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GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES OBTAINED FROM *THYMUS VULGARIS* L. HYDROLAT

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Abstract: *The essential oil industry has grown over the last decade and is generating a lot of useful by-products with diverse industrial applications in food, cosmetics, textile, and more recently in phytosynthesis of metal nanoparticles. GC-MS analysis performed on the hydrolat identified 18 out of the 21 compounds detected, and the major terpenic class was represented by oxygenated monoterpenes 92.63% (1-octen-3-ol, endo-borneol, carvacrol methyl ether, thymol, carvacrol). Obtaining quasi-spherical silver nanoparticles from hydrolat and 0.1 mM $AgNO_3$ solution was simple, effective, and non-toxic, yielding stable nanoparticles with average particle size under 45 nm. UV-Vis spectroscopy revealed a strong signal at 438 nm indicating the formation of silver nanoparticles and SEM-EDX analysis revealed that elemental Ag was detected and confirmed by a strong signal at 3 keV with more than 91% silver.*

Keywords: bioactive compounds, by-products, green synthesis, hydrolat, silver nanoparticles.

INTRODUCTION

Throughout the years the preference for green synthesis of metal nanoparticles (Maťátková et al., 2022) (mNPs) like silver (AgNPs), gold (AuNPs), platinum (PtNPs), and copper (CuNPs), has been observed (Kumar et al., 2021). Therefore, the continuous demand of mNPs in several fields of applications like biotechnology, engineering, functional textiles, electronics, environmental engineering, pharmaceuticals, water, and wastewater treatments (Hitesh and Lata, 2018) has focused the attention on phytosynthesis from plant-based extracts and by-products as reducing and capping agents (Popa et al., 2021; Vilas et al., 2014).

One representative medicinal plant belonging to the *Lamiaceae* family is the perennial subshrub *Thymus vulgaris* L. frequently found in the southern part of Europe like the Mediterranean region but with a worldwide distribution (Nasrollahzadeh et al., 2016). It is harvested and processed for its valuable essential oil is rich in oxygenated and hydrocarbonated monoterpenes (*p*-cymene, *γ*-terpinene, linalool, thymol, and carvacrol), alongside its phenolic compounds and flavonoids (Popa et al., 2021).

During the distillation process of medicinal and aromatic plants, the essential oils are carried out by the water vapours and while passing through the condenser, a mixture of water and essential oil is collected. After the separation, two phases are obtained, namely essential oil and hydrolat (Stahl-Biskup and Saez, 2003). While essential oils represent complex mixtures of monoterpenes and sesquiterpenes, their corresponding hydrolats are composed of 99% water (Labadie et al., 2015; Šilha et al., 2020).

The slightly more water-soluble terpenes impregnate the hydrolat with its specific scent (Martínez-Gil et al., 2013; Smail et al., 2011) providing them with the potential of metal salts reduction in green NPs synthesis. Industrially, hydrolats are considered invaluable by-products and are discarded (Garzoli et al., 2020; Martínez-Gil et al., 2013), or have food and cosmetic applications (Paolini et al., 2008).

Taking into consideration that medicinal and aromatic plants are intensively used to this day for their bioactive compounds (Moisă et al., 2018; Moisa et al., 2019), understanding and using by-products (hydrolats) (Popa et al., 2021) resulted from the essential oil (EO) industry as reducing and capping agents for metal nanoparticles synthesis is a promising alternative to classical synthesis.

Therefore, the present study aimed to use *Thymus vulgaris* hydrolats containing traces of dissolved essential oils, as mediators for the green synthesis of silver nanoparticles. To our knowledge, AgNPs from thyme hydrolats has not been obtained.

MATERIALS AND METHODS

All utilized chemicals and reagents were of suitable analytical grade purchased from Sigma-Aldrich and Merck.

Plant material and source

Fresh herbs of *Thymus vulgaris* L. were obtained from a local producer in Arad County. After harvesting, the plant material was airdried and stored in paper bags until needed.

EO and hydrolat extraction

Dried thyme plants were steam-distilled and the water–essential oil mixture was separated by density.

Hydrolat volatile compounds extraction

The resulting hydrolat was prepared for analysis after a liquid-liquid extraction step as follows: 1 mL hexane and 25 mL hydrolat kept in an ultrasound bath for 1 hour using the Elmasonic TI-H5 (Elma, Schimdbauer GmbH, Germany). After sonication, the mixture was separated using a Hettich ultracentrifuge (Rotina 380 R, Hettich GmbH, Tuttlingen, Germany) and the hexane layer was prepared for GC-MS analysis.

Chemical composition determined by GC-MS analysis

The resulting extract was analyzed by gas chromatography (GC, Shimadzu 2010, Kyoto, Japan) coupled with a mass spectrometer (MS, TQ 8040, Shimadzu, Kyoto, Japan). The GC-MS analysis method is described by (Chambre et al., 2020; Moisa et al., 2019).

Extracts preparation for Ag-NPs synthesis

The hydrolat was used immediately alongside 0.1 mM $AgNO_3$ solution with pH 8. Therefore, to 30 mL of boiling 0.1 mM $AgNO_3$ solution, 2 mL of hydrolat were added dropwise and mixed vigorously for 10 minutes on a hotplate magnetic stirrer. The reaction mixture was then transferred to another magnetic stirrer for 20 minutes without heat, to allow it to reach room temperature. The formation of AgNPs was confirmed visually by the colour change from a clear pale-yellow to a reddish-yellow or brown mixture.

UV-Vis analysis

The bio-reduction of $AgNO_3$ was monitored by scanning in the range of λ=300-800 nm using a UV-Vis spectrophotometer (Specord 200, Analytik Jena AG, Jena, Germany).

SEM-EDX analysis of AgNPs

To determine the morphology and size distribution of the obtained AgNPs, a scanning electron microscope was used (SEM, LYRA 3 XMU, Tescan, Czech Republic) coupled with an EDX - Energy Dispersive X-ray spectroscopy for elemental analysis (EDAX Inc., Mahwah, NJ, USA).

RESULTS AND DISCUSSIONS

Taking into consideration that hydro distillation is still the most intensively used method for obtaining essential oils, a large amount of hydrolat is generated in the process.

The hydrolat chemical composition is strongly related to that of the essential oil, and the main factor influencing the distribution of terpenic compounds in hydrolat is their polarity (Labadie et al., 2015; Popa et al., 2021). Thus, because the main compounds in thyme essential oil are phenolic based, its corresponding hydrolat has a high amount of dissolved volatiles and its potential for green nanoparticles synthesis is higher (Popa et al., 2021).

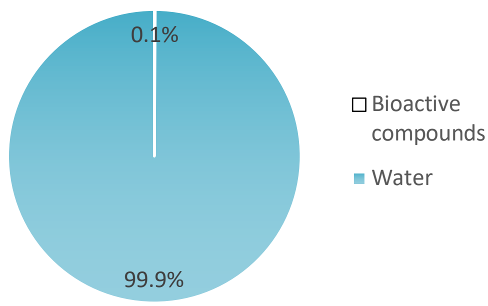

Figure 1. The average amount of bioactive compounds found in HD (Labadie et al., 2015)

Hydrolat chemical composition

After GC-MS analysis, 21 compounds were detected from which 18 were identified by comparing them with standards and by using the spectra libraries NIST 14, and Wiley 09.

The chemical composition of *Thymus vulgaris* hydrolat was composed mainly of oxygenated monoterpenes 92.63% (1-octen-3-ol, endo-borneol, carvacrol methyl ether, thymol, carvacrol). Hydrocarbonated monoterpenes and sesquiterpenes accounted only for 5.16%, and 2.2% of the compounds weren't identified.

As reported by other studies (Labadie et al., 2015; Popa et al., 2021) oxygenated compounds have a higher water solubility, therefore in thyme hydrolat the total amount of dissolved volatiles is higher making it suitable for Ag NPs synthesis.

Table 1. *Thymus vulgaris* hydrolat chemical composition

Nr. Crt.	RI	Compound name	Amount (%)
1	930	*α*-Thujene	0.07
2		ND	1.57
3	977	1-Octen-3-ol	1.7
4	980	3-Octanol	0.84
5	1024	*p*-cymene	2.33
6	1059	*γ*-terpinene	0.12
7	1070	*cis*-Sabinene hydrate	0.64
8	1096	Linalool	0.67
9	1169	endo-Borneol	1.76
10	1235	Thymol methyl ether	0.57
11	1244	Carvacrol methyl ether	1.68
12	1287	Bornyl acetate	0.22
13	1290	Thymol	63.77
14	1299	Carvacrol *	21.42
15	1376	*α*-Copaene	0.08
16	1419	*β*-Caryophyllene *	0.67
17	1441	Aromandrene	0.93
18	1481	Germacrene D	0.32
19		ND	0.56
20	1583	Caryophyllene oxide	0.01
21		ND	0.07

The RI was calculated compared to n-alkanes (C10 to C35) and the compounds marked with an asterisk (*) were identified using analytical standards.

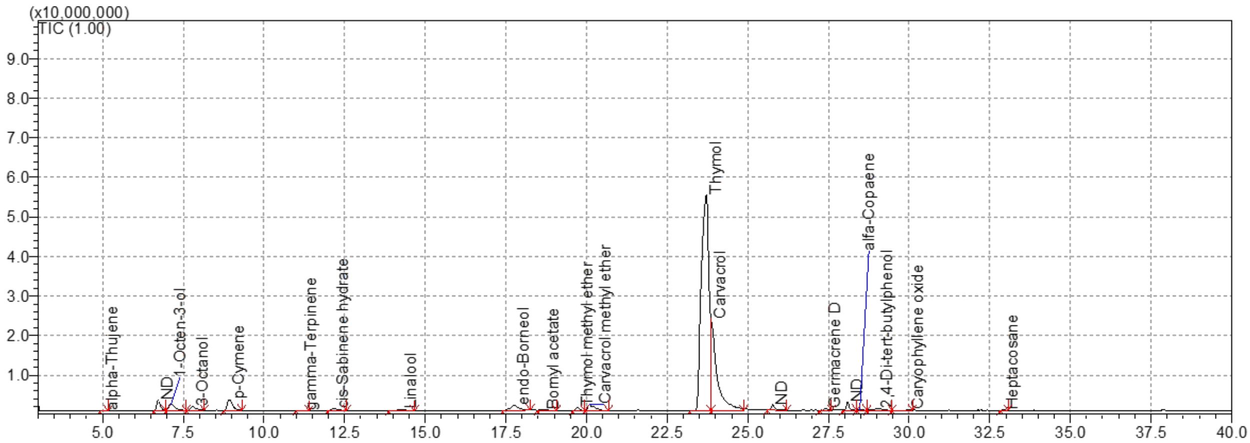

Figure 2. *Thymus vulgaris* hidrolat chromatorgram

UV-Vis Ag-NPs analysis

The bio-reduction of silver ions was mediated by the dissolved phenolic compounds present in the thyme hydrolat. This process was easily observed in the resulted reaction mixture by a visual colour change and confirmed by UV-Vis spectral analysis and a high value was recorded at 438 nm, specific for AgNPs with an average particle size of around 50-70 nm (Agnihotri et al., 2014).

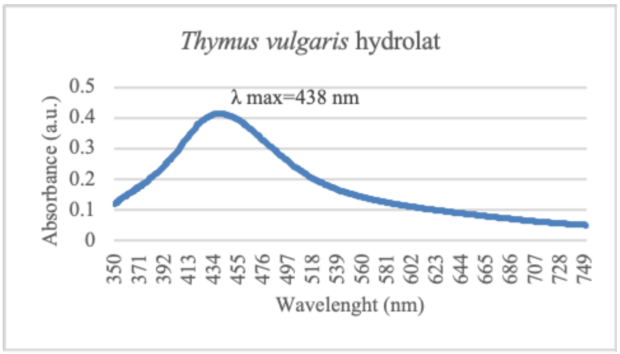

Figure 3. UV-Vis spectra of hydrolat biosynthesized AgNPs

SEM-EDX analysis of AgNPs

To analyse the particle size and surface morphology the obtained AgNPs mixture was centrifuged for 30 minutes at 10.000 rpm using a Hettich Rotina 380 R centrifuge and the AgNPs pellet was washed 3 times with ultrapure water. The purified AgNPs were placed on carbon tape, airdried and micrographs were recorded using a SEM at around 50kx magnification as presented in Figure 4.

The average particles shape was quasi-spherical and the size distribution was under 50 nm as presented in Table 2.

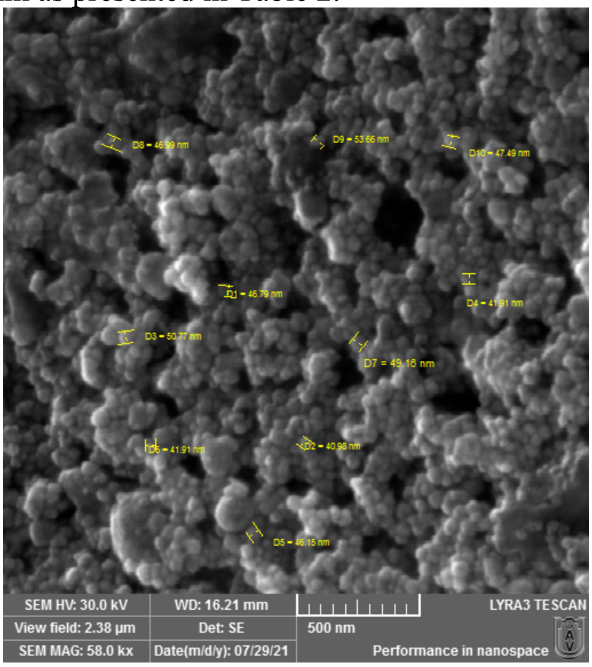

Figure 4. SEM micrograph of the hydrolat biosynthesized AgNPs

Table 2. Average size distribution of hydrolat biosynthesized AgNPs

Value	Hydrolat
	d [nm]
Obj. count	10
Summation	434.17
Min. value	36.28
Max. value	55.16
Mean value	43.42
Std. dev.	6.22

For further insight, the chemical composition of the resulted AgNPs was analysed by EDX, and elemental Ag was determined and confirmed by a strong signal at 3 keV representing more than 91% silver and minor quantities of carbon.

Figure 5 presents the EDX spectrum of the resulted AgNPs where no impurity peaks were detected. The EDX spectrum reveals the high purity of the resulted AgNPs.

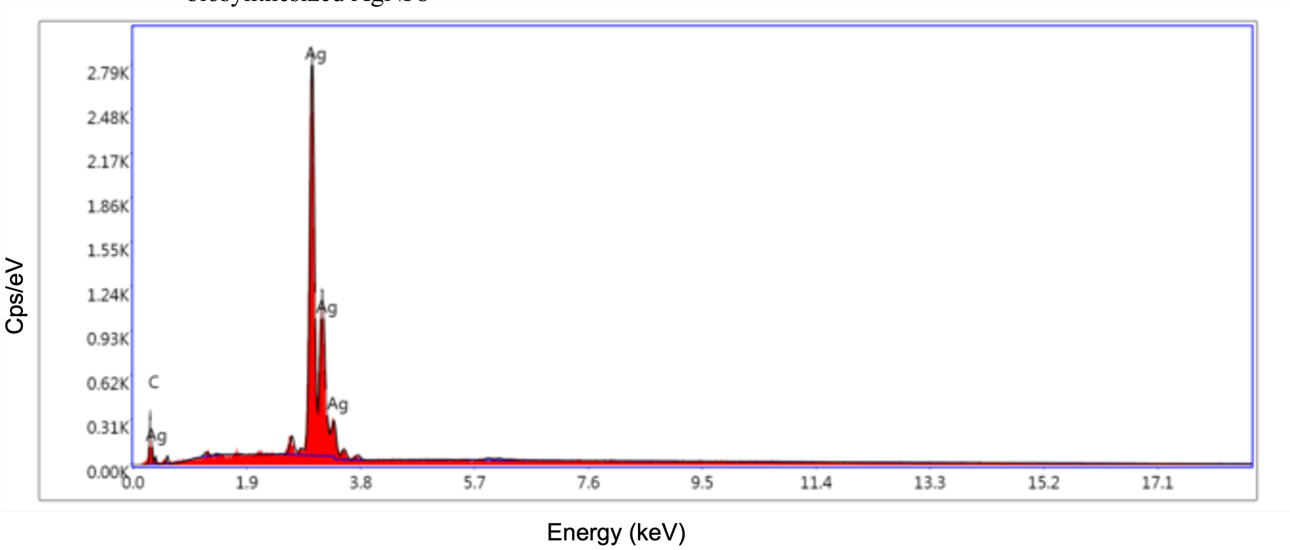

Figure 5. EDX spectrum of the hydrolat biosynthesized AgNP

CONCLUSIONS

The dissolved biomolecules present in the *Thymus vulgaris* hydrolat, are safe to handle and have mediated stable and eco-friendly quasi-spherical AgNPs with small average particle size.

SEM-EDX analysis confirmed the reduction of Ag^+ to Ag^0 with an average particle size of 43 nm and over 91% silver content.

Using plant extracts has many benefits from accessibility to a wide range of secondary metabolites useful for obtaining benign metal NPs with no side effects.

ACKNOWLEDGEMENTS

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