PROJECT FICHE

bilat BEL-VT R&D cooperation

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Project title :
CHEMICAL STUDIES AND BIOLOGICAL INVESTIGATIONS OF VIETNAMESE PLANTS AND HERBAL MEDICINES FROM THE EUPHORBIACEAE FAMILY TO DEVELOP HIGH-VALUE HEALTHCARE MATERIALS, AND THEIR QUALITY CONTROL

Context and objectives

Natural products are widely used in traditional medicine, and are nowadays also gaining interest in Western countries. Medicinal plants have a potential to be developed as high-value healthcare products. However, quality control is an important issue during development of such products to ensure the identity, safety and quality of the natural and synthetized products. In this project natural compounds of vegetable origin with interesting biological activities will be examined, in order to be utilised as new drugs or in the parapharmaceutic field, e.g. as non-nutritive and alternative sweeteners, nutraceuticals or ecological insecticides. Only a limited number of natural products will be focused on and considered as case studies. The main **objectives** of this project :

1) To find natural compounds or extracts from Euphorbiaceae plants used in Vietnam as herbal medicine with interesting biological activities to contribute as new material for the pharmaceutical or food industries, e.g. as food material, functional food, nutraceuticals, …

2) To survey medicinal plants from the Euphorbiaceae family used in traditional medicine in Vietnam. The study will focus mainly on *Phyllanthus,* but also on *Mallotus* species, with a special interest in endemic species.

3) To study the chemical composition and investigate biological activities of selected medicinal plants using chemical and molecular biological techniques.

4) To produce materials (food supplements) and/or to identify lead molecules with a potential interest for pharmaceutical or food industries.

5) To apply analytical aspects for quality control of plant materials or products from plants: chromatography fingerprints of crude extracts for identification and quality control purposes, and quantitative determination of specific active compounds.

Methodology

WP1: Find natural compounds or extracts from Euphorbiaceae plants used in Vietnam as herbal medicine with interesting biological activities

WP2: Survey medicinal plants from the Euphorbiaceae family used in traditional medicine in Vietnam
The plants were selected by the Vietnamese partner.

WP3: Study the chemical composition and investigate biological activities
At the UCL, the antioxidant, cytotoxic, antimicrobial and hepatoprotective activities of the selected samples were measured by the Walloon partner. From these results, interesting compounds for given activities were isolated and identified.

WP4: Produce materials (food supplements) and/or to identify lead molecules with a potential interest for pharmaceutical or food industries
This WP will be performed by Vietnamese partner in cooperation with a Vietnamese company.

WP5: Apply analytical aspects for quality control of plant materials or products from plants
At the VUB, methodology to develop HPLC fingerprints was defined by the Flemish partner. HPLC-UV and UFLC-UV fingerprints were developed and peaks responsible for given activities determined. From these results, interesting compounds for given activities were further examined by HPLC-MS. Besides also pCEC-UV fingerprints were developed and evaluated as a complementary technique to HPLC.
In Vietnam, HPLC-DAD-MS and HPLC-DAD-ELSD fingerprints were developed following the earlier defined methodology by the VUB with another HPLC column and using some compounds as marker.

Scientific Results

WP1: Find natural compounds or extracts from Euphorbiaceae plants used in Vietnam as herbal medicine with interesting biological activities

WP2: Survey medicinal plants from the Euphorbiaceae family used in traditional medicine in Vietnam
In **Vietnam**, 51 samples (23 Mallotus samples and 28 Phyllanthus samples from different species) were collected in different Vietnamese regions. Besides also 39 Mallotus samples from the previous bilateral project with Vietnam were still available. The research of the Vietnamese partner focused on the extraction, purification and identification of bioactive compounds from Mallotus and Phyllanthus genera (3 papers published (n° 4-5-7 in list below), paper in preparation (n°15)).

WP3: Study the chemical composition and investigate biological activities
At the **UCL**, a review was written about the phytochemistry and pharmacological activities of Mallotus species from Vietnamese mountainous areas (paper published (n° 6))
The antioxidant, cytotoxic, antimicrobial, and hepatoprotective activities of the 39 Mallotus and the 51 Mallotus and Phyllanthus samples were measured. Afterwards it was tried to identify in the antioxidant samples, compounds responsible for the activity. Samples were purified and fractions were tested for given activities (paper published (n° 10), paper submitted for publication (n° 14)).

WP4: Produce materials (food supplements) and/or to identify lead molecules with a potential interest for pharmaceutical or food industries
This WP will be performed by **Vietnamese partner** in cooperation with a Vietnamese company.

WP5: Apply analytical aspects for quality control of plant materials or products from plants
At the **VUB**, a methodology to develop HPLC fingerprints was defined (paper published (n° 3)). Afterwards, this methodology was used to develop HPLC-UV fingerprints of 39 *Mallotus* samples. From the fingerprints, peaks responsible for antioxidant (2 papers published (n° 1-2)) and cytotoxic (paper submitted (n° 12)) activity were determined using different linear multivariate calibration techniques. Interesting peaks for antioxidant activity were then further examined with HPLC-MS at the UCL (paper published (n° 11)).
HPLC-UV fingerprints of these 39 samples were then developed on dissimilar chromatographic systems, in order to indicate and identify peaks responsible for the antioxidant activity. LC-MS experiments were performed (at the UCL) to obtain additional information on the potentially antioxidant compounds (paper published (n° 9)).
pCEC-UV fingerprints of these 39 samples were also developed and evaluated as a complementary technique to HPLC (paper published (n° 8)).
The methodology was also used to develop HPLC-UV fingerprints on a monolithic column for 36 samples (10 Mallotus and 26 Phyllanthus samples). From the fingerprints, the samples were classified according to genera and species (paper in preparation (n° 17)) and peaks responsible for antioxidant activity were determined using different linear multivariate calibration techniques (paper submitted for publication (n° 13)).
Next, UFLC-UV fingerprints were developed on a fused-core column for 51 Mallotus and Phyllanthus samples. From the fingerprints, peaks responsible for antioxidant activity were determined using different linear multivariate calibration techniques (poster presentation n°5). The results obtained from the HPLC-UV and UFLC fingerprints of the 36 samples will be compared. However, this research is still ongoing.
Several oral and poster presentations were presented at various national and international congresses concerning the research on herbal fingerprints (see further).
In **Vietnam**, HPLC-DAD-MS fingerprints of Mallotus phillipinensis were developed (paper in preparation (n° 16)). Also HPLC-DAD-ELSD fingerprints were developed. However, the latter research is still ongoing.

Products and services

PUBLICATIONS

Published

1) N. Nguyen Hoai, B. Dejaegher, C. Tistaert, V. Nguyen Thi Hong, C. Rivière, G. Chataigné, K. Phan Van, M. Chau Van, J. Quetin-Leclercq, Y. Vander Heyden, Development of HPLC fingerprints for *Mallotus* species extracts and evaluation of the peaks responsible for their antioxidant activity, **Journal of Pharmaceutical and Biomedical Analysis 50 (2009) 753-763**

2) C. Tistaert, B. Dejaegher, N. Nguyen Hoai, G. Chataigné, C. Rivière, V. Nguyen Thi Hong, M. Chau Van, J. Quetin-Leclercq, Y. Vander Heyden, Potential antioxidant compounds in *Mallotus* species fingerprints. Part I: indication, using linear multivariate calibration techniques, **Analytica Chimica Acta 652 (2009) 189-197**

3) B. Dejaegher, G. Alaerts, and N. Matthijs, "Methodology to develop liquid chromatographic fingerprints for the quality control of herbal medicines", **Acta Chromatographica 22 (2010) 237-258**

4) Phan VK, Nguyen TM, Minh CV, Nguyen HK, Nguyen HD, Nguyen PT, Nguyen XC, Nguyen HN, Nguyen XN, Heyden YV, Quetin-Leclercq J, Kim GN, Jang HD, Kim YH, Two new C-glucosyl benzoic acids and flavonoids from *Mallotus nanus* and their antioxidant activity, **Arch. Pharm. Res. 33(2) (2010) 203-208**

5) Mai NT, Cuong NX, Thao NP, Nam NH, Khoi NH, Minh CV, Heyden YV, Thuan NT, Tuyen NV, Quetin-Leclercq J, Kiem PV, A new lignan dimer from *Mallotus philippensis*, **Nat. Prod. Commun. 5(3) (2010) 423-426**

6) C. Rivière, V. Nguyen Thi Hong, Q. Tran Hong, G. Chataigné, N. Nguyen Hoai, B. Dejaegher, C. Tistaert, T. Nguyen Thi Kim, Y. Vander Heyden, M. Chau Van, J. Quetin-Leclercq; Mallotus species from Vietnamese mountainous areas: phytochemistry and pharmacological activities; Phytochemistry Reviews 9 (2010) 217-253

7)Nguyen Hoai Nam, Phan Van Kiem, Ninh Khac Ban, Nguyen Phuong Thao, Nguyen Xuan Nhiem, Nguyen Xuan Cuong, Christophe Tistaert, Bieke Dejaegher, Yvan Vander Heyden, Joëlle Quetin-Leclercq, Do Thi Thao, Chau Van Minh, Chemical constituents of Mallotus macrostachyus growing in Vietnam and cytotoxic activity of some cycloartane derivatives, Phytochemistry Letters 4 (2011) 348-352

8) S. Pieters, C. Tistaert, G. Alaerts, K. Bodzioch, D. Mangelings, B. Dejaegher, C. Rivière, N. Nguyen Hoai, M. Chau Van, J. Quetin-Leclercq, Y. Vander Heyden; Pressurized Capillary Electrochromatography in a Screening for Possible Antioxidant Molecules in Mallotus Fingerprints: Challenges, Potentials and Prospects; Talanta 83 (2011) 1188-1197

9) C. Tistaert, B. Dejaegher, G. Chataigné, M. Chau Van, J. Leclercq-Quetin, Y. Vander Heyden; Dissimilar chromatographic systems to indicate and identify antioxidants from Mallotus species; Talanta 83 (2011) 1198-1208

10) V. Nguyen Thi Hong, C. Rivière, Q. Tran Hong, G. Chataigné, N. Nguyen Hoai, B. Dejaegher, C. Tistaert, T. Nguyen Thi Kim, K.P. Van, Y. Vander Heyden, M. Chau Van, J. Quetin-Leclercq; Identification by LC-ESI-MS of Flavonoids Responsible for the Antioxidant Properties of Mallotus Species from Vietnam; Natural Product Communications 6 (2011) 813-818

11) C. Tistaert , B. Dejaegher, G. Chataigné, N. Nguyen Hoai, C. Rivière, V. Nguyen Thi Hong, M. Chau Van, J. Quetin-Leclerq, Y. Vander Heyden, Potential antioxidant compounds in Mallotus species fingerprints. Part II: Fingerprint alignment, data analysis and peak identification, Analytica Chimica Acta 721 (2012) 35-43

Submitted for publication

12) C. Tistaert, G. Chataigné, B. Dejaegher, C. Rivière, N. Nguyen Hoai, M. Chau Van, J. Quetin-Leclerq, Y. Vander Heyden; Multivariate data analysis to evaluate the fingerprint peaks responsible for the cytotoxic activity of *Mallotus* species, Submitted for publication

13) S. Thiangthum, B. Dejaegher, M. Goodarzi, C. Tistaert, A.Y. Gordien, N. Nguyen Hoai, M. Chau Van, J. Quetin-Leclercq, L. Suntornsuk, Y. Vander Heyden, Potentially antioxidant compounds indicated from Mallotus and Phyllanthus species fingerprints, Submitted for publication

14) C. Rivière, V. Nguyen Thi Hong, N. Nguyen Hoai, B. Dejaegher, C. Tistaert, K.P. Van, Y. Vander Heyden, M. Chau Van, J. Quetin-Leclercq; N-methyl-5-carboxamide-2-pyridone from Mallotus barbatus: a chemosystematic marker in the sub-tribe Rottlerinae of the Euphorbiaceae, Submitted for publication

In preparation

15) Nguyen Hoai Nam, Phan Van Kiem, Chau Van Minh, Ninh Khac Ban, Nguyen Phuong Thao, Nguyen Xuan Cuong, Pham Hai Yen, Tran Hong Hanh, Bieke Dejaegher, Yvan Vander Heyden, Joëlle Quetin-Leclercq, Chemical investigations and biological studies of genus *Phyllanthus emblica,* in preparation

16) Nguyen Hoai Nam, Phan Van Kiem, Chau Van Minh, Ninh Khac Ban, Nguyen Phuong Thao, Nguyen Xuan Cuong, Pham Hai Yen, Tran Hong Hanh, Bieke Dejaegher, Yvan Vander Heyden, Joëlle Quetin-Leclercq, Chromatographic fingerprint analysis of *Mallotus philippensis* from various sources by HPLC-MS, in preparation.

17) M. Goodarzi, B. Dejaegher, C. Tistaert, A. Hoang Le Tuan, N. Nguyen Hoai, M. Chau Van, J. Quetin-Leclercq, Y. Vander Heyden, Development of HPLC fingerprints for a set of *Mallotus* and *Phyllanthus* samples and their classification, in preparation

ORAL PRESENTATIONS

1) "Indication and identification of potential antioxidant compounds in Mallotus species fingerprints", C. Tistaert, B. Dejaegher, N. Nguyen Hoai, M. Chau Van, V. Nguyen Thi Hong, G. Chataigné, C. Rivière, J. Quetin-Leclercq, J. Smeyers-Verbeke, Y. Vander Heyden, 11th Scandinavian Symposium on Chemometrics (SSC11) - June 8th-11th 2007 - Loen/Stryn – Norway

2) "Data handling of chromatographic herbal fingerprints", B. Dejaegher, Y. Vander Heyden, 11th International Symposium on Hyphenated Techniques in Chromatography and Hyphenated Chromatographic Analyzers (HTC-11) - January 27th-29th 2010 - Brugge - Belgium

3) "Identification of antioxidative compounds in Mallotus species combining chemometrical treated fingerprints with HPLC-MS", C. Tistaert, B. Dejaegher, G. Chataigné, N. Nguyen Hoai, C. Rivière, M. Chau Van, J. Quetin-Leclercq, Y. Vander Heyden, Belgian Society for Mass Spectrometry (BSMS) Annual Meeting 2010 - April 16th 2010 - Woluwé – Belgium

4) "Chromatographic fingerprints for herbal extracts: set-up and data analysis", G. Alaerts, M. Dumarey, J. van Erps, S. Pieters, M. Merino-Arévalo, N. Matthijs, B. Dejaegher, J. Smeyers-Verbeke, Y. Vander Heyden, ISCNP-ISDNP 2010, 7th International Symposium on Chromatography of Natural Products, combined with the 6th International Symposium of the International Society for the Development of Natural Products: The application of analytical methods for the development of natural products - June 14th-17th 2010 - Lublin – Poland

5) Data handling of chromatographic herbal fingerprints", B. Dejaegher, C. Tistaert, M. Dumarey, G. Alaerts, Y. Vander Heyden, 12th International Conference on Chemometrics in Analytical Chemistry (CAC-2010) - October 18th-21th 2010 - Antwerp - Belgium

6) "Chromatographic herbal fingerprints: Development, Validation, and Data Handling", B. Dejaegher, C. Tistaert, M. Dumarey, G. Alaerts, Y. Vander Heyden, Research on fingerprints of Chinese Materia Medica to develop standard and research protocols evaluating their identity, safety, and reproducibility - December 17th 2010 - Brussels - Belgium

7) "Basic unsupervised and supervised multivariate data analysis", B. Dejaegher, C. Tistaert, M. Goodarzi, M. Dumarey, G. Alaerts, Y. Vander Heyden, Workshop Metabolomics: Basics and Applications to Plant Sciences - April 11th-15th 2011 - Leiden - The Netherlands

8) "Data analysis of HPLC fingerprints from Mallotus and Phyllanthus samples", S. Thiangthum, B. Dejaegher, M. Goodarzi, C. Tistaert, Y. Vander Heyden, 15th Forum of Pharmaceutical Sciences - May 12th-13th 2011 - Spa - Belgium
9) "Chromatographic herbal fingerprints: development, validation and data handling", B. Dejaegher, C. Tistaert, M. Goodarzi, G. Alaerts, Y. Vander Heyden, Kwaliteitsaspecten van Geneeskrachtige Planten - Nederlandse Vereniging voor GeneeskruidenOnderzoek (NVGO) - May 27th 2011 - Strombeek-Bever – Belgium
10) "Herbal fingerprints: development and extraction of information", Y. Vander Heyden, G. Alaerts, M. Dumarey, C. Tistaert, B. Dejaegher, RDPA2011 - 14th International Meeting on Recent Developments in Pharmaceutical Analysis - September 21st-24th 2011 - Pavia – Italy
11) "Herbal Fingerprints: Extraction of Information", Y. Vander Heyden, C. Tistaert, G. Alaerts, B. Dejaegher, Twelfth International Symposium on Hyphenated Techniques in Chromatography and Hyphenated Chromatographic Analysers (HTC-12) - February 1st-3rd 2012 - Bruges - Belgium

POSTER PRESENTATIONS

1) "Identification of antioxidative compounds in Mallotus species combining chemometrical treated fingerprints with HPLC-MS ", C. Tistaert, B. Dejaegher, G. Chataigné, N. Nguyen Hoai, C. Rivière, V. Nguyen Thi Hong, M. Chau Van, J. Quetin-Leclercq, Y. Vander Heyden, 26th Annual Symposium on Chemometrics: Knowledge integration & visualization - May 20th 2010 - Utrecht - The Netherlands
2) "Identification of antioxidative compounds in Mallotus species combining chemometrical treated fingerprints with HPLC-MS ", C. Tistaert, B. Dejaegher, G. Chataigné, N. Nguyen Hoai, C. Rivière, V. Nguyen Thi Hong, M. Chau Van, J. Quetin-Leclercq, Y. Vander Heyden, PhD Research Day 2010 ("Dag van de Doctorandi 2010") - May 28th 2010 - VUB - Brussels – Belgium
3) "Data handling of chromatographic herbal fingerprints", B. Dejaegher, C. Tistaert, M. Dumarey, G. Alaerts, Y. Vander Heyden, 12th Scandinavian Symposium on Chemometrics (SSC12) - June 7th-10th 2011 - Billund – Denmark
4) "Classification of HPLC fingerprints for a set of Mallotus and Phyllanthus samples", M. Goodarzi, B. Dejaegher, C. Tistaert, A. Hoang Le Tuan, N. Nguyen Hoai, M. Chau Van, J. Quetin-Leclercq, Y. Vander Heyden, Fifth International Chemometrics Research Meeting (ICRM 2011) - September 25th-29th 2011 - Berg en Dal - The Netherlands
5) "Fused-core stationary phases for fingerprint development of Phyllanthus and Mallotus species", Greet Parewyck, Christophe Tistaert, Bieke Dejaegher, Debby Mangelings, Yvan Vander Heyden, PhD Research Day 2012 ("Dag van de Doctorandi 2012") - March 27th 2012 - VUB - Brussels - Belgium

---------- Ideas for future research----------
The research on the development and data analysis of UFLC-UV fingerprints of herbal samples with fused-core columns will be continued in order to speed-up analysis.

Execution

Period: 2010 – 2012
Laboratory/network Belgium: (coordinator and divers partners)
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Discipline

Medecine /Drugs (pharmacopy)