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The Antimicrobial Activity of Flavonoids Nanoparticles Obtained from *Salvia officinalis* Extract

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Abstract

Nanoparticles have recently been applied to increase the antimicrobial activity and the spectrum of action of existing, well-known therapeutic medications or herbal products. The present study sought to synthesize and investigate the antimicrobial effects of *Salvia officinalis* flavonoid extract and its corresponding flavonoid nanoparticles. The study also aimed to detect and quantify the flavonoids present in *Salvia officinalis*, assess their antioxidant activity, and identify the active chemical constituents using Fourier Transform Infrared (FTIR) spectroscopy. Additionally, Scanning Electron Microscopy (SEM) test was conducted to investigate the size of the produced nanoparticles. The extracted *Salvia officinalis* flavonoids demonstrated notable antioxidant activity compared to the control (vitamin C). FTIR spectroscopy demonstrated the presence of multiple functional groups, including amine (–NH), aliphatic (–CH_2), olefinic (–C=C–), and carbonyl (C=O) groups. The flavonoid nanoparticles exhibited stronger antimicrobial activity than the crude flavonoid extract against the three reference microbial strains tested in this study.

Keywords: Antimicrobial activity, Flavonoids, Nanoparticles, *Salvia officinalis*

Introduction

Anciently and recently, *Salvia officinalis* (sage) was used in food as flavor and preservative; however, *Salvia officinalis* was used in medicine due to its powerful antimicrobial and antioxidant activity in addition to its application in industries (El-Feky & Aboulthana, 2016). Excessive consumption of antimicrobial agents has led to increasing microbial resistance and necessitates the finding of new compounds from plant extracts with less toxic side effects (Dallal *et al*., 2011).

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The effectiveness of various plant extracts has been studied worldwide. Many studies have attributed their antimicrobial potential to their content of various phytochemical compounds. A number of these studies showed that extracts from plants in the Lamiaceae family, such as Salvia officinalis, exhibit biological activity against bacteria and yeasts (Mosafa *et al*., 2014; Jakovljević *et al*., 2019).

Nanoparticles of *Salvia officinalis* have shown unique properties for controlling infections, owing to their activity against bacteria, fungi, and viruses, as well as their anticancer and anti-inflammatory activities. Additionally, natural antioxidants present in *Salvia officinalis,* belonging to phytochemicals and phenolic compounds such as flavonoids, tannins, and lignin, may supplement the need in the human body for antioxidants that neutralize the free radicals released from the endogenous metabolic activity (Behravan *et al*., 2019; Hashim & Qasim, 2022; Qasim & Hashim, 2023).

This research aimed to: extract *Salvia officinalis* flavonoids and synthesize flavonoid nanoparticles, measure antioxidant activity of *Salvia officinalis* flavonoids, detect the functional chemical compounds via FTIR, synthesize *Salvia officinalis* nanoparticles with characterization of size by SEM, in addition to the assessment of the antimicrobial Activity of *Salvia officinalis* flavonoids and its nanoparticles.

Materials and Methods

Extraction Method

The dried leaves of *Salvia officinalis* were purchased from India. In the lab, *Salvia officinalis* leaves were crumbled into small pieces, then finally reduced to powder by an electrical grinder. One hundred grams of *Salvia officinalis* fine powder was used for flavonoid extraction with 1000 ml of 70% ethanol as a solvent. The dried plant and the extracting solvent were divided into flasks of equal amounts, then a sequence of flasks was dipped into an ultrasonic bath (40 kHz frequency, 40±1 °C, 20 min), when almost the highest amount of extracted materials in the liquid extracts was reached (Altemimi *et al*., 2017).

Detection of Flavonoids

Flavonoids were detected in this study according to Somnath and co-workers 2017 (Somnath De *et al*., 2017), which was achieved by adding 1 mL of ultrasonic methanolic extract of *Salvia*

officinalis to 1 mL of 10% Lead acetate, with shaking the solution. Yellow to brown precipitation formation indicated the detection of flavonoids and phenolic extracts.

Flavonoids Amount Determination

The total flavonoid component was investigated by spectrophotometer; 1 mL of *Salvia officinalis* flavonoid extract was mixed with 1% aluminum chloride (AlCl3) in ethanol, then the absorbance of the mixture (415 nm, 30 min) was measured against a blank (ethanol) to determine the flavonoid content using quercetin as the standard flavonoid.

Flavonoid Identification

The sonicated flavonoid extract was spotted distinctly with the quercetin standard on a 5×10 cm thin-layer chromatography (TLC) plate coated with silica gel. The mobile phase (1-Butanol, glacial acetic acid, and water) were used and retardation factor (Rf value) was estimated after 1 h from the air dried plate. The formed dots were harvested from the TLC plates and submerged in methanol; the solution was then tested separately using a UV-visible spectrophotometer and an FT-IR spectrometer for the detection of phenolic compounds and flavonoid derivatives (Abdul-Jalil *et al.*, 2010).

Nanoparticles Synthesis

In order to synthesize *Salvia officinalis* flavonoids in the form of silver nanoparticles, the extract was used as a reducing agents to prepare *Salvia officinalis* AgNPs. The nanoparticles were formed by the addition of 1 mL of *Salvia officinalis* flavonoids extract (drop by drop) to 2 mL of silver nitrate solution under heating (55 °C) for 25 min with continues stirring. A change in color was objectively observed turning into a brown colloidal dispersion following the addition of the AgNO3 solution, which indicated the formation of *Salvia officinalis* flavonoids nanoparticles. After that, the solution containing nanoparticles was centrifuges at 8000 rpm for 10 min. The dried sediment of AgNPs was stored in a labelled container in the refrigerator for subsequent nanoparticle size detection and antimicrobial activity assessment. *Salvia officinalis* flavonoids yielded 0.002 g of *Salvia officinalis* flavonoids nanoparticles (Guzman *et al*., 2012).

Antimicrobial Susceptibility Test

Reference strains used in this study, *Staphylococcus aureus* ATCC 3297*, Pseudomonas aeruginosa* 6422, and *Candida albicans* 7521, were kindly provided by the Microbiology Laboratory of College of Pharmacy, University of Mosul (Iraq). The antimicrobial Activity of *Salvia officinalis* flavonoids and its flavonoids nanoparticles was estimated using disc agar diffusion method. The bacterial strains were sub-cultured on the appropriate selective medium for each type of bacteria and handled aerobically for 24 h at 37 ˚C, while *Candida albicans* was sub-cultured on Sabouraud's agar with chloramphenicol and handled aerobically for 48-72 hours at 37 ˚C (Gudi *et al*., 2016).

Pure culture colonies of *S. aureus*, *P. aeruginosa*, and *Candida albicans* were separately inoculated into 3-5 mL of Mueller-Hinton broth; each suspension was then adjusted to a 0.5 MacFarland standard visually. A cotton swab was soaked in a bacterial and candidal inoculum suspension and then streaked across the entire surface of Muller-Hinton agar plates in triplicate (Hashim, 2022; Abid & Abachi, 2023).

Discs were made from sterile filter paper, Whatman No.1, primed with a range of concentrations of *Salvia officinalis* flavonoids and *Salvia officinalis* flavonoids nanoparticles prepared from stock concentrations (0.5,1.0, 1.5, 2, 2.5,3 mg/ml) and kept overnight for complete saturation (Mosafa *et al*., 2014). The discs were placed on the inoculated Muller-Hinton agar using sterile forceps, and the plates were incubated aerobically at 37 °C for 24 h (bacteria) and 48 h (*C. albicans*). At the end of this time, the inhibition zone diameters were measured for antimicrobial activity assessment. Chloramphenicol (10 µg) was employed as the positive control for bacteria and voriconazole (1 µg) for *Candida albicans*. The mean diameter of inhibition zones (mm) was calculated.

Antioxidant Activity Test

Antioxidant activity of flavonoids was calculated from the scavenging activity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. Three volumes (200-100-50 µl) were taken from *Salvia officinalis* flavonoids, then the volume was topped up to 1 ml with distilled water, and then 2 ml of DPPH was added to each test tube, followed by thorough mixing, and then the test tubes were incubated at room temperature for 30 min. The comparative control group was formulated from an ascorbic acid solution. The absorbance (A) was determined at 517 nm using spectrophotometer. Estimation of the inhibition of DPPH free radical in 1% was completed based on the following equation (Mutingatun *et al*., 2022):

$$1\% = \{ (Ac - As) / Ac\} * 100 \quad (1)$$

Fourier Transforms Infrared Spectroscopy (FTIR)

ATR-FTIR spectrophotometer (Bruker-Alpha, Germany) was used to print out the FTIR curves of sonicated active flavonoids, the extracted product was grounded and dried when employed to be examined at 800-4000 cm-1, band intensities expressed as transmittance as: strong (s), medium (m), and weak (w) (Gudi *et al*., 2016).

Scanning Electron Microscope (SEM)

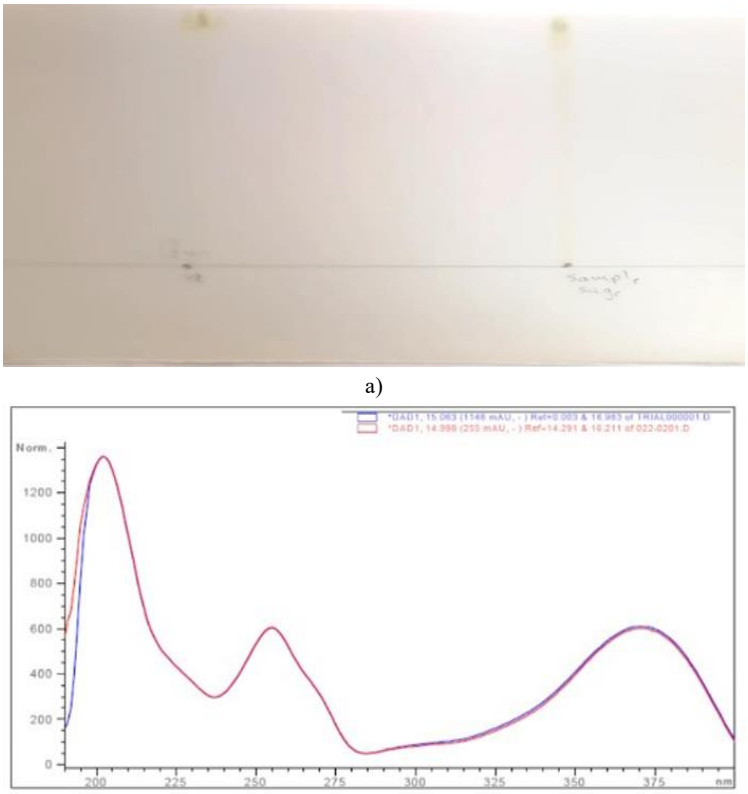
A TESCAN MIRA 3 (Czech Republic) was used to observe the size and morphology of Salvia officinalis flavonoid AgNPs (Zare, 2020).

Results and Discussion

Flavonoid isolation and determination: *Salvia officinalis* flavonoids had a deep yellow to brown color and a crystalline appearance. For each gram of *Salvia officinalis* leaves, 25 mg of flavonoids were obtained **(Table 1)**. Thin-layer chromatograph showed one spot with R_f value at 0.88 **(Figure 1a)**, in addition to a maximum absorption of *Salvia officinalis* flavonoids and quercetin at 225 nm **(Figure 1b)**.

Table 1. Thin-layer chromatography of separated compounds

Solvent: ratio	Test methods	R_f value
Butanol: 70	Naked eye	0.88, yellow
Acetic acid: 25	UV	0.88, fluorescent
Water: 5	Iodine and ammonia vapors	0.88, brown



a)

b)

Figure 1. a) TLC chromatograph of *Salvia officinalis* flavonoids and quercetin. b) Ultraviolet spectrum of *Salvia officinalis* flavonoids and quercetin.

Antioxidant Activity Test of Salvia officinalis

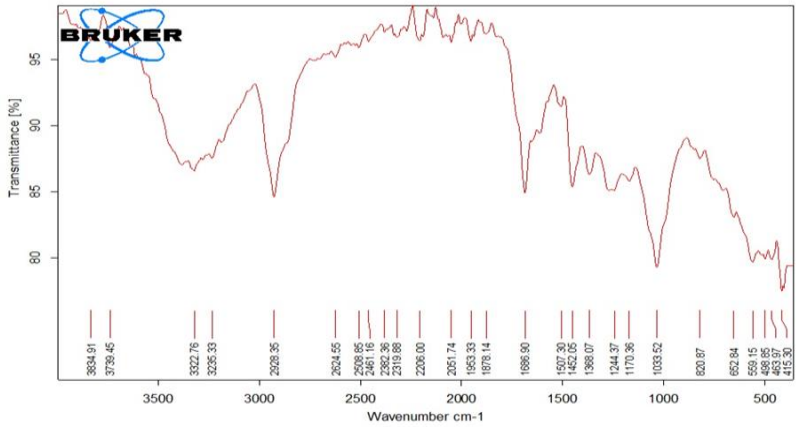
Scavenging activity of *Salvia officinalis* flavonoids was increased with increasing concentration. The antioxidant activity of *Salvia officinalis* flavonoids was comparable to that of ascorbic acid, used as a control in this study, as shown in **Table 2**.

Table 2. The antioxidant activity of *Salvia officinalis* flavonoids and ascorbic acid compared to DPPH radicals.

Extract	Concentration (ppm)	Scavenging activity %
Salvia officinalis flavonoids	2500	67
	5000	71
	7500	82
Ascorbic acid	7.5	92.6
	15	92.9
	23	95.5

FTIR Spectroscopy

Analytical methods, such as attenuated total reflectance Fourier Transform Infrared (ATR-FTIR), were used in this study to measure the wavelengths and band intensities of *Salvia officinalis* flavonoids. FTIR measurement showed the presence of a sharp band with different wavelengths, indicating the presence of multifunctional groups, such as amine group –N, Aliphatic –CH2, olefinic -C=C-, carbonyl C=O, and –OH phenolic bending **(Table 2 and Figure 1)**.



Stretching vibration band (cm-1)	Band shape	band	Functional group
3235-3322	sharp	N-H	Stretching of amine
2928	sharp	-CH2-	Aliphatic stretch
1452-1507	sharp	-C=C-	Stretching of olefinic
1685	sharp	C=O	Stretching C=O of -CHO Group
1386	sharp	O-H	Bending of phenolic -OH

Figure 2. FTIR spectra of *Salvia officinalis* flavonoids and the corresponding FTIR reading.

Antimicrobial Activity of Salvia officinalis Flavonoids and Its Silver Nanoparticle

In this study, the antimicrobial Activity of *Salvia officinalis* flavonoids and *Salvia officinalis flavonoids silver* nanoparticle **(Figure 4)** were determined by using disk diffusion method. The antimicrobial Activity of *Salvia officinalis* was increased when flavonoids extract was prepared as nanoparticles in comparison with control antimicrobial discs (chloramphenicol and voriconazole). Zone of inhibition of *Salvia officinalis* flavonoids was ranged from zero to 15 mm against *S. aureus* and *C. albicans,* respectively. No antimicrobial activity was reported against *P. aeruginosa*. However, zone of inhibition of *Salvia officinalis flavonoids silver* nanoparticle was ranged from (0-15), (10-20), and (0-25) mm against *S. aureus*, *C. albicans,* and *P. aeruginosa,* respectively and followed a dose-dependent manner **(Table 3 and Figure 3)**.

Table 3. Mean diameter and standard deviation of inhibition zone in millimeters against different concentrations of *Salvia officinalis* flavonoids and its nanoparticles.

Microbe	Concentrations mg/ml											
	0.5	1	1.5	2	2.5	3	0.5	1	1.5	2	2.5	3
	Mean ± SD zone of inhibition of *Salvia officinalis* flavonoids						Mean ± SD zone of inhibition of *Salvia officinalis* flavonoids silver nanoparticles					
P. aeruginosa	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	10±0.0	10±0.0	12±0.0	15±0.5
S. aureus	0±0.0	0±0.0	0±0.0	12±1.2	15±0.5	15±0.0	10±0.0	10±0.0	15±0.5	18±1.5	20±1.0	20±1.2
C. albicans	0±0.0	0±0.0	10±0.0	10±1. 0	12±0.0	15±0.0	0±0.0	0±0.0	10±0.0	15±0.5	20±1.0	25±0.0

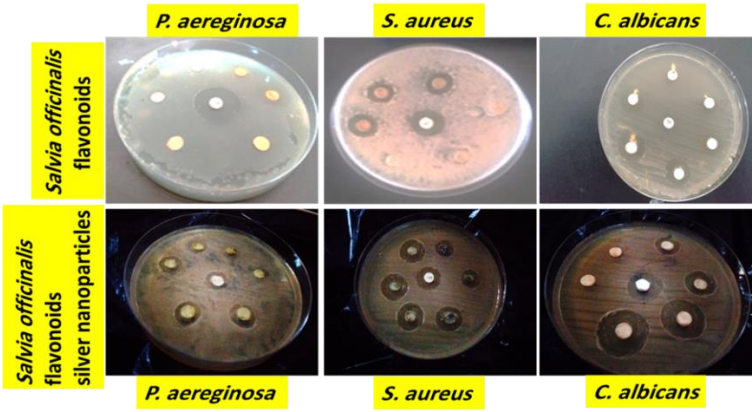


Figure 3. The Antimicrobial activity of *Salvia officinalis* flavonoids and its nanoparticles.

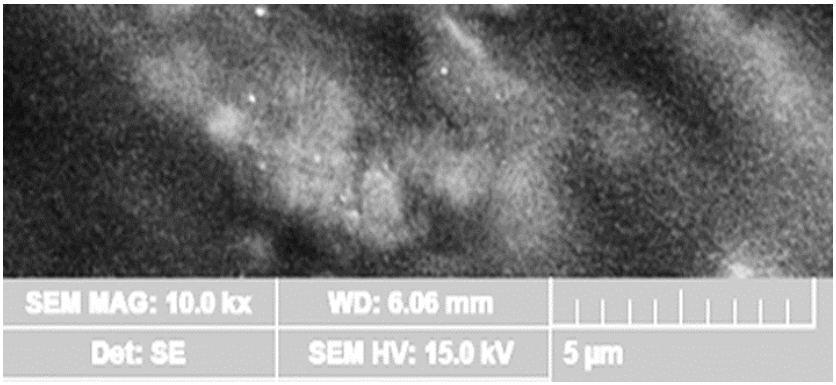


Figure 4. Nanoparticle size diameter of *Salvia officinalis* flavonoids by scan electron microscope.

Salvia officinalis (sage) leaves and its extracts have found applications in phytomedicine for different indications in many countries around the world. More than two thousand polyphenol flavonoids have been extracted from different plant species. Currently, there is increased concern in the identification and investigation of herbal products with confirmed therapeutic efficacy in the pharmaceutical industries, since synthetic drugs have been associated with several side effects. The result of the TLC chromatogram showed that the Rf values of *Salvia officinalis* flavonoids and quercetin, used as a standard flavonoid, were approximately the same (0.88), in addition to peak values at 225 nm obtained by using a UV spectrophotometer of quercetin and *Salvia officinalis* flavonoids, which indicates that *Salvia officinalis* flavonoids were identical to quercetin flavonoids (Sharifi *et al*., 2020).

Salvia officinalis flavonoids possess a significant antioxidant activity when compared to the positive control, ascorbic acid. The antioxidant activity increased in a concentration-dependent manner, which suggests that *Salvia officinalis* leaves contain antioxidant compounds with the ability to neutralize and scavenge free radicals resulting from oxidative stress, which consequently may induce tissue damage, cause various diseases, and weaken the immune system. This finding comes in accordance with the conclusion of other studies (Guzman *et al*., 2012; De *et al*., 2017).

Spectroscopic techniques such as FTIR showed that *Salvia officinalis* flavonoids have a variety of heterogeneous chemical compositions, such as amine groups (–NH), aliphatic groups (–CH2), olefinic groups (-C=C-), carbonyl groups (C=O), and phenolic groups (-OH). The qualitative use of FTIR to screen for the chemical composition of *Salvia officinalis* leaves extracts was shown to be rapid (the measurement time was 5 min per sample tested, and also showed the ability to efficiently describe the chemical functional components present in the sample in relation to wavelength (Zare, 2020). In the formation of silver nanoparticles using *Salvia officinalis* flavonoids in ethanolic extract, which acted as reducing agent and stabilizer, the color change from yellow to dark brown color with no precipitation during the interaction with the silver ion delivers a clear clue of the silver nanoparticles production and particles were small, however SEM used in this study detected nanoparticle size, silver nanoparticles measured at 81.2 ± 0.02 (mean diameter in nm ± SD) (Sharifi *et al*., 2020). Antimicrobial Activity of *Salvia officinalis* flavonoids was reported against *S. aureus* and *C. albicans*. On the other hand, no antimicrobial activity was observed against *P. aeruginosa*. In contrast, *Salvia officinalis* flavonoid nanoparticles demonstrated inhibitory activity against microorganisms, including *S. aureus*, *C. albicans*, and *P. aeruginosa*. Sharifi *et al*. (2020) investigated the antimicrobial effect of *Salvia officinalis* nanoparticles against four types of bacteria (*S. aureus*, *E. coli*, *B. subtilis*, and MRSA) utilizing the disc diffusion method (Sharifi *et al*., 2020). The study demonstrated that an effective antimicrobial inhibition was recorded against *B. subtilis* and MRSA, with zone of inhibition ranging from (0-10 nm) and (0-15 nm), respectively; however, no inhibition impact was observed against *S. aureus,* and *E. coli* was observed. Abdelkader *et al*. (2014) demonstrated that *Salvia officinalis* extract had antimicrobial action against *Candida albicans*, *E. coli*, and *B. subtilis* with inhibition zones ranging from (0-22 nm), (0-18 nm), and (0-12 nm), respectively (Abdelkader *et al*., 2014). Despite that, no antimicrobial action against *P. aeruginosa* and *S. aureus* was documented. The mechanism by which silver nanoparticles can harness to impart antimicrobial action is that silver nanoparticles form a collection of silver atoms with a 100 nm diameter. These collections can be internalized by the bacterial cells through their adherence to the sulfur-bearing proteins on the surface membrane of the microorganism. Hence, these particles initiate a set of changes in the shape, membrane penetrability, cellular respiration, and reproduction chain, leading to cell death (Umar Abdulkadir *et al*., n.d. ; Guzman *et al*., 2012; Abid & Abachi, 2023).

Conclusion

Salvia officinalis has a high content of polyphenol flavonoid compounds with polyfunctional groups, in addition to potent antioxidant activity that could be used in the prevention of free radical-associated diseases. Nanoparticle synthesis improves the antimicrobial activity, suggesting the potential application of *Salvia officinalis* flavonoids and their nanoparticles in different pharmaceutical formulations as herbal medicine.

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Ethics statement: None

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