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Research Paper

Biosynthesis of organic nanocomposite using Pistacia Vera L. hull: An efficient antimicrobial agent

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Abstract

In this study, *Pistacia Vera* hull essential oil analysis was performed by GC-MS method, in which α-Pinene, D-Limonen, Isobornyl acetate compounds constitute the highest percentage of *Pistacia Vera* hull essential oil. Phenolic compounds are one of the most important antioxidants that are involved in various fields, including pharmacy. *Pistacia Vera* hull is a rich source of phenolic compounds. In this study, the most phenolic compound in *Pistacia Vera* hull is gallic acid and rutin, which has been identified by HPLC analysis. Here presented a quick and easy synthesis of copper nanoparticles (CuNPs). Pistachio hull extract has been used as a reducing and stabilizing agent in the preparation of CuNPs. This biosynthesis is a kind of supporter of the environment because chemical agents were not used to making nanoparticles, and on the other hand, it prevents the release of pistachio waste in nature and its adverse effects on nature. The biosynthesized CuNPs and CuNPs/silver Schiff base nanocomposite (CSS NC) were characterized by UV-VIS spectroscopy, Fourier transforms infrared spectroscopy (FT-IR), X-ray diffraction (XRD), field emission scanning electron microscopy (FE-SEM), energy-dispersive X-ray spectroscopy (EDS). CSS NC antimicrobial activity was examined by both well diffusion and determination MIC methods against four bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa* and two fungi *Aspergillus nigero* and *Candida albicans*. CSS NC showed significant antimicrobial activity on the samples, preventing the growth of bacteria and fungi at very low concentrations. CSS NC had the greatest effect on *Escherichia coli* bacteria and *Aspergillus nigero* fungi.

Keywords: Nanocomposite, Biosynthesis, Antimicrobial activity, Anti fungi, Pistachio hull.

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Introduction

Nanotechnology is the careful and controlled modification of the atomic or molecular structure of materials at the nanoscale to provide particles with new features and specific applications.[1] Nanoparticles are particles having at least one dimension less than 100 nm.[2] Particles at this scale have unique properties that, if properly constructed, can be applied in the fields of medical sciences, biotechnology and the environment, electronics, and energy.[3] Composites are materials that come from a combination of two or more constituent which has better properties than any of the materials alone. Composite materials are made of two components: one phase matrix component and the other component is used as an amplifier. The composites are divided into three general categories: polymeric, ceramic, and metal-based on the type of base material.[4] Now, if the nanoparticle is a component of the composite amplifier, it is called a nanocomposite. Nanocomposites have better performance than composites because of the unique properties of nanoparticles.[5] The use of nanoparticles as an antimicrobial in medicine is particularly important because their surface-to-volume ratios are high and therefore highly sensitive.[6] The

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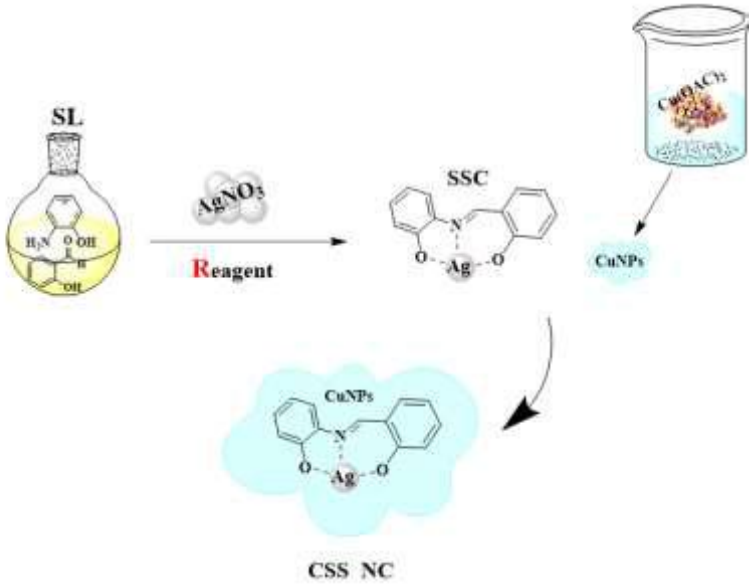
nanoparticles stick together over time and the reaction progresses, thus reducing their efficiency and also making it difficult to recover from the reaction mixture. These problems can be solved by placing the nanoparticles on a suitable bed and producing nanocomposites.[7]

Most of classic methods are expensive and toxic materials are used in the working process. [8] As well as sometimes these reactions are possible only at high temperatures and pressures. To overcome these shortcomings, the scientists turned to the biological synthesis of nanoparticles. This method is compatible with the environment. It does not use toxins and does not produce toxins. In addition, it achieves its goals with lower cost and higher efficiency. [9] Pistachio soft skin, because it has many biological polymers such as cellulose, tannic acid and

etc, has very good properties for use as a substrate in the preparation of nanocomposites, relatively high effective surface area, high chemical purity, high biocompatibility, good hydrophilicity, no need for harsh reaction conditions, non-adherence to nanoparticles, and reasonable price.[10] Metal nanocomposite produced by biological methods has useful properties such as high level, small size and high dispersion. The combination of these factors has led to a significant increase in the antimicrobial effects of metal nanocomposite compared to metals.[11] Metal nanocomposites can affect the metabolism reproductive processes of microorganisms by inhibiting the respiratory system of bacteria and causing damage to the bacterial cell membrane.[12] Using pistachio peel to make nanocomposites in addition to the benefits mentioned above will not leave these wastes in nature. Due to the abundant presence of phenolic compounds in pistachio soft skin, if they are abandoned in nature, it will damage the environment and soil, including such as lowering soil pH, thereby reducing soil fertility and lowering groundwater quality.[13] The aim of this study was to analyze pistachio skin essential oil and identify a group of phenolic compounds in pistachio skin and synthesize nanoparticles and nanocomposites using pistachio waste with the least pollution and cost. The function of synthesized nanocomposite was investigated as antibacterial.



Scheme 1: The synthesis procedure of CSS NC.

Experimental section and procedures

The completely dried pistachio hull powder was placed in Clevenger with deionized water for 6 hours. In this way, pistachio skin oil was obtained. The oil was then dehumidified with sodium sulfate and its GC-MS was taken. GC-MS results qualitatively identify pistachio skin oil components.

To prepare the desired extract for HPLC analysis, 15 g of the dried pistachio skin sample was transferred to an Erlenmeyer flask, 100 ml of distilled methanol solvent was added to it, and it was stirred on the shaker for 72 hours at room temperature. Extraction was performed. The extract was filtered and then analyzed by HPLC.

Nanocomposite synthesis steps

To prepare the extract required for the synthesis of copper nanoparticles (CuNPs) first, 15 grams of chopped dried pistachio skin was transferred to Erlenmeyer and 150 ml of deionized water was added to it. This extract for 20 minutes in an ultrasonic bath at a temperature of 45°C sonicated and then centrifuged at 6000 rpm for 30 minutes

to extract massive plant tissue be removed. Finally, the extract was filtered through a sinter funnel to remove particles of material and obtain a pure extract. The extract was stored in the refrigerator at 4 °C for later stages. Add of the above extract (8 mL) as a drop to solution of copper (II) acetate monohydrate (100 mL, 8 mmol) during stirring and at 70 °C water bath. In the first ten minutes the color changed from light sky blue to yellow-green and then to olive green and then to dark olive. This mixing was continued for 5 hours and then the solution was centrifuged at 6000 rpm and then the sediment was washed with distilled water and n-hexane and ethanol to remove uncoordinated phytochemical compounds. The blackish-brown powder obtained was dried overnight at room temperature and then dried for one hour in an oven at 65 °C and in this way, CuNPs is synthesized for (E)-2-((2-hydroxybenzylidene) amino) phenol Schiff base ligand (SL**)** synthesis, 2- aminophenol (1mmol) was combined with salicylaldehyde (1 mmol) in ethanol (5 ml). The resulting solution was refluxed at 80 °C. After 2 hours, the solution was centrifuged at 6000 rpm to the orange precipitate remove faster than the solution. This precipitate was washed three times, each time with 10 ml ethanol. Then the resulting precipitate was dried at room temperature.

In order to synthesize silver Schiff base complex (SSC), an ethanol solution of silver nitrate (5 mL, 0.2 M) dropwise to ethanol solution of SL (10 mL, 0.1 M) was added during sonicated for 15 minutes. The color of the solution changed from orange to blood red. The resulting solution was stirred at room temperature for 2 hours on a stirrer. After 2 hours, the solution was centrifuged and a silver-colored precipitate was obtained, which was washed three times, each time with 10 ml ethanol. The precipitate then dried at room temperature. For CuNPs/silver Schiff base nanocomposite (CSS NC) synthesis, the aqueous solution of SSC (800 µL, 0.01 M) was added to CuNPs (0.08 g) in water (10 mL) during sonicate for an hour and then centrifuged for 20 minutes.

The antibacterial and antifungal effects of (CSS NC) were investigated by the well diffusion method in agar and MIC. Microbial specimens using Luria Bertani and sabouraud dextrose cultivation medium and revived according to standard methods. To prevent the microbial suspension from 24-hour culture, each microorganism was inoculated separately into test tubes containing 3 ml of Mueller-Hinton Broths, and a suspension with turbidity equivalent to half McFarland was prepared. In the method of diffusion from the well in Agar from the suspension of each microbe for µl on a plate, containing Mueller Hinton Agar for bacteria and sabouraud dextrose agar for the fungi was poured and sterilized with sterile swap in three directions. Then, on the surface of each of the cultivated plates, wells with a diameter of approximately 6 mm and at a distance of 2 cm were created and inside each well, 50 µl was poured from each of the prepared CSS NC dilutions with a sampler. The series of dilutions used to determine the aura of non-growth in the propagation method from the well was 10, 20, 40, 60, and 100 micrograms per milliliter. In addition, the antibacterial antibiotic gentamicin and the antifungal antibiotic clotrimazole were considered positive controls. After completing the work, the bacterial culture medium in the incubator was 37 degrees for 24 hours and the fungal cultures in the 28-degree incubator were incubated for 48 hours. Finally, after 24-48 hours, microbial cultures were evaluated for the formation or non-formation of non-growth aura and the diameter of the formed auras was measured and reported in millimeters. To determine the minimum inhibitory consent (MIC) nanocomposite tubular dilution method was used. For this purpose, CSS NC prepared in test tubes containing 9 ml of Mueller-Hinton Broths culture medium of series of dilutions of 1.56, 3.125, 6.25, 12.5, 25, 50, 100, and 200 $mg.ml^{-1}$ was prepared. Then give each tube 1 ml of suspension prepared microbe was inoculated. It was used as a positive control of the tube containing the culture medium with the inoculated microbe, and for negative control and to ensure that the work steps were sterile, as well as the CSS NC suspension inside one tube, only the culture medium and in another tube the environment. Cultivation containing a solution with a concentration of half a microgram per milliliter of CSS NC was added. Finally, all test tubes were transferred to incubators at 37^{o}C for bacteria and 28^{o}C for fungi for 24-48 hours. After incubation, each tube was examined for turbidity due to the growth of the microorganism, and the lowest value in which the turbidity was not due to the inhibitory effect of nanocomposite was considered as MIC.

Result and discussion

The chemical composition of the essential oil (EO), which was identified by GC-MS analysis, is shown in the (Table 1).

The chemical composition of essential oils of *Pistacia vera* hull and *Pistacia khinjuk* is compared in (Table 2).

Table 1: Chemical composition of the essential oil from the Pistacia hull.

No	Compounds	Percentage
1	Ethyl acetate	1.85
2	Tricyclene	1.13
3	α-Pinene	45.62
4	Camphene	7.76
5	β-Pinene	1.11
6	3-Caren	2.97
7	D-Limonen	12.16
8	Terpinolene	4
9	Limonene-4-ol	4.24
١٠	α-Terpineol	5.38
11	Isobornyl acetate	13.78
12	total	100

Ethyl acetate, Limonene-4-ol, Isobornyl acetate are present in EO of *pistacia vera* hull, while they are not present in EO of *pistacia khinjuk.* Myrcene, β-Caryophyllene, α-Humulene are present in EO of *pistacia khinjuk* hull, while they are not present in EO of *pistacia vera.* The percentage of α-phenine and camphene in *Pistacia vera* hull EO is much higher than the percentage of these substances in *Pistacia khinjuk* hull EO, α-phenine is about 3 times and camphene about 7 times.[14]

Table 2: Comparison of EO chemical compounds in *Pistacia vera* hull and *Pistacia khinjuk*.

Compounds	Pistacia vera	Pistacia khinjuk
Tricyclene	1.13	1.8
α-Pinene	45.62	16.98
Camphene	7.76	1.48
β-Pinen	1.11	4.23
3-Caren	2.97	1.1
D-Limonen	12.16	11.85
Terpinolene	4	2.88
α-Terpineol	5.38	4.04
Ethyl acetate	1.85	-
Limonene-4-ol	4.24	-
Isobornyl acetate	13.78	-
Myrcene	-	20
β- Caryophyllene	-	29
α-Humulene	-	10
Total	100	100

As it turns out, the amount of EO chemical compounds varies in different types of pistachios. The results of measuring the phenolic compounds of pistachio skin methanolic extract using HPLC analysis are given in the (Table 3).

Table 3: The amount of phenolic compounds in the methanolic extract of pistachio skin.

Phenolic compounds	The amount of compounds (mg/g dried material)
Gallic acid	55
3,4-Dihydroxybenzoic acid	0.39
Catechin	1.17
Rutin	132.6
Quercetin	2.3

UV spectra at different times. The role of time is one of the important parameters in the process of reacting and forming copper nanoparticles (CuNPs). As shown in (Fig. 1), over time the intensity of the surface plasmon resonance peak increases in the range of 525 nm. Surface plasmon resonance is a feature of metal nanoparticles with sizes of 2-100 nm.

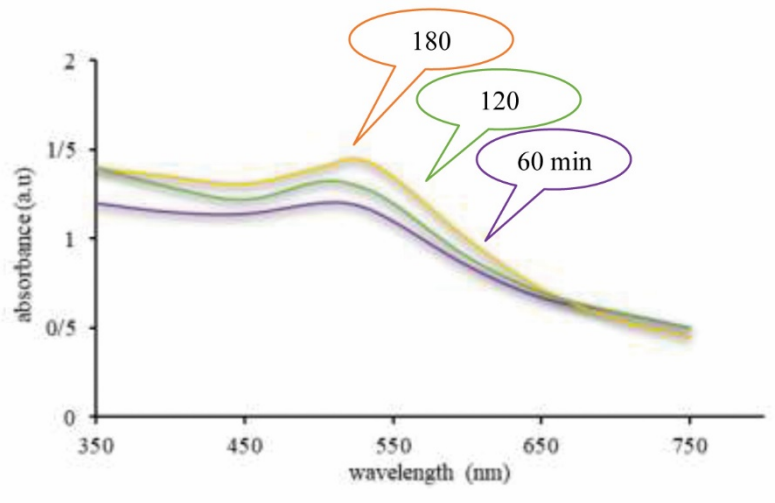


Figure 1: UV-Vis absorption spectrum of CuNPs biosynthesis at different time intervals.

SEM is used to determine the size of CuNPs. The SEM image of CuNPs is shown in (Fig. 2). According to this image, the diameter of the CuNPs is in the range of 26-51 nm.

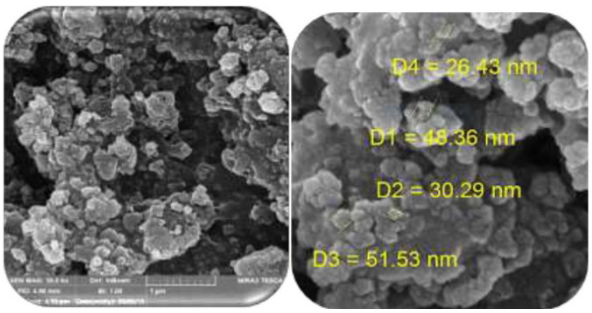


Fig 2: SEM image of the biosynthesized CuNPs by using pistachio hull extract.

The FT-IR method was used to identify silver Schiff base complex (SSC). The FT-IR spectra of E)-2-((2-hydroxybenzylidene) amino) phenol Schiff base ligand (SL) (a) and SSC (b) are shown in Fig. 3. In the SL spectrum, the peak observed at 3322 cm^{-1} corresponds to the stretching vibrations of O-H, and the peak observed at 1632 cm^{-1} indicates the double bond of carbon and hydrogen in SL structure. The absorption peak at 1464 cm^{-1} is related to aromatic C=C and the absorption peak in 3150 cm^{-1} is related to aromatic C-H in the structure of the benzene ring in SL. In the SL spectrum, the absorption peak at 2922 cm^{-1} is related to aliphatic C-H. As can be seen in the SSC spectrum, peaks have disappeared due to the interaction of oxygen and nitrogen in SL and the silver metal. [15]

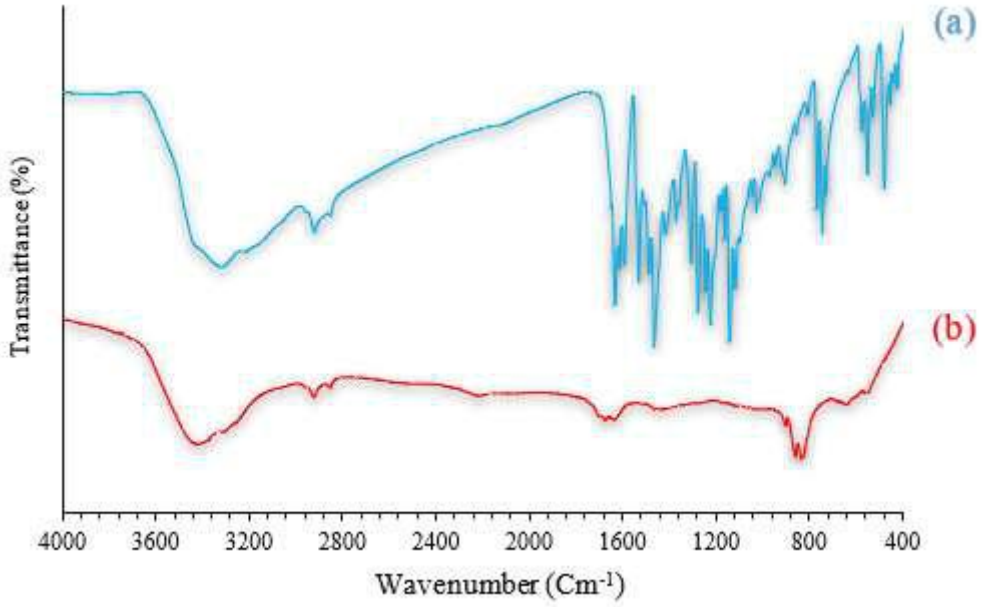


Fig 3: FT-IR of SL (a) and SSC (b).

The results of CuNPs/silver Schiff base nanocomposite (CSS NC) antimicrobial tests by well diffusion method and MIC determination are presented in (Table 4, 5), respectively. CSS NC has a significant microbial effect on case studies experiments showed that at very low concentrations it prevented the growth of bacteria and fungi. The results show that CSS NC has the greatest effect on *Escherichia coli* and *Aspergillus fungi* and had the least effect on *Staphylococcus aureus*.

Table 4: Non-microbial growth diameter at CSS NS various concentrations by diffusion from wells (in millimeters)

Microorganism	Different concentrations of CSS NC (µg.ml^{-1})						
	10	20	40	60	100	Gentamicin (50 µg/ml)	Clotrimazole (50 µg/ml)
Staphylococcus aureus	0	7	12	14	17	25	-
Bacillus cereus	7	9	11	14	18	24	-
Escherichia coli	9	11	14	17	22	23	-
Pseudomonas aeruginosa	8	11	13	17	20	25	-
Aspergillus niger	9	12	15	18	21	-	26
Candida albicans	9	11	14	17	20	-	25

Table 5: The minimum inhibitory concentration (MIC) of microbe's CSS NC in different concentrations (measured in $\mu g.ml^{-1}$).

Microorganism	Different concentrations of CSS NC ($\mu g.ml^{-1}$)									
	0.78	1.56	3.12	6.25	12.5	25	50	100	200	400
Staphylococcus aureus	+	+	+	+	+	-	-	-	-	-
Bacillus cereus	+	+	+	+	-	-	-	-	-	-
Escherichia coli	+	+	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	+	+	+	-	-	-	-	-	-	-
Aspergillus Niger	+	+	-	-	-	-	-	-	-	-
Candida albicans	+	+	+	-	-	-	-	-	-	-

Conclusion

Here's a simple, quick, and green way to make copper nanoparticles (CuNPs) with a size between 26-51 nm using pistachio skin extract as a reducing and stabilizing agent. We placed CuNPs on a bed of SSC to prevent their mobility and accumulation, thus not reducing their efficiency and also to be able to easily recover them from the reaction solution. Nanocomposites have high antimicrobial properties due to their unique properties such as high surface-to-volume ratio, high particle reactivity, etc., and can often destroy the microbe by destroying the membrane and the combination of these properties has led to the widespread use of nanocomposites in medicine and health, such as disinfection of drinking water, use in carbon filters and elimination of microbes in the air. The nanocomposite is also used today as an antimicrobial coating in medical equipment and the production of antimicrobial gels in the treatment of burns.

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