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STUDY OF SOME *SALVIA OFFICINALIS L.* (SAGE) COMPONENTS AND EFFECT OF THEIR AQUEOUS EXTRACT ON ANTIOXIDANT

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ABSTRACT

Antioxidants prevent the reaction of free radicals with biomolecules and can remind the nutritional values and physiological properties of foodstuffs. Nowadays there is an increasing trend among food technologists to replace the synthetic kind of antioxidants with the natural ones. Spices and herbs have been used not only for flavoring food but also for improving the overall quality of the product and to extend the shelf life of foods due to their antioxidant properties. Therefore, possible use aqueous extracts of *Salvia officinalis* to protect brain against the Lipid peroxidation. The present study was undertaken to evaluate the potential of *Salvia officinalis* against the DPPH free radical scavenging system and lipid peroxidation. The chemical components of the prepared aqueous extracts of *Salvia officinalis* 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. The study showed that the *Salvia officinalis* in the aqueous extracts contain: glycosides, proteins, saponins, tannins, phenolic compounds, flavonoids, alkaloids, steroids and vitamine C. Aqueous extracts were found effective in scavenging DPPH 48.2% in concentration 100 µL while a concentration 250 µL the extract showed activity 69.89%, as well as inhibiting the lipid peroxidation 20.92% in concentration 100 µL while 250 µL inhibited 31.79%. Our results suggest that *Salvia officinalis* treatment protects the rat brain against lipid peroxidation and DPPH free radical scavenging.

Key words: *Salvia officinalis*, Phytochemical, DPPH, Lipid peroxidation.

INTRODUCTION

The term ‘antioxidant’ refers to the activity of numerous vitamins, minerals and phytochemicals, which provide protection against the damage caused by ROS[1]. Antioxidants

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interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals and by acting as electron donors[2]. The natural antioxidant mechanisms may be insufficient in variety of conditions and hence dietary intake of antioxidant compounds are important[3]. 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[4]. 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[5].

Herbal treatment of many diseases including hepatopathy is increasing in many countries**.** Some plants have been shown to have protective antioxidant effects and are therefore hepatoprotective. Such plants include *Salvia officinalis* L (sage) is an aromatic and ornamental herb, known from Greeks and Romans ancient times and mentioned in the papers of Dioscorides and Galen. It is found in the spontaneous vegetation in Dalmatia, Croatia, Bosnia, Herzegovina, Serbia, Bulgaria, Albania, Macedonia, Greece and the Iberian peninsula[6]. Some species have economic importance while others are used as spices or flavors in perfumery and cosmetics[7].

In Romania, from the approximately 500 species of the genus Salvia, 3 species are occurring, from which only 2 species are cultivated (*Salvia officinalis*, *Salvia splendens*-grown as an ornamental), both a spontaneous and a cultivated species (*Salvia sclarea* L.-*Serlai*) and 10 spontaneous species, many of them common throughout the country (*Salvia Glutionsa* L. *Cinstet* – shady places, woods, *Salvia pratensis* – on fields, *Salvia nemorosa* L. and *Salvia nutans* L., *Salvia austriaca* Jacq, *Salvia verticillata* L.- in open places). One of these species – *Salvia transilvanica Schur*- is endemic in Romania[6].

The medicinal Salvia has the main importance. The essential oils contained in Salvia officinalis are used due to their healing properties in a wide range of diseases of the nervous system, heart and circulatory system, the respiratory illness, digestive, metabolic and endocrine diseases, while Salvia officinalis infusion is used for its haemostatic effect, estrogen, antiperspirant, antiseptic, hypoglycemia and for many other therapeutic properties[8] Phytochemical investigation of Salvia officinalis revealed a great number of bioactive compounds possesing a variety of biological activities. The main bioactive ingredient of Salvia officinalis is its essentials oil[9]. Essential oils of Salvia stenophyla, Salvia runcinata and Salvia repens exhibit anti-inflammatory and antimalarian properties[10]. Essentials oils from Salvia officinalis and Salvia trilobata were found to have antibacterial action[7]. Salvia

milthiorrhiza is one of the most important and popular Chinese medicinal plants and is used for the prevention and tratment of stasis, pains, dysmenorrhea, heart disease, liver and intumescente of the spleen[11] Membrane processes, such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) represent processes having potentiality for the concentration of medicinal plant extracts. Ultrafiltration processes offer many advantages over the conventional technologies. When compared with other classical methods, the membrane processes have the advantages of separation, purification and concentration of a certain compound, in a single phase, at the ambient temperature, without the interference of any other chemical reactive[12-16].

EXPERIMENTAL

Materials and methods

Collection and treatment of samples

The *Salvia officinalis* were collected from market of Baghdad, Iraq. The herb 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 25-30ºC 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[17].

Preparation of extracts

Air dried root 50 g were suspended in one liter of distilled water and left for 24 hrs at 35ºC with continuous stirring in shaking incubator. Then the mixture was filtered by filter paper, the filtrate was centrifuged for 10 min. at 2500 rpm, and the extract evaporated to dryness at 40ºC in the incubator[18].

Chemical detection of the plant components

The chemical components of the prepared watery extract were detected as shown in Table 1. They included: glycosides, alkaloids, saponins, phenolic compounds, tannins, flavonoids, proteins, steroids and Vitamine C[17,19].

DPPH Free radical scavenging system

The effect of plant extracts on (2 diphenyl 2 picryl hydrazyl hydrate) DPPH radical was estimated according to the method of Blois. The absorbance of the resulting solution

was measured spectro photometrically at 520 nm. Results were expressed as percentage inhibition of DPPH by comparing with blank[20].

$$\text{Scavenging effect (\%)} = (Ac\text{-}At)/Ac \times 100$$

Lipid peroxidation

The brain isolated from healthy albino rat (200-230 g) was used as lipid source. Brain homogenate (10% w/v) was prepared in 150 mM KCl and centrifuged at 800 g for 10 mins. The supernatant was collected and used immediately to study *in vitro* lipid peroxidation. Briefly, the reaction mixture contained 0.3 mL of brain homogenate, KCl (100) M), ascorbic acid (100) M), ferric chloride (100) M), 0.5 mL of graded concentrations of extracts and final volume was made with buffer. After incubating at room temperature for 20 mins, 1.0 ml of thiobarbituric acid-trichloroacetic acid (TBATCA) reagent was added. The resulted mixtures were heated at 800C for 20 mins, cool and centrifuged for 10 mins at 1000 rpm and by using a digital UV/VIS spectrophotometer recorded the absorbance at 532 nm. Control and standard (curcumin 10) 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 (21):

$$\text{\% inhibition} = (Ac\text{-}At)/Ac \times 100$$

RESULTS AND DISCUSSION

The results showed in Table 1, 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.

Table 1: Chemical components analysis for aqueous extracts of Salvia officinalis

Components	Reagents	Note	Result
Glycosides	Iodine test	Blue ppt.	Ve+
	Molish test	Violet ring	Ve+
	Benedict test	Orange ppt.	Ve+
Proteins	Folin-ciocalteau reagent	Blue color	Ve+

Cont…

Components	Reagents	Note	Result
Saponins	Fast stirring	Dense foam for long time	Ve+
	Mercuric chloride	White ppt.	Ve+
Phenolic compounds	1% Aqueous ferric chloride	Green ppt.	Ve+
Tannins	1% Aqueous ferric chloride	Green ppt.	Ve+
	Lead acetate%1	Preface yellow ppt.	Ve+
Flavonoids	1% Aqueous ferric chloride	Green ppt.	Ve+
	Ethanol hydroxide alcohol	Yellow ppt.	Ve+
Alkaloids	Mayer's reagent	white ppt.	Ve+
	Wagner reagent	Brown ppt.	Ve+
	Picric acid	Yellow ppt.	Ve+
Steroids	Libermann-burchar	Green ppt.	Ve+
	Libermann's reagent	Blue color	Ve+
Test for fats and oils	Solubility test		Ve+
Test for Vitamine C	Ascorbic acid	Yellow ppt	Ve+

As indicated by Table 2 aqueous extract of *Salvia officinalis* scavenged the DPPH stable free radicals in a concentration 100 µL the extract showed activity 48.2% while a concentration 250 µL the extract showed activity 69.89%

Table 2: Effect aqueous extracts of *Salvia officinalis*on DPPH Radical

Conc. *Salvia officinalis* (µg/mL)	Ab	Scavenging effect (%)
Control	558 ± 0.16	00.00
100	289 ± 0.34	48.2
250	168 ± 0.41	69.89

As shown in Table 3 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 *Salvia officinalis* (100 µL) inhibited 20.92% while (250 µL) inhibited 31.79%

Table 3: Effect aqueous extracts of *Salvia officinalis* on lipid peroxidation

Conc. *Salvia officinalis* (µg/mL)	Ab	Inhibition (%)
Control	717 ± 0.39	00.00
100	567 ± 0.24	20.92
250	489 ± 0.29	31.79

Plants are a source of different chemical compounds, which make them of a medicinal importance. These compounds are divided in to two types (inert and active constituents) depending on their activity. The inert constituents are defined as compounds that have no medicinal or physiological effects; for instance cellulose, lignin and subrine, while active constituents have these effects. The active constituents in turn are divided into other types (glycosides, tannins, saponins ,proteins, various phenolic compounds ,alkaloids , flavonoids, steroids and vitamine C) depending on their chemical and physical characteristics (22) and the chemical analysis of sage (*S. officinalis*) extract aqueous revealed some of these constituents (steroids, tanins, glycosides, flavonoids, saponines and terpens). A water-soluble polysaccharides complex from *S. officinalis* composed of galactose, glucose, mannose, xylose, and fructose have shown an immunomodulatory activity in the comitogenic thymocyte test, which is interpreted as being an *in vitro* correlate of adjuvant activity in addition to their mitogenic activity (23).

Literature survey reveals that flavonoids and other phenolic compounds are present in plant *Salvia officinalis*, 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 *Salvia officinalis*.

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 (24). The ability of aqueous extracts of *Salvia officinalis* to reduce DPPH radicals (62.45%), supports its free radical scavenging activity. Our study indicates the proton donating property may be responsible for free radical scavenging activity of sage. Antioxidant compounds for example, gallic acid poly-phenols reduce the Fe^{3+} to Fe^{2+} and are considered as chain breaking antioxidant for their proton donating activity (25).

Lipid per-oxidation is an oxidative change of polyunsaturated fatty acids in the cell membranes that generates a number of degradation products. TBARS, one of the products of

lipid per-oxidation, has been studied widely as an index of lipid peroxidation and as a marker of oxidative stress. We observed incubation of *Salvia officinalis* extract with brain homogenate reduced the lipid per-oxidation at large extent, which indicates the defensive effect of *Salvia officinalis* against lipid per-oxidation and TBARS formation.

CONCLUSION

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

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