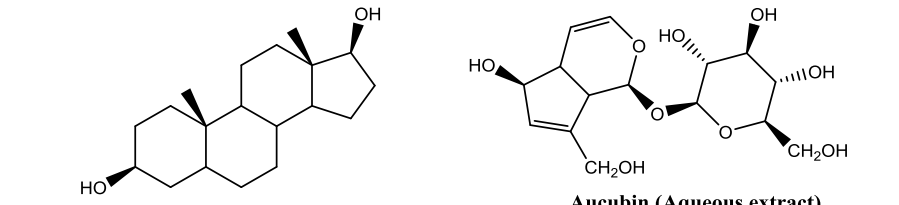


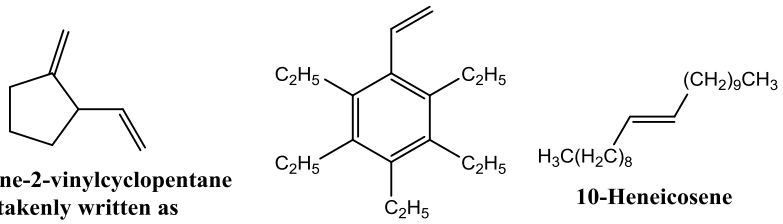
Figure 11: Major components of 80% aqueous ethanolic extract of *M. syriaca* leaves.[95]



3β,17β,5α-Androstane-diol (*n*-Butanolic extract) (Mistakenly written as "3b,17b,5a-Androstane" in article)

Aucubin (Aqueous extract) (Mistakenly written as "Acubin" in article)

Figure 12: Selected major components of extracts of *M. sylvestris* leaves.[109]



1-Methylene-2-vinylcyclopentane (Mistakenly written as 2-Methylene-2-vinylcyclopentane in article)

Pentaethylstyrene

10-Heneicosene

Figure 13: Major components of *S. fruticosa* extracts and EO.[136]

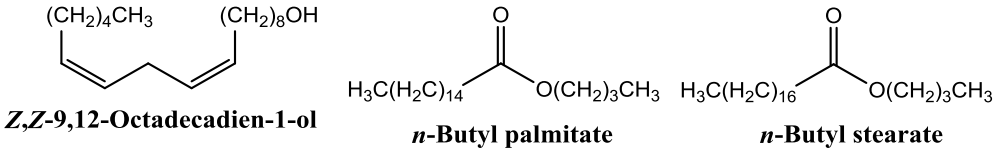


Figure 14: Major three components of seeds *n*-hexane extract of *S. alba*.[142]

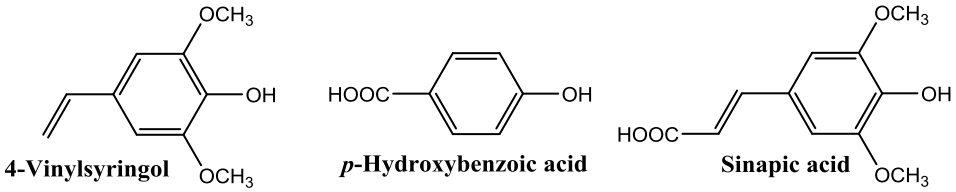


Figure 15: Major three components of seeds extract (three solvents) of *S. alba*.[147]

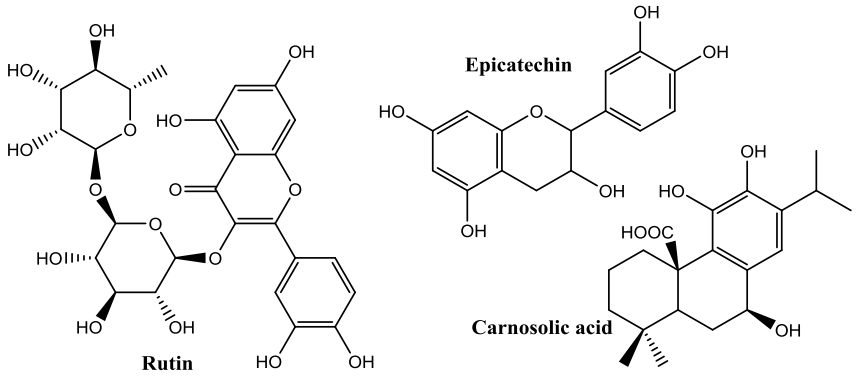


Figure 16: Major components of seeds extract (80% aqueous methanol) of *S. alba*.[150]

4. DISCUSSION

One of the key terms and methods in the estimation of antioxidant activity of plant materials is “total phenolic content”, TPC. In our current article, we mentioned this property only once (Table 1) when we presented the work of S. Doğan and her colleagues about the antioxidant activity of *Cichorium pumilum* seeds aqueous extract.[25] They present the antioxidant capacity as equal or at least direct proportional with TPC. This link or correlation is widely accepted and was reported in large number of publications.[151-153] S. Doğan and her colleagues use the Folin-Ciocalteu (F-C) method to quantify TPC, which is the most used method but not the only one.[154]

TPC was reported by most of the publications that we cited in Table 1[14-150], but we chose not to include in this review article for three reasons. First, the publications we cited presented other experimental methods for measurement of antioxidant activity. Second, the results of the measurements of TPC of plant extracts are mostly interfered by the presence of other reducing compounds such as ascorbic acid (AA), free carbohydrates and carotenoids. In such

cases, results corrections must be made after the tests or removal of these reductants must be performed before the tests.[155] Third, compounds like ascorbic acid are even more powerful antioxidants than gallic acid (GA), the most used standard in F-C method and other antioxidant activity tests. This can be understood with the values of the redox potentials (E^0) of both compounds: 0.35 V for ascorbic acid and 0.38 V for gallic acid.[156,157] So, even though AA is more powerful reductant than GA by E^0, AA has only 0.662 of antioxidant capacity according to F-C method.[158] But even the redox potential is very dependent on many factors such as other components of the system, mainly solvent/s, as can be seen in the case of E^0 values of β-carotene (0.634-1.06 V), which is practically insoluble in water.[159,160]

In the previous four parts (anticancer, anti-inflammatory, antidiabetic and antimicrobial and antiviral activities of the Deca-Plants) of this article series we have extensively discussed the inconsistency of reported results concerning the activities of plants extracts and EOs. Such reports are considered inconsistent when same plant parts are used, same extraction methods and same testing methods. In many cases, this seemingly contradiction emerges from differences in the plant materials that are affected by seasonality, stage of maturity, growing conditions and locality.

An example of this inconsistency is noticed in the following two reports of the antioxidant activity of *Arum palaestinum*, that used leaves ethanolic extract and used DPPH method, but published clear different results. A. Aboul-Enein *et al*. weak activity[16], A.I. Husein *et al*. strong activity.[17] But the work of N.A. Khalaf and his colleagues can provide an explanation to such "contradiction": leaves from two locations were extracted with 96% aqueous ethanol, and extracts had moderate and weak activities (DPPH) as can be seen by IC_{50} (µg/mL): Jordan valley 37 and Palestine 70.[18]

Foeniculum vulgare is one of the most powerful and active medicinal plants, as we have seen this in the first five parts of this review article series, including this part. Among the interesting reports about the antioxidant activities of this plant, the work of A.F. Ahmed and his colleagues that reported the differences between *F. vulgare* seeds compositions and activities from China and Egypt.[59] These results are shown in **Table 2**.

Table 2: Differences between Chinese and Egyptian *F. vulgare* seeds.[59]

Property	Chinese Seeds	Egyptian Seeds
Major Components	Limonene 2.5, L-fenchone 7.4,	Limonene 11.6, L-fenchone 8.2,

of EO (%)	estragole 20.3, *E*-anethole 54.3	estragole 51.1, *E*-anethole 3.6
AEE IC_{50} (mg/g) in DPPH test*	7.17	6.34
EO IC_{50} (mg/g) in DPPH test*	15.66	141.82

AEE, 70% aqueous ethanolic extract * Reference: BHT, 6.80

Similar differences in activities and compositions were also reported by F. Kalleli and her colleagues[60], that used EOs obtained from plants grown in three locations in Morocco and two locations in France. An explanation for these differences can be possibly found in the work of K.R. Ahmad and her colleagues.[161] The have cultivated *F. vulgare* (and *Pimpinella anisum*) under semi-arid conditions in two different locations. They found clear differences in chemical compositions and activities. This can be explained by the clear difference of *E*-anethole (Figure 8) concentrations: 34.51 and 29.61%. A much larger difference in *E*-anethole concentrations: 34.51 and 29.61%. A much larger difference in the concentrations of *E*-anethole is shown in Table 2: Chinese seeds EO 54.3% and Egyptian seeds EO 3.6%, resulting IC_{50} (mg/g), 15.66 and 141.82, respectively.

N. Sadeghpour and her colleagues reported the positive effect of seeds 70% aqueous ethanolic extract of *F. vulgare* on SOD and GPX concentrations in female mice blood.[53] They conclude that this extract "can decrease the serum level of oxidative factors in female mice; it can be introduced as a novel medicine for treatment of infertility". These findings enforce the result of the same group that published another study earlier in the same year, reporting the positive direct effect of the same extract on fertility biomarkers (estrogen, progesterone and prolactin) in female mice.[162]

As we have mentioned above, *F. vulgare* is one of the most active medicinal plants, and despite being very common in some of is natural habitats, it is rare in others due to over harvesting. As a result, it is cultivated in many parts of the world, and attempts and experiments are done to improve the cultivation yields, amounts and properties. N-Y. Jo and her colleagues studied the effect of elevated carbon dioxide concentration on *F. vulgare* growth genetics and antioxidant capacity, finding that this activity (DPPH, FRAP, NO_3^- reduction and TAC methods) was decreased.[163] A. Akbari and his colleagues developed three new cultivars by breeding, and these contained higher TPC and had higher concentrations of antioxidant compounds, such as ascorbic acid, chlorogenic acid, ferulic

acid, salicylic acid, caffeic acid, coniferyl alcohol and sinapyl alcohol (**Figure 17**, the alcohols).[164]

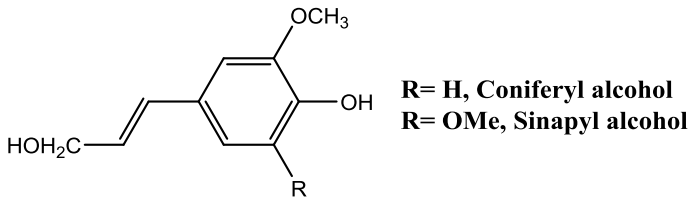


Figure 17: Phenolic alcohols from of *F. vulgare* new breeds.[164]

A.S. Abdelbaky and his colleagues used compost as bio-organic fertilizer when the grew *F. vulgare* and detected an increase of major components concentrations (*trans*-anethole, fenchone, limonene and estragole) and antioxidant activity (DPPH).[165] I. Bettaieb Rebey and her colleagues investigated the effect of salinity on seed yields, composition and antioxidant activity (DPPH, FRAP and ferrous ion chelating), and they found that moderate salinity improved all these properties.[166] A.F. Ahmad whom we cited earlier[59] and his colleagues studied the influence of benzyladenine (synthetic cytokinin) spraying of Chinese and Egyptian *F. vulgare*.[167] They discovered that it increased the concentration of *E*-anethole and the antioxidant activity [DPPH].

It is also very important to indicate that after obtaining a plant product, an extract or and EO, the properties of this material are clearly influenced by storage conditions, containers and duration, found F. Babakhani and her colleagues.[168] For example, the concentrations of *E*-anethole in in *F. vulgare* EO obtained from fresh fruits, can be 75.4% when prepared, and decrease to 72.5% after two months or 71.7% after four months. All other components of this EO were affected, and consequently, the antioxidant activity (DPPH).

Due to its pleasant smell and antioxidant activity, *F. vulgare* EO is added to edible oils, olive, sunflower, soybean and corn, as we have presented in Table 1.[46,48,51] *M. syriaca* EO is also useful for the same purpose.[89] When the stabilized oil is used for cooking, especially deep frying, it undergoes several chemical and physical changes, including oxidation, which is also the fate of the stabilizing EO, reported H. Wu and colleagues.[169] They used *tert*-butylhydroquinone (2-*t*-Butyl-1,4-dihydroxybenzene, TBHQ, a powerful antioxidant) as a reference and DPPH as testing method, and they found out that *F. vulgare* EO was oxidized more than Chinese Maye (food), and thus, it is an efficient stabilizer.

Some of the reports about the antioxidant activity of *F. vulgare* had some special importance because the included additional information that was not included in publications that we cited in Table 1. For example, S. Noreen and her colleagues obtained seeds extracts (80% aqueous ethanol, 80% aqueous acetone and water), tested their antioxidant activities (DPPH, FRAP) and analyzed their chemical compositions.[170] They found that acetone extract had the highest antioxidant activity, and the also report detailed chemical and nutritional compositions: TPC, total flavonoid content (TFC), soluble and insoluble fiber content, mineral profile, wet digestion sampling and amino acid profile. In a follow up study, S. Noreen and her colleagues focused on the determination of *trans*-anethole and antioxidant activity (ABTS, β-carotene bleaching) of seed extracts.[171] The third report that deserves special attention was published by E. Mansouri and his colleagues: they found that supplementation of seeds 80% aqueous ethanolic extracts to male rats increased numbers of red and white blood cells and reduced the effects of ROS.[172]

As we have presented in Table 1, there is a limited number of *Arum palaestinum* antioxidant activity publications.[14-22] But another aspect of this activity can be found in the report of Z. Ayyad and his colleagues.[173] They extracted leaves with olive oil and added the extract to yoghurt, which increased its stability against oxidation.

Young leaves and stems of *Cichorium pumilum* are considered a delicacy food in Eastern Mediterranean region, its flowers and its leaves make healthy tea and its seeds substitute coffee beans. For these reasons, it is harvested in the wild, but in recent years it is also cultivated as commercial crop. And like other plants we discussed in this article, the qualities and the properties of the cultivated plants depend on many variables. K. Kowalczyk and her colleagues grew several cultivars in different conditions, resulting different chemical compositions and antioxidant (DPPH, FRAP) activities.[174] And the work of D. Giannino and his colleagues is way more comprehensive in terms of variable growing conditions and compositions tests, resulting clear dependance of antioxidant activity (oxygen radicals scavenging).[175] In addition, they found out that all properties depend on cultivar and storage time and conditions. O.Y. Kvasko and N.A. Matvieieva improved the antioxidant activity of (reduction of 2,6-dichlorophenolindophenol, production of SOD) by growing transgenic plants.[176] In the work of M. Flores and her colleagues light was used to improve yields and properties *C. pumilum*: high intensity red light and LED.[177] They found out that the antioxidant activity (DPPH), TPC and TFC were higher than in plants that were not

irradiated. M. Serna and his colleagues used synthetic brassinosteroid, 3*β*,5*α*,25*R*-3,5-Dihydroxyspirostan-6-one (**Figure 18**) to promote the growth of *C. pumilum* and improve its antioxidant activity (ABTS).[178]

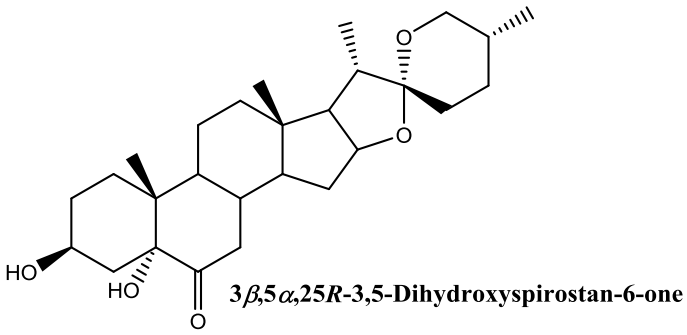


Figure 18: Brassinosteroid used to improve growth and properties of *C. pumilum*.[178]

Improvement of plant growth and properties are done also by using some plants to improve others, as reported by N.M. El-Shafey and H.R. Abdelgawad that mitigated salinity stress in Maize by leaves of *C. pumilum*.[179] Researchers proved that the improvement was an effect of antioxidant phenolics released by these leaves.

M. Farjadi-Shakib and his colleagues reported that applying salicylic acid in cultivation of *Cyclamen persicum* improved morphological and growth parameters and increased the production of SOD in leaves of the plant.[180]

Among the edible wild plants of Eastern Mediterranean region in general, and the D-P in particular, *Gundelia tournefortii* is considered the most delicious. Since only young stems and flowers are eaten, after difficult process of thorns removal, this plant is very expensive in the local markets. This brought the plant to near extinction in parts of natural habitats and forced local communities to start cultivating it.[181] In addition to several methods of cooking this plant, A. Ebrahimi and his colleagues used fresh aerial parts (after thorns removal) to improve the nutritional properties and expand shelf life of yoghurt.[182] Authors relate these improvements to antioxidant activity of this additive. It is also important to indicate that *G. tournefortii* is used to prepare different types of nanoparticles, that some of them have notable antioxidant activity (AuNPs, AgNPs, both tested with DPPH method).[183,184]

Majorana syriaca, like *G. tournefortii* has been overharvested few decades ago, and both plants have nearly disappeared from some of their traditional habitats.[185] And as in the case of *G. tournefortii*, *M. syriaca* is being cultivated for extended commercial use, and the

cultivation conditions influence yields and properties of the plant.[186,187] And due to water scarcity in the Middle East, creative irrigation methods are suggested and used[187], such as the work of M.S. Ali-Shtayeh and his colleagues, that used treated effluent irrigation.[188] They found out that this irrigation had no negative effect on chemical composition of leaves EO or on its antioxidant (DPPH, FRAP) and other activities.

In addition to the "classical" antioxidant activities of *M. syriaca*, that we presented in Table 1, two other reports need different attention. M. Shahwan and his colleagues fed rats with leaves aqueous extract and discovered improvement in all blood biomarkers, SOD and three others.[189] The remarkable aspect of this study that short term supplementation did not alter the animals behavior. M. Al-Hijazeen reports that adding the plant EO to chicken meat improved its taste and protected it against oxidation (TBRAS, protein oxidation), and consequently, extended its shelf life.[190]

Foods prepared from *Malva sylvestris* are among the most common and most delicious during late autumn and winter times of the reviewed region, since this plant is one of the most widespread. Most of these foods are also very easy to prepare. Anyhow, some applications of the antioxidant activity of this plant are of special interest.

M. Sadeghi-Kiakhani and his colleagues prepared silver nanoparticles (AgNPs) by reduction of $AgNO_{3\ (aq)}$ with flowers aqueous extract of *M. sylvestris*, and these AgNPs had strong antibacterial and antioxidant activities (DPPH).[191] In traditional medicines, this plant is very well known for its laxative and diuretic[192], and on this basis the report of M-A. Jabri and his colleagues confirms the traditional knowledge.[193] They found out that leaves aqueous extract preserved the antioxidant enzymes functioning and had laxative effect in loperamide-induced constipation in rats. D. Mravcáková and her colleagues used flowers methanolic extract to treat lambs with severe infections of *Haemonchus contortus* worms.[194] They also reported that the extract improved antioxidant activities of blood serum biomarkers. M. Contardi and his colleagues prepared self-adhesive poly(vinylpyrrolidone)/alginate-based bilayer films loaded with flowers aqueous extract, that proved to be wound healing.[195] It was also had strong antioxidant activity (ABTS) that promoted its medical property. M. Taha Nejad and his colleagues successfully stabilized soybean oil against oxidation (DPPH, ABTS, β-carotene bleaching, BHA and BHT as references) with *M. sylvestris* leaves aqueous extract.[196]

It was proved in a large number of studies that extraction methods, especially extraction solvents are crucial for the outcomes of the extraction and as a result, the activity of extract components and their activities. This is also true for cooking methods and the bioavailability of the active of components of the final food.[197] This effect was studied and published by M.E. El-Sayed and her colleagues, using different solvents (aqueous ethanol or methanol) to extract *M. sylvestris* leaves, which influenced the antioxidant activity of the extracts.[198]

In ethnomedicines of the reviewed region, *Micromeria fruticosa* teas are considered among the best for treatments of various health disorders[199], including calming and relaxation activities.[200] In the literature cited in Table 1, we found interesting reports. M. Al-Hamwi and his colleagues prepared ethanolic extract of aerial parts, tested it with DPPH method resulting moderate activity.[112] But E. Abu-Gharbieh and N.G. Ahmed that prepared whole plant extract (among others, to test them against CCl_4-induced hepatotoxicity in mice) and tested it with DPPH, found this extract excellently active.[113] This difference can result from various reasons, mainly including the roots of the plant in the second work. But it could be due to different growing locations, where the plants in Al-Hamwi work were harvested in Lebanon and in Abu-Gharbieh and Ahmed work in Jordan, keeping in mind that these countries have notably different climates. Moreover, R. Sharma and her colleagues tested the chemical compositions and antioxidant activities of plants of the same subspecies, growing in the same location (Newe Ya'ar, Israel).[201] They found that genetic differences are linked to different compositions, and vice versa, and consequently, different antioxidant activity [DPPH].

Another interesting aspect of *M. fruticosa* antioxidant activity was published by Koç and her colleagues.[202] They treated human U-87 MG cancer cell lines with aerial parts aqueous extract, resulting increased release of lactate dehydrogenase (LDH) enzyme, which catalyzes the reduction of pyruvate to L-lactate.

As in other D-P, the effect of extraction conditions has been extensively studied in regard of *Salvia fruticosa*. S. Grigorakis and his colleagues eco-friendly ultrasonicated extraction, especially designed for phenolic fraction.[203] They ultrasonicated aerial parts of the plant and extracted them with aqueous glycerol solutions of various concentrations. They tested antioxidant activity (DPPH) of the resulting extracts and found significantly higher than standard extraction methods, including ultrasound-assisted. In another study, the same group

tested the effect of citrate-based deep eutectic solvent of the stability of phenolic extract of the plant, resulting excellent stabilization, tested by its antioxidant activity (FRAP).[204]

In recent years some applications of *S. fruticosa* products were published, and some of them deserve special attention. N. Badalamenti and his colleagues analyzed the chemical composition of aerial parts EO, and its activity against oxidative stress caused by metal ion (Cd^{+2}, Cu^{+2}, Zn^{+2}, Pb^{+2}) in *Conocephalum conicum* (plant).[205] The major protection was observed in photosynthesis. A. Kofinas and his colleagues studied the protective activity of ultrasonic-assisted ethanolic aerial parts extract against Alzheimer's disease in human cell model.[206] They tested its activity against H_2O_2 and Aβ-peptide induced toxicities, reporting high activities. L. Risaliti and her colleagues loaded liposomes with commercial EO and tested their antioxidant activity (DPPH), reporting significant results compared with Trolox as a reference.[207] D. Erkakan and her colleagues prepared silver nanoparticles (AgNPs) by reduction of $Ag^{+}_{(aq)}$ with leaves aqueous extract, and these NPs had high antioxidant activity (DPPH).[208]

The attempts of increasing antioxidant natural products in plants are interesting and very diverse. J.F. Dat and his colleagues applied very low concentrations of salicylic acid while growing seeds of *Sinapis alba*.[209] Results showed notable increase in concentrations of salicylic acid way beyond the used amount for growth, ascorbic acid, glutathione and antioxidant enzymes. In addition, to increase the yield of the extracted antioxidants, extracted seeds were pretreated by heating to 45 °C for 1 h. J. Roasa and her colleagues solid-state fermented seeds bran, resulting significant increase of antioxidant activity (DPPH, FRAP, ORAC, oxygen radical absorption capacity) of 70% aqueous methanolic extract of fermented bran compared with non-fermented.[210] An increase of single antioxidant natural products (phenolics) was also detected. R. Torrijos and her colleagues investigated the chemical compositions and antioxidant activities of seeds flour and bran and found differences in both tests.[211] TPC and antioxidant activity in DPPH method were significantly higher for flour than bran, by 135 and 146%, respectively. To visualize the differences of chemical compositions, the average concentration of sinapic acid (Figure 15) in three fractions: in flour 1083 and in bran 684 mg/kg. W. Woch and B. Hawrylak-Nowak biofortified young plants with selenium, resulting increase of antioxidants concentrations (ascorbic acid and anthocyanins) and antioxidant activity tested with DPPH method.[212]

M-J. Xiao and her colleagues prepared seeds 75% aqueous ethanolic extract and supplemented it to mice with atherosclerosis resulting improvement of their health.[213] Authors report that the positive effect was due to antioxidant activity of the extract, which was measured by increase of superoxide dismutase (SOD), decrease of lipid peroxide (LPO) expression of CD36 gene and neutral fat accumulation. M.O. Salem and his colleagues enriched rainbow trout (*Oncorhynchus mykiss*) food with seeds EO, resulting improvements of several antioxidant biomarkers.[214]

5. Selected Wild Edible Non-Deca-Plants with Antioxidant activity

Antioxidant activity of most wild plants of Eastern Mediterranean region was published collectively in review articles[215], or separately in research articles. In **Table 3**, the antioxidant activity of five non-D-P wild edible plants is presented.

Table 3: Published Antioxidant Activities of the Non-D-P in Eastern Mediterranean region.

Testing Method, Results and Reference/s
Nasturtium officinale Arial parts were extracted with 70 and 80% aqueous ethanol and extracts were tested with DPPH, ABTS, FRAP, NO scavenging, H_2O_2 scavenging and Fe^{+2} chelating methods, resulting significant activities.[216]
Portulaca oleracea Aerial parts of fresh and steamed were separately ultrasound-assisted extracted with methanol and both extracts were tested with ABTS and DPPH methods. In both tests (and TPC) raw material showed higher activities. Very detailed chemical composition is presented.[217]
Rhus coriaria Fruits were extracted with 70% aqueous methanol and extract was tested with DPPH and FRAP methods with three references, showing high activity.[218]
Rosmarinus officinalis Aerial parts EO was prepared by hydrodistillation and tested with DPPH method and against CCl_4-induced hepatoxicity caused by oxidative stress in mice. In both tests significant activities were detected.[219]
Silybum marianum Cultivated seeds were successively extracted with petroleum ether and ethanol, and extract was tested with DPPH, TBRAS and FRAP methods, showing excellent activities.[220]

6. CONCLUSIONS

1) The Deca-Plants have moderate to significant antioxidant activities.

2) Some of the Deca-Plants contain high concentrations of antioxidants that can be promoted for human health.

3) The cultivation of the Deca-Plants should be largely expanded to meet the commercial demand and public health needs.

4) The is a need to perform more studies of antioxidant capacities of some Deca-Plants.

5) Publications of medicinal plants must include accurate information of used plant parts, location of harvest, cultivated or wild, maturity step, and detailed extraction procedures.

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