Application Note
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Extraction of eucalyptus for cosmetic use

UniversalExtractor E-800:
Extraction of plant material for active ingredients for cosmetics at the example of eucalyptus (*Eucalyptus nitens*)

Manon Trinel[1], Hortense Plainfossé[1,2], Pauline Burger[2], Xavier Fernandez[1,2], Claudia Blum[3], Aurélie Demont[3]
[1]Université Côte d'Azur, CNRS, ICN, Nice (France) ; [2]NissActive, Pépinière InnovaGrasse, Grasse (France), [3]BUCHI Labortechnik, Flawil (Switzerland)

1. Introduction

Today's consumers are increasingly attentive to the impact of their choices of products for daily use. They demand natural, sustainable and eco-designed products, in line with a more responsible lifestyle. Consequently, products of synthetic origin are voluntarily abandonded. [1] Natural extracts are therefore at the heart of the research and development strategy of many industrial sectors (pharmaceutical industry, nutraceuticals, etc.).
Cosmetics are no exception to this phenomenon. The research and development of natural and sustainable alternatives, just as effective as conventional synthetic ingredients, is a strong driving force behind innovation. The richness of biodiversity offers a wide choice of raw materials for the development of active ingredients [2].

Extraction is a crucial step in the development process of such natural ingredients. The dried and ground plant material is treated with different solvents (hydroalcoholic mixtures, glycols, oils, water, etc.) to extract the metabolite families of interest.
Traditional extraction methods as maceration are easy-to use and low in costs, but they are energy-, time- and solvent-consuming. In the so-called screening it is necessary to test a large number of extracts to identify a raw material of interest. Automated extraction methods allowing to process several raw materials simultaneously or a single raw material under different conditions, can considerably increase the efficiency of the screening.

This application note describes the extraction of plant material using BUCHI's UniversalExtactor by the example of eucaplytus.

2. Equipment

- UniversalExtractor E-800 with chamber heater and universal chamber
- Rotavapor R-3
- Recirculating chiller (Huber, Minichiller)1
- HPLC Agilent 1200 (Courtaboeuf, Ile-de-France, France) with diode array detector (DAD) and et un evaporative light scattering detector (ELSD)
- Analytical balance

3. Chemicals and Materials

Chemicals:

- Ethanol, analytical quality (Sigma-Aldrich)
- Acetonitrile, HPCL quality (Sigma-Aldrich)
- Isopropanol, HPCL quality (Sigma-Aldrich)

For a safe handling please pay attention to all corresponding MSDS!

Materials:

- Cellulose thimbles, Fioroni (41 x 123 mm), alternatively use BUCHI's cellulose thimbles 33 x 150 mm (11067446)

Samples:

Eucalyptus shoots, *Eucalyptus nitens* (Deane and Maiden) Maiden (family *Myrtaceae*)

This species of eucalyptus is naturally present in the humid forests of south-eastern Australia [5]. This species is very fragrant, and studies have revealed that the essential oil produced from the leaves has repellent and larvicidal activity against certain species of mosquitoes that carry the viruses of dengue or yellow fever [6, 7]. This species was studied as part of a screening of extracts with the aim of developing new cosmetic active ingredients with skin repair properties.

The samples were air-dried in the dark and ground using a household blender.

[1] BUCHI's Recirculating Chiller F-308 or F-314 are suitable for this application, too.

4. Procedure

The screening of the eucalypus shoots consist in the following steps

- Traditional maceration as reference method
- Extraction with UniversalExtractor E-800, using the methods Soxhlet, Soxhlet warm, Hot extraction and Twisselmann
- HPLC analysis of extract to determine the profile of extracted compounds

4.1. Maceration

The parameters for the maceration were the following:

- Ethanol, ratio 1:15 (plant material: solvent)
- Extraction 1 h 20 min under stirring, ambient temperature
- Concentration using Rotavapor R-3: bath temperature was 40 °C, rotation speed 500 rpm

4.2. Extraction of eucalyptus using the UniversalExtractor E-800

4.2.1. Preparation of the beakers

Always use dry and clean beakers for the extraction.

4.2.2. Extraction

Approx. 10 g of sample were placed in a cellulose thimble. The thimbles were placed into the extraction chamber. The level sensor was adjusted to the level of the sample, except for the method Twisselmann, there the level sensor must be below the level of the sample, at the lowest possible position. Fill the solvent into the beakers and place them on their corresponding heating plate. Activate the occupied positions and switch on the connected chiller.
The ratio sample:solvent was chosen to be the same as in the maceration process, so 150 ml of ethanol was used. The extractions were carried out using the parameters showed in Table 1. The extractions were done in triplicate.

Table 1: Parameters for the extractions with the UniversalExtractor E-800.

Step	Soxhlet	Soxhlet warm	Hot extraction	Twisselmann
Extraction				
Time / cycles	0 min / 6 cycles	0 min / 6 cycles	80 min	80 min
Heating level[2]	10	10	10	10
Heating level chamber	-	5	5	5
Rinse				
Time	10 min	10min	10min	10min
Heating level	10	10	10	10
Drying	☐AP 0 min	☐AP 0 min	☐AP 0 min	☐AP 0 min
Solvent [mL]	Ethanol 150 mL			
Total time[3]	78 min	81 min	80 min	80 min

Figure 1 show the extraction of eucalyptus shoots in the UniversalExtractor E-800

[2] The heating levels are lower then recommended by BUCHI on purpose to have a gentle extraction.
[3] Mean value of triplicate determination

Figure 1: Extraction of Eucalyptus nitens shoot using the UniversalExtractor E-800

4.3. Concentration of extracts

The UniversalExtractor E-800 is able to gently dry the extracts in the drying step. In this application note, the extracts were dried using a Rotavapor R-3, to use the same concentration method as for the maceration.

4.4. HPLC Analysis

The extracts were analysed on an HPLC under the following conditions :

Sample:	10 mg sample / mL ethanol
Column :	C18 (Phenomenex, Luna ® 5, 150 mm × 4.6 mm)
	Mobile phase: water (A) and acetonitrile, acidified with 0.1% formic acid (B) and isopropanol (C).
Gradient:	0 - 4 min, 2 % B ; 4 - 15 min, 2 – 98 % B ; 15 - 20 min, 98 % B ; 20 - 25 min, 98 - 2 % B et 2 - 98 % C, 25 - 30 min, 2 % B et 98 % C, 30 - 32 min, 0 - 98 % A, 2 % B et 98 - 0 % C ; elution flow : 1 mL/min.

5. Results

5.1. Maceration

The extract content was determined to be 20,7 ± 0,5 %.

5.2. Extraction using UniversalExtractor E-800

The results of the extract content determination are presented in Table 2 and Figure 2.

Table 2: Determined extract contents in Eucalyptus nitens (Deane and Maiden) Maiden shoots

	Soxhlet	Soxhlet warm	Hot extraction	Twisselmann
Extract content	**30.5**	**33.8**	**31.6**	**21.7**
rsd [%]	0.7	1.7	1.2	1.5

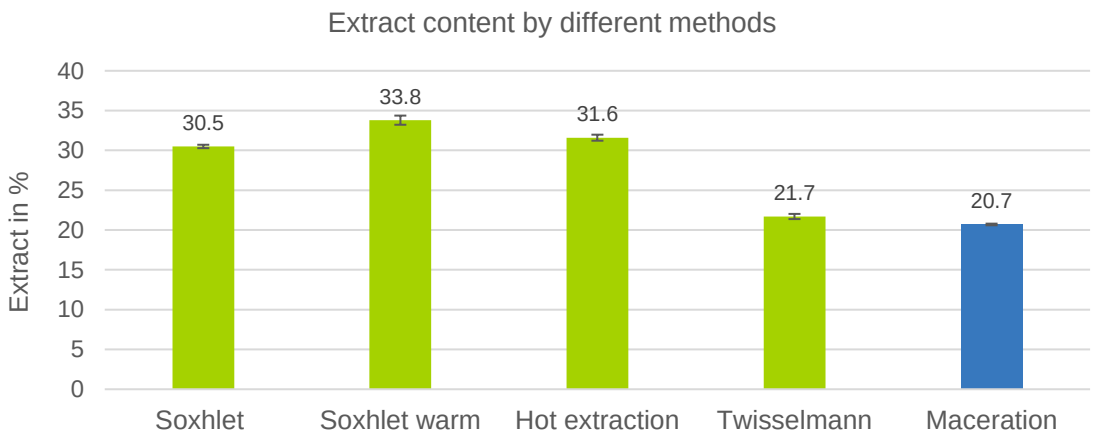

Figure 2: Determined extract contents in Eucalyptus nitens (Deane and Maiden) Maiden shoots, error bars indicate +/- standard deviation, n = 3.

The yielded extract content using the methods Soxhlet, Soxhlet warm or Hot extraction are in average 11.3 % higher than with the traditional maceration. Whereas the extraction method Twisselmann shows equivalent results to the traditional maceration.
In the extraction methods Soxhlet, Soxhlet warm and Hot extraction, the sample is both immersed in the solvent as in the maceration, as well as extracted by a solvent flow throught the sample. On the other hand, Twisselmann consists only of a solvent flow through the plant material, without immersion[4]. The immersion seems to have a beneficial effect on the extraction yield.

5.3. HPLC Analysis- Comparison of profiles

The extracts were analysed by HPLC to compare the profiles obtained by the different extraction methods and the traditional maceration. All extracts obtained by using the UniversalExtractor E-800 present similar chromatographic profiles. They are characterized by highly polar compounds (retention time between 1 and 3 min) and more lipophilic compounds (retention time between 9 and 12 min). However, it appears that the extractions performed by Soxhlet / Soxhlet warm and Hot extraction lead to a higher yield of metabolites than conventional maceration. The chromatograms are showed in figure 3.

[4] The recommended heating levels for Twisselmann extraction are higher than used for this application note. When the recommended heating levels [8] are used, the sample is heated up by the solvent vapor, which increased extraction efficiency resulting in equivalent results as the other extraction methods [9]. The heating level was chosen to be for better comparison of the extraction methods and to achieve a gentle extraction process.

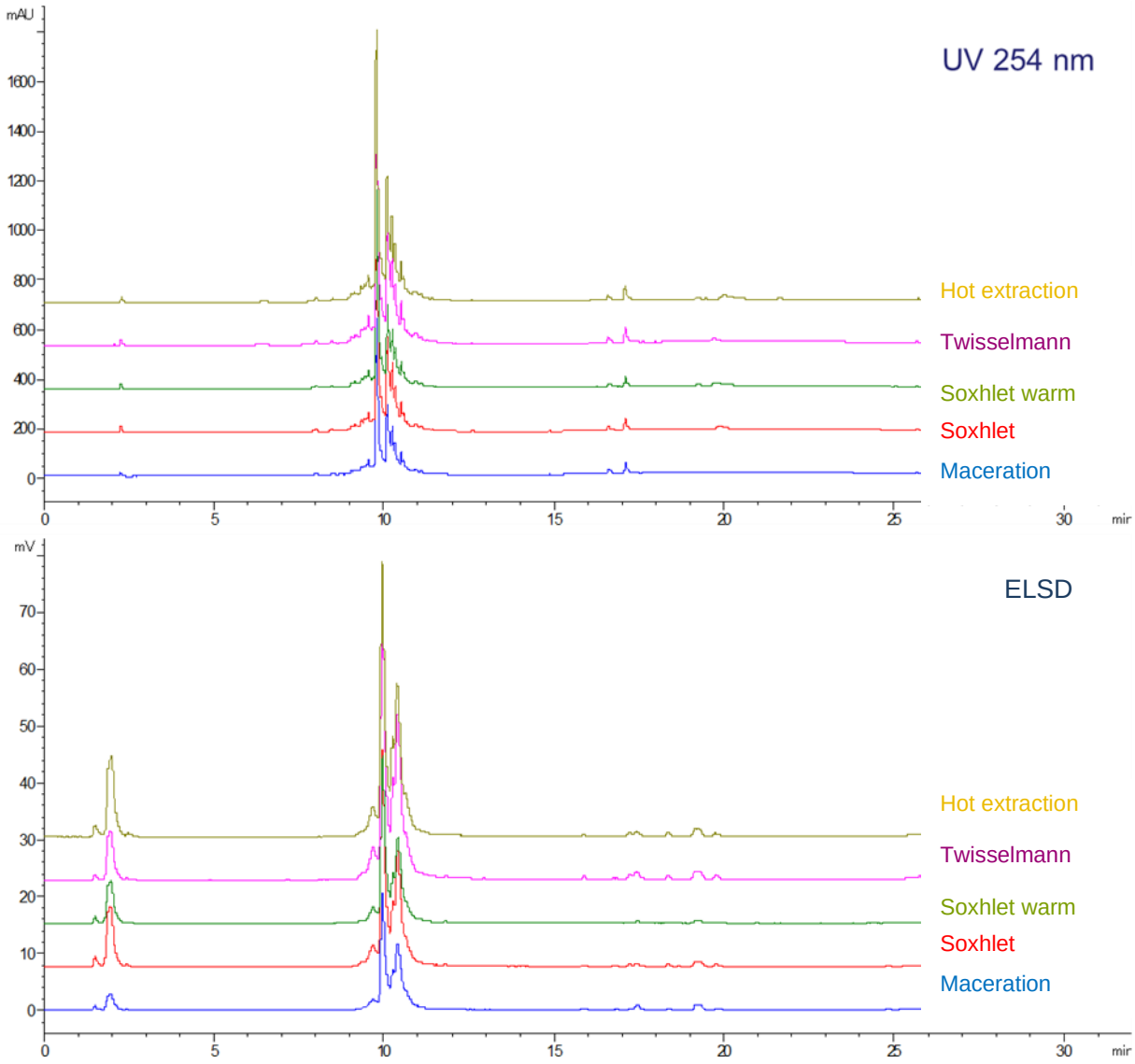

Figure 3: HPLC chromatograms of Eucalyptus nipens extracts obtained by different extraction method

6. Conclusion

The UniversalExtractor E-800 from Büchi is particularly suitable for the extraction of plant raw materials for the development of natural cosmetic active ingredients. The possibility of working in parallel with different methods allows to determine the most suitable method and extraction conditions for the efficient extraction of the metabolites of interest. The fast heaters, combined with the efficiency of the condensers, allow faster and more reproducible extractions. Finally, the intuitive user interface of the UniversalExtractor E-800 displaying all parameters, allow a very quick handling of the device.

7. Acknowledgements

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8. References

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[8] Technical note 363/2019. A comprehensive guide how to evaluate heating levels and drying settings for extractions in the UniversalExtractor E-800, available on www.buchi.com

[9] Application note 386/2019. Determination of total fat content in different food samples- Total fat determination in food samples according to Weibull-Stoldt by use of Soxhlet, Hot and Twisselmann Extraction, available on www.buchi.com

Extraction Reports App

Operation Manual of the UniversalExtractor E-800