Supplementary Information (SI) for Materials Advances.

Synthesis of Gold Nanoparticles Using *Eutrema japonicum* (Wasabi): Antioxidant and Anti-Inflammatory Studies

Christian Nanga Chick,[a,b,*] Mahiro Takano,[a] Francois Eya'ane Meva[b] and Toyonobu Usuki[a,*]

[a] *Department of Materials and Life Sciences, Faculty of Science and Technology, Sophia University, 7-1 Kioicho, Chiyoda-ku, Tokyo 102-8554, Japan*

[b] *Laboratory of Innovative Nanostructured Material (NANO: C), Faculty of Medicine and Pharmaceutical Sciences, The University of Douala, Cameroon*

*Corresponding authors: chicknanga0@gmail.com (CNC), t-usuki@sophia.ac.jp (TU)

Contents

Figure S1. *E. japonicum* stem and it grated paste.

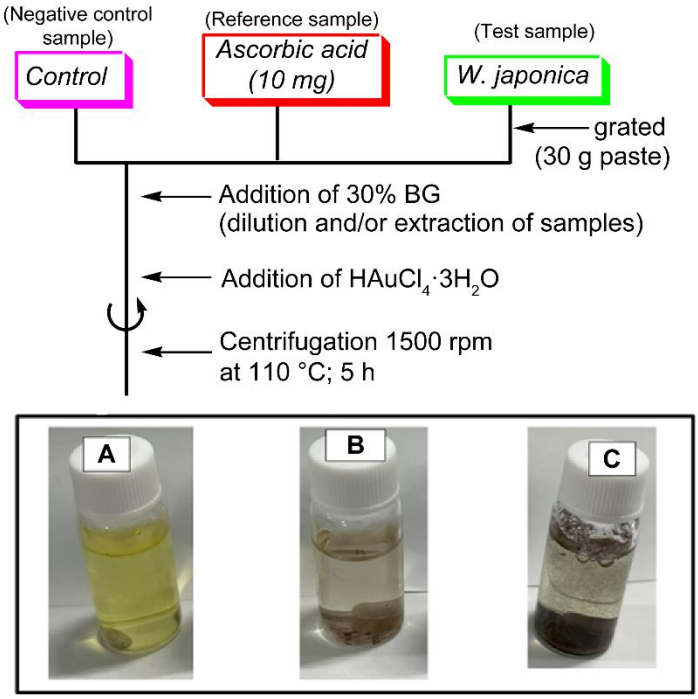


Figure S2. Samples preparation steps; Negative control 'A' (BG & $HAuCl_4 \cdot 3H_2O$ only), positive control 'B' (AuNP-A. a.), and test sample 'C' (AuNP-E.j.).

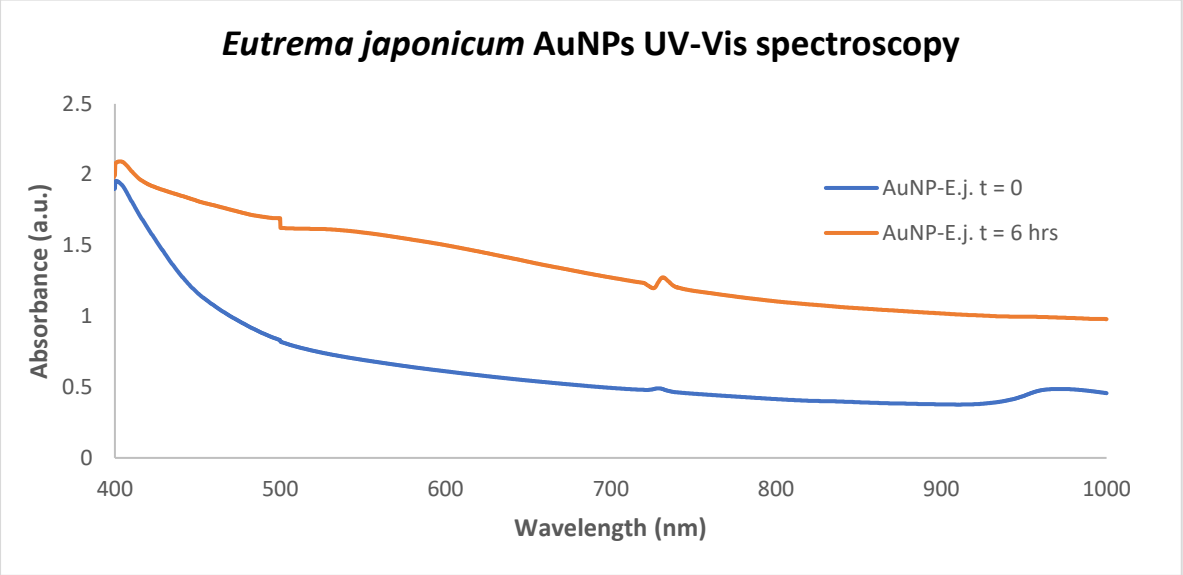


Figure S3. UV-vis spectral of AuNP-E.j taken at initial time of mixing (t = 0) and after 6 hours of incubation

Experimental

All reagents were obtained from commercial suppliers and used without further purification unless otherwise mentioned. Au(III) chloride trihydrate was obtained from Sigma-Aldrich. H_2O (HPLC grade) was purchased from Kishida Chemicals (Osaka, Japan). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from TCI (Tokyo, Japan). Phosphomolybdic acid hydrate was purchased from Kanto Chemical Co. (Tokyo, Japan). Wasabi (E.j.) was purchased from Daio Wasabi Nozyo (Nagano, Japan), and A.a. was purchased from FUJIFILM Wako Pure Chemicals (Osaka, Japan).

For the analysis, a JASCO (Tokyo Japan) V-730BIO device was used to measure the specific absorbance of the mixture for monitoring the reduction of Au ions to AuNPs. The JASCO FTIR-4100 spectrophotometer was used, and the spectra were recorded at room temperature by placing a drop of the prepared sample solution on the attenuated total reflectance compartment used for liquid/oil sample measurements. For DLS, a Malvern zetasizer Nano device was used; 50 and 900 µL liquid samples were transferred to cuvettes in ZEN 2112 (QS; 3.00 mm; low volume quartz batch) and DTS 1070 (for the measurement of ZP), respectively, to measure the mean hydrodiameter and ZP.

DPPH reduction assay

A buffer solution (pH 7.6), a DPPH solution (0.2 mM), and H_2O_2 were used. The DPPH solution (1 mL) and buffer solution (800 µL) were added to 200 µL of A.a. solutions at different concentrations (15.625–125 mg/mL). The samples were allowed to stand in the dark for 30 min, and their absorbance was measured at wavelengths between 516 and 520 nm; the results are shown in Figure S3.

For the calibration curve, measurements were performed at a wavelength of 517 nm, which was similar to that reported in the literature. From the curve, the A.a. equivalent DPPH reduction capacity was measured for AuNP-E.j., AuNP-A.a, and E.j..

For the bioactivity assay, carried out in triplicate, the percentage activity was calculated using *Equation 1*, as described in our previous reports.[1–4]

$$Percentage\ activity = \frac{A_{control} - A_{sample}}{A_{control}} \quad \cdots\cdots \quad Equation\ (1)$$

where, $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the extract or standard. Ascorbic acid measurements were used as the calibration. From the obtained equation *y* = *mx* + *c* of the calibration sample (A.a), the AAEAC of the test samples are calculated using their obtained % inhibition values. The AAEAC values for the test samples are expressed in mg/100 mL.

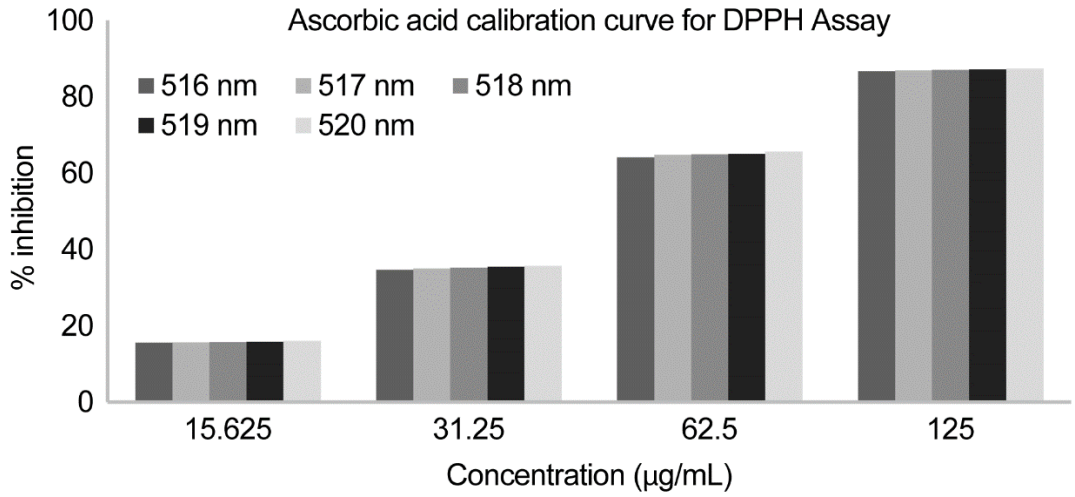


Figure S4. Histogram illustrating the DPPH reduction capacity of A.a. at different wavelengths.

Phosphomolybdenum reduction assay

The phosphomolybdenum solution (1 mL) and buffer solution (800 µL) were added to the A.a. solution (200 µL; 31.25–250 mg/mL). The sample was heated to 90 °C in the dark for 10 min, and subsequently its absorbance was measured at wavelengths between 695 and 800 nm, as illustrated in Figure S4. The efficacy to molybdenum reduction was calculated using equation (1): where '$A_{control}$' is the absorbance of the control (phosphomolydenum solution without the extract and/or AuNPs solution), and 'A_{sample}' is the absorbance of A.a or the test samples (phosphomolydenum solution with the extract and/or AuNPs solution). Triplicate measurements were performed to calculate the percentage inhibition using *Equation 1* with A.a. for calibration.[1–4] From the obtained equation $y = mx + c$ of the calibration sample (A.a), the AAEAC of the test samples are calculated using their obtained % inhibition values. The AAEAC values for the test samples are expressed in mg/100 mL.

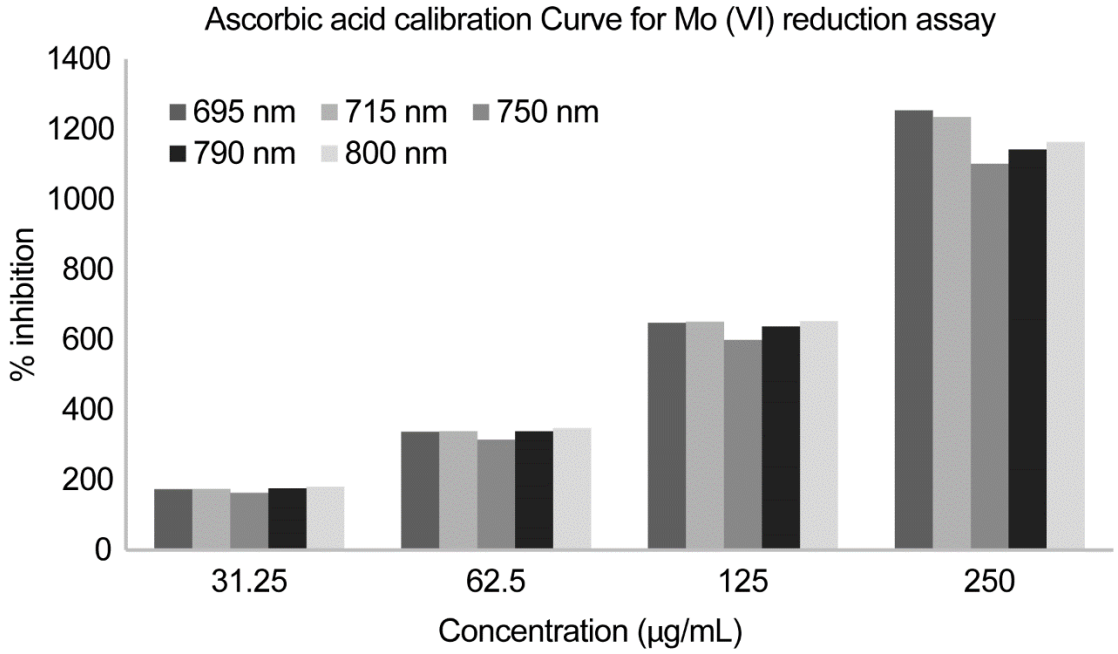


Figure S5. Histograms for the A.a. equivalent DPPH reduction capacity at different wavelengths.

Albumin denaturation assay

The samples were screened for anti-inflammatory properties using the inhibition of albumin denaturation technique, as previously described in previous works.[1,2] Briefly, the reaction mixture consisted of chick egg albumin prepared with PBS (pH 6.4) and the test samples and varying concentrations of standard compound diclofenac (100, 50, and 25 µg/mL). A similar volume of PBS was used as a control. The mixtures were heated for approximately 8 min at ~60 °C. After cooling, the absorbance was measured at 660 nm using a blank vehicle. The anti-inflammatory activity was estimated as the percentage inhibition or clearance of protein denaturation and calculated using Equation (1), where $A_{control}$ is the absorbance of the control (albumin-mixed PBS solution without the extract and/or AuNPs solution) and A_{sample} is the absorbance of the test sample (albumin-mixed PBS solution with the plant extract and/or AuNPs solution). All measurements were performed in triplicates, and diclofenac was used for the calibration. From the equation $y = mx + c$, the diclofenac equivalent egg albumin denaturation capacity of the test samples was calculated. The obtained % clearance for the test samples are extrapolated on the calibration curve to get the diclofenac equivalent egg albumin denaturation capacity of the test samples and are expressed in µg/mL

Table 1. UV absorbance at 660 nm for the measurement of diclofenac inhibition of egg albumin denaturation.

Test samples	**Concentration (µg/mL)**	**Mean wavelength (SD)**	**% clearance**
Control (PBS solution)	-	0.63 (0.13)	-
Diclofenac	100	0.56 (0.02)	44.44
	50	0.37 (0.15)	40.95
	25	0.35 (0.11)	11.59

Table 2. Albumin denaturation activity measurement for AuNP-E.j.

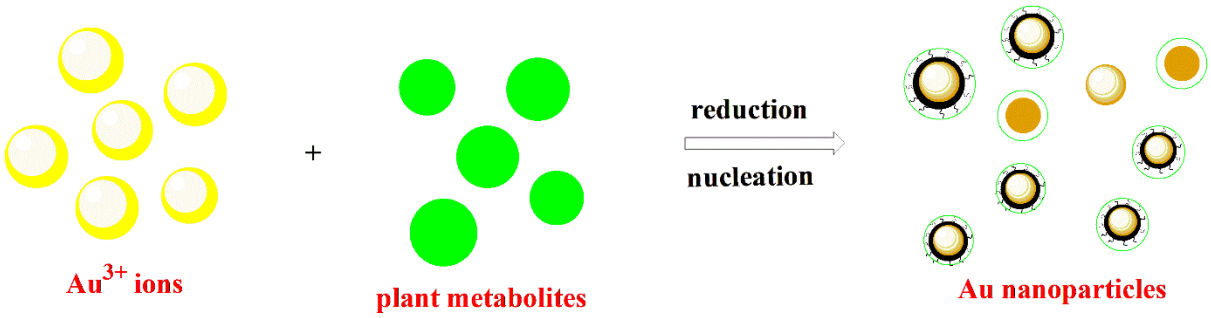
Test samples (2 mL)	**Absorbance (A) taken three times**	**Average (*SD*)**	**% clearance equivalence of diclofenac (**μg/100mL)
Control (PBS solution)	-	0.63 (0.130)	**-**
***E. japonicum* 30 % BG extract**	2.745	2.73 (0.014)	-
	2.721		
	2.721		
AuNP-E.j.	0.299	0.296 (0.003)	3.60
	0.297		
	0.292		
$HAuCl_4 \cdot 3H_2O$ solution	0.236	0.236 (0.004)	4.86
	0.240		
	0.232		

Data analysis

Analyses were conducted using Microsoft Excel (2023). Significant differences were observed using one-way analysis of variance procedure. Results with a 5% confidence level were considered statistically significant.

Gold nanoparticles formation

The image below illustrates the formation mechanism of gold nanoparticles from plant extract, wherein the plant secondary metabolites bring about the reduction of Au^{3+} to Au^{0} of the gold chloride solution for the formation of the AuNPs.



References

1 C. N. Chick, F. E. Meva, P. B. E. Kedi and T. Usuki, Green synthesis of silver nanoparticles with Cyclosorus dentatus and Nephrolepis biserrata and their antioxidant, anti-inflammatory studies, *Nano Select*, 2024, e202400093, Early View. https://doi.org/10.1002/nano.202400093.

2 C. N. Chick, M. Mahdaly, P. B. E. Kedi, K. Sonoko and F. E. Meva, Calculating the Crystallite Size of *Microsorum scolopendria* AgCl Nanoparticles and Their Biological Activities, *J. Biomater. Nanobiotechnol.,* 2024, **15**, 25–37. https://doi.org/10.4236/jbnb.2024.152002.

3 C. N. Chick, T. Misawa-Suzuki, Y. Suzuki and T. Usuki, Preparation and Antioxidant Study of Silver Nanoparticles of Microsorum pteropus Methanol Extract, *Bioorg. Med. Chem. Lett.*, 2020, **30**, 127526. https://doi.org/10.1016/j.bmcl.2020.127526.

4 P. Belle Ebanda Kedi, C. Christian Nanga, A. Paboudam Gbambie, V. Deli, F. Eya'ane Meva, H. Elsayed Ahmed Mohamed, A. Antoinette Ntoumba, M. H. Julien Nko'o, Ü. Kökam-Demir, B. Moll, H. Kouemegne Armelle Michelle, P. Teke Ndifon, E. Albert Mpondo Mpondo, A. B. Dongmo, M. Maaza, C. Janiak and M. Maaza, Biosynthesis of Silver Nanoparticles from Microsorum Punctatum (L.) Copel Fronds Extract and an In-vitro Anti-Inflammation Study, *J. Nanotechnol. Res.,* 2020, **2**, 025–041. https://doi.org/10.26502/jnr.2688-85210014.