Spectroscopic Study of Phytosynthesized Ag Nanoparticles and Their Activity as SERS Substrate

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Abstract: The affordable and scalable synthesis of noble metal nanoparticles that are biocompatible without additional functionalization steps has been a growing field of research, stimulated by numerous prospective applications of these NPs. In the case of phytosynthesized or biogenic noble metal NPs, the mechanism of NP stabilization by biomolecules contained in each particular plant extract or living organism determines the possible applications of these NPs. In this work, we investigated Ag NPs synthesized in water with plant extracts of common toothwort (Lathraea squamaria) and two species of pepper (Capsicum annuum and Capsicum chinense). From FTIR and XPS, we drew conclusions about the composition of the functional groups and molecules that stabilize NPs in each extract, such as polysaccharide compounds (pectins, cellulose, glycosides and phenolic acids). Distinct characteristic IR features of amide I and amide II proteins were observed, which are common in plant extracts, while features of amide III were not distinctly observed in our extracts. A Raman spectroscopy study revealed weak own-SERS activity of the biomolecules of the extract and high efficiency of the NPs in the enhancement of “external” analytes, such as dyes and antibodies. This is the first report of the efficient SERS application of phytosynthesized Ag NPs.

Keywords: Ag nanoparticles; phytosynthesis; plant extracts; colloidal synthesis; plasmon; SERS; FTIR; XPS

1. Introduction

Metal nanoparticles (NPs) possess a unique combination of antibacterial, optical, electrical, and catalytic properties, stimulating intense research on NP design and functionalization for various applications [1–5]. Most progress is currently made in the area of substrates for surface-enhanced Raman spectroscopy (SERS) [6–8] and anti-pathogen applications [2]. Due to the significant role of anticipated applications in biology and medicine, there has been a steep increase observed in the number of works devoted to the bio-mediated synthesis of NPs [2,4,5,9–16]. The “green” synthesis methods include mainly those using biomass derivatives as reducing agents for metal cations, for instance, in the
reaction of AgNO₃ reduction to metallic Ag. The biomass-related process for synthesizing silver nanoparticles is simple and scalable, and emerging as a viable method to compete with conventional physical and chemical methods [2]. However, using plant biomass poses challenges in terms of purification of the synthesized NP solutions for their subsequent applications. Hydroxyl groups of the biomolecules from the plant extract play key reductive roles in the formation of metal nanomaterials, but strong interactions between biomolecules and metal nanomaterials may deteriorate their stability [2]. Furthermore, the biomolecules and other organic molecules stabilizing the bio-synthesized metal NPs can serve as a proper biofunctionalization for applications that require binding of the NP to the target (cell, tissue, or other biomolecules), while other applications may be hindered by interference of the “own” biomolecules with the analyte (target) molecules [9]. The latter issue can be critical for SERS applications, for instance, when the detection of extremely low analyte concentrations is required. Therefore, an important task in the area of biosynthesized NPs, not only metals but also oxides and semiconductors, is a better understanding of the content of biomolecules, their interaction with NPs, and their potential contribution to certain NP applications.

For the Ag NPs based on common toothwort extracts, we recently reported a pronounced antipathogen activity and enhanced seed germination [17]. Ag NPs based on pepper extracts showed an antibacterial effect on the development of antibiotic resistant Pseudomonas aeruginosa, as reported by us recently in Ref. [18]. Here, we report the results of an extended spectroscopic characterization of Ag NPs synthesized using the above aqueous plant extracts and demonstrate their efficiency as SERS substrates. This is the first report of the efficient SERS application of phytosynthesized Ag NPs.

2. Materials and Methods

2.1. Synthesis of Ag NPs

The plant materials used in the Ag NP synthesis included the rhizomes (roots) and inflorescences (flowers) of common toothwort (Lathraea squamaria L.), as well as the fruits (pericarps) of two species of pepper (Capsicum annuum cv. Teja (S-17) and Capsicum chinense cv. Carolina Reaper). The plant material was washed several times in deionized water to remove soil particles and any organic impurities and then air dried at 60 °C to eliminate residual moisture. The cleaned and dried phytomaterial was cut into small pieces and powdered into finely dispersed flour. Two grams of flour were put in a flask with a flat bottom with 100 mL deionized water and heated for 20 min at 80 °C. The obtained phytoextracts were filtered at room temperature with filter paper.

With common toothwort extracts, silver nanoparticles were synthesized with the addition of 0.001 M AgNO₃ silver nitrate. For the reduction of silver ions, 10 mL of fruit extract was mixed with 40 mL of silver nitrate. The resulting solution was incubated for 1.5 h at room temperature to develop Ag NPs under a light-emitting diode lamp (Secret Jardin, 42 W, 6500 K) according to the protocol reported by us recently [18]. The extract preparation of the pepper material and corresponding synthesis of Ag NPs has been described in detail in our previous work [18].

2.2. Characterisation of Ag NPs

UV-vis spectra of the Ag NP solutions obtained were recorded with an UV-1800 Shimadzu (Kyoto, Japan) spectrophotometer at a spectral resolution of 1 nm. The mean size and size distribution were estimated by scanning electron microscopy (SEM, Tescan Mira 3 MLU).

X-ray photoemission spectroscopy (XPS) measurements were performed with an ESCALAB 250Xi X-ray Photoelectron Spectrometer Microprobe (Thermo Scientific, Waltham, MA, USA) equipped with a monochromatic Al Kα (hv = 1486.68 eV) X-ray source. A pass energy of 200 eV was used for survey spectra and 20 eV for high-resolution core-level spectra (providing a spectral resolution of 0.6 eV). Spectra deconvolution and quantification were performed using the Avantage Data System (Thermo Scientific, Waltham, MA, USA).
To prevent charging, the samples were measured using a built-in charge compensation system. Finally, the spectra were corrected to the C1s sp³ peak at 284.8 eV as a common internal standard for binding energy (BE) calibration.

Raman spectra were excited with 457, 514.7, and 532 nm solid-state lasers and acquired using a single-stage spectrometer MDR-23 (LOMO, USSR) or the LabRam HR800 (HORIBA Jobin Yvon GmbH, Bensheim, Germany) micro-Raman system, both equipped with cooled CCD detectors. The laser power density on the samples was less than $10^3$ W/cm², which was low enough to preclude any thermal or photo-induced modification of the samples. A spectral resolution of 6 cm⁻¹ was determined from the Si phonon peak width of a single crystal Si substrate. The Si phonon peak position of 520.5 cm⁻¹ was used as a reference for the frequency position of the Raman peaks.

Infrared (IR) absorption spectra were recorded from NPs dried on double-side polished Si wafers using the transmission geometry of a VERTEX 80v FTIR spectrometer (Bruker, Billerica, MA, USA) equipped with a DLaTGS detector and a KBr beam splitter.

Solutions of E.coli antibody for Raman/SERS measurements were prepared by dissolving commercial monoclonal anti E. coli J5 lipopolysaccharide antibody (stock solution 0.5 mg/mL, Invitrogen, Waltham, MA, USA) with PBS 1:100 (pH 7.4).

3. Results and Discussion

3.1. Confirmation of the Ag NP Formation

UV-vis absorption spectra acquired at different time intervals during the synthesis from the aqueous extracts of Common toothwort roots exhibited a pronounced absorption band with a maximum around 440 nm (Figure 1a), characteristic of the localized surface plasmon resonance (LSPR) in Ag NPs [19]. The formation of metal NPs was also confirmed directly by SEM (Figure 1b), revealing NPs with a mean size of 30 nm and a size distribution of 10–50 nm. Similar results were obtained for Ag NPs synthesized using peppers, as reported by us recently [18]. Notably, the relatively large size distribution did not deteriorate the sharpness and intensity of the LSPR—its FWHM (~100 nm) was comparable to the values reported for Ag NPs obtained with very sophisticated and more expensive fabrication techniques [19].

![Figure 1](image.png)

**Figure 1.** UV-vis absorption spectra (a) and SEM image (b) of Ag NPs phytosynthesized using aqueous extracts of common toothwort roots. The three curves in (a) correspond to different times after initiating the synthesis. The inset in (a) is a photograph of the final NP solution.

3.2. XPS

The elemental analysis of the phytosynthesized Ag NPs was performed by XPS. Figures 2 and 3 show the XPS spectra for the samples obtained from the pepper and common toothwort extracts, respectively. In addition to the main elements expected, namely Ag, C, O, and N, traces of K and S were also detected. The dominant contributions
to the sample composition stemmed from carbon (50%) and oxygen (37%). The Ag content inferred from XPS was less than 1%. However, taking into account that the Ag NP size was much larger than the size of the organic molecules and the XPS probing depth (several nanometers), we can assess the real Ag content in the sample to be several %. Minor contents of K of 2–3% and S around 1% are common for various plant extracts. It is unlikely that the observed sulfur belonged to Ag$_2$S that is known to form on the surface of Ag nanostructures during their storage in atmosphere (see also discussion in the Raman section) because the S2p binding energies of sulfides occurred at 161–162 eV [20].

![High-resolution XPS spectra](image)

**Figure 2.** High-resolution XPS spectra of the Ag NPs synthesized using pepper extracts: Ag (a), C (b), O (c), S (d), N (e).

The spectra of the pepper extract-stabilized NPs are shown for the precipitated part of the solution (after a certain time of storage) and for the remaining supernatant liquid. It is natural to expect that the precipitated part of the colloidal NP solution would contain organic molecules strongly bound to the NPs responsible for the NP stabilization and interaction with the environment. The supernatant, on the other hand, contained species not directly involved in the stabilization or molecules loosely bound to NPs. From the spectra in Figure 2, we can conclude that K and S were contained in the initial plant extracts, but their involvement in the molecular species responsible for the NP stabilization may have differed between the two extracts. Sulfur and potassium were not present in the precipitate of NP synthesized with Carolina reaper extract, but were found in the Teja-stabilized NPs. The binding energy of S species found in all extracts (168 eV) is characteristic of SO$_x$ groups [20]. Although K and S were found in both pepper and common toothwort extracts, nitrogen was present in a meaningful amount only in the former. It was most probably related to capsaicinoids, one of two main components of the pepper extract [21,22], while the major expected constituents of the common toothwort extract did not contain nitrogen (see next section about IR results and Figure S1). Note that the transfer of the NPs (precipitate) from the aqueous solution to DMSO does not affect the elemental composition.
(Figure 2), thus indicating a perspective broad range of media/environments for these NPs and rigid stabilization of these NPs by the components of the phytoextract.

![Graph](image-url)

**Figure 3.** High-resolution XPS spectra of the Ag NPs synthesized with *Common toothwort* extract: Ag/K (a), C/K (b), O (c), S (d), N (e). A survey spectrum in the range of all elements is shown in (f).

The C1s spectra showed the most complex structure including four components, corresponding to the different bonding of the carbon atoms, as shown in the representative fit performed for the common toothwort Ag NPs sample in Figure 3b. In view of the low nitrogen content in the samples, the component with a binding energy of 285.8 eV was most likely related to C=O bonding rather than to C-NH.

### 3.3. FTIR

The main type of molecular species expected in pepper extracts prepared as in this work was reported to be capsaicinoids and total phenolic compounds [18,23]. The phenolic compounds found in chili peppers are gallic, protocatechuic, ferulic, o-coumaric, p-coumaric, sinapinic, trans-cinnamic, and caffeic acids, quercetin, catechin, rutin, and vanillin [23]. Many of these compounds belong to phenylpropanoids, characteristic of chili species.

There is limited information in the literature about the chemical composition of *Common toothwort*. Phytochemical studies showed the presence of iridoid aglycones, phenolic acids [24], phenylethanoid glycosides, and benzoic acid [25] in the whole plant [26,27]. However, thorough research on quantitative and qualitative compound profiles is missing [28].

A transmission/absorption spectrum in the mid-IR spectral range is a rich source of information on the chemical bonds in the sample and their relative content [29]. This spectral range consists of four regions that can be generalized as the X–H stretching region (4000–2500 cm⁻¹), the triple-bond region (2500–2000 cm⁻¹), the double-bond region (2000–1500 cm⁻¹), and the fingerprint region (1500–600 cm⁻¹). The latter is normally
a complex area showing many bands specific to the molecular structure of the sample, frequently overlapping each other. The IR spectra of the phytosynthesized Ag NPs in this work exhibited absorption features in the fingerprint region and partially in the double-bond region, shown in Figure 4a, as well as several features in the X–H stretching region, shown in Figure 4b. The position of all observed features is marked in the figure and listed in Table S1 along with their proposed assignment based on literature data. The spectra of the common toothwort roots and flower extracts were very close to each other (Figure S2); therefore, only one of them is presented in Figure 1.

From a comparative analysis of the values in the summary table (Table S1) one can conclude that the best matching of the vibrational features observed in the spectra of our phytosynthesized Ag NPs was found for the following polysaccharide compounds: pectins, cellulose, xylloglucan (hemicellulose), glycosides and phenolic acids (featured by vibrations of benzoic ring). Notable was the presence of the characteristic features of amide I and amide II of proteins, frequently observed in the IR spectra of plant extracts [16,30–33], while features of amide III were not distinctly observed in our extracts.

It can be noticed from Figure 4 that the spectra of the phytoextracts became more structured upon the formation of Ag NPs. This trend was demonstrated in the case of Carolina Reaper extract, for which we compared the spectrum of the pure extract, Ag NPs separated by precipitation from the supernatant, as well as the precipitated NPs transferred to DMSO. The spectrum of the supernatant was closer to that of the pure extract than to that of the NP precipitate, indicating that the coordination to the NP surface induces noticeable changes in the spectra of the extract molecules. Transfer to DMSO did not change the spectrum notably. This result is in agreement with the observations made in XPS, and confirms a rigid stable binding of the stabilizing extract molecules to the NPs.

Comparing the spectra of two cultivars of peppers, Teja and Carolina Reaper, high resemblance between their supernatant and precipitate spectra was observed. A similar situation was observed for the common toothwort-stabilized NPs, for which only one spectrum of the non-precipitated colloidal sample is shown in Figure 2. However, it is obvious that it contained a set of absorption features similar to the spectra of pepper extracts, except for the band at 1658 cm⁻¹ (tentatively due to an amide I vibration, according to Table S1).
From our analysis of the literature on phytosynthesized metal NPs, we could infer that there was no common trend of how the IR spectrum changed upon NP formation. In some works, significant changes in the IR spectra upon Ag NP synthesis have been observed, for instance: using the extracts of *Malva parviflora* [34], *Plumbago auriculata* [35], leaves of *Stevia rebaudiana* [36], spruce bark extract [37], and various other medicinal plant extracts [38]. In other works, hardly any changes were observed, e.g., for water beech (*Fagus sylvatica* L.) bark extract in Ref. [39], *Cistus incanus* leaves [40], and *Cinnamomum camphora* leaf [41]. Of course, the different molecular content of the extracts from different species may be responsible for the different extent of their interaction with the NP surface. Moreover, in works with much lower NP concentrations, the spectral effect of the NP–extract interaction can be simply masked by a prevailing amount of non-interacting molecules. Nevertheless, a preliminary conclusion that can be made here is that the extent of the NP–extract interaction and the rigidity of the stabilizing shell around the NPs are important factors for subsequent applications of such NPs. Regarding the samples studied in this work, for instance, the NPs stabilized with common toothwort exhibited much better long-term colloidal stability (for many months) and a pronounced SERS enhancement of external analytes (discussed in the next section). Most likely, the set of molecular species contained in the common toothwort extract was optimum for both high colloidal stability of the NPs and enabling access of the analyte molecules to the NP surface.

From the IR data presented above, we can assume which functional groups or molecular species may be involved in the NP stabilization. First, the absence of the amide I band (1658 cm\(^{-1}\)) in the sample with good SERS efficiency indicates that this bonding might be (at least partially) related to the poor SERS activity of the NPs. Note that the 1658 cm\(^{-1}\) band was also one of the strongest features present in the spectra of NP precipitates and absent in the supernatant (Figure 4a), further confirming the involvement of the corresponding bond in the NP stabilization by pepper extracts. On the other hand, the band at 1355 cm\(^{-1}\) was stronger in supernatants than in precipitates of the pepper extract-stabilized NPs, and was pronounced in the SERS-active NPs (synthesized from common toothwort). The closest match for this frequency is the N=O of the aliphatic nitro group or C–H bending (Table S1). As the nitrogen content detected by XPS was very low in all samples and lower for common toothwort than for peppers (see the previous section), a more probable assignment seems to be the C-H bending.

In Ref. [34] the peaks at 1671 and 1621 cm\(^{-1}\), characteristic of amide I, merged into one peak centered at 1648 cm\(^{-1}\), and these structural changes were interpreted by the authors as Ag reduction and stabilization of their Ag NPs proceeding via coordination between N of the amide group and Ag ions. Even though we also observed the most pronounced changes in this spectral range, they may be indicative of a different stabilization mechanism, because our XPS spectra showed no significant content of nitrogen in our extracts.

FTIR spectra of an alcohol extract of *Azadirachta indica* (neem) leaves reported in Ref. [31] exhibited a similar overall line shape to that of our spectra (Figure 4). In particular, the main absorption features were located around 1070, 1400, 1600, 3000, and 3250 cm\(^{-1}\) [31]. Based on their FTIR data, it was suggested in Ref. [31] that the water-soluble phenolic compounds present in the ethanolic extract acted as the reducing, capping, and stabilizing agents for the fabricated nanoparticles. The considerable reduction of the absorption band intensities upon formation of the nanoparticles was assumed to be an indication of a strong interaction between the silver ions and the biomass during the reduction process. In some works, the IR spectra were found to be very poor, not in intensity but in the number of absorption features. Thus, only one distinct band at 1632 cm\(^{-1}\), attributed to C=O stretching or tertiary amide linkages in the amino acid or protein residues, was observed in the whole range of 1000–2500 cm\(^{-1}\) for *Panax ginseng* root extract in Ref. [42]. The weak peak at 2108 cm\(^{-1}\) and the very broad and strong one at 636 cm\(^{-1}\) were attributed to C≡C stretching and the C-Cl group of alkyl halides, respectively [42]. None of them were affected by the Ag NP formation, even not the broad band at 3276 cm\(^{-1}\) attributed to the O-H stretching vibration of hydroxyl groups.
3.4. SERS

In terms of the enhancement of Raman signals by plasmon excitation localized in the colloidal Ag NPs stabilized by any sort of organic molecules, we should consider two cases (which can take place simultaneously): (i) “self-SERS”, i.e., SERS of molecules contained in the plant extract and serving as a stabilizer, bound physically or chemically to the NP surface; and (ii) SERS of the molecules added to the NP solution (or deposited on the NPs dried on a substrate) as an “external” analyte. Both (i) and (ii) were so far only very rarely reported for biosynthesized Ag (or Au) NPs [9,41]. In Ref. [9], coffee was used as a biogenic reductant for producing Ag NPs and weak characteristic vibrational peaks of organic molecules contained in coffee were detected in the Raman spectra as a result of the SERS effect. However, those biogenic adsorbates blocked catalytic surface sites and affected the nanoparticle functionality. In Ref. [43], Ag NPs produced by fungi *Rhodotorula glutinis* and *Rhodotorula mucilaginosa* were studied, for which the protein-capping layer structure was found to be easily disturbed, and consequently the SERS spectra were unstable. The thickness of the protein capping layer measured by atomic force microscopy was 21–24 nm, which is apparently too thick for applications such as SERS.

In this work, we used dye analytes such as rhodamine 6G (R6G), crystal violet (CV), and malachite green (MG), and *E.coli* antibodies as probes of the SERS efficiency of the Ag NPs. A pronounced enhancement of the analyte spectra was obtained for Ag NPs synthesized with common toothwort (Figure 5). A substantial enhancement was achieved for both root and flower extracts (Figure 5a), and observed not only for freshly synthesized NPs, but also for colloids stored several months after synthesis (Figure 5b). These results corroborate the rigid stabilization and very good colloidal stability of these Ag NPs.

Even though the detected concentrations of $10^{-4}$–$10^{-5}$ of such standard SERS analytes as R6G, CV, and MG were below the record value obtained with much more sophisticated and expensive SERS substrates reported thus far [44–47], this is the best result reported for affordable phytosynthesized Ag NPs to date. The SERS properties of these NPs are quite versatile, as can be concluded from a pronounced enhancement obtained also for a poor Raman scatterer such as an antibody (Figure 5d).

Besides the prospective of SERS application itself, the SERS experiments demonstrated that this particular (phyto)-functionalization of Ag NPs allows other molecules to approach the NP surface close enough to “feel” the plasmon field of the NP, i.e., within a distance of a few nm. This information is of primary importance for numerous other applications of bio-synthesized NPs.

Other issues, which are important for any SERS substrate but can especially be critical for bio-synthesized ones, are the self-SERS effect and fluctuation of the SERS signal. The bio-synthesized NPs possessing strong own-SERS spectra (of their stabilizer) are not suitable for SERS application, as with the case, e.g., in Ref. [48], where Ag NPs were prepared using different flavonoids (not extracted but commercially supplied flavonoids were used).

In some works, the Raman spectra were reported to vary with time for acquisitions at the same sample spot, even for small acquisition times, such as 0.1 s for each acquisition and 1 s between acquisitions [43]. The affected peaks at around 500, 900–1000, and 1600 cm$^{-1}$ might be assigned to a shift of the NH$_2$ twisting, wagging, rocking, and scissoring modes, respectively, indicating an amine–silver interaction [43]. The stabilization of biogenic Ag NPs results from the capping of protein structures, usually oligopeptides lying on the surface of the nanoparticle, thus providing stability of the NP surface and preventing the oxidation of surface Ag$^0$ to Ag$^+$. Various oxidizing or reducing agents, (laser) light, or heat can lead to the collapse of the NP because of altering the conformation structure of the oligopeptides or altering their load content, destabilizing the protein and consequently the silver nanoparticle.
For Ag NPs synthesized in this work, no significant contribution of self-SERS was observed (Figure S3). Even though we did not observe noticeable SERS of R6G and CV with pepper-derived Ag NPs, experiments with other analytes and phytosynthesized NPs are in progress. These studies aim to better understand the relation between the molecular composition of the biomaterials used for NPs synthesis and their SERS efficiency concerning certain analytes.

To finalize the SERS section of this work, we only mention one more effect that accompanies Raman experiments with Ag-based SERS substrates. It is related to observing vibrational features due to Ag–S vibrations, between 200 and 250 cm\(^{-1}\) (see, e.g., Figure 5c) which can originate from the Ag–O [49] and/or Ag–S [50,51] bonds on the Ag NP surface due to the reaction of the surface silver atoms with oxygen and/or sulfur present in the plant extracts and lab environment. Observing this vibration is another indication of the strong SERS efficiency of the NPs obtained in this work.

Figure 5. (a) Raman spectra of 10\(^{-4}\) M rhodamine 6G (R6G) with two freshly prepared Ag NP–common toothwort (L. squamaria) samples (roots and flowers). The spectra of bare NPs and the same concentration of R6G on glass were measured for comparison (as reference spectra). (b) Raman spectra of 10\(^{-5}\) M Rhodamine 6G (R6G) and crystal violet (CV) enhanced by the Ag NP common toothwort (root) after six months storage of the NP solution after synthesis. The spectra of bare NPs and the same concentration of R6G and CV on glass were measured for comparison (as reference spectra), as well as a 100 times higher concentration of R6G (10\(^{-3}\)), at which the Raman peaks start to be detected without enhancement. (c) Raman spectra of 10\(^{-3}\) M malachite green (MG) with two Ag NP–common toothwort samples (roots and flowers). (d) Raman spectra of E.coli antibody with Ag NP–common toothwort (roots), monitored with 1 s time intervals. The spectrum for the same antibody with ordinary Ag–citrate NPs is shown for comparison, illustrating that the same set of antibody-related features was observed. The schemes of the analyte molecule are shown for R6G (a), CV (b), and MG (c).

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4. Conclusions

Colloidal Ag NPs synthesized in water with plant extracts of common toothwort (*L. squamaria*) and pepper (*Capsicum annuum* and *Capsicum chinense*) were investigated using FTIR, Raman, and XPS spectroscopies. From the FTIR and XPS, we reached conclusions regarding the composition of the functional groups and molecules that stabilize NPs in each extract. The Raman spectroscopy study revealed only a weak own-SERS activity of the biomolecules of the extract, while a high efficiency in the enhancement of commonly used “external” analytes was detected for NPs synthesized from common toothwort extract. In addition, the latter NPs exhibited better long-term colloidal stability. From the comparison of the tentative composition of the extracts and IR and XPS spectroscopic data, we conclude that the difference in the SERS and colloidal behavior of the common toothwort- and pepper-stabilized NPs may be related to the different molecular composition of the extracts. Nonetheless, owing to the weak self-SERS effect, both sorts of NPs/phytoextracts are considered promising for SERS applications, and require more extended studies involving other analytes and phytoextracts. Taking into account the noticeable antibacterial effect on the development of antibiotical species observed for these NPs before, a potential new level of application of phytosynthesized NPs can be merging SERS with investigations of the NP effect on bacterial or other bio-objects.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/chemosensors10040129/s1, Figure S1: Schematic of the molecules reported in *L. squamaria* and *Capsicum chinense*; Figure S2. Spectra of IR absorption of common toothwort roots and flowers extracts; Figure S3: variation of the Raman spectra with time.

Author Contributions: Conceptualization, V.Y. and O.S.; methodology, M.K.; investigation, N.M., S.P. and O.H.; data curation, V.D.; writing—original draft preparation, V.D.; writing—review and editing, N.T. and D.R.T.Z.; supervision, V.Y.; project administration, V.D. and Y.P.; funding acquisition, V.D. and D.R.T.Z.; A.Y. reviewed and edited the manuscript, analyzed the manuscript contents and made the manuscript corrections. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially funded by National Research Foundation of Ukraine, grant number 2020.02/0204 (2020–2022), for S.P., Y.P., V.Y., V.D.; in particular, SERS substrate fabrication and Raman (SERS) and IR spectroscopy studies. V.D. acknowledges Visiting Scholar Program of the TU Chemnitz for funding his research stay at TU Chemnitz.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank Mykola Skoryk (G.V. Kurdyumov Institute for Metal Physics, NAS of Ukraine) for SEM measurements.

Conflicts of Interest: The authors declare no conflict of interest.

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