Study of Some Miswak *(Salvadora persica L)* Components and Effect of Their Aqueous Extract on Antioxidant

Mustafa Taha Mohammed

ABSTRACT:

BACKGROUND:

Oxidative stress defines that, the level of Reactive Oxygen Species (ROS) exists in excess of antioxidant defenses. This imbalance in the redox milieu results in a switch from ROS-stimulated ambient signaling processes to ROS-mediated pathophysiological consequences. Oxidative stress has been implicated in the installation and progression of several degenerative diseases via DNA mutation, protein oxidation and/or lipid peroxidation. Therefore, possible use aqueous extracts of Miswak to protect brain against the Lipid peroxidation**.**

OBJECTIVE:

The present study was undertaken to evaluate the potential of *Salvadora persica* L. (miswak) against the DPPH Free Radical Scavenging System and Lipid peroxidation.

METHODS:

The chemical components of the prepared aqueous extracts of Miswak were detected as: glycosides, alkaloids, saponins, phenolic compounds, tannins, flavonoids , proteins ,steroids and Vitamine C. and then estimate DPPH Free Radical Scavenging System and Lipid peroxidation

RESULTS:

The study showed that the Miswak *(Salvadora persica* L.) in the aqueous extracts contain : glycosides , proteins, saponins ,tannins ,phenolic compounds, flavonoids , alkaloids , steroids and vitamine C . Aqueas extracts were found effective in scavenging DPPH(٦٢,٤٥%) in concentration (٢٥٠µl/ml), as well as inhibiting the lipid peroxidation (٤٢,٠٤%).

CONCLUSION:

Our results suggest that Miswak *(Salvadora persica* L.) treatment protects the rat brain against lipid peroxidation and DPPH free radical scavenging.

KEY WORDS : miswak , DPPH, lipid peroxidation.

INTRODUCTION:

Reactive oxygen species [ROS], sometimes called as active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions ($O_2^{\cdot-}$) and hydroxyl radicals ($OH^{\cdot}$) as well as non-free radical species such as hydrogen peroxide (H_2O_2). [١] These ROS play an important role in degenerative or pathological processes, such as aging, cancers, coronary heart diseases, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammations. [٢] Living organisms have antioxidant defence systems that protects against oxidative damage by removal or repair of damaged molecules[٣]. The term 'antioxidant' refers to the activity of numerous vitamins, minerals and phytochemicals which provide protection against the damage caused by ROS[٤].

Department of Chemistry, College of Science, Al-Mustansiriyah University, Baghdad, Iraq.

Antioxidants interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals and by acting as electron donors[٥]. The natural antioxidant mechanisms maybe insufficient in variety of conditions and hence dietary intake of antioxidant compounds are important[٦]. The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals. It has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and incidence of human diseases[١]. Plant based antioxidants are preferred to the synthetic ones because of their multiple mechanisms of actions and non-toxic nature. These facts have inspired widespread screening of plants for possible medicinal and antioxidant properties; the isolation and characterization of diverse phytochemicals and the utilization to antioxidants of natural origin to prevent the diseases[٧].

The Miswak *(Salvadora persica* L.) is a large shrub with opposite branches, sometimes growing as dense thickets on sand hummock, belonging to family Salvadoraceae, commonly known as 'Pilu', 'Jal' and 'Tooth brush tree' and is widely distributed in India, Africa, Saudi Arabia, Iran, Israel and Pakistan. It has been claimed in traditional literature to be valuable against a wide variety of diseases [٨،٩،١٠]. The Miswak (*S. persica* L.), also known as tooth brush tree ٤–٦ m tall with a short trunk, white bark and smooth green leaves, with a life span of ٢٥ years, belonging to the Salvadoraceae family, has been used as a brushing stick for more than ١،٣٠٠ years. Because these natural tooth brushes are so commonly used in different areas of the world by millions of people, a variety of studies have been performed on the antimicrobial effect of these sticks[١١] . Pharmacological studies indicated that *S. persica* L. plant possess anti-microbial, anti-plaque, aphrodisiac, alexiteric, analgesic, anti-inflammatory, anti-pyretic, astringent, diuretic and bitter stomachic activities [١٢]. It has great medicinal use in the treatment of nose troubles, piles, scabies, leucoderma, scurvy, gnorrhea, boils and toothache, to treat hook worm, venereal diseases, for teeth cleaning, in rheumatism, cough and asthma, to lower cholesterol plasma levels, reestablishment of the components of gastric mucosa, and as a laxative[١٣]. It contains important phytoconstituents such as vitamin C, salvadorine, salvadourea, alkaloids, trimethylamine, cyanogenic glycosides, tannins, saponins and salts mostly as chlorides[١٤]. *S. persica* seed contains about ٤٠٪ oil with a fatty acid composition (lauric -٢٠٪, myristic - ٥٥٪, palmitic ٢٠٪ and oleic -٥٪) which can make an excellent soap. Radical reactions in vivo can damage life essential molecules such as nucleic acids and proteins[١٥]. Phenolic compounds, particularly flavonoids, have been shown to possess an important antioxidant activity towards these radicals, which is principally based on their structural characteristics (number and position of phenolic hydroxyls, other groups, conjugation) [١٦]. The history and the use of miswak (tooth stick) as an oral tool as well as the biological effects of *S. persica* extracts are reviewed. Besides medicinal claims, various parts of this plant are of great ethno botanical importance and play a vital role in the livelihood of local communities[١٧] .

MATERIALS AND METHODS:

Collection and treatment of samples:

The root of *Salvadora persica* were collected from market of Baghdad, Iraq. The root were transported to the laboratory biochemistry in department of chemistry /College of Science /Al-Mustansiryih University, washed, cleaned with filter paper or soft clothes to remove all traces of dust and insects, then dried in shade ٢٥-٣٠ºC for one week, with continuous overturn to prevent mould .weighed, ground in a mortar and pestle, placed in airtight bottles and stored in dissicator to be used for extraction[١٨].

Preparation of extracts:

Air dried root ٥٠ g were suspended in one liter of distilled water and left for ٢٤ hrs at ٣٥ºC with continuous stirring in shaking incubator .Then the mixture was filtered by filter paper ,the filtrate was centrifuged for ١٠ min. at ٢٥٠٠ rpm ,and the extract evaporated to dryness at ٤٠ºC in the incubator[١٩] .

Chemical detection of the plant components:

The chemical components of the prepared watery extract were detected as shown in table .١.They included: glycosides, alkaloids, saponins, phenolic compounds, tannins, flavonoids , proteins ,steroids and Vitamine C [٢٠،٢١] .

DPPH Free Radical Scavenging System:

The effect of plant extracts on(٢ diphenyl ٢ picryl hydrazyl hydrate) DPPH radical was estimated according to the method of Blois. The absorbance of the resulting solution was measured spectro photometrically at ٥٢٠ nm, Results were expressed as percentage inhibition of DPPH by comparing with blank[٢٢].

scavenging effect (%) = (Ac-At)/Ac ×١٠٠

Lipid peroxidation:

The brain isolated from healthy albino rat (١٨٠-٢٠٠ gm) was used as lipid source. Brain homogenate (١٠٪ w/v) was prepared in ١٥٠ mM KCl and centrifuged at ٨٠٠ g for ١٠ minutes. The supernatant was collected and used immediately to study *in-vitro* lipid peroxidation. Briefly, the reaction mixture contained ٠,٣ ml of brain homogenate, KCl (١٠٠)M), ascorbic acid (١٠٠)M), ferricchloride (١٠٠)M), ٠,٥ ml of graded concentrations of extracts and final volume was made with buffer. After incubating at room temperature for ٢٠ minutes, ١,٠ ml of thiobarbituric acid-trichloroacetic acid (TBATCA) reagent was added. The resulted mixtures were heated at ٨٠٠C for ٢٠ minutes,

cool and centrifuged for ١٠ minutes at ١٠٠٠ rpm and by using a digital UV/VIS spectrophotometer recorded the absorbance at ٥٣٢ nm. Control and standard (curcumin ١٠)M) were carried out at similar manner. Percentage inhibition of thiobarbituric acid reactive substance (TBARS) formation by extract/standard drug (curcumin) was calculated by comparing with control. All experiments were carried out in triplicate and results are the means of one such individual experiment. Percentage inhibition of lipid peroxidation by test compound[٢٣].

% inhibition = (Ac-At)/Ac ×١٠٠

RESULTS AND DISCUSSION:

The results showed in Table.١, the extract gave positive tests for (glycosides, proteins, saponins, tannins, various phenolic compounds alkaloids , flavonoids, steroids and vitamine C) similar results are also obtained by other studies[١٣،١٤] .

Table ١: Chemical components analysis for aqueous extracts of Miswak.

Components	Reagents	Note	Result aqueous extract
Glycosides	Iodine test Molish test Benedict test	Blue ppt. Violet ring Orange ppt.	+Ve +Ve +Ve
Proteins	Folin-Ciocalteau reagent	Blue color	+Ve
Saponins	Fast stirring Mercuric Chloride	Dense foam for long time White ppt.	+Ve +Ve
Phenolic compounds	١%Aqueous Ferric chloride	Green ppt.	+Ve
Tannins	١%Aqueous Ferric chloride ١%Lead acetate	Green ppt. Preface yellow ppt.	+Ve +Ve
Flavonoids	١%aqueous Ferric chloride Ethanol hydroxide alcohol	Green ppt. Yellow ppt.	+Ve +Ve
Alkaloids	Mayer's reagent Wagner reagent Picric acid	white ppt. Brown ppt. Yellow ppt.	+Ve +Ve +Ve
Steroids	Libermann-burchar Libermann's reagent	Green ppt. Blue color	+Ve +Ve
Test for Fats and Oils	Solubility test		+Ve
Test for Vitamine C	Ascorbic acid	Yellow ppt	+Ve

As indicated by (Table٢) aqueous extracts of *Salvadora persica* root scavenged the DPPH stable free radicals in a concentration ٢٥٠µl the extract showed activity ٦٢,٤٥%

Table ٢:Effect aqueous extracts of Miswak on DPPH Radical.

Conc. Miswak µg/ml	Ab	scavenging effect (%)
Control	٥٧٨±٠,٢٣	٠٠,٠٠
٢٥٠	٢١٧±٠,١٤	٦٢,٤٥

As shown in Table ٣ The amount of thiobarbituric acid reactive substance (TBARS) was calculated and percentage inhibition of TBARS formed was compared with control and standard drug (curcumin). The aqueous extracts of Salvadora persica root (٢٥٠µl) inhibited ٤٢,٠٤%.

Table ٣:Effect aqueous extracts of Miswak on Lipid peroxidation.

Conc. Miswak µg/ml	Ab	inhibition (%)
Control	٧٢٣±٠,٣٤	٠٠,٠٠
٢٥٠	٤١٩±٠,١٢	٤٢,٠٤

The aqueous extract of Miswak *(Salvadora persica* L.) contains glycosides, proteins, saponins, tannins, various phenolic compounds alkaloids , flavonoids, steroids and vitamine C [٢٤،٢٥،٢٦]. Free radicals are atoms or groups of atoms with an odd number of electrons and can damage important cellular components such as DNA or cell membranes. To prevent free radical damage, the body has a defense system of antioxidants [٢٧]. Scientific literature reveals that the carbonyl groups present in the flavonoids and phenolic compounds are responsible for the free radical scavenging activity [٢٨]. Literature survey reveals that flavonoids and other phenolic compounds are present in plant *S. persica* L. which are known to be responsible for the antioxidant activity; since it has phytoconstituents of antioxidant interest, thus present research concluded comparative evaluation of antioxidant activity of different extracts of *Salvadora persica* L..

DPPH radical is usually used as a form to study the scavenging potential of several natural compounds such as phenolic or crude extract of plants[٢٩]. The ability of aqueous extracts of *Salvadora persica L* to reduce DPPH radicals(٦٢,٤٥%), supports its free radical scavenging activity. Our study indicates the proton donating property may be responsible for free radical scavenging activity of arak . Antioxidant compounds for example, sesamol, gallic acid poly-phenols reduce the Fe^{3+} to Fe^{2+} and are considered as chain breaking antioxidant for their proton donating activity[٣٠].

Lipid peroxidation is an oxidative change of polyunsaturated fatty acids in the cell membranes that generates a number of degradation products[٣١]. TBARS, one of the products of lipid peroxidation, has been studied widely as an index of lipid peroxidation and as a marker of oxidative stress[٣٢]. We observed incubation of *S. persica L* extracts with brain homogenate reduced the lipid peroxidation(٤٠,٠٤%) at large extent which indicates the defensive effect of Miswak against lipid peroxidation and TBARS formation.

CONCLUSION:

The present study confirm that the aqueous extracts of *Salvadora persica L*. posses *in vitro* antioxidant activity because of its content (glycosides , tannins , saponins ,proteins ,various phenolic compounds ,alkaloids , flavonoids, steroids and vitamine C).

REFERENCES:

١. Yildrim A, Oktay M, Bilaloğlu V, The antioxidant activity of leaves of *Cydonia vulgaris*. Turkish Journal of Medical Science ٢٠٠١; ٣١:٢٣-٢٧.

٢. Huang DH, Chen C, Lin C, Lin Y, Antioxidant and antiproliferative activities of water spinach (*Ipomoea aquatica* Forsk.) constituents. Botanical Bulletin of Academic Science ٢٠٠٥; ٤٦:٩٩-١٠٦.

٣. Sun J, Chen Y, Li M, Ge Z. Role of antioxidant enzymes on ionizing radiation resistance. Free Radical Biology and Medicine ١٩٩٨; ٤٢:٥٨٦-٩٢.

٤. Khilfi S, Hachimi E, Khalil A, Es-Safi A, Belahyam A, Tellal A, El Abbouyi A. *In-vitro* antioxidant properties of *Salvia verbenaca*. L. hydromethanolic extract. Indian Journal of Pharmacology ٢٠٠٦; ٣٨:٢٧٦-٨٠.

٥. Gulcin I, Alici HA, Cesur M. Determination of *in- vitro* antioxidant and radical scavenging activities of propofol. Pharmacology Bulletin ٢٠٠٥; ٥٣:٢٨١-٨٥.

٦. Terao J, Piscula M, Yao MC. Protective effect of epicatechin, epicatechin gallate and quercetin on lipid peroxidation in phoshpolipid bilayers. Archives of Biochemistry and Biophysics ١٩٩٤; ٣٠٨:٢٧٨-٨٤.

٧. Akinmoladun AC, Ibukun EO, Afor E, Akinrinlola BL, Onibon TR, Akinboboye AO, Obutor EM, Farombi EO. Chemical constituents and antioxidant activity of *Alstonia boonei*. African Journal of Biotechnology ٢٠٠٧; ٦:١١٩٧-٢٠١.

٨. Khare C.P., Indian Medicinal Plants: An Illustrated Dictionary, Springer Publishers, ٢٠٠٧; ٥٧٤.

٩. Bhandari M. M., Flora of Indian desert, MPS. Repros, Jodhpur, ١٩٩٠; ١٩٣.

١٠. The Wealth of India, Publication and Information Directorate CSIR, Lucknow,١٩٧٢: ١٩٤–٩٥.

١١. Al-lafi T, Ababneh H . The effect of the extract of the Miswak (chewing sticks) used in Jordan and the Middle East on oral bacteria. Int Dent J١٩٩٥; ٤٥: ٢١٨–٢٢.

١٢. Kamil M, Jayaraj A F, Ahmad F, Gunasekhar C, Samuel S, Habibullah M & Chan K. A., Rare natural product from *Salvadora Persica* leaves; Natural Products Research; Amsterdam, ١٩٩٩; ٥٦٢.

١٣. Alali F, Hudaib M, Aburjai T, Khairallah K, Al-Hadidi N . GC-MS Analysis and Antimicrobial Activity of the Essential Oil from the Stem of the Jordanian Toothbrush Tree *Salvadora persica*. Pharmaceu. Biol.,٢٠٠٤; ٤٢: ٥٧٧-٨٠.

١٤. Rajesh V, Suresh P, Anil B, Brijesh K, Priyanka P . *Salvadora persica L* (Tooth Brush Tree)*:* A Review. J. P.R.,٢٠٠٩;٢: ١٨٠٩-١٢.

١٥. Saez TG, Oliva MR, Muniz P, Valls V, Iradi A, Ramos M . Oxidative stress and genetic damage. In: Health and orange, Valencia, Spain: Fundacion Valenciana de Estudios Avanzados,١٩٩٤;٥١–٦٠.

١٦. Bors W, Hellers W, Michel C, Saran M . Radical chemistry of flavonoids antioxidant. In: Emerit (ed) Antioxidants in therapy and preventive medicine,. Plenum, New York, ١٩٩٩;١: ١٦٥–٧٠.

١٧. Abuzinada AH, Al-Wetaid YI, Al-Basyouni SZM . The National Strategy for Conservation of Biodiversity in the Kingdom of Saudi Arabia. Prepared and issued by: The National Commission for Wildlife Conservation and Development. Conservation of Biological Diversity, Riyadh, Saudi Arabia.٢٠٠٥

١٨. Jassim,A.M.N. ," Study of Some *Cucurbita moschata* Duchesne ex poiret Leaves Components and Effect of Its Extracts on Different Microorganisms and Identification of Some Flavonoids by HPLC" ,Al-Mustansirya , J.Sci.,٢٠١٠;٢١:١٠١-١٠.

١٩. Al-Bayati,R.I.H., Naji,N.A. and Al-Sedah,M.M.M., "Study on The Effect of*Capparis Spinosa* Fruits Extracts on Acetylcholinesterase Activity in Human Blood",Mustansiriya J.Sci.,٢٠٠٢;١٣:١٤٦-٣١.

٢٠. Mohammed, M. T., " Study of Some *Mirabilis jalapa* L. Leaves Components and Effect of Their Extracts on Growth of Pathogenic bacteria" Al-Mustansirya J.Sci.٢٠١٢;٢٣:٦١١٧-٢٤.

٢١. Khandalwal KR. Practical pharmacognosy.New Delhi:Vallabh prakashan ١٩٩٤:١٤٩-٥٩.

٢٢. Blois,M.S Antioxidant determination by the use of stable free radical. Nature. ١٩٥٨; ٢٦: ١١٩٩-١٢٠٠.

٢٣. Rajakumar DV, Rao MNA. Antioxidant properties of dehydrozingerone and curcumin in rat brain homogenates. Molecular and cellular Biochemistry ١٩٩٤;١٤٠:٧٣-٧٩ .

٢٤. Kamil M, Jayaraj AF, Ahmad F, Gunasekhar C, Samuel S, Habibullah M & Chan K., Useof high-speed counter current chromatography for separation of flavonol glycoside from the leaves of *Salvadora persica*, Natural Products and Medicinal Chemistry, ٢٠٠٠; ٤٣: ١٤٠- ٥٥.

٢٥. Kamil M, Jayaraj A F, Ahmad F, Gunasekhar C, Samuel S, Habibullah M & Chan K. Isolation and identification of a flavonol glycoside using high speed counter current chromatographic technique from the leaves of *Salvadora persica*, Pakistan Journal of Scientific and Industrial Research, ٢٠٠٠; ٤٣:٢٢٥-٢٧.

٢٦. Kamil M, Jayaraj A F, Ahmed F, Gunasekhar C, Samuel S, Habibullah M & Chan K., Pharmacognostic and phytochemical studies on *Salvadora persica* L., Journal of Pharmacy and Pharmacology,١٩٩٩; ٥١: ٢٢٧.

٢٧. Thomas M. J., The role of free radical and antioxidant, Nutrition, ٢٠٠٠; ١٦: ٧١٦.

٢٨. Nicholls D.G. & Budd S. L, Mitochondria and neuronal survival, Physiological Reviews, ٢٠٠٠;٨٠:٣١٥–٦٠.

٢٩. Veerapur VP, Prabhakar KR, Parihar VK, Kandadi MR, Ramakrishana S, Mishra B, Satish Rao SB, Srinivasan KK, Priyadarsini KI, Unnikrishnan MK. *Ficus racemosa* stem bark extract: a potent antioxidant and a probable natural radioprotector. Evid. Based Complement Alternat. Med.٢٠٠٩; ٦:٣١٧-٢٤ .

٣٠. Parihar VK, Prabhakar KR, Veerapur VP, Sudheer KM, Rosi Reddy Y, Joshi R, Unnikrishnan MK, Rao MC. Effect of sesamol on radiation induced cytotoxicity in Swiss albino mice. Mutation Research٢٠٠٦; ٦١١: ١-٩ .

٣١. Pratt DA, Tallman KA, Porter NA. Free radical oxidation of polyunsaturated lipids: New mechanistic insights and the development of peroxyl radical clocks. Acc. Chem. Res.٢٠١١; ٤٤: ٤٥٨-٦٧.

٣٢. Prabhakar KR, Veerapur VP, Parihar VK, Priyadarsini KI, Rao BSS, Unnikrishnan MK. Evaluation and optimization of radioprotective activity of Coronopus didymus Linn. in gamma-irradiated mice. Int. J. Radiat. Biol.٢٠٠٦;٨٢:٥٢٥-٣٦ .