Review on Iranian Medicinal Plants with Antioxidant Properties

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Abstract

Antioxidants have important preventive roles, not only on undesirable changes in the flavor and nutritional quality of food, but also on tissue damage in various human diseases. Some of Iranian plants, despite wide spread use of them as medicines in Iran; have been investigated for their beneficial use as an antioxidants or source of antioxidants. The present review deals with a list of such plants based on information collected from various literatures dealing with herbs found in Iran having antioxidant properties. Also a brief review on common methods for evaluating anti-oxidant capacity is presented.

Keywords: Antioxidant, Medicinal Plants

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Introduction

Almost all organisms are well protected against free radical damage by either enzymes or compounds, such as ascorbic acid, -α- tocopherol and gluthione. When the mechanism of antioxidant protection unbalanced by the deterioration of different factors, physiological functions can occur which result in diseases or accelerated aging. Consequently, it is important to find compounds that prevent oxidation. Antioxidants have important preventive roles, not only on undesirable changes in the flavor and nutritional quality of food, but also on tissue damage in various human diseases [1].

Currently there has been an increased interest globally to identify antioxidant compounds and low or no side effects for use in preventive medicine and food industry.

Iran has great possibilities in product and export of medicinal plants. Iran has various climates and over 7500 plant species, (that claimed %10 to %15 of them are medicinal plants) and this is a great possibility. In these various plants can be harvested or can be cultivated [2].

Some of Iranian plants, despite wide spread use of them as medicines in Iran; have been investigated for their beneficial use as an antioxidants or source of antioxidants.

The present review deals with a list of such plants based on information collected form various literatures dealing with herbs found in Iran having antioxidant properties.

Assay methods for antioxidants

Several methods are used to measure the antioxidant activity of a biological material. The most commonly used ones are those involving chromogen compounds of radical nature that stimulate the reductive oxygen species. These methods are popular due to their ease, speed and sensitivity [3].

The presence of antioxidants leads to the disappearance of these radical chromogens; the most widely used ones being the ABTS and DPPH methods. Some other commonly used assays like FRAP assay, ORAC assay etc as are mentioned below

DPPH method

This is the most widely reported method for screening of antioxidant activity of many plant drugs. DPPH assay method is based on the reduction of methanolic solution of colored free radical DPPH by free radical scavenger. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 516 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. The activity is expressed as effective concentration EC_{50} [4 – 7]

FRAP Method

FRAP (Ferric Reducing Ability of Plasma) is one of the most rapid test and very useful for routine analysis. The antioxidative activity is estimated by measuring the increase in absorbance caused by the formation of ferrous ions from FRAP reagent containing TPTZ (2,4,6– tri (2 – pyridyl)–s–triazine) and $FeCl_3 6H_2O$. The absorbance is measured spectrophotometrically at 595 nm 23 [8, 9].

TRAP Method

This method is defined as total radical trapping antioxidant parameter. The fluorescence of R-Phycoerythrin is quenched by ABAP (2,2-azo–bis (2-amidino-propane) hydrochloride) as a radical generator. This quenching reaction is measured in presence of

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antioxidants. The antioxidative potential is evaluated by measuring the delay in decoloration [10, 11].

Super oxide radical scavenging activity

In-vitro super oxide radical scavenging activity is measured by riboflavin/light/NBT (Nitro blue tetrazolium) reduction. Reduction of NBT is the most popular method. The method is based on generation of super oxide radical by auto oxidation of riboflavin in presence of light. The super oxide radical reduces NBT to a blue colored formazon that can be measured at 560 nm. The capacity of extracts to inhibit the colour to 50% is measured in terms of EC50. Antioxidant activity of Ailanthus, flavanoids and Triphala has been reported in terms of super oxide radical scavenging activity. The super oxide radical can also be detected by oxidation of hydroxylamine, yielding nitrite which is measured colorimetric reaction [12 – 15].

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. This method involves in-vitro generation of hydroxyl

Radicals using Fe^{3+} /ascorbate/EDTA/H_2O_2 system using Fenton reaction.

Scavenging of this hydroxyl radical in presence of antioxidant is measured. In one of the methods, the hydroxyl radicals formed by the oxidation is made to react with DMSO (dimethyl sulphoxide) to yield formaldehyde. Formaldehyde formed produces intense yellow color with Nash reagent (2M ammonium acetate with 0.05M acetic acid and 0.02M acetyl acetone in distilled water). The intensity of yellow color formed is measured at 412nm spectrophotometrically against reagent blank. The activity is expressed as % hydroxyl radical scavenging [16, 17].

Nitric oxide radical inhibition activity

Nitric oxide, because of its unpaired electron, is classified as a free radical and displays important reactivity's with certain types of proteins and other free radicals. In vitro inhibition of nitric oxide radical is also a measure of anti oxidant activity. This method is based on the inhibition of nitric oxide radical generated from sodium nitroprusside in buffer saline and measured by Griess reagent. In presence of scavengers, the absorbance of the chromophore is evaluated at 546nm. The activity is expressed as % reduction of nitric oxide [18, 19].

ABTS (2,2-azinobis(3-ethyl benzothiazoline-6-sulfonicacid) diamonium salt Method

This is a measure of antioxidant activity as opposed to antioxidant concentration which might include a proportion of biologically inactive antioxidants. It also permits the measurement of antioxidant activity of mixtures of substances and hence helps to distinguish between additive and synergistic effects. The antioxidant activity of wines was measured by using this method. The assay is based on interaction between antioxidant and ABTS+ radical cation which has a characteristic color showing maxima at 645, 734 and 815 nm [20 – 24].

DMPD (N, N-dimethyl-p-phenylene diamine dihydrochloride) Method

This assay is based on the reduction of buffered solution of colored DMPD in acetate buffer and ferric chloride. The procedure involves measurement of decrease in absorbance of DMPD in presence of scavengers at its absorption maxima of 505nm. The antioxidant activity of wines was measured by using this method. The activity was expressed as percentage reduction of DMPD [25, 26].

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Oxygen Radical Absorbance Capacity (ORAC)

ORAC is an exciting and revolutionary new test tube analysis that can be utilized to test "Antioxidant Power" of foods and other chemical substances. It calculates the ability of a product or chemical to protect against potentially damaging free radicals. This analytical procedure measures the ability of a food, vitamin, nutritional supplement, or other chemicals to protect against the attack by free radicals, or to act as an Antioxidant. The test is performed using Trolox (a water-soluble analog of Vitamin E) as a standard to determine the Trolox Equivalent (TE). The ORAC value is then calculated from the Trolox Equivalent and expressed as ORAC units or value. The higher the ORAC value, the greater the "Antioxidant Power".

This assay is based on generation of free radical using AAPH (2,2-azobis 2-amido propane dihydrochloride) and measurement of decrease in fluorescence in presence of free radical scavengers. In this assay b-phycoerythrin (b-PE) was used as target free radical damage, AAPH as a peroxy radical generator and Trolox as a standard control. After addition of AAPH to the test solution, the fluorescence is recorded and the antioxidant activity is expressed as trolox equivalent [27 – 30].

β -Carotene Linoleate model

This is one of the rapid method to screen antioxidants, which is mainly based on the principle that Linoleic acid, which is an unsaturated fatty acid, gets oxidized by "Reactive Oxygen Species" (ROS) produced by oxygenated water. The products formed will initiate the b-carotene oxidation, which will lead to discoloration. Antioxidants decrease the extent of discoloration, which is measured at 434nm and the activity is measured [31, 32].

Microsomal lipid peroxidation or Thiobarbituric acid (TBA) assay

TBA test is one of the most frequently used tests for measuring the peroxidation of lipids. Method involves isolation of microsomes from rat liver and induction of lipid peroxides with ferric ions leading to the production of small amount of Malonaldehyde (MDA). TBA reacts with MDA to form a pink chromagen, which can be detected spectrophotometricaly at 532 nm [33].

Iraninan plants with antioxidant activity

1- *Lavandula officinalis*

Lavandula officinalis chaix family labiatae is an evergreen bushy shrub with straight, woody branches, the lower of which are leafless, putting out numerous herbaceous stems to a height of about 1 meter. The plant is indigenous to southern Europe and is sometimes found growing wild in the Mediterranean area between the coast and the lower mountain slopes. It is cultivated throughout Europe as well as indifferent parts of Iran.

Flowers of the plant have carminative, antispasmodic, antidepressant, antirheumatic, antiseptic and tonic properties and are indicated in flatulent dyspepsia, colic and depressive headache. Essential oil of the flowers in rubfacient and is used topically in rheumatic pain. Essential oil of *L. officinalis* contains linalool (34.1%), 1, 8 – cineole (18.5%1, borneole (14.5%) and comphor (10.2%) as main components [34].

The ability of *L. officinalis* extract to act as a free radical scavenger or hydrogen donor was revealed by DPPH radical – scavenging activity assay [35].

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2- *Melilouts officinaliss* (fabaceae)

Melilouts officinalis (L.) is a biennial legume (fabaceae) native to Eurasia that has become naturalized throughout the world hexadeanoic acid, Lupanone, lupeol

Betulinic acid, oleanolic, kamp ferol-3-o-B- glu.

The result of pormoradi study showed that the extract of M. officinalis, which contain highest amount of flavoniod and phenolic compounds, exhibited the greatest antioxidant activity. the high scavenging property of M. officinalis may be due to hydroxyl group's existing in the phenolic compounds chemical structure that can provide the necessary component as a radical scavenger [36].

3- *Olea europaeal*

Olea europaeal (syn. Olive, zaytoon) belonging to the family oleaceae is an important medicinal plant which is used all over the world. It is a traditional tree crop of the meditrranen Basin.

The oil of olea fruit consisted of fourteen saturated fatty acid (22.56%) and sixteen unsaturated fatty acids (73.32%), oleic acid (68.07), palmitic acid (12%1) and arachidic acids (9.7%) were the major components of oil [37].

Antioxidant activity of methanoilc (50%) extracts of olive pulp (*olea europael*) was investigated. Total antioxidant activity, phenolic contents and reducing power in the six itanian olive cultivavs were determined the highest antioxidant activity of Iranian olive cultivars were determined as 28.69 mmol fell (100 gr).

Results showed Iranian olives possess relatively high antioxidant activity due to contribution of phenolic compounds and can be considered as good sources of natural antioxidants for medicinal and commercial uses [38].

4- *Chrozophora tinctoria* (L.)

Chrozophora tinctoria (L.) belong to the subtribe chrozophoriane, and family Euphorbiaceae. This species is native to number of countries in afica, Temperate and tropical Asia (Kuwait, Saudi Arabia, Afganistan, Iran).

Chrozophora tinctoria is well known for producing dye substances and flavonoids, alkaloids, coumarins, chromones, etc.

Ethanolic extract of *C. tinctoria* has been found to have antioxidant effects, shown by the inhibition of lipid peroxidation and DPPH assay [39].

5- ***Echium amoenum***

E. amoenum is one of the most important medicinal plants in Iranian traditional medicine. The flower of this plant have been used as demulcent, anti- inflammatory, analgesic and sedative in folk medicine of Iran. Extract of this plant has been shown to contain flavonoids, saponins, unsaturated terponoids and sterols

Phytochemical studies on *E. amoenum* revealed presence of many chemicals such as flanoids, & - linolenic acid and trace amount of alkaloids. the antioxidant properties of flavonoid have been well established.

Results showed that E. amoenum decoction has very good potential to improve human antioxidant status and prevent normal oxidative stress that happens dialy due to normal exposure to many causal chemicals and conditions [40].

6- *Salvia mirzayani* Rech (labiatae)

Salvia mirzayani Rech (labiatae) is a medicinal plant that is used for diabetes in local medicine.

Bicyclogermacrene (31.3%), a - pinene (13.2%), B- pinene (10.3%) and sabinene (11.7%) are main componet of salvia oil [41].

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The free radical scavenging capacity, and reducing power and prevented B- carotene peroxidation was established. These results were confirmed by a protective effect on H_2O_2- induced fibroblast more over, this experimental antioxidant activity.

This extract may be useful in the treatment of human pathologies in which free radical production plays a key role [42].

7- *Achillea species*

The genus *Achillea* of the asteraceae family is diversely distributed in Iran and represented by 42 species of which are endemic for Iran.

Antinociceptive and anti- inflammatory human erythrocyte and leukocyte protective, antispasmodic and antimicrobial activities of different achillea species are proved monoterpenes, such as pinene, 1, 8- cineole, camphor, Artemisia ketone and sesquiterpenes, such as caryophylene, germacrene, azulene and derivatives as well as sesquiterpene lacton, polyenes, alkamides, flavonoids, lignans and trierpenes are considered as important major constituents and their amounts vary within different species [11].

The free radical scavenging activity of extracts was characterized by the DPPH scavenging test. Results showed *A. micrantha* with (IC 50= 32.92. Mgr/ml) was significantly lower than the other [43, 44].

8- *Silybum marianml* (Asteraceae)

Silybum marianum is a milk thisle is an annual or biannual plant of the Asteraceae family.

Silymarim is a purified extract from the seeds of *silybun marinum* L. (Asteraceae) also called "milk thistle" that consist of a large number of flavolignans, includiney silybin, which is the most active components, isosilybin, silydianin, and sily chreistin.

Currently silymarim is widely used as hepatoprotectant and as supportive therapy of liver disorders such as a cirrhosis, hepatitis and fatty acid infiltration due to the alchol and toxic chemicals. many of these properties is related to the antioxidant and free radical scavenging activity of silymarin enzymes and reduced glutathione in alloxaninduced diabetes in rat pancreas. Result of this study showed, FRAP assay shows high antioxidant capacity of silymarin compared with other standard and green tea. DPPH scavenging activity of silymarin also indicated high antioxidant power of this compound. The present study suggests that silymarin may be used in preventing free radical related diseases as a dietary natural antioxidant supplement [45].

9- Basil (*Ocimum basilicm*)

Basil (*Ocimum basilicum*), of the family lamiaceae (mints) is a tender low – growing herb that plays a major role in the Iranian cuisines. The plant tastes somewhat like anise, with a strong, pungent sweet smell. Basil is originally native to Iran, India and other topical regions of Asia, having been cultivated there for more than 5000 years.

Basil (*ocimum basilicm*) is used in traditional medicine, as a culinary herb and a well – known source of flavouring principles. Iranian basils possess valuable antioxidant properties for culinary and possible medicinal use [46].

10 - *Teucrium polium*

Teucrium polium (known popularly as **felty germander**) is a sub-shrub and herb native to the Mediterranean region and the Middle East. Its flowers are small and range from pink to white, and its leaves are used in cooking and for medicinal purposes, particularly for the treatment of stomach ailments. It has also shown some promise in

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the treatment of visceral pain. In traditional Persian medicine, *T. polium* (locally called 'kalpooreh') is used as an anti-hypertensive, anti-bacterial, carminative, anti-nociceptive, anti-inflammatory, anti-diarrhea, anti-diabetes and anti-convulsant agent [47].

The therapeutic benefits of *T. polium* extracts are usually attributed to their ability to suppress oxidative processes.

For example, some studied reported that an alcoholic extract of T. polium could suppress hydrogen peroxide-induced lipid peroxidation in red blood cells in a concentration-dependent manner and aqueous extract of T. polium suppressed iron (Fe^{2+})—induced lipid peroxidation in rat liver homogenates [48].

11- *Achillea wilhelmsii*

Achillea wilhelmsii C. Koch (Asteraceae), locally known as "boomadaran" (Lavander cotton) is widely found in different part of Iran. This plant is full of flavonoids and sesquiterpene lactones, which have been shown to be effective in lowering blood lipids and hypertension [49, 50].

The test method for screening the antioxidant activity of this plant by linoleic acid peroxidation test using 1, 3- diethyl -2 – Thiobarbituric acid as the reagent reveled good antioxidant property for this plant [51].

12- *Green tea*

Green tea is a made solely with the leaves of camellia sinensis that has undergone minimal oxidation during processing. *Green tea* contains caffeine [52] and also green teas contain two caffeine metabolites: theophlline, which is stronger stimulant than caffeine, and theobromine, which is slightly weaker than caffine.

Antioxidant activity of green tea extract in comparison with commercial antioxidants was evaluated. Green tea was tested for antioxidant activity with comparison with sodium metabisulfite and butilated hydroxyl toluene.

Results suggested the possibility of using a green tea extract as effective natural antioxidant for substances that are oxidation – susceptible [53].

13 - *Anethum graveolens* Boiss.

Anethum graveolens Boiss belong to the Apiaceae family. This plant, in Iran, naturally in many areas such as Tabriz, Khorasan and Tafresh was grown.

Carven and limonene as a main component of *Anethum graveolens* Boiss have antioxidant and antibacterial properties [54].

The antioxidant activity was investigated with two methods, DPPH free radical scavenging and β- carotene / linoleic acid. Also antioxidant activity of essential oil was determined by measuring peroxide and thiobarbituric acid values in crude soy bean oil in DPPH system, the Ec50 value of antethum graveolens essential oil determined by measuring peroxide and thiobarbituric acid values in crude soybean oil in Dpph system, the EC50 value of *Anethum graveolens* essential oil was determined as 2.57 ± 1.52 mg/ml.

Results showed that the essential oil of *Anethum graveolens* could be used as a natural antioxidant in food stuffs.

14- *Iranian Taxons*

Iranian conifers are evergreen and aromatic plants are widely spread and grow in different parts of many countries including Iran. Each of them has its own Persian name [55 - 57].

Most of these trees are medicinal plants and seeds, dried leaves and fruits are used to treat various diseases like bronchitis, common cold, nose bleeds, hypertension, inflammation and gout, and used as expectorant,

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contraceptive, diuretics, for rheumatic symptoms, to regulate menstruation and to relieve menstrual pain [58].

Emami and cooperators evaluated antioxidant activity of leaves and fruits of 11 different taxons. The leaves of both male and female, and fruits of these plants were collected from different areas of the country. Methanol extract of leaves and fruits of these taxons were prepared. Antioxidant activity of each extracts was measured using two different tests of the ferric thiocyanate method and thiobarbituric acid. Results indicated that the methanol extracts of leaves, of male and female, and fruits of all these species (27 samples) possessed antioxidant activity when tested with both methods. The antioxidant activity was then compared with those of -tocopherol (a natural antioxidant) and butylated hydroxytoluene (a synthetic antioxidant). Methanol extract of fruits of *C. semipervirens* cv. Cereifeormis showed the highest antioxidant activity while the methanol extract of leaves of *C. semipervirens* var. *semipervirens* possessed the lowest antioxidant activity. However, our finding showed that most of the tested extracts were showing strong antioxidant activity even higher than -tocopherol [59].

15- *Zea mays*

Traditionally corn silk (CS) has been used as diuretic, antilithiasic, uricosuric, and antiseptic. It is used for the treatment of edema as well as for cystitis, gout, kidney stones, nephritis, and prostatitis. The ability of *Zea mays* extract to act as a antioxidant source were estimated by different methods. Also phenol and flavonoid content of the extract were measured by Folin Ciocalteu and $AlCl_3$ assays. CS extract contained a significant amount of phenol and flavonoids. The percentage of DPPH radical scavenged by CS extract was 92.6 at a concentration of 1.6 mg ml–1. IC50 of the extract and the standard compounds butylated hydroxytoluene (BHA) and quercetin was 0.59, 0.053, and 0.025 mg ml–1, respectively. Iron chelating activity of the extract was less than the standard compounds. CS extract showed nitric oxide-scavenging effect less than the reference agent (quercetin). The extract showed a high reducing ability. According to ferric thiocyanate (FTC) method, the extract showed more than 88% inhibition of linoleic acid peroxidation. It might be concluded that some of the properties of CS in traditional medicine is due to its antioxidant ability [60].

16- *Zhumeria majdae*

Z. majdae is one of the members of Labiatae family which has a limited geographic range in southern region of Iran (near Persian Golf). This plant is known as Mohre-khosh in Hormozgan Province and is used for the treatment of a wide range of disorders such as diarrhoea, cold, reflux and headache. It is also used as carminative and for wound healing [62, 63].

The reducing power of the ethyl acetate fraction of plant extract was possessed the great radical scavenging activity (IC50 = 41.85 ± 0.61 µg/ml) No significant differences exist between the IC50 of this subfraction and quercetin (IC50 =38.84 ± 0.84 µg/ml), $p > 0.05$. The greatest amount of phenolic compounds (1.98 ± 0.01 mg/g) and flavonoids (357.4 ± 18.7 µg/ml) were detected in ethyl acetate subfraction by Moein [64].

17- *Nepeta ispahanica*

The genus of *Nepeta* L. belongs to Lamiaceae and is distributed in Europe, Asia and some areas of Africa. One of the species of this genus is *Nepeta ispahanica* Boiss.

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That is endemic to Iran. In this paper the essential oil composition of *N. ispahanica* and antibacterial and antioxidant activities of the oil and its various extracts are reported [65].

The major constituents of the oil were found to be 1,8-cineol (64%), β-pinene (6.4%), germacrene-D (3.7%) and α-pinene (2.3%).

Also, the antioxidant activity of the oil and extracts was investigated by DPPH assay and considerable antioxidant effect was observed from the essential oil.

18 - *Lippia citriodora*, *thymus daenensis*

Essential oil of Thymus daenensis is a rich source of thymol and carvacrol which has been reported to possess the highest antioxidant activity [66]. In Iran, it is predominantly found in the north of the country. It is used as a food ingredient, as a tea, as an herbal drug for its reputed medicinal properties [67, 68].

Thymol (54.68%) and gama-terpinene (12.9%), pcymene (11.25%) were reported as main component of the essential oil of this plant.

The total phenolic contents of L. citriodora (385.36 mg/l) and t. daenensis (307.96 mg/l) are determined. The antioxidant activities of essential oils are studied by two methods such as ß-caroten linoleic acid and DPPH assays. The decrease in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radial by hydrogen donation.

20 - *Ferulago angulata*

Ferulago is a genus belonging to the Apiaceae family. It has 35 species, of which seven grow wildin Iran. Ferulago angulata is one of these species that are found in their natural state in Iran [69], basically belongs to west of Iran. Traditionally this plant was added to different products to prevent from decay as well as give them a pleasant taste. Different concentration of essential oil and extract were added to vegetable oil. Peroxide and Thiobarbituric indexes of samples were determined and compared with blanks samples (without any antioxidant and with TBHQ) showed that minimum concentration of extract for conserving of vegetable oil is about% 0.02 under excremental conditions. Extract with 0.5% concentration is more effective than TBHQ. [70].

21 - *Artemisia haussknechtii*

Artemisia species with common Persian name of Dermaneh are found all over Iran. A. haussknechtii is used in dyspepsia and other gastrointestinal disorders by local people in the western part of Iran; province of Kermanshah.

Forty-eight components were identified constituting 98.35 of total oil. Camphor (12.4%), α-Terpineol (9.93%), Davana ether (6/24%), and Bornyl acetate (3.77%) were the major components. Good antioxidant activity of extract; increasing with the increment of concentration of plant extract was revealed. This ability was revalued by DPPH and FTC method [71].

22 - *Smyrnium cordifolium*

Genus Smyrnium belongs to umbelliferae family. Smyrnium cordifolium Boiss. is a native of Iran and eaten as a green by some people in west part of Iran. It is offen used internally for bladder and kindly swelling.

The ethanolic extract was subjected to evaluation for antioxidant activity using 2,2-diphenyl, 1- picryl hydrazil for measurement of free radical scavenging activity with ferric ammonium thiocynate method for evaluation of lipid peroxidation properties. The results of these assays agree that the extract of this plant displayed high antioxidant [72].

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23 - *Alium latifolium*

Allium latifolium Gilib. (Liliaceae) is a Persian native plant, grown in Cool regions of Iran. It is used by natives as additive and preservative for prolonged preserving of some of the foods and utilized in folk medicine for treatment of such varied physical disorders as burns, wounds, headaches, colds and rheumatism.

Result showed Thymol (22.8%), Dimethyl trisulfide (17.9%), 4-methyl-5-thiazo ethanol (7.5%), z-β-ocimene (5.8%) and Carvacrol (5.1%) were the major components of the essential oil *Allium latifolium*.

Antioxidant activity of the extract of this plant, espicialy , in the DPPH assay and FTC method exhibited a notable dose dependent inhibition of DPPH activity, with a 50% inhibition (IC_{50}) at a concentration of 0.145 mg/ml. were investigated due to effects of sulfur-containing compounds, which occur in *Allium* species such as onion and garlic,. It is elucidated that the alk (en) yl substituents and some of sulfur atoms in the compounds were important for the antioxidative activities [73].

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