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Research paper

Extraction, characterization, and assessment of the antioxidant activity of salvia officinalis extracts

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Abstract

Background : One of the biggest and most significant fragrant and medicinal genera in the Lamiaceae family is salvia (sage). Sage infusion can have numerous health advantages, including anti-mycotic, anti-carcinogenic, antidiabetic, antibacterial, antioxidant, anti-inflammatory, and anti-proliferative effects. By lowering free radicals, antioxidants are the chemical compounds that stop or postpone the oxidation process. Lipid oxidation causes color changes, texture problems, odd flavors, nutrient loss, and the creation of hazardous chemicals. Aim of study evaluation of the activity of Salvia officinalis extracts as antioxidants., Methods: Investigated was the chemical detection of active substances. the existence of an active substances and the use of aqueous and alcoholic extracts separately for toxicity test in mice in three concentrations: (10, 20,40 %) it was given orally and the dose ranged between (0.1-0.2)ml twice a day. Leave the mice for 72 h with monitoring to determine the toxicity of the extracts or not, and evaluation of oxidative stress efficiency by using the DDPH method, Results: The presence of these compounds in extracts of the sage plant, which are compounds that have an effectiveness against cancer cells, which lies in the removal of free radicals (OH. O) they contribute to protecting cells and tissues from active oxygen, The toxicity test results of the aqueous and alcoholic extract showed that they were free of toxicity after oral dosing of mice, The active compounds in the prepared extracts have different polarity, in addition to the use of two different solvents, (water and ethanol), which lead differing results of the effectiveness of the aqueous and alcoholic extracts as antioxidants compared to the gallic acid. Conclusion: The tests for the detection of the active groups of the extracts showed that they are affected by the nature of the solvent in terms of polarity, which affects the type and concentration of the groups extracted and the nature of the extraction, The toxicity test results showed that the aqueous and alcoholic extracts were non-toxic, the results of the antioxidant activity test showed that the alcoholic extract of the sage plant is more effective than the aqueous extract.

Keywords: *Phytochemical; Sage; Antioxidant Activity.*

1. Introduction

Salvia (sage), one of the biggest and most significant fragrant and medicinal genera of the Lamiaceae family, has around 1000 species that are scattered throughout the world. It has a long history of usage in medicine and cuisine, and it is also utilized as an ornamental garden plant today. There are numerous related and unrelated species that go by the common name "sage.". [1], [2]. Sage infusion can have numerous health advantages, including anti-mycotic, anti-carcinogenic, antidiabetic, antibacterial, antioxidant, anti-inflammatory, and anti-proliferative properties [3]. Sage infusion also has other effects, including antiradical activity, which is highly correlated with their high level of total phenolic content [4], [5]. By lowering free radicals, antioxidants are the chemical compounds that stop or postpone the oxidation process. Lipid oxidation causes color deterioration, texture degradation, bad flavors, nutritional loss, and hazardous chemical production [6]. Additionally, free fatty acids are produced as lipids are digested, and these acids might go through additional secondary oxidative modifications. Reactive oxygen species (ROS) and free radicals are well-known initiators of cellular and tissue pathogenesis that result in a number of human diseases, including atherosclerosis, cancer, and cardiovascular diseases [7], [8]. The phenolic chemicals in spices are responsible for their antioxidant activity, which is why they behave similarly to synthetic antioxidants.

2. Materials and methods

2.1. Sample collection

The sage plant was obtained from the local Jordanian market. The plant variety was obtained from Baghdad University - College of Science - Herbal plant and it was found that it is a salvia officinalis type .

The leaves were washed thoroughly with water to remove dust and impurities, and allowed to dry in the shade with Constant flipping to prevent rot. After drying, the leaves are ground with an electric grinder to obtain a fine powder.

2.2. Preparation of the extract

a) Water extract

We take 100 g. From sage leaf powder, immerse it in 400 ml of distilled water in a 1000 ml conical flask, then place it in a vibratory incubator at 37 ° C for 6 hours, then filter it with several layers of clean cloth and then filter it with the solution using a Buechner funnel with filtration Leaf-type (watt man No. 1). the solution was then dried with a spray dryer at 60 ° C, the final dry weight obtained was 7 g. [9]

b) Alcoholic extract

We take 100 g. From sage leaf powder, immerse it in 5 00 ml of 80 ml ethanol alcohol in a 1000 ml conical flask, then place it in a vibratory incubator at 37 ° C for 6 hours, then filter it with several layers of clean cloth and then line it with a clean cloth. The solution was then dried with a spray dryer at 60 ° C, and the final dry weight obtained was 6 g. [9]

We take a concentration of 10% of each product by dissolving 1 g of dry powder in 10 ml of distilled water and then prepare the other concentrations in the toxicity test (20%, 40%).

2.3. Chemical detection of active compounds:

Chemical detection techniques have been used to look for the presence of active substances in the following ways:

a) Mix 1 ml of extract with 1 ml of water lead acetate (1%); if a white deposit appears, the test is successful and tannins are present.
b) Monitoring for carbs Mix 1 ml of the form and 5 drops of Alpha Naphthol Alcoholic in a tube with Molisch's reagent. Including a blue ring to denote the presence of carbohydrates and 2.5 mL of sulfuric acid.[11]
c) c. Glycoside detection Test: The Fehling reagent's ability to identify glycosides and the appearance of a crimson deposit both point to the presence of glycosides. [10]
d) d- Phenol's test: Mix (0.1 g) of the extract with (1-2) drops of FeCl3 solution in 1 ml of distilled water. When the color turns blue or green, it's a good sign that there are phenols present. [11]
e) e- Lead acetate test: To 1 ml of the extract, add 1 ml of lead acetate (1%). When white precipitation is discovered, the test is successful and resins are present. [11]
f) Detection of Flavonoids Test: When a yellow deposit forms after adding 1 ml of the Alcoholic potassium hydroxide reagent to 1 ml of the extract, the test is positive and shows the presence of flavonoids. [12]
g) Test for the presence of saponins: Mix 1 ml of the extract with 5 percent Mercury Water Chloride Reagent. Finding white precipitation is a sign that soap is present, which is a good sign. [12]
h) Alkaloid test: Wagner's reagent is used to detect alkaloids by adding a few drops to one milliliter of extract; if an acorn forms, the test is successful and alkaloids are present. [10]
i) Protein detection: Detection of proteins employing a papurite detection composed of 1 ml of a 10% reagent and (80%) copper sulphate diluted in distilled water. When the presence of proteins is shown by the color violet. [12]
j) Coumarins test: Put some of the sample in a test tube, cover it with filter paper that has been soaked with diluted sodium hydroxide solution, heat it on a boiling water bath for a few minutes, and then look for coumarins by exposing the filter paper to ultraviolet light. Bright green and yellow coloration on the paper indicates the presence of coumarin. [10]
k) steroids and terpene testing Test: One gram from the model, one drop of strong sulfuric acid, and one drop of acetic anhydride are added to a little amount of chloroform. When compared to the brown color, which looks to symbolize the containment of the soil model, the dark blue Fidel hue holds the steroids. [12].

2.3.1. Toxicity test

The extracted materials were tested using (mice) in the laboratory, where they were divided into three groups, and each group contained three injected mice with different concentrations and an amount of 4 replicates.

The experiment was conducted at Al-Nahrain University / Biotechnology Research Center to examine the toxicity of aqueous and alcoholic sage extracts and it was as follows:

The use of aqueous and alcoholic extracts separately in three concentrations: (10, 20,40 %) it was given orally and the dose ranged between (0.1-0.2) ml twice a day. Leave the mice for 72 h with monitoring to determine the toxicity of the extracts or not.

2.3.2. Evaluation of oxidative stress efficiency by using the DDPH method

An amount (5 ml) of (DPPH) 2,2-diphenyl-1-picryhydrazyl was prepared at a concentration of (0.004%) in methanol and mixed with (50 µl) for different concentrations of the extract to be evaluated for its effectiveness as an antioxidant is as follows (5, 10, 15 25, 35, 50) mg / ml, mix each concentration with a solution of prepared DPPH, leave for (30 minutes). The absorbance of each of them was measured at a wavelength (517 nm) using a Spectrophotometer, and methanol alcohol (Blank) was used to zero the device.

The process was repeated using Gallic acid as a natural antioxidant, the tests were repeated [3] times and the percentage of reductase DPPH, that showed the ability to reduce free radicals was calculated from the following equation:

Reduction %= [Abs (DPPH) – Abs Dil.]/Abs (DPPH)] *100

Whereas

Abs (DPPH)= Absorbance rate of a solution DPPH

Abs Dil.= The absorbance rate of a dilute solution from the extract whose effectiveness as an antioxidant is to be measured with DPPH. [16]

3. Result and discussion

Chemical identification of sage's primary constituents in alcoholic and aqueous extracts According to Table 1, chemical analysis using the aforementioned reagents revealed that the sage plant's extract contains tannins, carbohydrates, phenols, glycosides, flavonoids, soap, alkaloids, and coumarins in both its alcoholic and water extracts, but did not detect resins, proteins, or terpenes.

Table 1: Chemical Components of the Extract of Alcohol and Water for Sage

Active chemical compounds	Sage plant extract	
	Water	Alcoholic
Carbohydrate	Positive	positive
Resins	negative	negative
Phenols	negative	negative
Proteins	negative	negative
Tannins	positive	positive
Terpenes	negative	negative
Soap	positive	positive
Alkaloids	positive	positive
Glycoside	positive	- positive
Coumarins	positive	positive
Flavonoids	positive	positive

(+)=The substance is present in the plant. (-)=the substance is not present in the plant

Phenols and flavonoids are characterized by their effectiveness against cancer cells [17], which lies in the removal of free radicals (OH. O) [18] they contribute to protecting cells and tissues from active oxygen [19].
The toxicity test results of the aqueous and alcoholic extract showed that they were free of toxicity after oral dosing of mice with several concentrations of each extract and following them up for 72 h as shown in the table [2].

Table 1: Toxicity Study of Aqueous and Alcoholic Extract

Type of extract	Con.	No. of mice	The amount of extract given	Times	Results
aqueous and alcoholic extract	10%	3	(0.1-0.2)ml	72 h	Non-toxic
	20%	3	(0.1-0.2)ml	72 h	Non-toxic
	40%	3	(0.1-0.2)ml	72 h	Non-toxic

The active compounds in the prepared extracts have different polarity, in addition to the use of two different solvents, (water and ethanol), which lead differing results of the effectiveness of the aqueous and alcoholic extracts as antioxidants compared to the gallic acid shown in the table 3.
The effectiveness of the compounds depends on the efficiency of losing the hydrogen atom, as the values of IC50 showed that the highest effectiveness of the alcoholic extract of the sage plant compared with the aqueous extract is due to the alcoholic extract containing flavonoids, phenols and alkaloids with a higher concentration than (++), where there is a direct relationship between Polyphenolic compounds and their IC50values which increase the ability to capture free radicals. [20]

Table 3: The Ability to Capture Free Radicals IC50 for Alcoholic and Aqueous Extract of the Sage Plant

Con. DPPH (mg/ml)	Aqueous extract	Alcoholic extract	Gallic acid
50	29.6	37.88	70.2
100	37.23	48.1	84.1
200	47.5	55	88
300	54.9	62.3	91.8
400	63.1	71.3	94.07

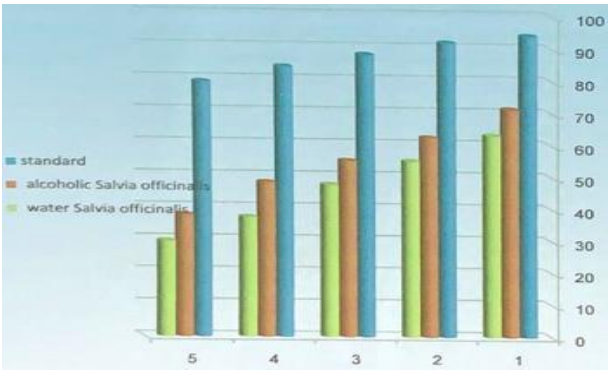

Fig. 1: The Percentage of A Compound Reductase DPPH by Using the Extracts of the Sage Plant, Alcoholic and Aqueous, with Antioxidant (Gallic Acid) as A Control, After 30 Min. of Exposure and the Corresponding Value IC50.

The process of removing free radicals from the important activities of vital systems through which the body is eliminate of the dangerous effects resulting from their formation inside the body that occur naturally as a result of metabolism or as a result of immune activities as a defense against some pathogens that can attack the body and there are different ways to measure the effectiveness of antioxidant The method of using a substance DPPH is considered one of the accepted methods in evaluating the efficiency of antioxidant activity of various substances and plant extracts, as the compound DPPH is one of the main free radicals that can receive an electron or hydrogen root to form a molecule. [21 - 23]

4. Conclusion

The tests for the detection of the active groups of the extracts showed that they are affected by the nature of the solvent in terms of polarity, which affects the type and concentration of the groups extracted and the nature of the extraction. The toxicity test results showed that the aqueous and alcoholic extracts were non-toxic. The findings of the antioxidant activity test revealed that the sage plant's alcoholic extract is superior to the plant's aqueous extract.

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