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Literally green chemical synthesis of artemisinin from plant extracts

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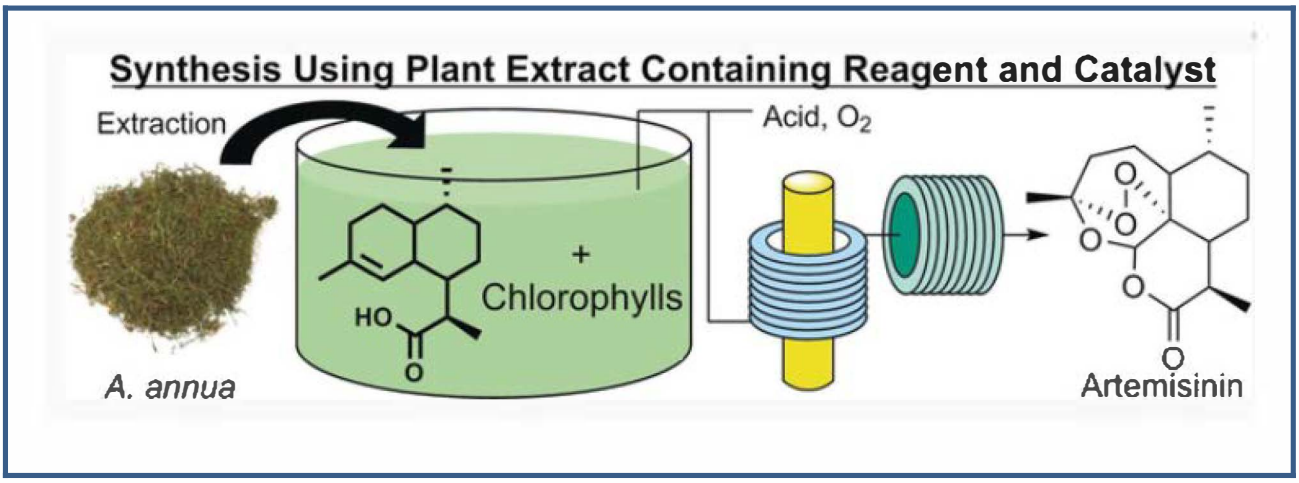

Crude plant extracts contain a large variety of chemically active compounds, which can be utilized for natural product synthesis. This approach is illustrated by the partial synthesis of artemisinin with crude chlorophyll as the photosensitizer.

Literally green chemical synthesis of artemisinin from plant extracts

Susann Triemer,[a] Kerry Gilmore,[b]* Giang T. Vu,[a] Peter H. Seeberger,[b,c] Andreas Seidel-Morgenstern[a,d]

Abstract: Active pharmaceutical ingredients are either extracted from biological sources – where they are synthesized in complex, dynamic environments – or prepared via step-wise chemical syntheses by reacting pure reagents and catalysts under controlled conditions. A combination of these two approaches, where plant extracts containing reagents *and* catalysts are utilized in intensified chemical syntheses, creates expedient and sustainable processes. We illustrate the principle by reacting crude plant extract, oxygen, acid, and light to produce artemisinin, a key active pharmaceutical ingredient of the most powerful anti-malaria drugs. The traditionally discarded extract of *Artemisia annua* plants contains dihydroartemisinic acid - the final biosynthetic precursor - as well as chlorophyll, which acts as a photosensitizer. Efficient illumination with visible light in a continuous flow setup produces artemisinin in high yield, and the artificial biosynthetic process outperforms syntheses using pure reagents.

Nature has evolved efficient processes to synthesize complex molecules exploiting a myriad of parallel and competing chemical pathways. Enzymatic and non-enzymatic reactions proceed in solutions that contain all reagents, auxiliaries, intermediates, and products. In striking contrast, synthetic chemists perform single reactions with pure reagents in a controlled environment. Chemicals that are isolated from natural sources are purified prior to use, the parameters for each step are optimized, and the products are purified (Figure 1). Ideally, reagents and catalysts found in nature could be used directly from their sources to perform the desired transformations. Intensified natural processes that are not merely based on enzymes, but rather fueled with natural extracts may accelerate, simplify, and improve the efficiency of natural product synthesis. We illustrate this principle by the photocatalyzed synthesis of the complex natural product artemisinin from the discarded components of the extract of *Artemisia annua* leaves.

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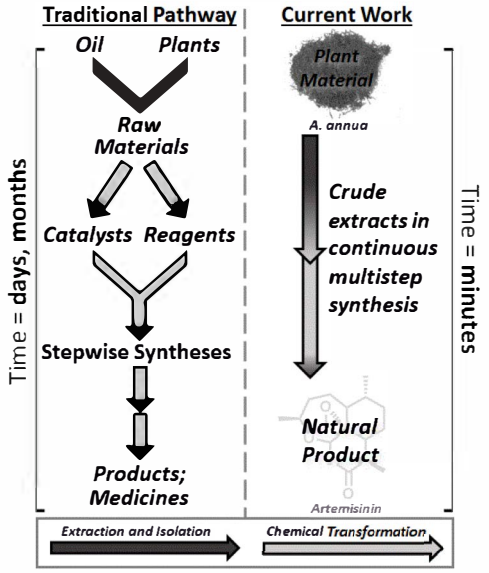


Figure 1: Chemical synthesis is accelerated and simplified when the number of purification steps for reagents and intermediates is reduced.

Artemisia annua plants contain about 1% of the metabolite artemisinin (**5**, Figure 2)[1] that can be converted to the most powerful active pharmaceutical ingredients (APIs)[2] to treat malaria, a disease that kills over 500,000 people each year.[3] Artemisinin production and prices vary greatly as they depend on plant production,[4] while total synthesis[5,6] is too complicated and expensive to contribute significant amounts. Semi-syntheses from the biosynthetic precursors artemisinic acid (AA, **2**) and dihydroartemisinic acid (DHAA, **4**), obtained either from modified yeast[7] or the discarded waste after artemisinin extraction, are an attractive alternative to increase API production (Figure 1).[8-10] DHAA, contained in the plant in about the same amounts as artemisinin,[11] can be converted efficiently to artemisinin by drying the harvested plant for a few weeks in the sun.[12] The conversion process in the plant proceeds via a $^1O_2$ ene reaction to generate a peroxide intermediate, followed by an acid induced Hock cleavage and a cyclization cascade (Figure S7 in SI).[13] Chemical conversion of DHAA to artemisinin proceeds in 69% yield in twelve minutes when singlet oxygen is produced in the presence of a dye in a continuous flow photoreactor.[14,15] Process intensification in flow reactors allows for higher irradiation efficiencies and increased gas-liquid mass transfer as compared to batch reactors.[16,17]

The flow process mimics the biosynthetic pathway, using 5,10,15,20-tetraphenylporphyrin or 9,10-dicyanoanthracene (DCA) as a photosensitizer instead of chlorophyll.[18-22] Although plentiful in nature, chlorophyll has rarely been used for synthetic transformations.[23,24] A crude mixture of artemisinin, DHAA, as well as chlorophyll derivatives extracted from *A. annua* leaves

can be fed directly into a flow photooxidation reactor in the presence of acid to convert DHAA into artemisinin. Stirring dried, ground *A. annua* leaves in toluene at different temperatures yields the key compounds in a temperature dependent manner (Figure 3a, Figure S2 in SI). DHAA and artemisinin levels remain constant after ten minutes at temperatures below 90 °C while chlorophyll extraction reaches equilibrium only after about 24 h. Extraction of artemisinin and chlorophyll increases at higher temperatures while DHAA levels decline. Chlorophyll extraction is the most temperature sensitive and mainly pheophytin A is co-extracted, while little chlorophyll A and B are obtained (Table S1 in SI).

Extraction for 10 min at 50 °C is best to obtain both reagent and catalyst. For the *A. annua* extracts we examined, the DHAA concentration was only approximately one third of that of artemisinin (Table S1 in SI). The extract broadly absorbs with maxima at 416 nm and 672 nm (Figure S4 in SI) such that a range of light sources can be used for the photooxidation. The photooxidation was optimized using crude toluene extract containing additional pure DHAA to facilitate observation of the reaction progress. The reaction conditions were based on our earlier work.[2, 15] Crude extract containing DHAA (0.5 M concentration) was combined with pure oxygen (1:4 vol/vol) via a T-mixer prior to entering the photoreactor for irradiation with 420 nm or 660 nm LED light at –20 °C (Figure S8 in SI). The reactor outflow was collected and analyzed by ^{1}H NMR.

The extract photosensitizes similar to DCA (2.5 mM) in pure toluene (Figure 3b), even though the chlorophyll concentration was *six times* lower. With DCA as photoactivator, the desired hydroperoxide intermediate **PO1** was obtained in 91% yield after two minutes of irradiation with blue light. Similar yields were obtained when the extract was irradiated for three minutes with red light (87%) or five minutes with blue light (88%) (for further information, see SI).

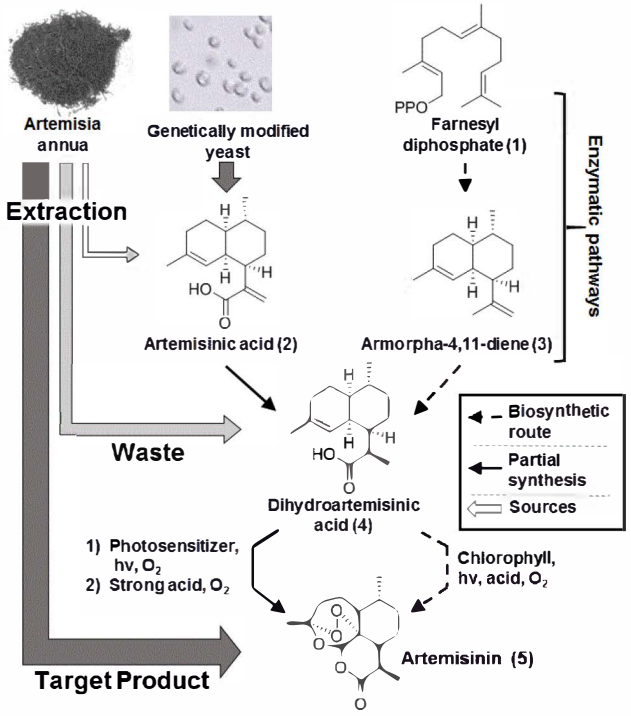


Figure 2: Pathways to produce artemisinin from different sources.[7,8, 15, 22]

To directly compare the photosensitizing activity of extract with both isolated chlorophylls and DCA, the photooxidation was performed with pure photosensitizer solution and crude extract of equal photosensitizer concentration (30 μM, which corresponds to tenfold diluted extract). Pure pheophytin A and chlorophyll A greatly outperform the anthracene derivative in converting pure DHAA (Figure 3c). However, the crude extract performs better, with up to 90% yield of the desired intermediate after seven minutes (pure chlorophylls = 83%, DCA = 20%), even under these highly dilute conditions.

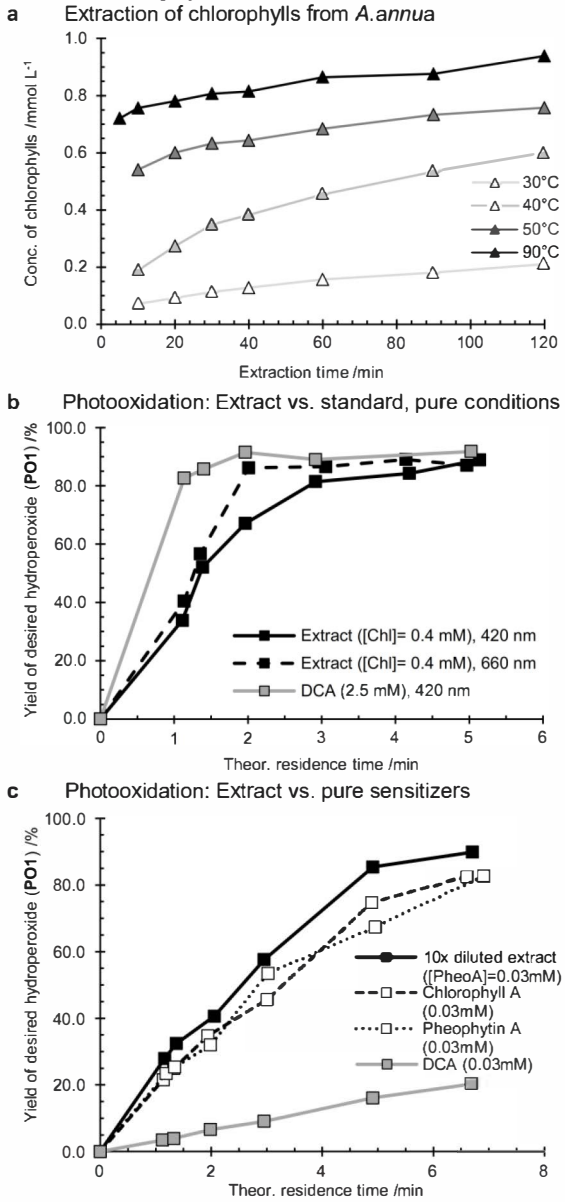
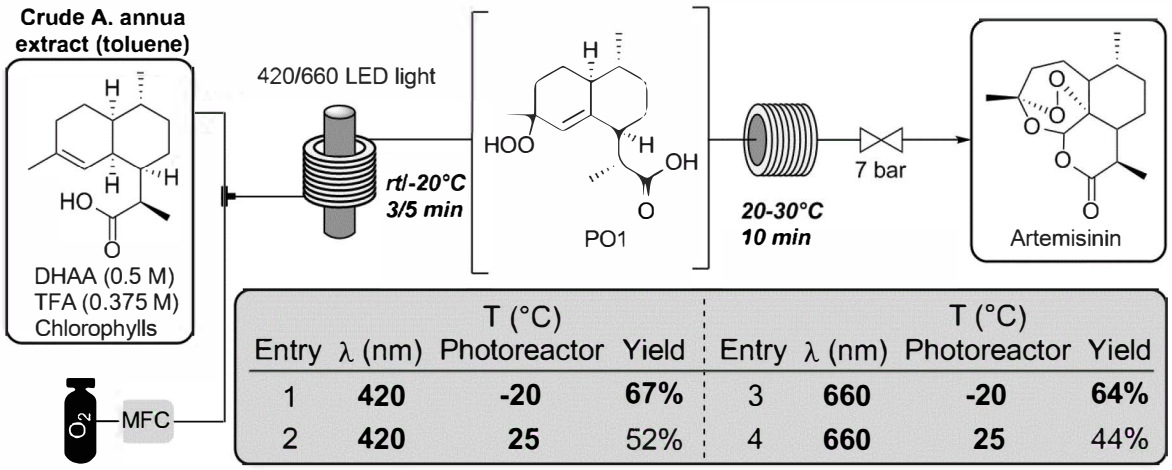


Figure 3: **a.** Overall concentration of co-extracted chlorophyll species (chlorophyll A, B, pheophytin A) during extraction of dried *A. annua* leaves (12 g) in toluene (104 mL), determined by taking samples over the course of the extraction, and quantitative analysis by UV/Vis; **b.** Photooxidation of dihydroartemisinic acid (DHAA) to the tertiary hydroperoxide intermediate (**PO1**, Figure 4), photosensitized in the flow photooxidation reactor by chlorophyll species present in the plant extract in comparison to 2.5 mM 9,10-dicyanoanthracene (DCA) as photosensitizer (-20 °C, 7 bar O_2, 0.5 M DHAA). **c.** Direct comparison of the photosensitizing performance of extract (10 times diluted) to pure ChlA, PheoA, and DCA in toluene at equal photosensitizer concentration of 0.03 mM (-20 °C, 7 bar O_2, 420 nm, 0.5 M DHAA).



Entry	λ (nm)	T (°C) Photoreactor	Yield	Entry	λ (nm)	T (°C) Photoreactor	Yield
1	**420**	**-20**	**67%**	3	**660**	**-20**	**64%**
2	**420**	**25**	52%	4	**660**	**25**	44%

Figure 4: Continuous flow synthesis of artemisinin from crude *A. annua* extract.

The optimized photooxidation conditions were applied to the synthesis of artemisinin from an extract containing DHAA. During the acid-catalyzed Hock cleavage of the hydroperoxide, best induced by trifluoroacetic acid (TFA),[8, 10, 14] an enol forms that is oxidized by a second equivalent of oxygen before the subsequent reaction cascade furnishes artemisinin (Figure S7 in SI). TFA does not affect the photooxidation step and can be added directly to the feed solution. A second reaction loop (ID. 1.58 mm) was connected to the photoreactor and an optimal residence time of ten minutes was determined for the second step (Figure S15a, in SI). A fully continuous process using crude extract, to which TFA (0.25 M) and DHAA (0.5 M) are added, yielded artemisinin in 52%. The addition of more TFA (0.375 M) helped to protonate any basic residues present in the crude extract and increased the yield to 58% (Figure S15b in SI). Quenching of the product stream with saturated aqueous solutions of inorganic bases such as $NaHCO_3$ gave better results than triethylamine (Table S9 in SI).

The continuous process at –20 °C yielded 67% of artemisinin when starting with crude extract (Figure 4), the same yield as was obtained starting from pure reagents and catalysts.[15] When red light was used instead of blue light for the photooxidation step, the volume of the first reactor can be reduced due to the faster reaction with similar yields (64%), resulting in a space-time yield of 2.1 kg L^{-1} day^{-1}. Attempts to further simplify the photooxidation step by conducting it at room temperature resulted in a 15-20% decrease in yield due to the selective degradation of the hydroperoxide intermediate, which was noted with pure starting materials as well.[15] One notable difference between the developed process and those previously published is the increased amount of impurities present as a result of crude extract solution used. While this could potentially complicate the final crystallization,[25,26] this solution is akin to that from which artemisinin is currently purified.[27,28]

In summary, we demonstrate that complex natural products such as artemisinin can be prepared from crude plant extracts that contain reactants and catalysts. The simple continuous photoflow process using crude extract, oxygen, and acid produces artemisinin as efficiently as state-of-the-art techniques that use purified reagents. Crude chlorophyll, a widely available, non-toxic and highly reactive dye with a strong and broad absorption in the visible range is an attractive photosensitizer for other reactions as well.

Acknowledgements

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Conflict of interest

The authors declare no conflict of interest.

Keywords: Chlorophyll • Artemisinin • Extraction• Photooxidation • Natural Product Synthesis

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