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Article

Ultrafast and Highly Efficient Laser Extraction of Matrine and Oxymatrine from *Sophora flavescens* for the Anticancer Activity

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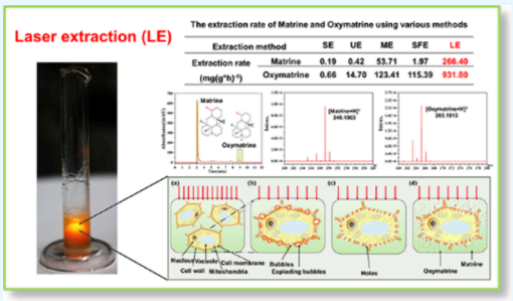
ABSTRACT: Matrine and oxymatrine are mainly obtained from *Sophora flavescens* using the high-temperature and prolonged solvent extraction methods currently employed in industries. In this study, an ultrafast and highly efficient method for extracting matrine and oxymatrine from *S. flavescens* at room temperature using laser technology, specifically, laser extraction, was demonstrated. The laser extraction rates for matrine and oxymatrine from *S. flavescens* at room temperature for 1 min were 266.40 and 936.80 mg(g·h)$^{-1}$, respectively. These rates were 1400 times higher than those achieved with conventional solvent extraction. These results mean that 1 min of laser extraction is equivalent to 24 h of solvent extraction. The reason for such a high efficiency is that laser-induced cavitation can accelerate the rapid release of alkaloid molecules in plant cells. Mass spectrum, nuclear magnetic resonance, and Fourier-transform infrared spectrum analyses of the extracted matrine and oxymatrine compounds confirmed that they are the same as the products of solvent extraction. Furthermore, it was found that the anticancer activity of laser-extracted compounds is slightly better than that of conventionally solvent-extracted ones, likely due to the slight change in the microstructure or conformation of these compounds under laser irradiation. These findings demonstrated that the laser extraction method was ultrafast and highly efficient, unveiling a novel approach to alkaloid extraction. This discovery will have significant implications for the extraction and utilization of alkaloids from plants.



INTRODUCTION

Colorectal cancer ranks among the second or third most common cancers globally, posing a serious threat to human health.[1] In recent years, matrine and oxymatrine have been extensively studied in both in vivo and in vitro models of colorectal cancer.[2−6] These alkaloids have demonstrated considerable anticancer potential due to their unique chemical structures and biological activities.[3,4] Notably, in the treatment of colorectal cancer, matrine and oxymatrine have been shown to effectively inhibit cancer cell proliferation and metastasis while inducing apoptosis in cancer cells.[5,6] Additionally, these compounds exhibit a range of biological functions, including anti-inflammatory, immunomodulatory, and antiviral properties, making them noteworthy as natural medicinal components.[3,7] Since 1992, China has approved the use of Compound Kushen Injection (CKI) for the treatment of various cancers, including colorectal cancer, with matrine and oxymatrine being the primary components.[8] CKI has not only been widely applied in clinical practice but also been proven to have significant therapeutic effects in numerous studies. These compounds have been extensively shown to markedly inhibit SW480 cells,[9−11] a specific colorectal cancer cell line, indicating their potential application in colorectal cancer treatment.

However, traditional extraction methods for matrine and oxymatrine have certain limitations. The current primary method is solvent extraction (SE), which is typically time-consuming and inefficient.[12] Other extraction methods, such as ultrasonic extraction (UE),[13] microwave extraction (ME),[14] and supercritical fluid extraction (SFE),[15] although showing certain advantages under laboratory conditions, are challenging to apply on a large industrial scale due to their complex operating conditions and high costs.[7]

Therefore, developing a rapid and efficient extraction method to obtain matrine and oxymatrine from plants is crucial for enhancing their clinical application value. In recent years, with advancements in technology, laser technology has increasingly been applied in the fields of medicine and food,[16−19] offering new possibilities for the extraction of natural products. Studies have shown that pulsed laser irradiation can promote the release and dissolution of compounds in a short period, with the advantages of simple operation, short time, and high efficiency.[20,21]

Herein, it is reported for the first time that ultrafast and highly efficient extraction of matrine and oxymatrine from *S. flavescens*

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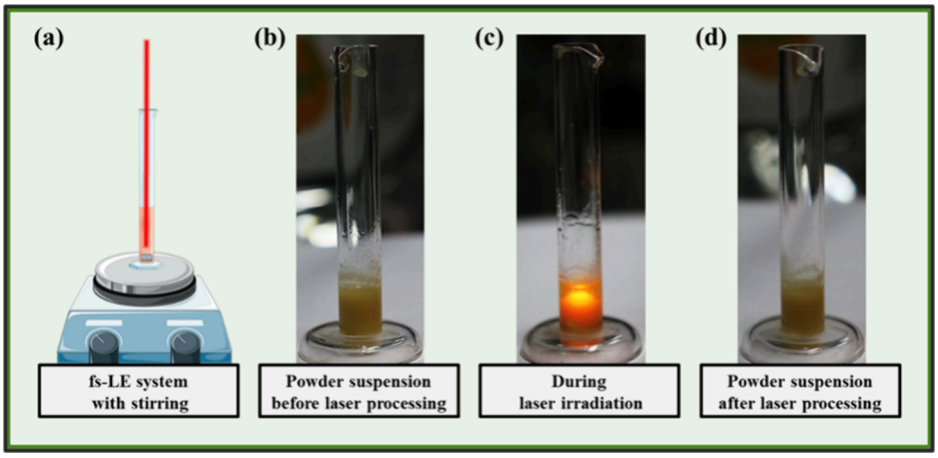

Figure 1. (a) fs-LE process with a stirring system, (b) *S. flavescens* powder suspension before laser irradiation, (c) *S. flavescens* powder suspension during laser irradiation, and (d) *S. flavescens* powder suspension after laser irradiation.

powder suspension was achieved by pulsed laser irradiation in liquids at room temperature. This method is named laser extraction (LE). This study aims to explore the application of the LE method for extracting matrine and oxymatrine, evaluate their efficiency and anticancer properties, and compare them with traditional extraction methods. The goal is to provide a novel solution for the extraction of alkaloids from plants.

EXPERIMENTAL SECTION

Materials. Flaky *S. flavescens* roots (origin: Shanxi Province, China) were purchased from Guangdong Shizhen Pharmaceutical Co., Ltd. The standard compounds matrine and oxymatrine were imported from Shanghai Macklin Biochemical Technology Co., Ltd. Ethanol (EtOH), methanol (MeOH), chloroform, and phosphoric acid were purchased from Fisher Chemical, USA. Cell culture materials were supplied by Gibco, Grand Island, NY, USA.

Plant Pretreatment, Extraction, Isolation, and Alkaloid Purification. The flaky *S. flavescens* roots were crushed into powder by a grinder, passed through a 200-mesh screen, and then sealed in a dry environment. Root powder (500 mg) was accurately weighed and placed in a special quartz tube and mixed with 3 mL of 60% aqueous ethanol solution.

For SE, the above-mentioned *S. flavescens* suspension was soaked at room temperature and stirred for 24 h.

For LE, femtosecond pulsed laser irradiation of the aforementioned *S. flavescens* suspension was performed with a Vision-S laser (Chameleon Ultra, Vision) centered at 753 nm (this process is called fs-LE). An fs-laser with a pulse duration of 140 fs, a repetition rate of 80 MHz, and a laser output power of 3.4 W was used in the experiments. Continuously applying femtosecond-pulsed laser in the solution generates consistent thermal effects. The experiment was restricted to a duration of 1 min to regulate and minimize uncertain thermal impacts. To ensure thorough extraction, a smaller quartz tube reactor equipped with a stirring system was employed. This configuration facilitated complete contact between the loaded suspension and the pulsed laser within 1 min, thereby enhancing the extraction process. Ultimately, to achieve efficient extraction without inducing excessive thermal effects, loading 0.5 mL was determined as the optimal volume. In short, the experiments were carried out in a smaller quartz tube (only 0.5 mL suspension) irradiated for 1 min and equipped with a stirring system. The schematic procedure is shown in Figure 1a. Finally, a total of 3 mL of irradiated suspension was collected in a single experiment for follow-up treatment. After extraction, the supernatant was collected by centrifugation and filtered by a 0.44 μm filter membrane for subsequent analysis, isolation, and purification. A sufficient amount of each extraction solution was collected under the two extraction methods for the isolation and purification of alkaloid monomer. The isolation and purification were performed by Nanjing DASF Biotechnology Co., Ltd., using the following steps: the ethanol from the extract of *S. flavescens* was recovered, and the concentrated solution was diluted with six times its volume of water and left to stand overnight. The supernatant was collected and processed with a D101 resin column. The column was eluted sequentially with three column volumes of water, 20% ethanol, and 70% ethanol. The 70% ethanol eluent was collected, and after recovering the ethanol, the concentrated solution was dried. The dried concentrate was mixed with silica gel and applied to a silica gel column, where fractions containing matrine and oxymatrine with a purity above 80% were collected. These crude products were further purified using a preparative column to obtain matrine and oxymatrine monomers with 98% purity. In addition, particle size analysis of *S. flavescens* powder was conducted before and after extraction using a Laser Particle Sizer (Malvern Mastersizer 3000, UK) under wet conditions.[22] Morphological characterization of the powders pre- and postextraction was performed via field emission scanning electron microscopy (SU8010, Japan) utilizing an acceleration voltage of 10 kV and operating in secondary electron mode.

HPLC Analysis. The extraction solution was diluted five times and packed into a 2 mL special vial for high-performance liquid chromatography (HPLC) analysis performed on an UltiMate3000 (Thermo Fisher, USA) system equipped with an Agilent Zorbax Extend-C18 column (250 mm × 4.6 mm × 5 μm).[23] The UV spectra were recorded between 200 and 400 nm to determine the peak characteristics. Matrine and oxymatrine exhibit significant UV absorption near 217 nm, so the detection wavelength was set at this value. The content of matrine and oxymatrine was calculated using the standard curve method.

Monomer's Structure Elucidations. Before the isolated monomers were elucidated, solutions of each monomer were prepared as a single solution, and their purity was checked according to the HPLC analysis method mentioned above. The chemical structure of the monomer was then determined using

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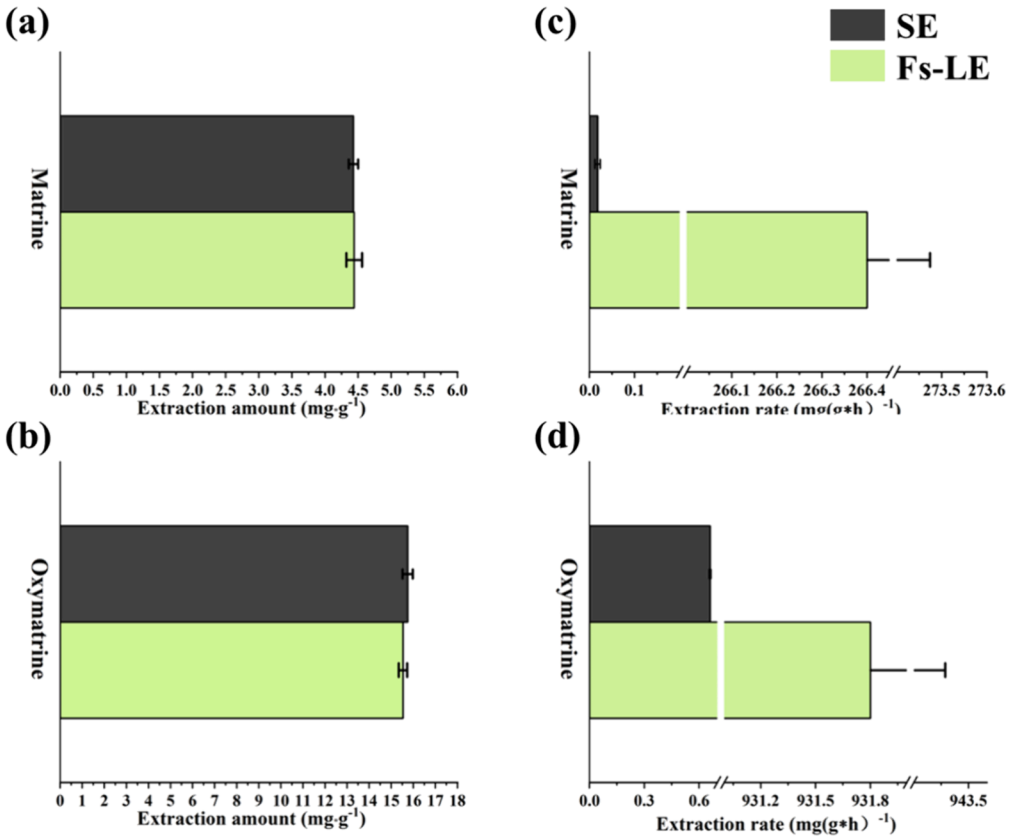

Figure 2. Extraction efficiencies of SE and fs-LE: (a, b) the extraction amount and (c, d) the extraction rate.

FTIR, MS, and NMR (^{13}C NMR and ^{1}H NMR) spectroscopic techniques.[24]

For the FTIR, the KBr pellet technique was employed on a Bruker spectrograph (Bruker, Billerica, MA, USA). The spectra were obtained at a coaddition of 256 interferograms collected at 4.0 cm^{-1} resolution and with a wavenumber range of 400−4000 cm^{-1}.

For the MS, a Fourier-transform ion cyclotron resonance (FT-ICR) apparatus (Perkin-Elmer, Waltham, MA, USA) at room temperature was used to obtain the compound mass by the electrospray ionization (ESI) method.

The NMR was performed on a Bruker Advance III 500 MHz instrument, and samples were measured with $CDCl_3$ (δ_H 7.26 s; δ_C 77.16 s). The resulting signals were demonstrated on a ppm scale using tetramethylsilane (TMS) as the internal standard.

***In Vitro* Anticancer Test.** The identified monomers were used for *in vitro* cell anticancer evaluation. SW480 cells (colon adenocarcinoma SW480 cells) were chosen for the experiment. SW480 cells were cultured in DMEM containing 10% fetal bovine serum (Invitrogen) 100 U/mL penicillin, and 100 mg/mL streptomycin. The cells were cultured in a standard incubator at 37 °C, 21% O_2, and 5% CO_2.

The cell viability was assessed using the MTT assay.[25] SW480 cells were seeded in 96-well plates overnight at a density of 6000 cells per well. The medium was then replaced with 200 μL of fresh DMEM containing various concentrations of monomers individually. After incubating at 37 °C for 24 h, the medium was replaced with a 10% MTT solution. Following that, 100 μL of DMSO was added, and the plates were analyzed with a microplate reader at an absorbance of 575 nm. Each experiment was performed at least three times.

To evaluate the number of apoptotic cells, Annexin V/PI staining was performed.[25] SW480 cells were individually treated with 2 mg/mL monomers. After 18 h, the cell pellet was resuspended in 100 μL of 1× Annexin binding buffer containing 10 μL of Annexin V. After a 15 min incubation at room temperature, 2 μL of PI and 900 μL of binding buffer were added. Following a 10−15 min incubation, the cells were analyzed by flow cytometry. Each experiment was performed at least three times.

Furthermore, Calcein-AM/PI double stain was used to detect cell viability.[26] SW480 cells were treated with 2 mg/mL monomers individually for 24 h and then incubated with Calcein-AM (10 μM) and PI (10 μM) at 37 °C for 20 min. The cells were subsequently analyzed by flow cytometry. Each experiment was performed at least three times.

Statistical Analysis. All experimental results were derived from three replicated experiments, and Origin 2021 was used to obtain the mean ± standard deviation (mean ± SD).

RESULTS AND DISCUSSION

LE Process. It is well-known that the concentration of the organic solvent plays an important role in the extraction of alkaloids from plant materials,[27,28] and 60% ethanol was found to be highly suitable for the extraction of alkaloids from *S. flavescens*.[23,29] Therefore, it was reasonably chosen as the extracting solvent in this work. The ratio of liquid to material was set to 10:1 based on previous reports and pre-experiments.[14,23,29] The selection of powder mesh was similar to the ratio of the liquid to the material. The influence of output power from the femtosecond-pulsed laser, laser wavelength, and irradiation time on the extraction process was considered. Experimental data on these effects on extraction amount are

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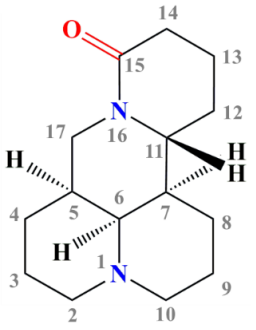
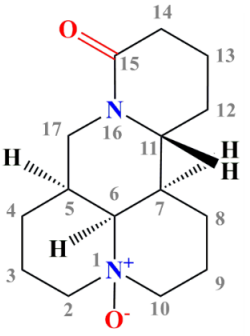
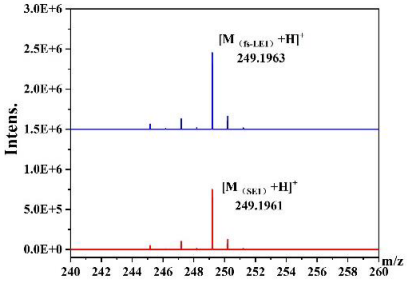
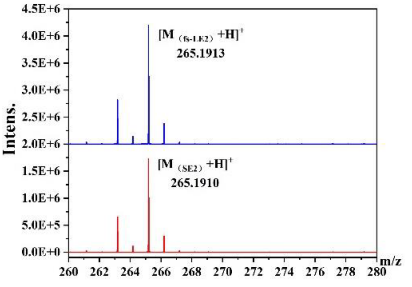
Table 1. Extraction Rates of Matrine and Oxymatrine Using Various Methods[a]

extraction method		SE	UE[13]	ME[14]	SFE[15]	LE
extraction rate $(mg(g\cdot h)^{-1})$	matrine	0.19	0.42	53.71	1.97	266.40
	oxymatrine	0.66	14.70	123.41	115.39	931.80

[a]SE and LE were the data in this study.

Table 2. Chemical Properties in Terms of the Mass (Measured by Mass Spectrometry (MS)) and the Infrared Spectra (FTIR) of the Monomers (fs-LE1, SE1, fs-LE2, SE2) from *S. flavescens*[a]

Alkaloid	Matrine		Oxymatrine	
Chemical formula	$C_{15}H_{24}N_2O$		$C_{15}H_{24}N_2O_2$	
Chemical structure				
Monomer	fs-L1	SE1	fs-LE2	SE2
Mass (MS)				
	MS: m/z = 249.1963 [M + H]$^+$ Theory: 249.1955	MS: m/z = 249.1961 [M + H]$^+$ Theory: 249.1955	MS: m/z = 265.1913 [M + H]$^+$ Theory: 265.1898	MS: m/z = 265.1910 [M + H]$^+$ Theory: 265.1898

[a]fs-LE1 and fs-LE2 were extracted by the fs-LE method; SE1 and SE2 were extracted by the SE method.

described in the Supporting Information (Figure S1). The extraction yield is primarily determined by laser power and irradiation time. Insufficient power may lead to inadequate extraction, whereas excessive power could potentially damage the alkaloid structure, reducing the alkaloid content. Therefore, selecting the appropriate extraction power is crucial. Laser irradiation generates heat, and increasing the duration of laser exposure raises the temperature of the extraction solution. High temperatures may damage the structure of bioactive alkaloids, thereby affecting the extraction efficiency. Shorter laser irradiation periods could quickly complete the extraction process. Ultimately, fs-LE for extracting *S. flavescens* alkaloids was established in Figure 1a. The process of femtosecond-pulsed laser irradiation in suspension is depicted in Figure 1c. The pulsed laser was fully applied to the suspension, and the reaction was facilitated by a stirring system, resulting in complete extraction within 1 min. Visually, there were no notable changes observed in the suspension before (Figure 1b) and after (Figure 1d) irradiation. LE is a simple method offering real-time extraction status visualization.

LE Efficiency. To verify the viability of fs-LE, the analysis focused on the yield of alkaloids in the extract. Among the alkaloids found in *S. flavescens*, matrine and oxymatrine are the primary bioactive compounds for disease treatment. Hence, the detection of these two alkaloids was given priority. The presence of matrine at 44.40 μg/mL and oxymatrine at 155.30 μg/mL in the extract is illustrated in Figure S2, indicating that a 1 min fs-LE process was adequate for extracting alkaloids from *S. flavescens*. The peak area was utilized for determining the yield of matrine and oxymatrine based on the standard curve. The extraction amount and extraction rate of matrine and oxy-

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matrine using fs-LE and SE were computed using eqsAand B, respectively.

$$\text{Extraction amount } (\text{mg}\cdot\text{g}^{-1}) = \frac{\text{weight of alkaloid (mg)}}{\text{weight of } \textit{Sophora flavescens} \text{ powder (g)}} \quad (A)$$

$$\text{Extraction rate } (\text{mg}(\text{g}\cdot\text{h})^{-1}) = \frac{\text{weight of alkaloid (mg)}}{\text{weight of } \textit{Sophora flavescens} \text{ powder (g)} \times \text{extraction time (h)}} \quad (B)$$

The outcomes are depicted in Figure 2. For fs-LE, the extraction amounts of 4.44 mg·g^{-1} for matrine and 15.53 mg·g^{-1} for oxymatrine were essentially the same as for SE of 4.43 mg·g^{-1} for matrine and 15.74 mg·g^{-1} for oxymatrine (Figure 2a,b). It is indicated that fs-LE and SE have consistent extraction amounts for matrine and oxymatrine. The extraction rate of fs-LE of matrine and oxymatrine in 1 min was 266.40 and 936.80 mg(g·h)$^{-1}$, respectively. Compared to the traditional SE that needs 24 h, the extraction rates of matrine and oxymatrine were 1402 and 1419 times higher (only 0.19 mg(g·h)$^{-1}$ for matrine and only 0.66 mg(g·h)$^{-1}$ for oxymatrine). Thus, this difference was significant (Figure 2c,d). It was evident that fs-LE was a more efficient extraction method compared to SE. However, it should be noted that even without comparing it to SE, fs-LE was still highly efficient on its own (Table 1).

Monomer's Structure Identification. A satisfactory quantity of samples was extracted using both the fs-LE and SE methods, facilitating subsequent experimental studies and comparisons. This ensures the reliability and accuracy of the experiments, leading to more compelling findings. After isolation and purification, the monomers obtained from the fs-LE method (fs-LE1 and fs-LE2) and SE methods (SE1 and SE2) exhibited high purity. This was confirmed by the presence of only one characterized peak in the HPLC chromatograms (see Supporting Information, Figure S3 and Table S1), indicating their suitability for chemical determinations and biological evaluation. Then, these structures of monomers were fully identified based on the FTIR, MS, and NMR methods. The FTIR spectra of fs-LE1 and SE1 are identical, and so are those of fs-LE2 and SE2 (Table 2). The MS spectra and fragmentation of the $[M + H]^+$ ion of each monomer are detailed in Table 2. The chemical shifts of H NMR and C NMR are detailed in Table 3 and correspond to the number of hydrogen and carbon atoms.

All data of these monomers are consistent with the literature.[24,30,31] Therefore, fs-LE1 and SE1 were determined as matrine, named fs-LE-M and SE-M, respectively. fs-LE2 and SE2 were determined as oxymatrine, named fs-LE-OM and SE-OM, respectively. These indicated that the fundamental structures of the compounds have not been altered by laser irradiation during the fs-LE process. However, the microscopic structures or conformations of these compounds might undergo slight alterations under laser irradiation, which structural characterization methods such as NMR, IR, and mass spectrometry cannot detect. Nevertheless, this does not exclude the possibility of subtle structural differences.

Anticancer Assays. To evaluate the anticancer effects of the obtained matrine and oxymatrine, SW480 cells were exposed to different concentrations of these compounds to observe their impact on cell proliferation, cell viability, and apoptosis processes. The cytotoxicity of matrine and oxymatrine toward SW480 cells was evaluated using the classical MTT assay (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide) at different concentrations for 24 h. As shown in Figure 3a,b, matrine and oxymatrine compounds obtained by fs-LE or SE exhibited remarkable cytotoxicity against SW480 cells. In the case of matrine treatment, the cell viability of SE-M and fs-LE-M groups was sharply decreased to 18 and 15% at a concentration of 4 mg/mL, respectively, indicating an effective and strong dose-dependent cytotoxicity. However, oxymatrine demonstrated lower toxicity toward SW480 cells. The cell viability of SE-OM and fs-LE-OM groups was decreased to 73 and 70% at a concentration of 4 mg/mL, respectively. Moreover, the results of Annexin V/PI staining and dead/live cell staining (Figure 3c−g and h−i) consistently confirmed that both matrine and

Table 3. Nuclear Magnetic Resonance (NMR) Chemical Shifts (Carbon and Hydrogen) of the Monomers[a]

	fs-LE1 (matrine)			SE1 (matrine)		
monomer	δ_C (fs-LE1)	δ_H (fs-LE1)		δ_C (SE1)	δ_H (SE1)	
2	57.58	2.72−2.85	2.72−2.85	57.57	2.71−2.84	2.71−2.84
3	21.47	1.31−1.99	1.31−1.99	21.46	1.32−1.99	1.32−1.99
4	27.44	1.31−1.99	1.31−1.99	27.43	1.32−1.99	1.32−1.99
5	35.63	1.31−1.99		35.62	1.32−1.99	
6	64.06	1.31−1.99		64.05	1.32−1.99	
7	41.76	1.31−1.99		41.75	1.32−1.99	
8	26.74	1.31−1.99	1.31−1.99	26.73	1.32−1.99	1.32−1.99
9	21.06	1.31−1.99	1.31−1.99	21.04	1.32−1.99	1.32−1.99
10	57.51	2.72−2.85	2.72−2.85	57.50	2.71−2.84	2.71−2.84
11	53.49	3.79		53.48	3.79	
12	28.04	1.31−1.99	1.31−1.99	28.03	1.32−1.99	1.32−1.99
13	19.25	1.31−1.99	1.31−1.99	19.24	1.32−1.99	1.32−1.99
14	33.11	2.35−2.45	2.35−2.45	33.10	2.36−2.46	2.36−2.46
15	169.81			169.79		
17	43.52	4.37	3.79	43.50	4.37	3.79
	fs-LE2 (oxymatrine)			SE2 (oxymatrine)		
monomer	δ_C (fs-LE2)	δ_H (fs-LE2)		δ_C (SE2)	δ_H (SE2)	
2	69.73	3.06	3.12	69.71	3.05	3.14
3	17.51	2.71	1.54	17.49	2.70	1.54
4	26.39	1.67	1.78	26.38	1.67	1.78
5	34.82	1.82		34.80	1.82	
6	67.47	3.00		67.46	3.00	
7	42.96	1.60		42.95	1.60	
8	24.96	1.52	2.01	24.95	1.52	2.00
9	17.46	2.62	1.49	17.45	2.62	1.49
10	69.37	3.03	3.10	69.35	3.03	3.12
11	53.26	5.04		53.25	5.04	
12	28.82	1.22	2.15	28.81	1.21	2.15
13	18.92	1.64	1.74	18.91	1.64	1.74
14	33.18	2.21	2.40	33.17	2.21	2.40
15	170.47			170.47		
17	41.97	4.13	4.36	41.96	4.12	4.36

[a]fs-LE1 and SE1 were identified as matrine, and fs-LE2 and SE2 were identified as oxymatrine. Corresponding references.[30,31]

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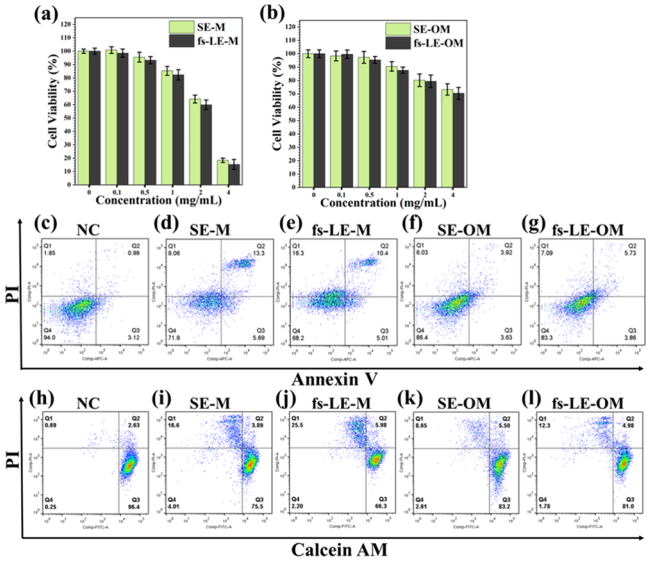

Figure 3. (a, b) Cell viability of SW480 cells treated with different concentrations of matrine and oxymatrine compounds obtained by the LE method or fs-LE method. (c–g) Annexin V/ PI (propidium Iodide) staining. SW480 cells were treated with marine or oxymatrine compounds obtained by the LE method or fs-LE method and stained with Annexin V-APC (allophycocyanin) and PI. (h–l) Dead/live cell staining of SW480 cells treated with marine or oxymatrine compounds obtained by the LE method or fs-LE method.

oxymatrine compounds obtained by the fs-LE or SE method induced apoptosis after 18 or 24 h of incubation. Results of apoptosis tests showed that 28.5, 31.8, 13.5, and 16.68% of sw480 cells were involved in the apoptosis and necrosis pathway after treatment with SE-M, fs-LE-M, SE-OM, and fs-LE-OM, respectively. These results indicate that both matrine and oxymatrine obtained from the fs-LE and SE methods exhibit significant inhibitory effects on SW480 cells. The inhibitory effects of matrine and oxymatrine on SW480 cells were due to the fact that, on the one hand, matrine and oxymatrine can disrupt the cell cycle regulation of SW480 cells, thereby preventing their entry into the proliferative phase, and on the other hand, matrine and oxymatrine can modulate the expression of genes associated with tumorigenesis, inhibit the proliferation signaling pathways of cancer cells, and promote apoptosis.[6,9,32] It is worth noting that the anticancer properties of the matrine and oxymatrine compounds obtained through the fs-LE method are like those obtained through the SE method. This suggests that the alkaloids obtained through this specific extraction process retain their original medicinal activity. It should be noted that the inhibitory effects of matrine and oxymatrine obtained through the fs-LE method were slightly superior to those obtained through the SE method (although there is no significant difference between the two). These differences may be attributed to variations in the compound's microstructure or conformation. During laser irradiation, fluctuations in the microenvironment, such as rapid changes in local temperature and transient high energy density, can induce subtle alterations in the microstructure, thereby affecting the activity of the substance.[33] In summary, matrine and oxymatrine obtained through the fs-LE method exhibit good anticancer properties, and this extraction process does not affect the medicinal activity of the alkaloids.

LE Mechanism. The particle size of the powder was assessed both before and after extraction, revealing a noticeable reduction in size following both SE and fs-LE methods (Figure S4). Notably, the SE method showed a more substantial decrease. Moreover, the comparison of images depicting the morphology of the powder before and after extraction aligns with the observed variations in the particle size (Figure S5). This provides further validation to the findings and offers additional supporting evidence for the observed changes in particle size distribution. The SE method adopts the principle of similarity and compatibility, which is a slow impregnation and infiltration process.[34] Powder was broken smaller due to impregnation and osmotic pressure, allowing matrine and oxymatrine to leach from the powder to the solution. Laser fragmentation is extensively employed in the production of inorganic nanoparticles.[35–38] Additionally, specific drugs like fenofibrate and naproxen can undergo fragmentation using femtosecond lasers in aqueous environments.[20,39] Laser fragmentation has demonstrated enhanced dissolution kinetics.[20] In the context of this study, the decrease in powder size after fs-LE may be attributed to laser fragmentation, which facilitates the release of the components matrine and oxymatrine from the powder into the solution.

The powder, derived from the pulverized roots of *S. flavescens*, comprises xylem cells, phloem cells, duct cells, sieve cells, and other cellular components. These *S. flavescens* powders can be viewed as an aggregation of the various cells found in the plant's roots. For the sake of clarity and to enhance understanding of the LE mechanism, the powder was regarded as intact cells throughout this study. The formation of cavitation bubbles during pulsed laser irradiation in the solution is well-documented.[40–43] Femtosecond laser irradiation in a cell suspension leads to the formation of cavitation bubbles around the cells. These bubbles promptly implode and directly collide with the cells, causing mechanical damage and potentially

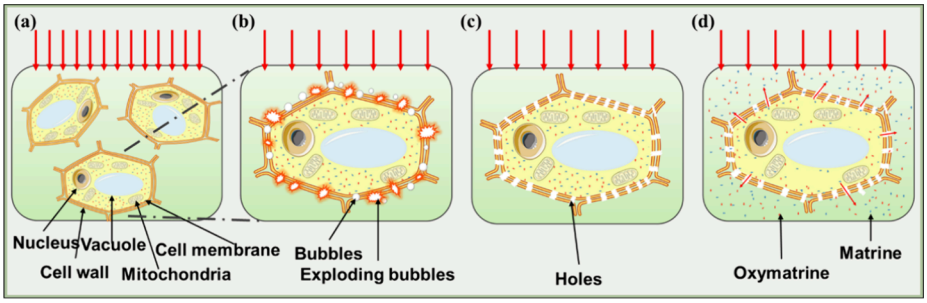

Figure 4. LE schematic. (a) Laser irradiated cell (powder) suspension. (b) Cavitation bubble formation and explosion. (c) Hole punctuation. (d) Matrine and oxymatrine were released from the broken site and the holes.

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creating puncture holes.[44,45] This phenomenon is known as the femtosecond laser-induced cavitation effect. The generation and collapse of cavitation bubbles release a tremendous amount of energy, subjecting the cells to high-pressure and high-velocity impacts, resulting in cell damage. Hence, the cells might have been fragmented and punctuated by successive explosions of cavitation bubbles during the fs-LE process, which might be the reason for the reduced particle size mentioned above. Interestingly, femtosecond lasers can also perforate cells.[45−47] When femtosecond laser irradiates the cell surface, pores form in the cell wall, creating a connection between the cell's interior and exterior.[45] This continuous high-frequency pulsed laser not only creates holes on the cell surface due to cavitation bubble explosions but may also penetrate the cell, forming thousands of minuscule pores. Therefore, during the process involving femtosecond lasers, the powder undergoes significant structural changes. More importantly, the femtosecond laser perforation of cells drastically decreases the Young's modulus of the cell wall and weakens the cell stiffness,[46] which probably allows the mechanical force damage of the cavitation bubble in the fs-LE process to easily cause hole punctuation and cell fragmentation.

In summary, the LE mechanism (Figure 4) is as follows: during the fs-LE process, the femtosecond laser can perforate the cells (powder), creating countless channels on the cells and decreasing their stiffness. At the same time, the mechanical force of the cavitation bubble explosion generated by the femtosecond laser in the solution will fracture the cells and punctuate holes (Figure 4b,c). Because the cells are perforated at the same time, the explosive force of those cavitation bubbles makes the cells' fragmentation more efficient, and the cell fragments will continue to be perforated. Perforation and fragmentation continue to occur uninterruptedly. Eventually, many holes in the cells' surface appeared within a short time (1 min), and the cells were also constantly breaking into smaller pieces (Figure 4d). The intracellular (intrapowderous) substances such as matrine and oxymatrine were then rapidly released from the broken site and the holes into the solution.

CONCLUSIONS

In summary, a simple, ultrafast, and highly efficient method of extracting matrine and oxymatrine from *S. flavescens* using laser technology has been developed. Surprisingly, LE rates are more than 1400 times higher than those of SE, which imply that 1 min LE is equivalent to 24 h SE. The structure of the compounds extracted via LE was consistent with that obtained through SE, yet the anticancer activity of the LE-extracted compounds was superior. These results thus showed that the pulsed laser has an efficient and nondestructive capability for extracting alkaloids while maintaining their structural integrity and biological activity. Compared to the traditional SE method, the LE process concludes within 1 min, thus significantly reducing time costs. The LE method operates without the need for heating, aligning with a low-carbon, environmentally friendly paradigm. Although the relatively high cost of lasers may constrain the wide application of the LE method for alkaloid extraction, LE as a novel extraction method offers tremendous potential for further research and optimization due to its exceptional performance.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c05003.

(1) The results of HPLC analysis for matrine and oxymatrine obtained from SE or fs-LE, including their retention times and purity data. (2) The amount of matrine and oxymatrine under varying output powers, different wavelengths, and different irradiation times for the LE method. (3) The powder particle size and powder morphology images of *S. flavescens* before and after LE or SE (PDF)

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Author Contributions

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Author Contributions

P.W. and P.W.: experimental work. W.C. and N.L.: data analysis. H.Z. and G.Y: project planning.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Sung, H.; Ferlay, J.; Siegel, R. L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Ca-Cancer J. Clin.* **2021**, *71*, 209−249.

(2) Chen, M.-H.; Gu, Y.-Y.; Zhang, A. L.; Sze, D. M. -y.; Mo, S.-L.; May, B. H. Biological effects and mechanisms of matrine and other constituents of *Sophora flavescens* in colorectal cancer. *Pharmacol. Res.* **2021**, *171*, No. 105778.

(3) Lan, X.; Chen, Y.; Duan, J. J.; Xu, J. Study on oxymatrine-based research from 2001 to 2022: A bibliometric analysis. *Acs Omega* **2024**, *9* (8), 9633−9643.
(4) Rashid, H. U.; Xu, Y. M.; Muhammad, Y.; Wang, L. S.; Jiang, J. Research advances on anticancer activities of matrine and its derivatives: An updated overview. *Eur. J. Med. Chem.* **2019**, *161*, 205−238.
(5) Zhang, S.; Cheng, B.; Li, H.; Xu, W.; Zhai, B.; Pan, S.; Wang, L.; Liu, M.; Sun, X. Matrine inhibits proliferation and induces apoptosis of human colon cancer LoVo cells by inactivating akt pathway. *Mol. Biol. Rep.* **2014**, *41* (4), 2101−2108.
(6) Wang, X.; Liu, C.; Wang, J.; Fan, Y.; Wang, Z.; Wang, Y. Oxymatrine inhibits the migration of human colorectal carcinoma RKO cells via inhibition of PAI-1 and the TGF-beta1/Smad signaling pathway. *Oncol. Rep.* **2017**, *37* (2), 747−753.
(7) Sun, X. Y.; Jia, L. Y.; Rong, Z.; Zhou, X.; Cao, L. Q.; Li, A. H.; Guo, M.; Jin, J.; Wang, Y. D.; Huang, L.; Li, Y-H; He, Z. J.; Li, L.; Ma, R. K.; Lv, Y. F.; Shao, K. K.; Zhang, J.; Cao, H. L. Research advances on matrine. *Front. Chem.* **2022**, *10*, No. 867318.
(8) Sun, M. Y.; Cao, H. Y.; Sun, L.; Dong, S.; Bian, Y. Q.; Han, J.; Zhang, L. J.; Ren, S.; Hu, Y. Y.; Liu, C. H.; Xu, L. M.; Liu, P. Antitumor activities of kushen: literature review. *Evid. Based Complement. Altern. Med.* **2012**, *2012*, No. 373219.
(9) Zhang, Y.; Wang, M.; Xu, X.; Liu, Y.; Xiao, C. Matrine promotes apoptosis in SW480 colorectal cancer cells via elevating MIEF1-related mitochondrial division in a manner dependent on LATS2-Hippo pathway. *J. Cell. Physiol.* **2019**, *234* (12), 22731−22741.
(10) Liu, Y.; Bi, T.; Wang, Z.; Wu, G.; Qian, L.; Gao, Q.; Shen, G. Oxymatrine synergistically enhances antitumor activity of oxaliplatin in colon carcinoma through PI3K/AKT/mTOR pathway. *Apoptosis* **2016**, *21* (12), 1398−1407.
(11) Liu, J.; Guo, Y.; Cao, J. Matrine triggers colon cancer cell apoptosis and G0/G1 cell cycle arrest via mediation of microRNA-22. *Pharmacol. Res.* **2020**, *34* (7), 1619−1628.
(12) Cui, Y.; Zhang, Y.; Hu, Ch. M. Extraction and separation of *sophora flavescens* alkaloid. *J. Chongqing Inst. Technol.* **2007**, *21*, 114−116.
(13) Wang, H.; Tong, Y.; Li, W.; Zhang, X.; Gao, X.; Yong, J.; Zhao, J.; Koike, K. Enhanced ultrasound-assisted enzymatic hydrolysis extraction of quinolizidine alkaloids from *Sophora alopecuroides L.* seeds. *J. Nat. Med.* **2018**, *72*, 424−432.
(14) Zhou, S.; Wu, X.; Huang, Y.; Xie, X.; Lin, Y.; Fan, H.; Luo, L.; Zhang, W.; Tang, J. Z. Microwave-assisted aqueous two-phase extraction of alkaloids from *Radix Sophorae Tonkinensis* with an ethanol/Na_2HPO_4 system: Process optimization, composition identification and quantification analysis. *Ind. Crops Prod.* **2018**, *122*, 316−328.
(15) Ling, J. Y.; Zhang, G. Y.; Cui, Z. J.; Zhang, C. K. Supercritical fluid extraction of quinolizidine alkaloids from *Sophora flavescens Ait.* and purification by high-speed counter-current chromatography. *J. Chromatogr. A* **2007**, *1145*, 123−127.
(16) Salim, A. A.; Bakhtiar, H.; Bidin, N.; Ghoshal, S. K. Antibacterial activity of decahedral cinnamon nanoparticles prepared in honey using PLAL technique. *Mater. Lett.* **2018**, *232*, 183−186.
(17) Teng, X.; Zhang, M.; Mujumdar, A. S. Potential application of laser technology in food processing. *Trends Food Sci. Tech.* **2021**, *118*, 711−722.
(18) Tagami, T.; Imao, Y.; Ito, S.; Nakada, A.; Ozeki, T. Simple and effective preparation of nano-pulverized curcumin by femtosecond laser ablation and the cytotoxic effect on C6 rat glioma cells in vitro. *Int. J. Pharm.* **2014**, *468* (1−2), 91−96.
(19) Panchev, I. N.; Kirtchev, N. A.; Dimitrov, D. D. Possibilities for application of laser ablation in food technologies. *Innov. Food Sci. Emerg. Technol.* **2011**, *12* (3), 369−374.
(20) Ding, W.; Sylvestre, J.-P.; Bouvier, E.; Leclair, G.; Meunier, M. Ultrafast laser processing of drug particles in water for pharmaceutical discovery. *Appl. Phys. A: Mater. Sci. Process.* **2014**, *114* (1), 267−276.
(21) Ziefuss, A. R.; Hupfeld, T.; Meckelmann, S. W.; Meyer, M.; Schmitz, O. J.; Kaziur-Cegla, W.; Tintrop, L. K.; Schmidt, T. C.; Gokce, B.; Barcikowski, S. Ultrafast cold-brewing of coffee by picosecond-pulsed laser extraction. *npj Sci. Food* **2022**, *6* (1), 19.
(22) Lin, Q. Y.; Mei, Y. J.; Huang, W.; Zhang, B.; Liu, K. Understanding the role of polyvinylpyrrolidone on ultrafine low-rank coal flotation. *Acs Omega* **2022**, *7* (12), 10196−10204.
(23) Xia, E. Q.; Cui, B.; Xu, X. R.; Song, Y.; Ai, X. X.; Li, H. B. Microwave-assisted extraction of oxymatrine from *Sophora flavescens*. *Molecules* **2011**, *16* (9), 7391−7400.
(24) Thang, P. N. T.; Tran, V. H.; Vu, T. A.; Vinh, N. N.; Huynh, D. T. M.; Pham, D. T.Determination of antioxidant, cytotoxicity, and acetylcholinesterase inhibitory activities of alkaloids isolated from *Sophora flavescens Ait.* grown in dak nong, vietnam. *Pharmaceuticals***2022**, *15* (11).
(25) Antoszczak, M.; Otto-Slusarczyk, D.; Kordylas, M.; Struga, M.; Huczynski, A. Synthesis of lasalocid-based bioconjugates and evaluation of their anticancer activity. *Acs Omega* **2022**, *7* (2), 1943−1955.
(26) Tian, R. R.; Li, X. D.; Zhang, H.; Ma, L. A.; Zhang, H. M.; Wang, Z. X. Ulex Europaeus agglutinin-i-based magnetic isolation for the efficient and specific capture of SW480 circulating colorectal tumor cells. *Acs Omega* **2022**, *7* (34), 30405−30411.
(27) Dehkharghanian, M.; Adenier, H.; Vijayalakshmi, M. A. Study of *flavonoids* in aqueous spinach extract using positive electrospray ionisation tandem quadrupole mass spectrometry. *Food Chem.* **2010**, *121* (3), 863−870.
(28) Xia, E. Q.; Ai, X. X.; Zang, S. Y.; Guan, T. T.; Xu, X. R.; Li, H. B. Ultrasound-assisted extraction of phillyrin from *Forsythia suspensa*. *Ultrason. Sonochem.* **2011**, *18* (2), 549−552.
(29) Cao, Q.; Wang, L.; Ur Rashid, H.; Liang, H.; Liu, X.; Xie, P. Ultrasonic-assisted reductive extraction of matrine from *sophorae tonkinesis* and its purification by macroporous resin column chromatography. *Sep. Sci. Technol.* **2018**, *53* (5), 745−755.
(30) Bai, G. Y.; Wang, D. Q.; Ye, C. H.; Liu, M. L. ^1H and ^{13}C chemical shift assignments and stereochemistry of matrine and oxymatrine. *Appl. Magn. Reson.* **2002**, *23* (2), 113−121.
(31) Gonnella, N. C.; Chen, J. Stereochemical analysis and complete $^1H/^{13}C$ NMR chemical shift assignments of matrine. Application of NOE and two-dimensional NMR techniques. *Magn. Reson. Chem.* **1988**, *26* (3), 185−190.
(32) Liang, L.; Huang, J. Oxymatrine inhibits epithelial-mesenchymal transition through regulation of NF-kappaB signaling in colorectal cancer cells. *Oncol. Rep.* **2016**, *36* (3), 1333−1338.
(33) Hashemi, F.; Hormozi-Nezhad, M. R.; Corbo, C.; Farvadi, F.; Shokrgozar, M. A.; Mehrjoo, M.; Atyabi, F.; Ghahremani, M. H.; Mahmoudi, M.; Dinarvand, R. Laser irradiation affects the biological identity and cellular uptake of plasmonic nanoparticles. *Nanoscale* **2019**, *11* (13), 5974−5981.
(34) Vieira, P. G.; de Melo, M. M. R.; Şen, A.; Simões, M. M. Q.; Portugal, I.; Pereira, H.; Silva, C. M. Quercus cerris extracts obtained by distinct separation methods and solvents: Total and friedelin extraction yields, and chemical similarity analysis by multidimensional scaling. *Sep. Purif. Technol.* **2020**, *232*, No. 115924.
(35) Torrisi, A.; Cutroneo, M.; Torrisi, L.; Vacík, J. Biocompatible nanoparticles production by pulsed laser ablation in liquids. *J. Instrum.* **2020**, *15* (03), C03053−C03053.
(36) Shinonaga, T.; Kinoshita, S.; Okamoto, Y.; Tsukamoto, M.; Okada, A. Formation of periodic nanostructures with femtosecond laser for creation of new functional biomaterials. *Procedia CIRP* **2016**, *42*, 57−61.
(37) Al Baroot, A.; Alheshibri, M.; Drmosh, Q. A.; Akhtar, S.; Kotb, E.; Elsayed, K. A. A novel approach for fabrication ZnO/CuO nanocomposite via laser ablation in liquid and its antibacterial activity. *Arab. J. Chem.* **2022**, *15* (2), No. 103606.
(38) Gonzalez-Rubio, G.; Guerrero-Martinez, A.; Liz-Marzan, L. M. Reshaping, fragmentation, and assembly of gold nanoparticles assisted by pulse lasers. *Acc. Chem. Res.* **2016**, *49* (4), 678−686.
(39) Pan, S.; Takebe, G.; Suzuki, M.; Takamoto, H.; Ge, J.; Liu, C.; Hiramatsu, M. Nanonization of poorly water-soluble drug clobetasone

butyrate by using femtosecond laser. *Opt. Commun.* **2014**, *313*, 152−156.
(40) Cao, W.; Li, Y.; Yan, B.; Zeng, Z.; Liu, P.; Ke, Z.; Yang, G. Laser-induced methanol decomposition for ultrafast hydrogen production. *Research* **2023**, *2023* (5), No. 0132.
(41) Cao, W.; Li, Y.; Liu, P.; Ke, Z.; Yang, G. Laser-induced activation of N_2 and catalyst-free synthesis of HCN. *ACS Sustain. Chem. Eng.* **2023**, *11* (20), 7874−7881.
(42) Lee, S. J.; Theerthagiri, J.; Choi, M. Y.Time-resolved dynamics of laser-induced cavitation bubbles during production of Ni nanoparticles via pulsed laser ablation in different solvents and their electrocatalytic activity for determination of toxic nitroaromatics. *Chem. Eng. J.***2022**, *427*.
(43) Lü, T.; Li, Z. Underwater holmium-laser-pulse-induced complete cavitation bubble movements and acoustic transients. *Sci. Bull.* **2011**, *56* (12), 1226−1229.
(44) Rukmana, T. I.; Moran, G.; Méallet-Renault, R.; Clavier, G.; Kunieda, T.; Ohtani, M.; Demura, T.; Yasukuni, R.; Hosokawa, Y. Photoinjection of fluorescent nanoparticles into intact plant cells using femtosecond laser amplifier. *APL Photon.* **2020**, *5* (6), No. 066104.
(45) Rukmana, T. I.; Moran, G.; Meallet-Renault, R.; Ohtani, M.; Demura, T.; Yasukuni, R.; Hosokawa, Y. Enzyme-assisted photo-injection of megadalton molecules into intact plant cells using femtosecond laser amplifier. *Sci. Rep.* **2019**, *9*, 17530.
(46) Yamasaki, Y.; Tsugawa, S.; Ito, K.; Okano, K.; Hosokawa, Y. Femtosecond laser perforation of plant cells for evaluation of cell stiffness. *Appl. Phys. A: Mater. Sci. Process.* **2022**, *128* (9), 838.
(47) Schinkel, H.; Jacobs, P.; Schillberg, S.; Wehner, M. Infrared picosecond laser for perforation of single plant cells. *Biotechnol. Bioeng.* **2008**, *99* (1), 244−248.

https://doi.org/10.1021/acsomega.4c05003
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