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Original Research Article

Effect of secondary metabolite compounds of *Dracocephalum kotschyi* Boiss plant on green synthesis of Cu nanoparticles

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ABSTRACT

Medicinal plants are a rich source of secondary metabolites. In the present study, the dried aerial parts of *Dracocephalum kotschyi* Boiss were extracted using the digestion method. Copper nanoparticles were synthesized by combining the extract with a copper chloride solution at a ratio of 1:4. The characteristics of copper nanoparticles were analyzed using various techniques including ultraviolet-visible spectrometry (UV-Vis), X-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS), Brunauer-Emmett-Teller (BET), and Fourier transform infrared spectroscopy (FT-IR). The SEM analysis predicted the size of the copper nanoparticles to be 63.28 nm. The EDS spectrum confirmed the presence of copper nanoparticles. BET analysis predicted the mesoporous structure of copper nanoparticles. Thirty-one (31) compounds were identified in the essential oil of the plant, constituting 93.1% of the total composition. The eight major compounds were perilla acetate (49.3%), 2-methyl-1-octen-3-yne (17.2%), limonene (15.0%), 1,8-cineole (5.2%), *trans*-α-ocimene (2.4%), *p*-mentha-1(7),8(10)-dien-9-ol (1.1%), sabinene (1.4%), and 4-terpineol (1.5%).

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1. Introduction

Due to the new properties and characteristics exhibited by materials with nanometer dimensions in various industries, there is currently a strong interest in their processing and utilization. Nanotechnology refers to the design, analysis, fabrication, and utilization of structures, tools, and systems with precise control over shape and size at the nanometer scale. Chemical and physical properties, such as optical, electronic, and antibacterial properties of nanoparticles can be altered due to their small size (Husen and Siddiqi, 2014). Among metal nanoparticles, copper nanoparticles (CuNPs) are highly significant due to their affordability, widespread availability, and superior electrical conductivity compared to gold and silver nanoparticles. The application of CuNPs in various fields, including catalysts (Ali et al., 2018), gas sensors (Dewangan et al., 2022), lubricants (Zhou et al., 2000), nanofluids (Khamliche et al., 2021), heat transfer, nanocomposite coatings (Guzman et al., 2015), and the dyeing industry, is of interest. Metal vapor synthesis (Tegeder et al., 2018), radiation (Abedini et al., 2013), aeroemulsion techniques (Malik et al., 2012), sonochemical methods (Malik et al., 2012), thermal decomposition (Kim et al., 2006), and chemical reduction (Aguilar et al., 2019) have been employed in the synthesis of copper nanoparticles. High costs, pollution caused by chemicals, production of hazardous byproducts, and the need for non-toxic and environmentally friendly methods for synthesizing nanoparticles have been taken into account. Medicinal plants are a rich source of compounds derived from secondary metabolites. Green synthesis of various nanomaterials and nanoparticles, utilizing organic or aqueous extracts from plants, has been extensively studied for a wide range of applications, as reported in several literature sources (Mahdavi et al., 2022; Zhang et al., 2021). Due to its simplicity, cost-effectiveness,

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minimal toxicity, and the potential of medicinal plants in Iran, it is necessary to investigate the production of metal nanoparticles using the green synthesis method. The metal nanoparticles synthesized by the green method are capped by biomolecules such as phenols, tannins, flavonoids, and ascorbate found in plant materials. They increase the stability of nanoparticles and also prevent their interaction with atmospheric oxygen. Therefore, it is very important to select a plant that has a reducing and capping agent effect.

Plants of Lamiaceae are herbs, shrubs, trees, or rarely vines in habit and are widely distributed in tropical, subtropical and coastal areas of China to tropical Asia (Palariya et al., 2019). The main group of secondary metabolites of various herbs belong to the Lamiaceae (Labiatae) family, especially *D. kotschyi* Boiss, a valuable plant endemic to Iran. This plant is known as "Badrandjboie-Dennaie" or "Zarrin-Giah" (golden-plant) and "Semsa" (In the Laki dialect), which has the highest distribution in the highlands of Alborz and Zagros areas in Northern and Western Iran. In traditional medicine and native culture, it is used as an additive to enhance the taste and aroma of beverages and foods, as well as to alleviate pain and inflammation among indigenous people. According to the international union for conservation of nature (IUCN) policy statement and convention on the trade in endangered species of wild fauna and flora, no information has been recorded about this plant.

Most of the past research is on the green synthesis of copper oxide (Cuong et al., 2022) and few results were reported for CuNPs by plant extract of *Azadirachta indica* A.Juss. leaves (Nagar and Devra, 2018), *Tinospora cordifolia* (Sharma et al., 2018), *Citrus medica* Linn. (Idilimbu) Juice (Shende et al., 2015), *Ziziphus spina-christi* (L.) Willd (Khani et al., 2018), *Asparagus adscendens* Roxb (Thakur et al., 2018) and *Eclipta prostrata* leaves (Chung et al., 2017).

In the the first phase of the present research, CuNPs are synthesized using the plant extract of *D. kotschyi* Boiss through the digestion method. The characteristics of the synthesized CuNPs were analyzed using various techniques including ultraviolet-visible spectroscopy (UV-Vis), X-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS), Brunauer-Emmett-Teller (BET), and Fourier transform infrared spectroscopy (FT-IR). The antimicrobial properties of the CuNPs against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) bacteria were evaluated using the well agar diffusion method. Furthermore, in the second phase of this study, an essential oil was extracted from the aerial parts of the *D. kotschyi* Boiss through hydrodistillation using a Clevenger device. The essential oil compounds were identified using a gas chromatography device connected to a mass spectrometer (GC-MS).

2. Experimental

2.1. Materials

Copper (II) chloride dihydrate ($CuCl_2.2H_2O$), *n*-hexane (C_6H_{14}), ethanol (C_2H_5OH), ammonia solution (NH_4OH), barium (II) chloride ($BaCl_2$) were purchased form Merck. Anhydrous sodium sulfate (Na_2SO_4) of analytical grade is purchased from Sigma-Aldrich (St. Louis, MO, USA). The green synthesis of CuNPs was confirmed using the ultraviolet-visible spectroscopy (UVD-2900 model, equipped with UVWIN software version LABOMED Inc., USA), X-ray diffraction (Philips Analytical PW1730, CuLFF, λ = 1.540598 Å), energy dispersive X-ray (EDS) spectroscopy (JEM-2100) and FT-IR spectroscopy (NiColet iSlet, Thermo Electron Corp., Madison, VVI, USA). FT-IR analysis was also done on the pellet sample made from KBr (99.99%). Morphology and size of CuNPs synthesized were investigated by the scanning electron microscope (SEMXL30E Philips, Netherlands). The Brunauer-Emmett-Teller (BET, ASAP 2020 Model, Micromeritics) method is applied to calculate the specific surface of CuNPs on the basis of nitrogen adsorption isotherm measurements at 77 K. The GC-MS (Agilent 5977C) is used to identify its constituent compounds quantitatively and qualitatively in essential oil of *D. kotschyi* Boiss.

2.2. Methods

2.2.1. Preparation of *D. kotschyi* Boiss extract

D. kotschyi Boiss is one of the native medicinal plants of Iran and belongs to the Lamiaceae family, which is highly valuable from a medicinal perspective (Fig. 1a). The plant under study was collected from the highlands of Aladagh Mountain, which is situated in North Khorasan-Shogan. The geographic coordinates of the collection site are 37.346°N and 56.895°E (Fig. 1a). The collection was made during the spring season. A voucher specimen (No. 36-1-2) was authenticated and then deposited in the herbarium of the Faculty of Pharmacy at Tehran University of Medical Sciences for future reference.

An amount of 10 g of powdered *D. kotschyi* Boiss plant was transferred into an Erlenmeyer flask containing 200 mL of distilled water. Then, the contents of the flask were heated in a water bath at 60 °C for 30 minutes. After reaching room temperature, the mixture was filtered using Whatman No.1 filter paper. The resulting extract was then refrigerated at 4 °C for subsequent tests.

2.2.2. Synthesis of CuNPs

To 100 mL of a 0.01 M copper chloride dihydrate solution, various volumes of freshly prepared extract (25, 50, 75, and 100 mL) were added. The solution was then stirred for 24 h at a temperature of 60 °C with a speed of 300 rpm. Additionally, to prevent the solution from losing volume, the container was covered with aluminum foil. Then, the solution was brought to room temperature. The synthesized CuNPs are separated using a centrifuge device (160 g for 5 min). After washing the synthesized nanoparticles with distilled water three times, the filtrate was tested with an ammonia solution to check for the absence of Cu^{2+}. Finally, the plant extract was washed with hexane and ethanol solvents to remove

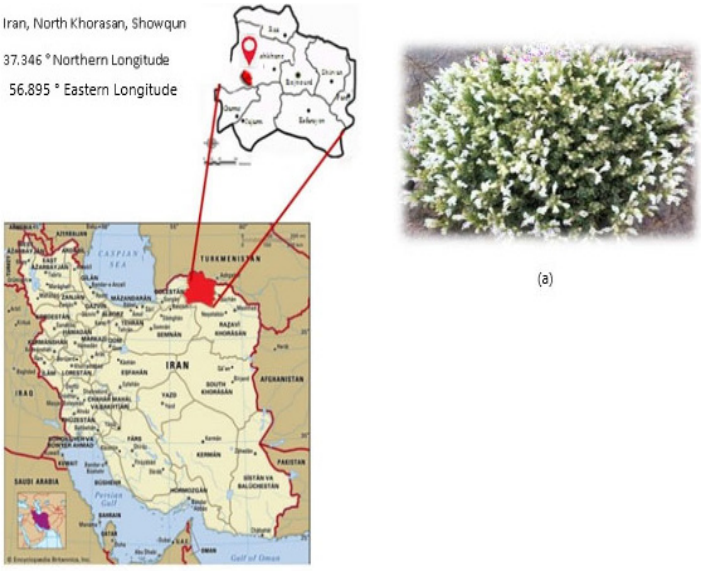

Fig. 1. a) The photograph of *D. kotschyi* Boiss, b) The geographical map of the sampling area of *D. kotschyi* Boiss.

the organic compounds. The synthesized nanoparticles were dried in an oven at 185 °C for 5 minutes.

2.2.3. Preparation *of D. kotschyi* Boiss essential oil

About 120 g of dried and ground *D. kotschyi* Boiss plant material was weighed. The essential oil was obtained through a two-hour distillation process using a Clevenger device. Anhydrous sodium sulfate was utilized to remove any water from the collected essential oil. For further testing, the essential oil was collected in a closed and dark container and stored at a temperature of 4 °C. The essential oil of *D. kotschyi* Boiss was injected into the GC-MS device to identify its constituent quantitatively and qualitatively. GC-MS was performed using an HP-5MS capillary column, which had a length of 30 m, an inner diameter of 0.25 mm, and a film thickness of 0.25 µm. The temperature of the column started at 50 °C. After remaining at this temperature for 5 minutes, the temperature increased at a rate of 3 °C per minute until it reached 250 °C. The injection port temperature was set at 280 °C. Helium gas was used as a carrier gas at a speed of 1 mm min^{-1}. Then, under the same conditions, C_9 to C_{22} series alkanes were injected into the GC-MS device and their retention time was obtained. The Kovats index was calculated based on Eqn. 1 (Jonsson, 1987).

$$KI = 100\frac{t_r - t_n}{t_{n+1} - t_n} + 100n \qquad \text{(Eqn. 1)}$$

Where KI, n, t_r, t_n and t_r are Kovats index, the number of carbon atoms in the smaller alkane, the retention time of the compound of interest; the retention time of the smaller alkane and the retention time of the larger alkane, respectively.

2.2.4. Activation of microbial strains

Escherichia coli (PTCC 1399) and *Staphylococcus aureus* (PTCC 1431) were prepared in the form of lyophilized ampoules from the Central Veterinary Laboratory of North Khorasan. using of a Pasteur pipette, 0.4 mL of physiological serum as a sterile solution was added to the dry substance in the ampoule and a microbial suspension is obtained after mixing.

2.2.4.1. Preparation of half McFarland standard

The standard was half McFarland. 1.175 g of barium chloride was dissolved in distilled water and diluted to a volume of 100 mL. Then, 0.5 mL of barium chloride solution prepared using sulfuric acid (1%) was diluted to a volume of 100 mL.

2.2.4.2. Preparation of culture medium

Muller Hinton Agar (MHA) culture medium was used for microbial tests. For this purpose, 3.8 g of culture medium was weighed and dissolved in 100 mL of distilled water and then heated to boiling. After cooling, the contents were transferred to two test tubes and then these tubes were placed in an autoclave for 15 min with a pressure of 121 atm for sterilization and disinfection. After transferring the microbial suspension to the culture medium prepared with a sterile swab, they were incubated at 37 °C for 24 h.

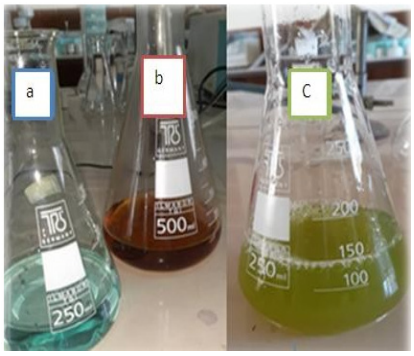

Fig. 2. a) Copper chloride solution (0.01 M), b) The *D. kotschyi* Boiss extract and c) The synthesized CuNPs before separation.

2.2.4.3. Antibacterial activity of CuNPs

Serial dilutions of CuNPs (0.100, 0.050, 0.030, 0.01, 0.006, 0.003 and 0.002 mg mL^{-1}) were prepared using a 96-well microplate and dimethyl sulfoxide solvent. It should be noted that if the concentration of bacteria exceeds half of the McFarland standard, nanoparticles are unable to prevent bacterial growth, rendering the results invalid. Dimethyl sulfoxide solvent was considered as negative control and gentamicin antibiotic as positive control.

3. Results and discussion

3.1. Characterization of the synthesized CuNPs nanoparticles

In this report, CuNPs were synthesized by combining of different volumes of *D. kotschyi* Boiss extract with a copper chloride solution, and the optimum condition was obtained at a ratio of 1:4. The color change of the solution to amber, green, or yellow is due to the synthesis of CuNPs (Fig. 2) (Khatami et al., 2020).

3.1.1. Characterization by UV-Vis spectroscopy

In order to investigate the absorption of synthesized CuNPs, their absorption spectrum was examined in the range of 200-900 nm. The maximum absorption wavelength was observed at 570 nm (Fig. 3). The absorption bands for CuNPs have been reported to be in the range of 500-600 nm (Shikha et al., 2015). Rahman et al. (2011) obtained similar results in the synthesis of copper nanoparticles. Metal nanoparticles contain free electrons, and the vibration of these electrons enhances the surface plasmon resonance, resulting in the creation of an absorption band. Previous research has shown that the width of the absorption peak depends on the size of the particles and the quantity of agglomerated nanoparticles (Srivastava and Dwivedi, 2018).

3.1.2. Characterization by scanning electron microscopy (SEM)

SEM of synthesized CuNPs using *D. kotschyi* Boiss extract at different magnifications is shown in Fig. 4. As can be seen in this figure, the synthesized CuNPs have a spherical morphology, and the particle size is 63.28 nm.

3.1.3. Characterization by energy dispersive X-ray spectroscopy (EDS)

EDS spectrum related to the synthesized CuNPs is shown in Fig. 5, confirming the presence of copper element. The peaks related to carbon, oxygen, and nitrogen are likely associated with the active compounds present in the extract of *D. kotschyi* Boiss. These compounds act as reducing and masking agents during the production of copper nanoparticles.

3.1.4. Characterization by energy X-ray diffraction (XRD)

In order to determine the crystalline structure of the CuNPs, XRD analysis was performed in the angular range of 2θ = 0-85°. The X-ray diffraction pattern of CuNPs is shown in Fig. 6. Bragg's reflections at a 2θ value of 43.5°, 50.30°, and 73.9° correspond to the (111), (200), and (220) planes of metal copper (JCPDS No. 71-4610), respectively. These reflections indicate the formation of CuNPs synthesized using the extract of *D. kotschyi* Boiss plant. Scherer's equation was used to estimate the mean size of nanoparticles.

$$d = \frac{0.9\lambda}{\beta \cos\theta} \quad \text{(Eqn. 2)}$$

Where d is the mean diameter of nanoparticles, λ denotes the wavelength of the X-ray radiation source, and β represents the angular full width at half maximum (FWHM) of the X-ray diffraction peak at the diffraction angle (Patterson, 1939). The mean particle size of the CuNPs, as estimated by SEM, was approximately 63.28 nm. This value closely matched the estimation obtained from XRD data using Scherer's equation (62.12 nm).

3.1.5. Characterization by Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectrum of plant extract and CuNPs are shown in Fig. 7, respectively. The absorption peak 3326.39 cm^{-1} is related to O-H and N-H stretching

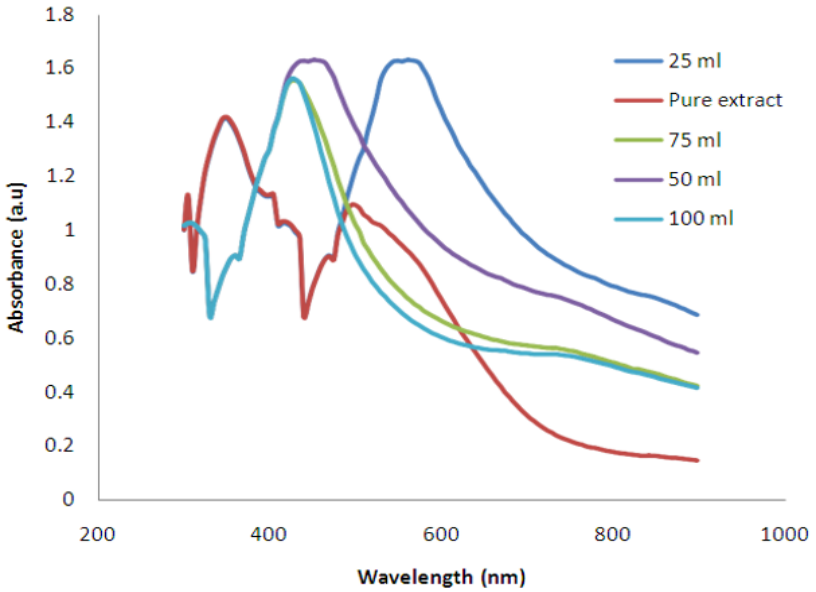

Fig. 3. UV-Vis spectrum of the synthesized copper nanoparticles (CuNPs) versus different volumes of *D. kotschyi* Boiss extract.

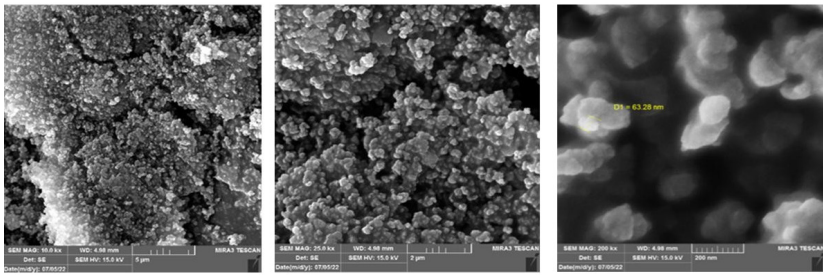

Fig. 4. SEM of synthesized copper nanoparticles (CuNPs) using *D. kotschyi* Boiss extract in different magnifications.

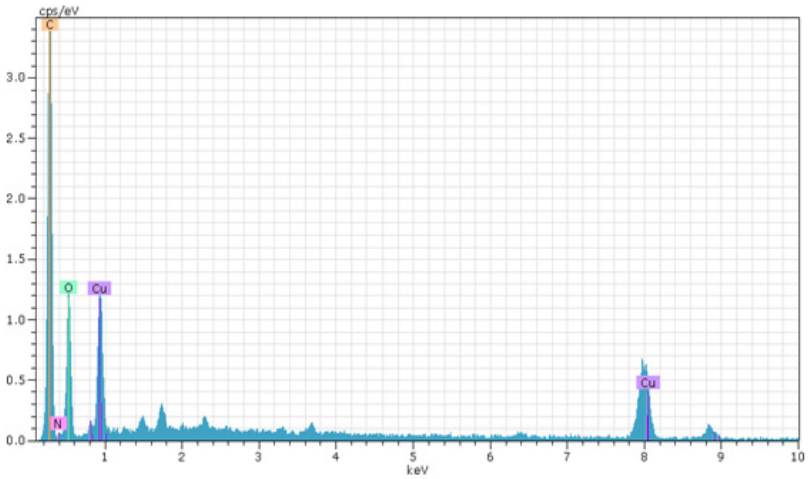

Fig. 5. EDS of synthesized copper nanoparticles (CuNPs) using *D. kotschyi* Boiss extract.

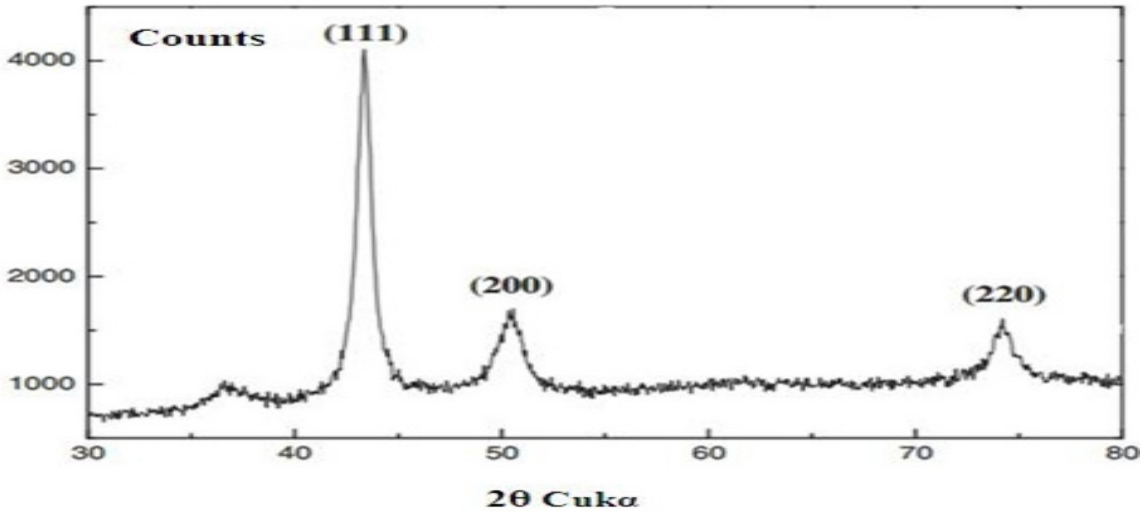

Fig. 6. XRD of synthesized copper nanoparticles (CuNPs) using *D. kotschyi* Boiss extract.

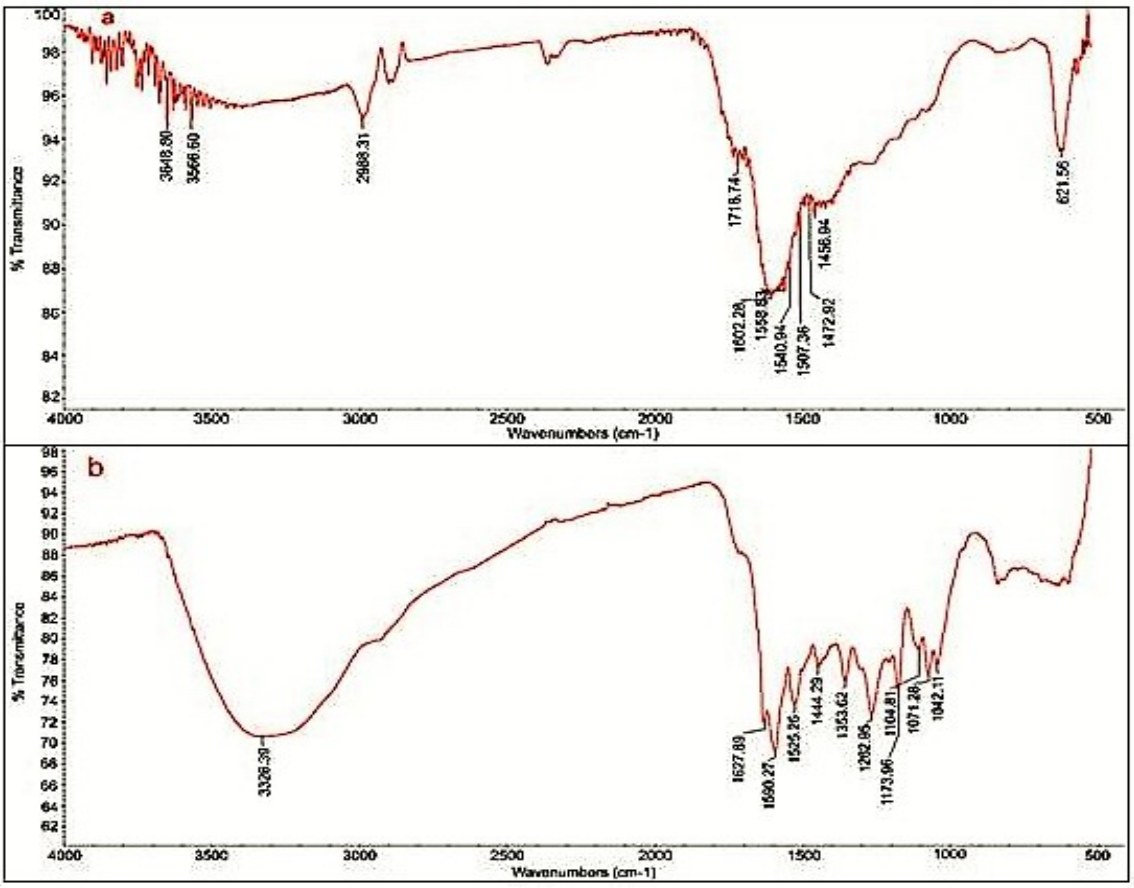

Fig. 7. FT-IR spectrum: (a) The synthesized CuNPs, (b) Extract of *D. kotschyi* Boiss.

vibrations, peaks in the range of 1500 to 11700 cm^{-1} can be related to C=C stretching vibrations in alkenes and aromatic ring, as well as C=O stretching vibration. These bands indicate the presence of proteins and phenolic compounds in the extract of *D. kotschyi* Boiss, which play a reducing and masking. As can be seen in these figures, the FT-IR spectrum of the extract is similar to that of CuNPs. However, in the FT-IR spectrum of the synthesized copper nanoparticles, the intensity of the peaks is weaker compared to the plant extract, which proves that the functional groups in the extract were effective for the formation of copper nanoparticles. Also, the peaks at 2285 cm^{-1} and 530 cm cm^{-1}, which are related to Cu-H and Cu-O vibrations, respectively (Shankar et al., 2004), are not detected in the spectrum of copper nanoparticles. This indicates the formation of CuNPs.

3.1.5. Characterization by Brunauer-Emmett-Teller (BET)

Accurate measurement of surface area and porosity is important in many applications, such as catalysts and metal nanoparticle adsorbents. The results of the BET analysis for CuNPs are given in Table 1. According to the IUPAC classification, the structure of the porous medium can be classified based on the average size of the pores. Pores smaller than 2 nm are called micropores, pores between 2 and 50 nm are called mesopores, and pores larger than 50 nm are called macropores. Based on the results mentioned in Table 1, the synthesized CuNPs are found to be mesoporous.

Table 1
BET parameters of the copper nanoparticles (CuNPs) synthesized.

Parameter	Amount
as,BET	24.6 (m^2g^{-1})
Total pore volume (p/p0=0.982)	0.3 (cm^3g^{-1})
Average pore diameter	41.4 (nm)

Adsorptive, N_2; Apparatus temperature, 0 °C; and adsorption temperature, 77 K.

3.2. Antibacterial activity of the synthesized CuNPs

In antibacterial experiments, no growth for *E. coli* was observed in the presence of copper nanoparticles. The results of the investigation of the zone of inhibition (ZOI), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of CuNPs synthesized using the agar well diffusion method on *Staphylococcus aureus* and *Escherichia coli* are showed in Table 2. According to the results of this table, the MIC in nanoparticles is related to *Staphylococcus aureus* bacteria and the MBC is related to the same bacteria and is equal to 0.1 mg mL^{-1}.

3.3. Chemical profile of the essential oil isolated from the aerial parts of *D. kotschyi* Boiss

Essential oils are complex mixtures of volatile and lipophilic compounds, including terpenes, particularly monoterpenes, sesquiterpenes, and phenylpropanoids. The quantity and composition of these compounds vary depending on environmental conditions. The biological activity of plant essential oils, such as antioxidant, antimicrobial, and anti-inflammatory properties, is related to their chemical constituents and structure (Camilo et al., 2017). The chromatogram of the essential oil of *D. kotschyi* Boiss is shown in Fig. 8. By comparing the mass spectra of unknown compounds in the essential oil with the mass spectra of standard compounds, the constituents in the essential oil were identified.

The essential oil contains volatile compounds such as monoterpenes hydrocarbons (MTC), oxygenated monoterpenes (OMT), sesquiterpene hydrocarbons (STC), and their oxygenated sesquiterpenes (OST), as well as phenolic derivatives. Table 3 displays the relative percentage and the Kovats index of the constituent components that make up the essential oil. The results of Table 3 show that 31 compounds have been identified in the essential oil, constituting 93.1% of the total essential oil. Among these compounds, the eight major ones are perilla acetate (**25**) (49.3%), 2-methyl-1-octen-3-yne (**6**) (17.2%), limonene (**9**) (15.0%), 1,8-cineole (**10**) (5.2%), *trans*-α-ocimene (**11**) (2.4%), *p*-mentha-1(7),8(10)-dien-9-ol (**19**) (1.1%), sabinene (**3**) (1.4%), and 4-terpineol (**15**) (1.5%) (Fig. 9). A comparison of the main components of *D. kotschyi* Boiss essential oils from this research and other studies reveals both similarities and differences in the reported components. These differences might be due to varying geographic conditions (Pavlović et al., 2006).

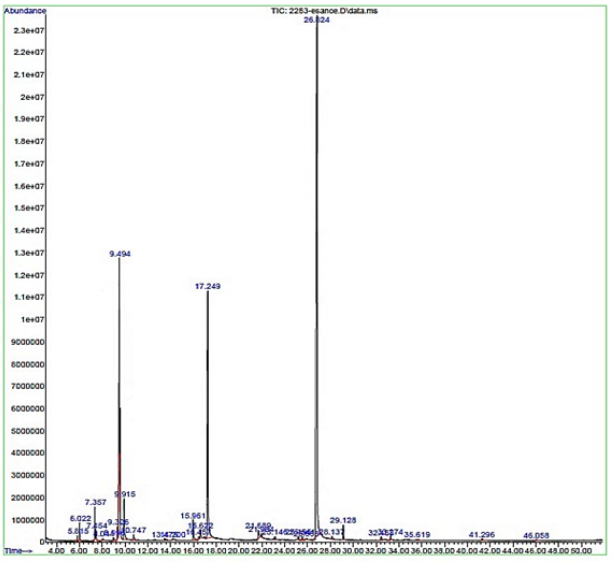

Fig. 8. The chromatogram the essential oil of *D. kotschyi* Boiss.

Table 2

The results of the antibacterial activities of copper nanoparticles (CuNPs) and gentamicin on *E. coli* and *S. aureus*.

		E. coli			*S. aureus*		
	Conc.	**ZOI**	**MIC**	**MBC**	**ZOI**	**MIC**	**MBC**
	(mg mL^{-1})	(mm)	(mg mL^{-1})	(mg mL^{-1})	(mm)	(mg mL^{-1})	(mg mL^{-1})
CuNPs	0.5×10^{-1}	***	+	+	24	-	-
	0.3×10^{-1}	***	+	+	17	-	-
	0.1×10^{-1}	***	+	+	***	+	+
	0.6×10^{-2}	***	+	+	***	+	+
	0.3×10^{-2}	***	+	+	***	+	+
	0.2×10^{-2}	***	+	+	***	+	+
Gentamicin	0.5×10^{-1}	32	-	-	45	-	-
	0.3×10^{-1}	29	-	-	42	-	-
	0.1×10^{-1}	24	-	-	40	-	-
	0.6×10^{-2}	20.3	-	-	33	-	-
	0.3×10^{-2}	18.8	-	-	24	-	-
	0.2×10^{-2}	10	-	-	19	-	-

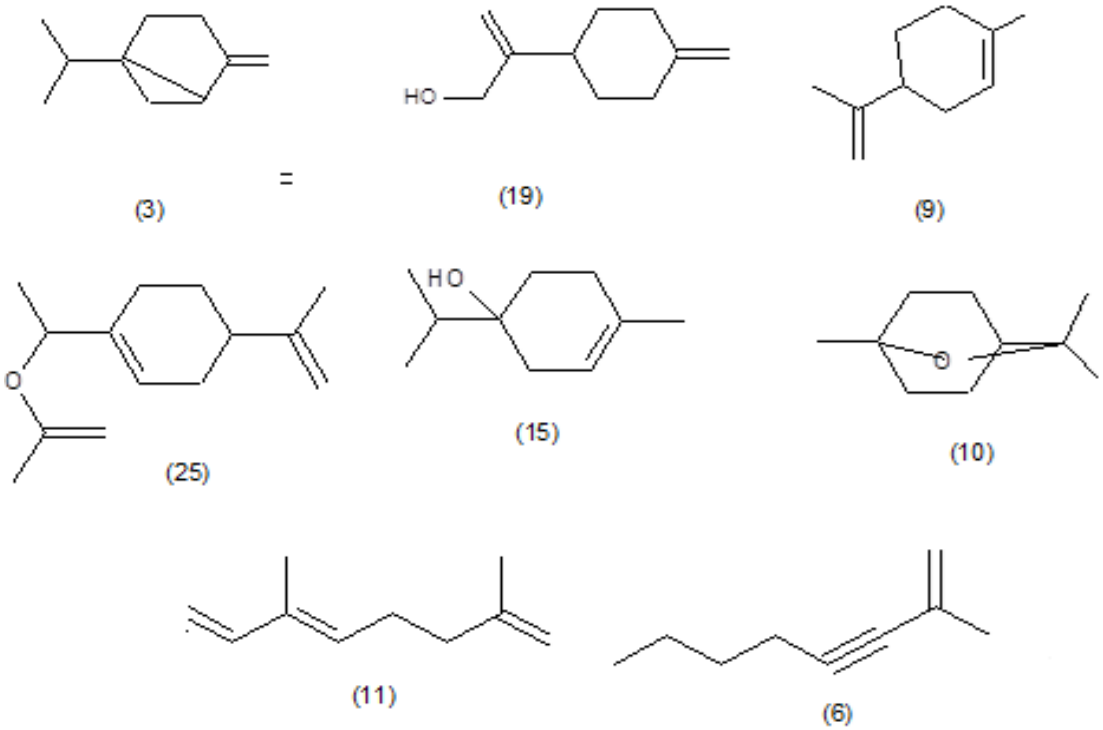

Fig. 9. Chemical structure of the main components characterized in essential oils from the aerial parts of of *D. kotschyi* Boiss.

Table 3
GC-MS analysis of the composition of essential oil of *D. kotschyi* Boiss.

Sr. No	Compound	KIcal[a]	KIlit[b]	Area%	Ref
1	α-Thujene (MTC)	908.6	911	0.2	(Kartaletal.,2007)
2	α-Pinene (MTC)	915.2	917	0.7	(Kartaletal.,2007)
3	Sabinene (MTC)	958.1	961	1.4	(Kartaletal.,2007)
4	β-Pinene (MTC)	961.3	964	0.5	(Kartaletal.,2007)
5	Myrcene (MTC)	980.4	981	0.2	(Safaei-GhomiandBatooli,2010)
6	2-Methyl-1-octen-3-yne (OMT)	981	981	17.2	(Wangetal.,2006)
7	α-Terpinene (MTC)	1007.5	1003	0.2	(Couladisetal.,2003)
8	Cymene (MTC)	1014.8	1010	0.6	(Couladisetal.,2003)
9	Limonene (MTC)	1018.6	1014	15	(Couladisetal.,2003)
10	1,8-Cineole (OMT)	1019.9	1015	5.2	(Wangetal.,2011)
11	*trans*-α-Ocimene (MTC)	1027.9	1027	2.4	(Wangetal.,2011)
12	γ-Terpinene (MTC)	1064.3	1064	0.4	(Kartaletal.,2007)
13	*cis*-Sabinene hydrate (OMT)	1106.7	1106.6	0.1	(Andriamaharavo,2014)
14	*cis*-Limonene oxide (OMT)	1110.9	1120	0.1	(Smadja,2005)
15	4-Terpineol (OMT)	1161.2	1157	1.5	(Kovacevicetal.,2006)
16	3,9-epoxy-p-Mentha-1,8(10)-diene (OMT)	1175.1	-[c]	0.1	-
17	α-Terpineol (OMT)	1175.7	1179	0.6	(Kundakovicetal.,2007)
18	Perillaalcohol (OMT)	1171.5		0.1	(Dharmawanetal.,2009)
19	*p*-Mentha-1(7),8(10)-dien-9-ol (OMT)	1278.1	-	1.1	-
20	*trans*-Pinocarvyl acetate (OMT)	1283.6	1281	0.4	(Cavallietal.,2003)
21	Myrtenyl acetate (OMT)	1308.5	1305	0.2	(Ningetal.,2008)
22	α-Copaene (STC)	1357.9	1359	0.2	(Couladisetal.,2003)
23	β-Bourbonene (STC)	1406.2	1406	0.2	(Jalali-Heravietal.,2006)
24	7-*epi*-*cis*-Sesquisabinene hydrate (OMT)	1374.5	-	0.3	-
25	Perilla acetate (OMT)	1436.5	1436.1	49.3	(Andriamaharavo,2014)
26	α-Caryophyllene (STC)	1433.6	1434	0.1	(Couladisetal.,2003)
27	GermacreneD (STC)	1461.5	1461	0.8	(Couladisetal.,2003)
28	Caryophyllene oxide (OST)	1561.9	1561	0.2	(Couladisetal.,2003)
29	Humulene epoxidell (OST)	1589.9	1586	0.2	(Fernandezetal.,2006)
30	*ent*-Germacra-4(15),5,10(14)-trien-1β-ol (OST)	1694..5	1694.5	0.2	(Andriamaharavo,2014)
31	Eicosane (STC)	1879	-	0.3	-
Monoterpenes hydrocarbons		21.6			
Oxygenated monoterpenes		76.2			
Sesquiterpene hydrocarbons		1.6			
Oxygenated sesquiterpene		0.6			

[a] The calculated retention Kovats indices; [b] The literature retention Kovats indices; [c] The result has not been reported.

4. Concluding remarks

In this research, the synthesis of copper nanoparticles (CuNPs) was carried out using a green chemistry method. The extract of *D. kotschyi Boiss demonstrated a high efficacy in the synthesis of CuNPs*. CuNPs were synthesized by combining the extract with a copper chloride solution at a ratio of 1:4. GC-MS analysis of the essential oil of *D. kotschyi Boiss showed that environmental conditions have an impact on the composition and quantity of constituents*. Most of the constituents found in the essential oil of *D. kotschyi Boiss were oxygenated monoterpenes and monoterpene hydrocarbons*. The results of this research are promising for the use of CuNPs in the treatment and prevention of Staphylococcus infections. Additionally, the prevalence of infections caused by this strain could be decreased by using methods such as coating medical equipment with CuNPs.

Author contribution statement

Malihe Samadi Kazemi contributed to the project in various roles, including supervision, conceptualization, funding acquisition, resource management, project administration, validation, visualization, writing review and editing. Zohre Imani contributed to the conceptualization, data curation, investigation, methodology, funding acquisition, validation, visualization, and writing-both the original draft and the review and editing process. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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