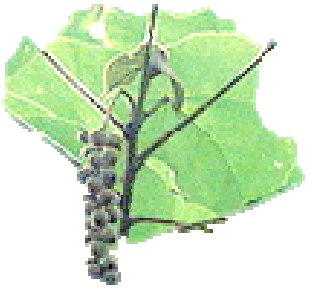

Final Report

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 (BL/03/V21)

Period December 2009 – September 2012

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CONTENT

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GENERAL INTRODUCTION

General Introduction

1 Scientific background

Natural products have served mankind as a source of medicine since or even before the recorded history existed. Vietnam, with its vast geographical diversity, boasts diverse botanical resources and possesses a strong ethno-botanical tradition. About 3200 medicinal plants grow in Vietnam with a third regarded as medicinally effective and the remaining two third as dubious to mildly effective. Those resources have a great potential to be developed as high-value healthcare products (food supplements). Nowadays, natural products are also gaining more interest in Western countries.

Quality control is an important issue during development of such products to ensure the identity, safety and quality of the natural and derived products. In this regard we have made efforts to survey useful medicinal plants, and other bio-resources from Vietnam and to evaluate their potential economic value using state-of-the-art chemical and molecular biological techniques. Natural compounds of vegetable origin with interesting biological activities were examined, in order to be possibly utilized as new drugs or in the para-pharmaceutical field, e.g. as non-nutritive and alternative sweeteners, nutraceuticals or ecological insecticides. Only a limited number of natural products was focused on and considered as case studies.

The search for bio-active components from natural materials as a source of lead compounds in drug development is a major endeavor in natural products chemical research. Some species belonging to the Euphorbiaceae family, for example *Phyllanthus and Mallotus* species, are very important in the Vietnamese traditional medicine and have diverse activities.

In a herb and its extracts, there are hundreds of unknown components, many of them are in low amounts and usually variability exists within the same herbal material. Moreover, the chemical components in the herbal medicinal products may vary depending on the harvest season, plant origins, drying processes and other factors. Therefore assaying only some isolated compounds and/or pharmacologically active constituents hardly represents the complex herbal extracts and is not reliable enough for the quality control of these herbs.

The actual situation is that many herbs are used in traditional medicine, but that their active principles (or derived compounds) are not commercialized because either they were not identified yet or their efficacy and safety is insufficiently examined and documented yet or because a synergy or additivity of effects exists between several compounds or classes of compounds, needing the use of more crude extracts (polytherapy).

The project focused on the chemical studies and biological investigations of Vietnamese plants and herbal medicines from the Euphorbiaceae family to develop healthcare materials (e.g. food supplements), and on their quality control.

2 Overall objectives

A number of natural products from the Euphorbiaceae family, i.e. *Mallotus* and *Phyllanthus* species, were focused on and considered as case studies. Therefore, the main aims of this project, entitled "Chemical and biological investigations of Vietnamese herbal medicines from the Euphorbiaceae family to develop high-value healthcare materials and their quality control", were:

1) To find natural compounds or extracts from Euphorbiaceae plants used in Vietnam as herbal medicines 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 Vietnamese medicinal plants from the Euphorbiaceae family used in traditional medicine in Vietnam. The study focused 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: chromatographic fingerprints of crude extracts for identification and quality control purposes, and quantitative determination of specific active compounds.

3 Specific tasks

3.1 VAST-IMBC

- To survey and collect potentially medicinal plants of the *Phyllanthus* species. This occasionally also will be done for *Mallotus* species.
- Preparation of crude and/or purified extracts of selected plant samples and primary screening extracts. Including defined and modified methodology to produce *Phyllanthus* and *Mallotus* extracts.
- Isolation and structure determination of compounds from the selected plants.
- Chromatographic fingerprint with isolated compounds as markers.

3.2 UCL-CHAM

- Evaluation of some biological activities of *Mallotus* and *Phyllanthus* species.
- Isolation and identification of active constituents.
- Analysis of extracts by HPLC-MS.

3.3 VUB-FABI

- Define a methodology to develop HPLC fingerprints.
- Develop HPLC-DAD fingerprints of a set of *Mallotus* and/or *Phyllanthus* samples, herbs commonly used in Vietnamese traditional medicine, and link the HPLC fingerprints to measured activities, such as antioxidant, antimicrobial and cytotoxic activity. For this purpose, prediction models will be build using linear multivariate calibration techniques and it was aspired to indicate and identify the peaks and their underlying compounds that are potentially responsible for the measured activity.
- Evaluate pressure-assisted capillary electrochromatography (pCEC-UV) as a complementary technique to HPLC for the indication of possible antioxidant compounds in *Mallotus* samples from different species.
- Discriminate between *Mallotus* and *Phyllanthus* species (two classes) and their sub-species (six classes).
- Develop UFLC-DAD fingerprints of a set of *Mallotus* and/or *Phyllanthus* samples on a fused-core column, and link the HPLC fingerprints to measured activities, such as antioxidant, antimicrobial and cytotoxic activity. For this purpose, prediction models will be build using

linear multivariate calibration techniques and it was aspired to indicate and identify the peaks and their underlying compounds that are potentially responsible for the measured activity.

FINAL REPORT

Bilateral scientific cooperation

W&T-cooperation with Vietnam

BL/03/V21

Final Report
Period December 2009 – September 2012

1. Administrative information

1.1. Project nr.	BL/03/V21
1.2. End date of the Project	September 30th 2012
1.3. Partner country	Vietnam (Prof. Chau van Minh)
1.4. Flemish promotor - *Name*	Prof. Yvan Vander Heyden
1.5.Participating Waloon universities	Université Catholique de Louvain (Prof. Joelle Leclercq-Quetin)

2. Summary of the Research Results

A number of natural products from the Euphorbiaceae family, i.e. *Mallotus* and *Phyllantus* species, were focused on and considered as case studies. Therefore, the main aims of this project, entitled "Chemical and biological investigations of Vietnamese herbal medicines from the Euphorbiaceae family to develop high-value healthcare materials and their quality control", were:

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, … and

2) To survey Vietnamese 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.

2.1. VAST

Objectives

- To survey and collect potentially medicinal plants of the *Phyllanthus* species. This occasionally also will be done for *Mallotus* species (see research topic ***(1)***)
- Preparation of crude and/or purified extracts of selected plant samples and primary screening extracts. Including defined and modified methodology to produce *Mallotus* and *Phyllanthus* extracts (see research topic ***(2)***)
- Isolation and structure determination of compounds from the selected plants (see research topic ***(3)***)
- Chromatographic fingerprint with isolated compounds as markers (see research topic ***(4)***)

Research

(1)* Survey and collect potentially medicinal plants** (ANNEX IMBC1***).

The *Mallotus* and *Phyllanthus* genera, both belonging to the Euphorbiaceae family, are widely distributed in Vietnam and the south of China. In Vietnam, roots, stem barks, leaves and fruits of *Mallotus* species have been used for hundreds of years in traditional medicine for the treatment of chronic hepatitis and enteritis. Besides, *Phyllanthus* species possess more potential bioactivities than *M.* species. For example, *P. amarus* is used in the traditional medicine to treat hepatitis under the name "Diephachau". In the project we continued to focus on *M.* species (*M. philipinensis, M. japonicus, M.macrostachyus*) and selected some *P.* species which are used in Vietnamese folklore medicine (*P. amarus, P. reticulatus, P. emblica, P. urinaria)*. The survey was carried out in 16 different areas of Vietnam. Then extracts were made of the leaves of 51 *M.* and *P.* samples. The collected samples are presented in Table 1 and some pictures of them are given in Figure 1.

Table 1. *Mallotus and Phyllanthus samples*

No	Code	Species	Collection time	Origin	Part of plant
1	VNB_01	*Mallotus apelta*	Agust, 2009	VanBan-LaoCai	leaves
2	VNB_02	*Phyllanthus emblica*	Agust, 2009	VanBan-LaoCai	leaves
3	VNB_03	*P. emblica*	November,2009	DongDang-Langson	leaves
4	VNB_04	*M. apelta*	November,2009	TamDao-Vinhphuc	leaves
5	VNB_05	*M. apelta*	November,2009	DongDang-Langson	leaves
6	VNB_06	*M. paniculatus*	December,2009	DongVan-Hagiang	leaves
7	VNB_07	*P. emblica*	December,2009	DongVan-Hagiang	leaves
8	VNB_08	*M. apelta*	December,2009	HamYen-TuyenQuang	leaves
9	VNB_09	*P. reticulatus*	February,2010	NghiaTrai-HungYen	leaves
10	VNB_10	*P. urinaria L*	February,2010	NghiaTrai-HungYen	leaves
11	VNB_11	*P. amarus*	February,2010	NghiaTrai-HungYen	leaves
12	VNB_12	*P. amarus*	March,2010	VanDien-Hanoi	leaves
13	VNB_13	*M. paniculatus*	March,2010	HuongHoa-Quangtri	leaves
14	VNB_14	*P. reticulatus*	March,2010	VanDien-Hanoi	leaves
15	VNB_15	*P. emblica*	March,2010	HuongHoa-Quangtri	leaves
16	VNB_16	*P. urinaria L*	March,2010	VanDien-Hanoi	leaves
17	VNB_17	*M. paniculatus*	April, 2010	MeLinh-Vinhphuc	leaves
18	VNB_18	*P. reticulatus*	April, 2010	MeLinh-Vinhphuc	leaves
19	VNB_19	*P. amarus*	April, 2010	MeLinh-Vinhphuc	leaves
20	VNB_20	*P. reticulatus*	April, 2010	LanOng-Hanoi	leaves
21	VNB_21	*P. emblica*	April, 2010	MeLinh-Vinhphuc	leaves
22	VNB_22	*P. amarus*	April, 2010	LanOng-Hanoi	leaves
23	VNB_23	*P. urinaria L*	April, 2010	MeLinh-Vinhphuc	leaves
24	VNB_24	*P. urinaria L*	April, 2010	LanOng-Hanoi	leaves
25	VNB_25	*P. reticulatus*	May, 2010	NinhHiep-Hanoi	leaves
26	VNB_26	*P. reticulatus*	May, 2010	DongAnh-Hanoi	leaves
27	VNB_27	*P. amarus*	May, 2010	QueVo-Bacninh	leaves
28	VNB_28	*P. emblica*	May, 2010	DongAnh-Hanoi	leaves
29	VNB_29	*P. amarus*	May, 2010	DongAnh-Hanoi	leaves
30	VNB_30	*P. urinaria L*	May, 2010	QueVo-Bacninh	leaves
31	VNB_31	*P. urinaria L*	May, 2010	NinhHiep-Hanoi	leaves
32	VNB_32	*P. amarus*	May, 2010	NinhHiep-Hanoi	leaves
33	VNB_33	*P. urinaria L*	May, 2010	DongAnh-Hanoi	leaves
34	VNB_34	*M. apelta*	June,2010	PaCo-HoaBinh	leaves
35	VNB_35	*M. paniculatus*	June, 2010	VQG-Pumat	leaves

36	VNB_36	*M. paniculatus*	July,2010	Cucphuong-NinhBinh	leaves
37	VNB_37	*M. barbatus*	May,2010	Dackrong-Quangtri	leaves
38	VNB_38	*M. barbatus*	May,2010	KyAnh-Hatinh	leaves
39	VNB_39	*M. barbatus*	December,2009	HamYen-TuyenQuang	leaves
40	VNB_40	*P. acidus*	March,2010	PhanRang-NinhThuan	leaves
41	VNB_41	*M. sp*	Ferbuary, 2011	KyAnh_HaTinh	leaves
42	VNB42	*M.macrostachyus*	Agust, 2010	VanBan-LaoCai	leaves
43	VNB_43	*M.macrostachyus*	November,2009	DongDang-Langson	leaves
44	VNB_44	*M. repandus*	June,2010	PaCo-HoaBinh	leaves
45	VNB_45	*M. repandus*	April, 2010	MeLinh-Vinhphuc	leaves
46	VNB_46	*M. resinosus*	March,2010	TuyenHoa_QuangBinh	leaves
47	VNB_47	*M. sp*	Ferbuary, 2011	DeoNgang_HaTinh	leaves
48	VNB_48	*M. sp*	Ferbuary, 2011	DeoNgang_HaTinh	leaves
49	VNB_49	*P. acidus*	March,2010	KhanhHoa	leaves
50	VNB_50	*M. japonicus*	May,2010	Sapa_Laocai	leaves
51	VNB_51	*M. microcapus*	March,2010	TuyenHoa_QuangBinh	leaves

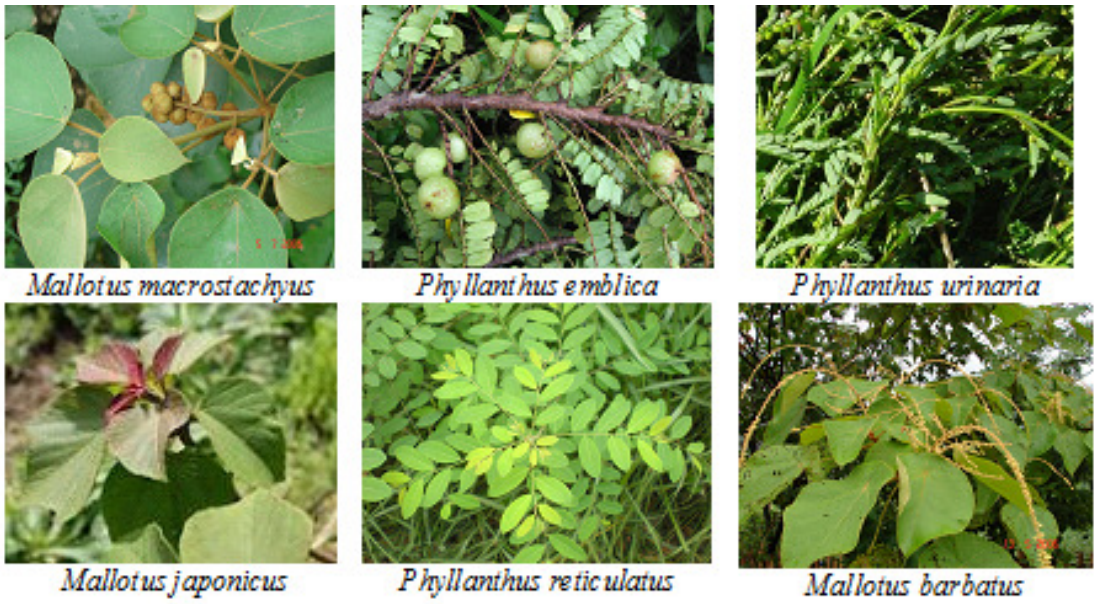


Figure 1. *Mallotus* and *Phyllanthus samples*

(2) **Preparation of crude and/or purified extracts of selected plant samples and primary screening** (***ANNEX IMBC2***).

A methodology to prepare *Mallotus* and *Phyllanthus* extracts was defined and modified based on the experience from the previous project and some Vietnamese national projects. To prepare the herbal extract, 10,0 g plant sample (leaves) was weighed and extracted three times with 100 mL methanol in an ultrasonic bath (Branson Ultrasonic Corporation, Connecticut, US), each time at a temperature between 30-45°C during 60 minutes. The combined extracts were filtered through a 240 nm pore size filter paper (Whatman, Hanoi, Vietnam) and evaporated at decreased pressure (60 Pa) at a temperature of 40°C. The samples preparation methodology is presented in Figure 2.

The obtained crude extracts were divided into 3 fractions, i.e. one for the UCL (activity assays), one for the VUB (HPLC analysis), and one was kept as a library sample for reference purposes.

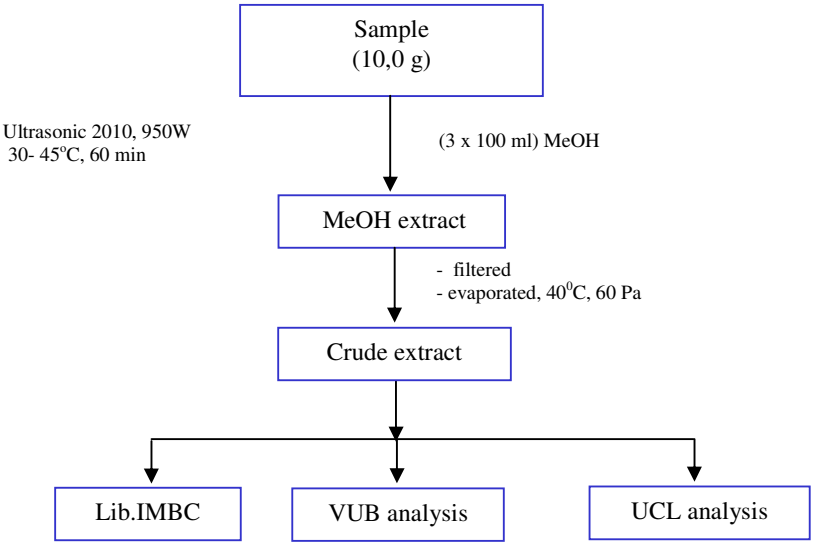


Figure 2. The extraction procedure.

***(3)* Isolation and structure determination of compounds from the selected plants (*ANNEX IMBC3A-3F*).**

In the context of phytochemical and biological studies on *Mallotus* and *Phyllanthus* genera, we selected *Mallotus philippensis*, *Mallotus macrostachyus*, *Phyllanthus emblica*, *Phyllanthus urinaria*, *Phyllanthus reticulates*, *and Mallotus japonicus* for the extraction, purification and identification of bioactive compounds.

Several interesting compounds were isolated and their biological activities were evaluated. Furthermore some new compounds have been isolated and characterized.

***3a) Study on chemical components of Mallotus philippensis* (*ANNEX IMBC3A*).**

An article concerning this work is **published: Nguyen Thi Mai, Nguyen Xuan Cuong, Nguyen Phuong Thao, Nguyen Hoai Nam, Nguyen Huu Khoi, Chau Van Minh, Yvan Vander Heyden, Ngo Thi Thuan, Nguyen Van Tuyen, Joëlle Quetin-Leclercq, and Phan Van Kiem, A new lignan dimer from *Mallotus philippensis*, Natural Product Communications 5(3) (2010) 423-426.**

***3b) Study on chemical components of Mallotus macrostachyus* (*ANNEX IMBC3B*).**

An article concerning this work is **published: 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 QL, Do Thi Thao, Chau Van Minh, Chemical constituents of *Mallotus macrostachyus* growing in Vietnam and cytotoxic activity of some cycloartane derivatives, Phytochemistry Letters 4(3) (2011) 348-352.**

***3c) Study on chemical components of Phyllanthus emblica* (*ANNEX IMBC3C*).**

In this study, 11 known compounds were isolated, i.e. Lupeol (1), 24R*)-24-Methyldammara-25-ene-3-one (2), 6''-p-Coumaroylprunin (3), 2-[5-Hydroxy-2,3-bis-(4-hydroxy-3-methoxy-bezyl)-pentyloxy]-6-hydroxy-methyl-tetrahydro-pyran-3,4,5-triol (4), Corchoionoside (6), (1R,2R)-methyl-5'-hydroxyjasmonate (7), 3,5-Dihydroxy-4-methoxy-benzoic acid (8), Multifidol glucoside 1-[(2methylbutyryl) phloroglucinyl]-β-D-glucopyranoside (9), 2''-O-Acetylquercitrin (10), and (-)-

isolariciresiol-4-O-β-D-glucopyranoside (11), by detailed analyses of their NMR and ESI-MS data and comparison of them with reported data. An article about this research is **in preparation: Nguyen Hoai Nam, Phan Van Kiem, Chau Van Minh[a,*], 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*.**

3d) Study on chemical components of Phyllanthus urinaria (***ANNEX IMBC3D***).
In this study, 7 compounds were isolated, i.e. 5,8-Dimethoxy-7-hydroxy-2-(4-methoxyphenyl)-4-oxo-4*H*- chromene-6-sulfonic acid (1) (a new compound), Dendranthemoside B (2), (1R,2R)-Methyl β-D-glucopyranosylepituberonate (3), 2 Heliobuphthalmin lactone (4), hypophyllanthin (5), Astragalin (6), and nirtetralin (7), by detailed analyses of their NMR and ESI-MS data and comparison of them with reported data. An article about this research is **in preparation: 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 urinaria*.**

3e) Study on chemical components of Phyllanthus reticulatus (***ANNEX IMBC3E***).
In this study, 8 compounds were isolated, i.e. (3*S*,5*R*,6*S*,9ξ)-megastigman-7-ene-3,6,9,10-tetrol (1) (new compound, Methyl brevifolincarbonxylate (2), 3-*O*-methyl-4′-*O*-α-L-rhamnopyranosylellagic acid (3), Isolariciresinol (4), (+)-Pinoresinol-β-D-glucoside (5), Quercetine (6), Gingerglycolipid A (7), and kaempferol 3-glucoside (8), by detailed analyses of their NMR and ESI-MS data and comparison of them with reported data. An article about this research is **in preparation: Nguyen Hoai Nam, Phan Van Kiem, Chau Van Minh, Ninh Thi Ngoc, Nguyen Phuong Thao, Nguyen Xuan Cuong, Vu Anh Tu, Le Duc Dat, Bieke Dejaegher, Yvan Vander Heyden, Joëlle Quetin-Leclercq, Chemical investigations and biological studies of genus *Phyllanthus reticulates*.**

3f) Study on chemical components of Mallotus japonicus (***ANNEX IMBC3F***).
In this study, 6 compounds were isolated, i.e. Bergenin (1), 25,26,27-trisnor-3-ketocycloartan-24-oic acid (2), 29-Norlupane-3,20-dione (3), 25,26,27-trisnor-24-hydroxycycloartan-3-one (4), 5,7-Dihydroxy-4'-methoxy-6-(3-methylbut-2-enyl)flavanone (5), and 29-Norlupane-3,20-dione (6) by detailed analyses of their NMR and ESI-MS data and comparison of them with reported data.
An article concerning this work is **published: Phan Thi Thanh Huong, Chau Ngoc Diep, Le Duc Dat, Vu Anh Tu, Ninh Thi Ngoc,Nguyen Phuong Thao, Nguyen Hoai Nam, Nguyen Xuan Cuong, Nguyen Thi Kim Thanh, Nguyen Nghia Thin2, Phan Van Kiem and Chau Van Minh, Chemical constituents of *Mallotus japonicus*, Vietnam Journal of Chemistry 50(4A) (2012) 183-186.**

(4) **Chromatographic fingerprint with isolated compounds as markers**

4a) In a first study, HPLC-DAD-MS fingerprints of *Mallotus philippensis* were developed following the earlier defined methodology to develop herbal fingerprints with a different HPLC column. An article about this research is **in preparation: 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.**

4b) In a second study, HPLC-DAD-ELSD fingerprints of *Mallotus macrostachyus, Mallotus japonicus, Phyllantus urinaria* using some compounds as markers were built following the earlier defined methodology to develop herbal fingerprints with the change in HPLC column. However, this research is still ongoing.

2.2. UCL-CHAM

Objectives

- Evaluation of some biological activities of *Mallotus* and *Phyllanthus* species (see research topic ***(1)***)
- Isolation and identification of active constituents (see research topic ***(2)***)
- Analysis of extracts by HPLC-MS (see research topic ***(3)***)

Results

(1) **Study of biological activities**

1a) Antioxidant activity of extracts
28 *Phyllanthus* and 23 *Mallotus* crude methanolic extracts were tested in duplicate to evaluate their DPPH antiradical activity. Mean IC_{50}'s with their 95% confidence intervals are compiled in ***ANNEX UCL1***. 13 extracts (12 *Phyllanthus*, 1 *Mallotus*) showed an antioxidant activity comparable to the activity of the reference compound tocopherol (IC_{50}=12.60µg/mL). Results were noticeably homogenous and promising for the six *P. emblica* extracts with IC_{50}'s ranging from 8.57 to 10.92µg/mL. For *P. amarus, P. reticulatus* and *P. urinaria*, more variations in IC_{50} values were observed between samples of a same species.

1b) Cytotoxic activity of extracts
Evaluation of the cytotoxic activity of the 26 *Phyllanthus* and 10 *Mallotus* extracts towards J774 and CHO cells shows that only 4 extracts (including 3 from *P. reticulatus*) showed cytotoxicity against CHO cells below 50µg/mL. Within a same species the toxicity varies according to collection place and time. None of the extracts showed cytotoxicity against J774 cells at 100 µg/ml (see ***ANNEX UCL1***).

1c) Hepatoprotective activity of extracts
These 36 extracts were also tested for hepatoprotective activity on rat liver slices against the hepatotoxicity of paracetamol. N-acetylcysteine and boldo extract were used as positive controls. The results obtained showed that at 1mg/mL most extracts were toxic to the liver slices and offered no protection against paracetamol toxicity (see ***ANNEX UCL1***).
At 0.5mg/mL several *P. emblica* and *P. urinaria* extracts showed some moderate hepatoprotective activity. VNB21 offered the best hepatoprotection against paracetamol toxicity (53%) but the extract itself is hepatotoxic (56%). VNB19 (*P. amarus*) is therefore the most promising extract.

1d) Cytotoxic activity of isolated compounds
The cytotoxic activity of several compounds, isolated in Vietnam from *Phyllanthus urinaria* and *Mallotus japonica*, was also tested. Results are given in ***ANNEX UCL1*** and a publication is **in preparation: A. Gordien, P. Buc Calderon, Nguyen Thi Hong Van, Nguyen Hoai Nam, M. Chau Van, Y. Vander Heyden, J. Quetin-Leclercq Evaluation of the hepatoprotective activity of *Phyllanthus* species from Vietnam on paracetamol induced toxiticy on rat liver slices**.

***(2)* Isolation of active compounds**

2a) Cytotoxic activity

A crude methanolic extract of *Phyllanthus amarus* (PAM), a crude methanolic extract of *Phyllanthus reticulatus* (PRM) and its chloroform (PRC) and ethyl acetate (PRE) fractions as well as the subfractions (PRW1 to PRW6) of its water fraction (PRW) were tested for cytotoxic activity towards J774 and CHO cells. IC_{50}'s are compiled in ***ANNEX UCL1***. PRC was the most cytotoxic sample to both cell lines. A vacuum liquid chromatographic (VLC) fractionation was therefore carried out on PRC affording 13 subfractions (PRC1-13).

2b) Antioxidant activity

PRC1-13 along with 3 compounds isolated from the water fraction by Mr. Nguyen Xuan Cuong during his stay at UCL were tested in duplicate to evaluate their DPPH antiradical activity. Mean IC_{50}'s with their 95% confidence intervals are compiled in ***ANNEX UCL1***. The antioxidant activity observed for PRC was mainly recovered in subfraction PRC5. The antiradical activity of W2E1A (IC_{50}=0.88µg/mL) characterised as gallic acid was identical to the activity of a commercial gallic acid sample (IC_{50}=0.89µg/mL) while W2G1 (IC_{50}=5.77µg/mL) characterised as isoquercitrin showed higher activity than tocopherol (IC_{50}=9.33µg/mL). W2E5 characterised as 3-*O*-methyl-4′-*O*-α-L-rhamnopyranosylellagic acid showed no antiradical activity. However, this is the first report of isolation of this compound from *P. reticulatus*.Other fractions were shown to contain quercitrin and rutin, already identified in the plant.

2c) Antimicorbial activity

PAM, PRC, PRE and PRM were tested for their antimicrobial activity against a panel of gram positive and gram negative bacteria. PAM, PRC and PRM showed no activity at 1mg/mL. PRE showed weak activity (mean ICs: 250-1000µg/mL) against *Morganella Morganii*, *Staphylococcus aureus* and *Yersinia enterocolitica*. Based on these unpromising results, it was decided that *Phyllanthus* extracts would not be further investigated for antimicrobial activity.

2d) Cytotoxic activity

During the stay of Nguyen Thi Hong Van, the chloroform fraction of *P. reticulatus* was further purified by VLC. As most fractions contained chlrorophyllic compounds, which are known to be cytotoxic, chlorophyll was removed and cytotoxicity was evaluated on both chlorophyllic and not chlorophyllic fractions. Results are given in ***ANNEX UCL1***.

Some of the most active fractions devoided of chlorophyll were further purified to give compounds PR01 to PR08. Structure determination is ongoing.

The hexane *Phyllanthus amarus* fraction was further purified by VLC and column chromatography on Si60 or RP-18 stationary phases and 4 compounds were isolated. Three were identified as stigmasterol, phyllanthin and (2S)-1-(3,4-dimethoxyphenyl)-3-methoxy-propan-2-amine. The structure determination of the last one is ongoing.

***(3)* Analysis of fingerprints by HPLC-HRMS**

Several *Mallotus* and *Phyllanthus* extracts were analyzed by HPLC-MS to identify compounds responsible for some biological activities. Up to now, focus was put on the antioxidant activity. Most results were published, in collaboration with the VUB team (see publications).

2.3. VUB-FABI

Objectives

- define a methodology to develop HPLC fingerprints (see below, research topic ***(1)***)
- develop HPLC-DAD fingerprints of a set of *Mallotus* and/or *Phyllanthus* samples, herbs commonly used in Vietnamese traditional medicine, and link the HPLC fingerprints to measured activities, such as antioxidant, antimicrobial and cytotoxic activity. For this purpose, prediction models were build using linear multivariate calibration techniques and it was aspired to indicate and identify the peaks and their underlying compounds that are potentially responsible for the measured activity (see below, research topics ***(2)-(6)*** and ***(11)***)
- evaluate pressure assisted capillary electrochromatography (pCEC-UV) as a complementary technique to HPLC for the indication of possible antioxidant compounds in *Mallotus* samples from different species (see below, research topic ***(7)***)
- discriminate between *Mallotus* and *Phyllanthus* species (two classes) and their sub-species (six classes) (see below, research topic ***(10)***)
- develop UFLC-DAD fingerprints of a set of *Mallotus* and/or *Phyllanthus* samples on a fused-core column, and link the HPLC fingerprints to measured activities, such as antioxidant, antimicrobial and cytotoxic activity. For this purpose, prediction models were build using linear multivariate calibration techniques and it was aspired to indicate and identify the peaks and their underlying compounds that are potentially responsible for the measured activity (see below, research topic ***(12)***)
- additionally, two reviews were written about the development and data handling of herbal fingerprints (see below, research topics ***(8)-(9)***)

Research

(1) A fingerprint methodology was defined. This methodology is **published: 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** (***ANNEX VUB 1***).

(2) The earlier defined fingerprint methodology was applied to develop HPLC fingerprints of *Mallotus species*. After an exploratory analysis using PCA, the fingerprints were used to evaluate the peaks responsible for the antioxidant activity, using PLS. An article concerning this work is **published: 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** (***ANNEX VUB 2***).

(3) It was tried to link the antioxidant activity (%DPPH) of the 39 *Mallotus* samples to the fingerprints, in order to determine peaks or groups of peaks responsible for the antioxidant activity. For this purpose, Step-MLR, PCR, PLS, UVE-PLS, and OPLS were applied. An article concerning this work is **published: 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** (***ANNEX VUB 3***).

(4) The 11 antioxidant *Mallotus* samples were then analysed by HPLC-MS at the UCL-CHAM (Waloon partner), in order to identify the antioxidant components. An article concerning this work is

published: C. Tistaert, B. Dejaegher, G. Chataigné, N. Nguyen Hoai, C. Rivière, M. Chau Van, J. Quetin-Leclercq, 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 (***ANNEX VUB 4***).

(5) Fingerprints were developed on dissimilar chromatographic systems, in order to indicate and identify peaks responsible for the antioxidant activity of the 39 *Mallotus* samples. LC-MS experiments were performed (at the UCL-CHAM) to obtain additional information on the potentially antioxidant compounds. An article concerning this work is **published: C. Tistaert, B. Dejaegher, G. Chataigné, N. Nguyen Hoai, M. Chau Van, J. Quetin-Leclercq, Y. Vander Heyden, Dissimilar chromatographic systems to indicate and identify antioxidants from *Mallotus* species, Talanta 83 (2011) 1198-1208** (***ANNEX VUB 5***).

(6) It was also tried to model other activities of the *Mallotus* samples, such as antimicrobial or cytotoxic activity. Modelling the antimicrobial activity seemed less interesting, since the antimicrobial properties of the 39 *Mallotus* samples are all quite low, which was concluded from measurements performed at the UCL. For the cytotoxic activity, it was tried to link the activity, measured at the UCL, to the fingerprints, in order to determine peaks or groups of peaks responsible for the cytotoxic activity. Both the cytotoxic activity of a non-cancerous and a cancerous cell line were evaluated. First, an exploratory analysis using PCA was performed. The effect of different preprocessing techniques was evaluated. Finally, to link the cytotoxic activity to the fingerprints, multivariate calibration techniques were applied, i.e. Step-MLR, PCR, PLS, UVE-PLS, and OPLS. An article concerning this work is **published: C. Tistaert, G. Chataigné, B. Dejaegher, C. Rivière, N. Nguyen Hoai, M. Chau Van, J. Quetin-Leclercq, Y. Vander Heyden, Multivariate data analysis to evaluate the fingerprint peaks responsible for the cytotoxic activity of Mallotus species, Journal of Chromatography B 910 (2012) 103-113 (*ANNEX VUB 6*).**

(7) Pressure assisted capillary electrochromatography (pCEC) was evaluated as a complementary technique to high-performance liquid chromatography (HPLC) for the indication of possible antioxidant compounds in *Mallotus* samples from different species. As pCEC is a rather new technique, not so strongly developed as HPLC, it is the intention to evaluate its potential, while exposing its analytical shortcomings met in this application. This way, the interest in upgrading the technique for fingerprint applications can be expanded. An article concerning this work is **published: S. Pieters, C. Tistaert , G. Alaerts, K. Bodzioch, D. Mangelings, B. Dejaegher, C. Rivière, N. Nguyen Hoai, M. Chau Van, J. Quetin-Leclerq, 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** (***ANNEX VUB 7***).

(8) A chromatographic fingerprint from a herbal product consists of the whole chromatographic profile. A fingerprint is an approach used to evaluate the quality of the investigated product.
This review discusses recent developments in the set-up and data analysis of chromatographic fingerprints for herbal products. First, different set-ups for fingerprint development are reviewed. Second, the data analysis is focused on in the context of different fingerprint applications. In this section, data pretreatment, unsupervised data analysis and supervised data analysis are discussed topics. Unsupervised data analysis is described in the context of similarity analysis, exploratory analysis, and curve resolution methods. Supervised data analysis techniques are divided into pattern recognition or classification methods and multivariate calibration methods. The different application areas are illustrated and discussed with several case studies.
This review is **published: G. Alaerts, B. Dejaegher, J. Smeyers-Verbeke, Y. Vander Heyden, Recent developments in chromatographic fingerprints from herbal products: set-up and data**

analysis, Combinatorial Chemistry & High Throughput Screening 13 (2010) 900-922 (***ANNEX VUB 8***).

(9) A review on the quality control of herbal medicines by chromatographic fingerprints and the data analysis of the obtained data was published. As herbal medicines have an important position in health care systems worldwide, their current assessment and quality control are a major bottleneck. Over the past decade, major steps were taken not only to improve the quality of the herbal products but also to develop analytical methods ensuring their quality. Nowadays, chromatographic fingerprinting is the generally accepted technique for the assessment and quality control of herbal products. This review briefly considers the evolution of the regulations and guidelines on the quality control of herbal medicines, and reviews the established analytical techniques for herbal fingerprinting with an emphasis on the most recent developments, such as miniaturized techniques, new stationary phases, analysis at high temperatures and multi-dimensional chromatography. Accessory to the new analytical techniques, the chemometric data handling techniques applied are discussed. Chemometrics provide scientists with useful tools in understanding the huge amounts of data generated by the analytical advances and prove to be valuable for quality control, classification and modeling of, and discrimination between herbal fingerprints.
This review is **published: C. Tistaert, B. Dejaegher, Y. Vander Heyden, Chromatographic separation techniques and data handling methods for herbal fingerprints: A review**, **Analytica Chimica Acta 690 (2011) 148–161** (***ANNEX VUB 9***).

(10) HPLC-DAD fingerprints were developed for all 36 Vietnamese samples (10 *Mallotus* samples, and 26 *Phyllantus* samples). The same experimental settings were used as for the previously developed fingerprints of the 39 *Mallotus* samples. These settings were obtained according to an earlier defined methodology to develop herbal fingerprints.
After the development of the fingerprints, the desired information is extracted from the multivariate data. The goal was to discriminate between *Mallotus* and *Phyllanthus* species (two classes) and their sub-species (six classes). First, the data was preprocessed. Three different approaches were followed: column centering, normalization followed by column centering, and Standard Normal Variate followed by column centering. Secondly, an Exploratory Analysis using Principal Component Analysis was performed to visualise the data and to look for groups in the data, e.g. a group with *Mallotus* samples and one with *Phyllantus* samples. Thirdly, supervised discrimination/classification techniques, such as Linear Discriminant Analysis (LDA), Quadratic Discriminant Analysis (QDA), Classification and Regression Trees (CART), and Soft Independent Modeling of Class Analogy (SIMCA), were used to discriminate between *Mallotus* and *Phyllanthus* species (two classes) and their sub-species (six classes). An article about this research is **in preparation: J. Viaene, 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 for discrimination and classification purposes** (***ANNEX VUB 10***).

(11) The same fingerprints (as in ***(9)***) were used to model the antioxidant activity of the 36 samples. The same three different preprocessing approaches as above were evaluated, and different multivariate calibration methods, such as Partial Least Squares (PLS), and Orthogonal Projections to Latent Structures (OPLS), were used to model an activity measured at the UCL as a function of the fingerprints, and to indicate in the fingerprints peaks possibly responsible for the considered activity. An article about this research is published: **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, Journal of Chromatography B 910 (2012) 114-121** (***ANNEX VUB 11***).
An **oral presentation** was given about the above data analysis at the **15th Forum of Pharmaceutical**

Sciences in Spa, Belgium, May 12th-13th 2011: S. Thiangthum, B. Dejaegher, M. Goodarzi, C. Tistaert, Y. Vander Heyden, Data analysis of HPLC fingerprints from *Mallotus* and *Phyllanthus* samples.

(12) In another study, UFLC-DAD fingerprints for 51 Vietnamese samples were developed applying a fused-core column. The same experimental settings were used as for the previously developed HPLC-DAD fingerprints of the 36 samples. These settings were obtained according to an earlier defined methodology to develop herbal fingerprints. A report about the development of the UFLC-DAD fingerprints is given in ***ANNEX VUB 12***.
After the development of the fingerprints, the desired information was extracted from the multivariate data using chemometric techniques. The same three different preprocessing approaches as above were evaluated, and Exploratory Analysis using Principal Component Analysis and Cluster Analysis was performed to visualise the data and to look for groups in the data. Then the multivariate calibration method Partial Least Squares (PLS) was used to model an activity measured at the UCL as a function of the fingerprints, and to indicate in the fingerprints peaks possibly responsible for the considered activity. However, this research is still ongoing. Also Orthogonal Projections to Latent Structures (OPLS) should be evaluated as modelling technique. A preliminary report about the data analysis is presented in ***ANNEX VUB 13***.
A **poster presentation** was presented at the **PhD Research Day 2012** at the **VUB** in **Brussels, Belgium** on **March 27th 2012: Greet Parewyck, Christophe Tistaert, Bieke Dejaegher, Debby Mangelings, Yvan Vander Heyden, Fused-core stationary phases for fingerprint development of *Phyllanthus* and *Mallotus* species**.

3. Scientific activities during the whole period

3.1. Exchanges from the partner country to Belgium

Name	From (date)	To (date)	Host institute
Mr. Nguyen Hoai Nam	1st August 2010	31st August 2010	VUB
Nguyen Xuan Cuong	1st July 2010	30th November 2010	UCL
Hoang Le Tuan Anh	17th September 2010	16th December 2010	VUB
Nguyen Xuan Cuong	5th April 2011	28th June 2011	VUB
Nguyen Hoai Nam	3rd June 2011	28th June 2011	VUB
Nguyen Thi Hong Van	27th May 2011	27th October 2011	UCL

3.2. Exchanges from Belgium to the partner country

Name	From (date)	To (date)	Host institute
Yvan Vander Heyden	19th November 2011	26th November 2011	VAST
Joëlle Quetin-Leclercq	19th November 2011	26th November 2011	VAST

3.3. Identification of members in the Flemish/Belgian project teams

Name	Institute / University	Title/function
Yvan Vander Heyden	VUB	Professor (Flemish Belgian partner)
Bieke Dejaegher	VUB	Post-doctoral researcher
Christophe Tistaert	VUB	Ph.D. student
Sigrid Pieters	VUB	Ph.D. student
Mohammad Goodarzi	VUB	Ph.D. student
Sumate Thiangthum	VUB	Ph.D. student
Joëlle Quetin-Leclercq	UCL	Professor (Waloon Belgian partner)
Céline Rivière	UCL	Post-doctoral researcher
Andrea Gordien	UCL	Post-doctoral researcher
Jessica Le Ven	UCL	Post-doctoral researcher

3.4. Identification of members in the partner's project teams

Name	Title/function
Chau Van Minh	Professor
Nguyen Hoai Nam	Ph.D
Nguyen Xuan Cuong	Ph.D. student
Hoang Le Tuan Anh	PhD
Pham Hai Yen	PhD
Phan Van Kiem	Professor
Nguyen Thi Mai	Ph.D. student
Nguyen Thi Hong Van	Ph.D. student

3.5. Project – related workshops.
Place: Université Catholique de Louvain (UCL) - CHAM Date: 20th September 2010 Title: Project kick-off meeting Number of Belgian participants: 3 Number of participants from abroad: 3
Place: Vietnam, VAST Date: 20th November 2011 Title: Meeting DWTC Bilateral Project Number of Belgian participants: 2 Number of participants from abroad: 25
Place: Date: Title: Number of Belgian participants: Number of participants from abroad:
Place: Date: Title: Number of Belgian participants: Number of participants from abroad:

3.6. Project-related publications

3.6.1. Published

* 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 (2010). Two new C-glucosyl benzoic acids and flavonoids from *Mallotus nanus* and their antioxidant activity, Arch Pharm Res. 33(2) (2010) 203-208

* 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 (*ANNEX IMBC3A*)

* Nguyen Hoai Nam, Phan Van Kiem, Chau Van Minh, Ninh Khac Ban, Nguyen Phuong Thao, Nguyen Xuan Cuong, Hoang Le Tuan Anh, Andréa Gordien, Yvan Vander Heyden, Bieke Dejaegher, Joëlle Quetin-Leclercq, Chemical constituents and biological activity of *Mallotus macrostachyus* growing in Vietnam, Phytochemistry Letters 4 (2011) 348-352 (*ANNEX IMBC3B*)

Phan Thi Thanh Huong, Chau Ngoc Diep, Le Duc Dat, Vu Anh Tu, Ninh Thi Ngoc,Nguyen Phuong Thao, Nguyen Hoai Nam, Nguyen Xuan Cuong, Nguyen Thi Kim Thanh, Nguyen Nghia Thin2, Phan Van Kiem and Chau Van Minh, Chemical constituents of *Mallotus japonicus*, Vietnam Journal of Chemistry 50(4A) (2012) 183-186 (*ANNEX IMBC3F*)

* 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 (*ANNEX UCL2*)

* 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 (*ANNEX UCL3*)

* 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, Biochemical Systematics and Ecology 44 (2012) 212–215 (*ANNEX UCL4*)

* 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 (*ANNEX VUB 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 (*ANNEX VUB 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 (*ANNEX VUB 3*)

* C. Tistaert, B. Dejaegher, G. Chataigné, N. Nguyen Hoai, C. Rivière, M. Chau Van, J. Quetin-Leclercq, 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 (*ANNEX VUB 4*)

* C. Tistaert, B. Dejaegher, G. Chataigné, N. Nguyen Hoai, M. Chau Van, J. Quetin-Leclercq, Y. Vander Heyden, Dissimilar chromatographic systems to indicate and identify antioxidants from *Mallotus* species, Talanta 83 (2011) 1198-1208 (*ANNEX VUB 5*)
* C. Tistaert, G. Chataigné, B. Dejaegher, C. Rivière, N. Nguyen Hoai, M. Chau Van, J. Quetin-Leclercq, Y. Vander Heyden, Multivariate data analysis to evaluate the fingerprint peaks responsible for the cytotoxic activity of Mallotus species, Journal of Chromatography B 910 (2012) 103-113 (*ANNEX VUB 6*)
* S. Pieters, C. Tistaert , G. Alaerts, K. Bodzioch, D. Mangelings, B. Dejaegher, C. Rivière, N. Nguyen Hoai, M. Chau Van, J. Quetin-Leclerq, 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 (*ANNEX VUB 7*)
* G. Alaerts, B. Dejaegher, J. Smeyers-Verbeke, Y. Vander Heyden, Recent developments in chromatographic fingerprints from herbal products: set-up and data analysis, Combinatorial Chemistry & High Throughput Screening 13 (2010) 900-922 (*ANNEX VUB 8*)
* C. Tistaert, B. Dejaegher, Y. Vander Heyden, Chromatographic separation techniques and data handling methods for herbal fingerprints: A review, Analytica Chimica Acta 690 (2011) 148–161 (*ANNEX VUB 9*)
* 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, Journal of Chromatography B 910 (2012) 114-121 (*ANNEX VUB 11*)

3.6.2. In print

None

3.6.3 In revision or submitted for publication

* A. Gordien, N Xuan Cuong, V. Nguyen Thi Hong, B. Dejaegher, N. Hoai Nam, C. Van Minh, P. Buc Calderon, Y. Vander Heyden, J. Quetin-Leclercq Variations of Hepatoprotective, Antioxidant, and Cytotoxic Activity within Vietnamese *Phyllanthus* species, submitted for publication (*ANNEX UCL5*)

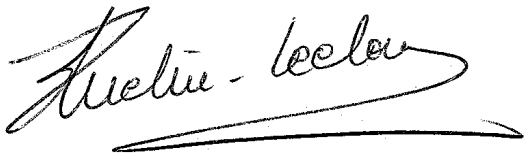
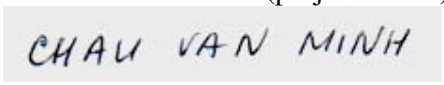
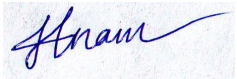
3.6.4. In preparation

* 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 (*ANNEX IMBC3C*)
***** 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 urinaria*, in preparation (*ANNEX IMBC3D*)
* Nguyen Hoai Nam, Phan Van Kiem, Chau Van Minh, Ninh Thi Ngoc, Nguyen Phuong Thao, Nguyen Xuan Cuong, Vu Anh Tu, Le Duc Dat, Bieke Dejaegher, Yvan Vander Heyden, Joëlle Quetin-Leclercq, Chemical investigations and biological studies of genus *Phyllanthus reticulates*, in preparation (*ANNEX IMBC3E*)
* 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

* A. Gordien, P. Buc Calderon, Nguyen Thi Hong Van, Nguyen Hoai Nam, M. Chau Van, Y. Vander Heyden, J. Quetin-Leclercq, Evaluation of the hepatoprotective activity of *Phyllanthus* species from Vietnam on paracetamol induced toxiticy on rat liver slices, in preparation

* J. Viaene, 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 for discrimination and classification purposes, in preparation (*ANNEX VUB 10*)

4. Signatures

The Flemish promotor Prof. Yvan Vander Heyden	
The Waloon promotor Prof. Joelle Quetin-Leclercq	
The promotor from the partner country Prof. Chau van Minh (project leader)  Nam Nguyen Hoai	 
Date	December 31st 2012

CONCLUSIONS

Conclusions

This project was entitled "Chemical and biological investigations of Vietnamese herbal medicines from the Euphorbiaceae family to develop high-value healthcare materials and their quality control".

The first two overall objectives were to find natural compounds or extracts from Euphorbiaceae plants used in Vietnam as herbal medicines with interesting biological activities to contribute as new material for the pharmaceutical or food industries, e.g. as food material, functional food, nutraceuticals, …, and to survey Vietnamese medicinal plants from the Euphorbiaceae family used in traditional medicine in Vietnam. The study focused mainly on *Phyllanthus*, but also on *Mallotus* species, with a special interest in endemic species. These objectives were performed by the Vietnamese partner in the project. In Vietnam, two sets of samples were collected in different Vietnamese regions, i.e. 39 *Mallotus* samples from different species, and 51 samples (23 *Mallotus* samples and 28 *Phyllanthus* samples) from different species. A methodology to produce *Mallotus* and *Phyllanthus* extracts was defined and modified based on the experience from the previous project and some Vietnamese national projects. The research of the Vietnamese partner focused on the extraction, purification and identification of given bioactive compounds from *Mallotus* and *Phyllanthus* genera.

A new lignan dimer, i.e. **bilariciresinol**, was isolated from the leaves of *Mallotus philippensis*, along with platanoside, isovitexin, dihydromyricetin, bergenin, 4-O-galloylbergenin, and pachysandiol A. Their structures were elucidated by spectroscopic experiments including 1D and 2D NMR and FTICR-MS.

Two new cycloartane derivatives, i.e. **macrostachyosides A and B**, and seventeen known compounds were isolated from the methanol extract of *Mallotus macrostachyus* leaves. Their structures were elucidated by NMR and MS data. Macrostachyosides A (1) and B (2) showed **significant cytotoxic activities** on KB (epidermoid carcinoma) and LU-1 (lung adenocarcinoma) human cancer cell lines with IC_{50} values ranging from 4.31 ± 0.09 to 7.12 ± 0.07 mg/mL.

Several known compounds, i.e. lupeol, 24R*)-24-methyldammara-25-ene-3-one, 6"-p-coumaroylprunin, 2-[5-hydroxy-2,3-bis-(4-hydroxy-3-methoxy-bezyl)-pentyloxy]-6-

hydroxymethyl-tetrahydro-pyran-3,4,5-triol, corchoionoside, (1R,2R)-methyl-5'-hydroxy-jasmonate, 3,5-dihydroxy-4-methoxy-benzoic acid, multifidol glucoside 1-[(2methyl-butyryl)phloroglucinyl]-β-D-glucopyranoside, 2"-O-acetylquercitrin, and (-)-isolariciresiol-4-O-β-D-glucopyranoside, were isolated from *Phyllanthus emblica*. Their structures were elucidated by their NMR and ESI-MS data.

Seven compounds, i.e. 5,8-dimethoxy-7-hydroxy-2-(4-methoxyphenyl)-4-oxo-4*H*chromene-6-sulfonic acid, dendranthemoside B, (1R,2R)-methyl b-D-glucopyrano-sylepituberonate, 2 heliobuphthalmin lactone, hypophyllanthin, astragalin, and nirtetralin, were isolated from *Phyllanthus urinaria*.

Further, eight compounds, i.e. (3*S*,5*R*,6*S*,9*x*)-megastigman-7-ene-3,6,9,10-tetrol, methyl brevifolin-carbonxylate, 3-*O*-methyl-4′-*O*-*a*-lrhamnopyranosylellagic acid, isolariciresinol, (+)-pinoresinol-β-D-glucoside, quercetine, gingerglycolipid A, and kaempferol 3-glucoside, were isolated from *Phyllanthus reticulatus*.

Several compounds were isolated from a methanol extract of *Mallotus japonicus* leaves by various chromatographic methods. By comparison of the spectroscopic data (one-dimensional nuclear magnetic resonance spectroscopy (1D-NMR):1H-NMR, 13C-NMR, and DEPT; 2D-NMR: HSQC and HMBC; and electrospray ionization mass spectrometry: ESI-MS) with reported values, their chemical structures were elucidated to be 5,7-dihydroxy-4'-methoxy-6-(3-methylbut-2-enyl)flavanone, 29-norlupane-3,20-dione, lupeol, 25,26,27-trisnor-24-hydroxycycloartan-3-one, and 25,26,27-trisnor-3-ketocycloartan-24-oic acid.

The third overall objective was to study the chemical composition and investigate biological activities of selected medicinal plants using chemical and molecular biological techniques, and was mainly performed by the Belgian Walloon partner. At the UCL, a review was written about the phytochemistry and pharmacological activities of *Mallotus* species from Vietnamese mountainous areas. 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 isolate and identify, compounds responsible for the antioxidant activity in the antioxidant active samples. For this purpose, samples were purified and fractions were tested for given activities. The same was done to evaluate the cytotoxic and antimicrobial activities of the samples, and to isolate cytotoxic compounds.

Flavonoids responsible for the antioxidant properties of *Mallotus* species from Vietnam were isolated by LC-ESI-MS. Identified compounds were kaempferol 3-*O*-α-L-rhamnoside, quercitrin, and astilbin.

Several components were isolated from *Mallotus barbatus*, i.e. quercitrin, 3-O-a-L-rhamnosyl kaempferol, N-methyl-2-pyridone-5-carboxamide, and friedelin. It was found that **N-methyl-2-pyridone-5-carboxamide** could used be a **chemotaxonomical indicator** of the genus *Mallotus*.

The hepatoprotective, antioxidant, and cytotoxic activities were examined for several Vietnamese *Phyllanthus* species. The hepatoprotective effect of 26 Phyllanthus leaves extracts from *P. amarus*, *P. emblica*, *P. reticulatus* and *P. urinaria* was tested on paracetamol injured precision cut rat liver slices. Antioxidant activity and cytotoxicity to CHO cells were also evaluated. Results were only homogenous within *P. emblica* extracts for which no influence of collection time or place was observed. *P. emblica* extracts showed moderate hepatoprotective activity, good antioxidant activity and no toxicity. More variations of activity were observed within extracts from the other species. The results confirmed *in vitro* the validity of some traditional use of *Phyllanthus* species. However fluctuation of activity with collection time and place within a same species was observed for 3 out of 4 species which emphasises the necessity of quality control and standardisation of extracts prior to their incorporation into herbal preparation.

The fourth overall objective was to produce materials (food supplements) and/or to identify lead molecules with a potential interest for pharmaceutical or food industries. This task was performed by the Vietnamese partner in cooperation with a Vietnamese company. At the moment, a formulation with *Mallotus apelta* is marketed in Vietnam.

The fifth overall objective was to apply analytical aspects for quality control of plant materials or products from plants. Chromatography fingerprints of crude extracts were developed for identification and quality control purposes, and for quantitative determination of specific active compounds. This task was mainly performed by the Belgian Flemisch partener. At the VUB, a methodology to develop HPLC fingerprints was defined. Afterwards, this methodology was used to develop HPLC-UV fingerprints of 39 *Mallotus* samples. From the fingerprints, peaks responsible for antioxidant and cytotoxic activity were determined using different linear multivariate calibration techniques. Interesting peaks for antioxidant activity were then further examined with HPLC-MS at the UCL. 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. pCEC-UV fingerprints of these 39 samples were also developed and evaluated as a complementary technique to HPLC. 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, in a first study, the samples were classified according to genera and species and in a second study, peaks responsible for antioxidant activity were determined using different linear multivariate calibration techniques. 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. The results obtained from the HPLC-UV and UFLC fingerprints of the 36 samples will be compared. However, this research is still ongoing.

However, also the Belgian Walloon partner and the Vietnamese partner have performed some research in this context. At the UCL, HPLC-HRMS fingerprints of several *Mallotus* and *Phyllanthus* extracts were developed in order to identify compounds responsible for some biological activities. This research, however, is still ongoing. In Vietnam, HPLC-DAD-MS fingerprints of *Mallotus phillipinensis* were developed. Also HPLC-DAD-ELSD fingerprints of *Mallotus macrostachyus, Mallotus japonicus, Phyllanthus urinaria* using some compounds as marker were developed following the earlier defined methodology by the VUB with another HPLC column and using some compounds as markers. This research, however, is also still ongoing.

Summarized, all five overall objectives were studied and lead to several publications in peer-reviewed journals and to several oral and/or poster presentations on international and national congresses.

RESULTS FICHE

PROJECT FICHE ....(bilat BEL-VT R&D cooperation-) Projectcode BL/03/V21
title of research project

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**
(Geographic) study area (country/region) : ...

BELSPO testsite (if applicable) **:**

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 were:
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 medicines with interesting biological activities, and WP2: Survey medicinal plants from the Euphorbiaceae family used in traditional medicine in Vietnam
The plants were selected by the Vietnamese partner, where extraction, purification and identification of given bioactive compounds from *Mallotus* and *Phyllanthus* genera were performed.
- 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. From these results, interesting compounds with 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 was performed by the 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. 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 in collaboration with the UCL. Besides also pCEC-UV fingerprints were developed and evaluated as a complementary technique to HPLC. HPLC-UV fingerprints were also used to discriminate between *Mallotus* and *Phyllanthus* species (two classes) and their sub-species (six classes).
At the UCL, HPLC-HRMS fingerprints of several *Mallotus* and *Phyllanthus* extracts were analyzed to identify compound(s) responsible for a given activity.
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 markers.

Scientific Results max 25 lines

- WP1: Find natural compounds or extracts from Euphorbiaceae plants used in Vietnam as herbal medicines with interesting biological activities, and WP2: Survey medicinal plants from the Euphorbiaceae family used in traditional medicine in Vietnam

In **Vietnam**, two sets of samples were collected in different Vietnamese regions, i.e. 39 *Mallotus* samples from different species, and 51 samples (23 *Mallotus* samples and 28 *Phyllanthus* samples) from different species. A methodology to produce *Mallotus* and *Phyllanthus* extracts was defined and modified based on the experience from the previous project and some Vietnamese national projects. The research of the Vietnamese partner focused on the extraction, purification and identification of given bioactive compounds from *Mallotus* and *Phyllanthus* genera. This research has resulted in several papers.

- 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. 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 isolate and identify, compounds responsible for the antioxidant activity in the antioxidant active samples. For this purpose, samples were purified and fractions were tested for given activities. The same was done to evaluate the cytotoxic and antimicrobial activities of the samples, and to isolate cytotoxic compounds. This research has resulted in several papers.

- WP4: Produce materials (food supplements) and/or to identify lead molecules with a potential interest for pharmaceutical or food industries

This WP was performed by the Vietnamese partner in cooperation with a Vietnamese company. At the moment, a formulation with *Mallotus apelta* is marketed in Vietnam.

- 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. Afterwards, this methodology was used to develop HPLC-UV fingerprints of 39 *Mallotus* samples. From the fingerprints, peaks responsible for antioxidant and cytotoxic activity were determined using different linear multivariate calibration techniques. Interesting peaks for antioxidant activity were then further examined with HPLC-MS at the UCL. 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. pCEC-UV fingerprints of these 39 samples were also developed and evaluated as a complementary technique to HPLC. 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, in a first study, the samples were classified according to genera and species and in a second study, peaks responsible for antioxidant activity were determined using different linear multivariate calibration techniques. 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. The results obtained from the HPLC-UV and UFLC fingerprints of the 36 samples will be compared. However, this research is still ongoing. This research has resulted in several papers.

At the **UCL**, HPLC-HRMS fingerprints of several *Mallotus* and *Phyllanthus* extracts were developed in order to identify compounds responsible for some biological activities. However, this research is still ongoing.

In **Vietnam**, HPLC-DAD-MS fingerprints of *Mallotus phillipinensis* were developed. Also HPLC-DAD-ELSD fingerprints of *Mallotus macrostachyus, Mallotus japonicus, Phyllanthus urinaria* using some compounds as marker were developed following the earlier defined methodology by the VUB with another HPLC column and using some compounds as markers. However, this research is still ongoing. This research has resulted in several papers.

Products and services (if applicable: maps, database, peer reviewed article(s),weblink...)

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) 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

4) 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

5) 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

6) 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

7) G. Alaerts, B. Dejaegher, J. Smeyers-Verbeke, Y. Vander Heyden, Recent developments in chromatographic fingerprints from herbal products: set-up and data analysis, Combinatorial Chemistry & High Throughput Screening 13 (2010) 900-922
8) 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
9) 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
10) 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
11) 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
12) C. Tistaert, B. Dejaegher, Y. Vander Heyden, Chromatographic separation techniques and data handling methods for herbal fingerprints: A review, Analytica Chimica Acta 690 (2011) 148–161
13) Phan Thi Thanh Huong et al, Chemical constituents of *Mallotus japonicus*, Vietnamese Journal of Chemistry 50 (4A) (2012) 183-186
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, Biochem. Syst. Ecol. 44 (2012) 212-215
15) 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
16) C. Tistaert, G. Chataigné, B. Dejaegher, C. Rivière, N. Nguyen Hoai, M. Chau Van, J. Quetin-Leclercq, Y. Vander Heyden, Multivariate data analysis to evaluate the fingerprint peaks responsible for the cytotoxic activity of Mallotus species, Journal of Chromatography B 910 (2012) 103-113
17) 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, Journal of Chromatography B 910 (2012) 114-121

Submitted for publication

In preparation
18) 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
19) 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.
20) Nguyen Hoai Nam et al, Chemical investigations and biological studies of genus *Phyllanthus unaria*, in preparation
21) Nguyen Hoai Nam et al, Chemical investigations and biological studies of genus *Phyllanthus reticulatus*, in preparation
22) Nguyen Hoai Nam et al, Chromatographic fingerprint analysis of *Mallotus philippensis* from various sources by HPLC-MS, in preparation
23) A. Gordien et al, Variations of hepatoprotective, antioxidant and cytotoxic activities of Vietnamese *Phyllanthus* species, in preparation
24) J. Viane, 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 for discrimination and classification purposes, in preparation

ORAL PRESENATIONS
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
12) "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 23th-27th 2012 - Leiden - The Netherlands
13) "Fused-core stationary phases for fingerprint development of Phyllanthus and Mallotus species", G. Parewyck, C. Tistaert, B. Dejaegher, D. Mangelings, Y. Vander Heyden, 16th Forum of Pharmaceutical Sciences – May 7th-8th 2012 – Blankenberge – Belgium
14) "Data handling of chromatographic herbal fingerprints", B. Dejaegher, M. Goodarzi, G. Alaerts, C. Tistaert, M. Dumarey, Y. Vander Heyden, 13th International Conference on Chemometrics in Analytical Chemistry (CAC-2012) - June 25th-29th 2012 - Budapest – Hungary
15) "Data handling of chromatographic herbal fingerprints", B. Dejaegher, S. Thiangthum, M. Goodarzi, G. Alaerts, C. Tistaert, and Y. Vander Heyden, 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 – Université Catholique de Louvain - September 25th 2012 – Brussels – Belgium
16) "Herbal Fingerprints: Extraction of Information, Focussing on Similarity Analyses", Y. Vander Heyden, G. Alaerts, M. Dumarey, M. Goodarzi, C. Tistaert, B. Dejaegher, 19th International Symposium on Electro- and Liquid Phase-separation Techniques (ITP2012) – September 30th – October 3th 2012 – Baltimore – Maryland – USA

POSTER PRESENATIONS
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
6) "Fused-core stationary phases for fingerprint development of Phyllanthus and Mallotus species", Greet Parewyck, Christophe Tistaert, Bieke Dejaegher, Debby Mangelings, Yvan Vander Heyden, The XXXVth Symposium 'Chromatographic Methods of Investigating the Organic Compounds' - May 30th – June 1 2012 - Katowice – Szczyrk – Poland
7) "Fused-core stationary phases for fingerprint development of Phyllanthus and Mallotus species", G. Parewyck, J. Viaene, C. Tistaert, B. Dejaegher, D. Mangelings, Y. Vander Heyden, 29th International Symposium on Chromatography (ISC 2012) – September 9th-13th 2012 – Torun – Poland

---------- 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 (promotor names, institutes, mail-adresses, web-site) :

Belgium: (coordinator and divers partners)
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Discipline (select one or more appropriate disciplines)

Medecine /Drugs (pharmacopy)

ANNEXES VAST-IMBC

***ANNEX IMBC1*- Nguyen Nghia Thin, Nguyen Thi Kim Thanh, Nguyen The Cuong, Nguyen Hoai Nam**
Collect Mallotus samples and identify sciencetific name of the plants

Introduction

The *Mallotus* and *Phyllanthus* genus, belonging to the Euphorbiaceae family, is widely distributed in Vietnam and the south of China. In Vietnam, roots, stem barks, leaves and fruits of *M.* species are for hundreds of years used in traditional medicine for the treatment of chronic hepatitis and enteritis. Besides, The *P.* species have more potential bioactivities than *M.* species. Among them there is *P. amarus,* used in the traditional medicine to treat hepatitis under the name "Diephachau". In the project we continue to focuse on *M.* species (*M. philipinensis, M. japonicus, M.macrostachyus*) and selected some P. species which used in Vietnamese folklore medicine (*P. amarus, P.reticulatus, P.emblica, P.urinaria).* These survey carried out on 16 different areas of Vietnam. Then extracts were made of the leaves of 51 *Mallotus* and *Phyllanthus* samples. The *M.* and *P.* samples and some pictures of them (see table 1, figure 1). To idenfify the sciencetific of the *Mallotus* and *Phyllanthus* samples, several botanic steps had to be carried out.

Results

In this report we briefly introduce some *Mallotus* and *Phyllanthus* species which related to 51 crude extracts of *Mallotus* and *Phyllanthus* species (Table 1).

Table 1. *Mallotus and Phyllanthus samples species, origin, collection time, plant part, and voucher number*

No	Code	Species	Collection time	Origin	Part of plant
1	VNB_01	*Mallotus apelta*	Agust, 2009	VanBan-LaoCai	leaves
2	VNB_02	*Phyllanthus emblica*	Agust, 2009	VanBan-LaoCai	leaves
3	VNB_03	*P. emblica*	November,2009	DongDang-Langson	leaves
4	VNB_04	*M. apelta*	November,2009	TamDao-Vinhphuc	leaves
5	VNB_05	*M. apelta*	November,2009	DongDang-Langson	leaves
6	VNB_06	*M. paniculatus*	December,2009	DongVan-Hagiang	leaves
7	VNB_07	*P. emblica*	December,2009	DongVan-Hagiang	leaves
8	VNB_08	*M. apelta*	December,2009	HamYen-TuyenQuang	leaves
9	VNB_09	*P. reticulatus*	February,2010	NghiaTrai-HungYen	leaves
10	VNB_10	*P. urinaria L*	February,2010	NghiaTrai-HungYen	leaves
11	VNB_11	*P. amarus*	February,2010	NghiaTrai-HungYen	leaves
12	VNB_12	*P. amarus*	March,2010	VanDien-Hanoi	leaves

13	VNB_13	*M. paniculatus*	March,2010	HuongHoa-Quangtri	leaves
14	VNB_14	*P. reticulatus*	March,2010	VanDien-Hanoi	leaves
15	VNB_15	*P. emblica*	March,2010	HuongHoa-Quangtri	leaves
16	VNB_16	*P. urinaria L*	March,2010	VanDien-Hanoi	leaves
17	VNB_17	*M. paniculatus*	April, 2010	MeLinh-Vinhphuc	leaves
18	VNB_18	*P. reticulatus*	April, 2010	MeLinh-Vinhphuc	leaves
19	VNB_19	*P. amarus*	April, 2010	MeLinh-Vinhphuc	leaves
20	VNB_20	*P. reticulatus*	April, 2010	LanOng-Hanoi	leaves
21	VNB_21	*P. emblica*	April, 2010	MeLinh-Vinhphuc	leaves
22	VNB_22	*P. amarus*	April, 2010	LanOng-Hanoi	leaves
23	VNB_23	*P. urinaria L*	April, 2010	MeLinh-Vinhphuc	leaves
24	VNB_24	*P. urinaria L*	April, 2010	LanOng-Hanoi	leaves
25	VNB_25	*P. reticulatus*	May, 2010	NinhHiep-Hanoi	leaves
26	VNB_26	*P. reticulatus*	May, 2010	DongAnh-Hanoi	leaves
27	VNB_27	*P. amarus*	May, 2010	QueVo-Bacninh	leaves
28	VNB_28	*P. emblica*	May, 2010	DongAnh-Hanoi	leaves
29	VNB_29	*P. amarus*	May, 2010	DongAnh-Hanoi	leaves
30	VNB_30	*P. urinaria L*	May, 2010	QueVo-Bacninh	leaves
31	VNB_31	*P. urinaria L*	May, 2010	NinhHiep-Hanoi	leaves
32	VNB_32	*P. amarus*	May, 2010	NinhHiep-Hanoi	leaves
33	VNB_33	*P. urinaria L*	May, 2010	DongAnh-Hanoi	leaves
34	VNB_34	*M. apelta*	June,2010	PaCo-HoaBinh	leaves
35	VNB_35	*M. paniculatus*	June, 2010	VQG-Pumat	leaves
36	VNB_36	*M. paniculatus*	July,2010	Cucphuong-NinhBinh	leaves
37	VNB_37	*M. barbatus*	May,2010	Dackrong-Quangtri	leaves
38	VNB_38	*M. barbatus*	May,2010	KyAnh-Hatinh	leaves
39	VNB_39	*M. barbatus*	December,2009	HamYen-TuyenQuang	leaves
40	VNB_40	*P. acidus*	March,2010	PhanRang-NinhThuan	leaves
41	VNB_41	*M. sp*	Ferbuary, 2011	KyAnh_HaTinh	leaves
42	VNB42	*M.macrostachyus*	Agust, 2010	VanBan-LaoCai	leaves
43	VNB_43	*M.macrostachyus*	November,2009	DongDang-Langson	leaves
44	VNB_44	*M. repandus*	June,2010	PaCo-HoaBinh	leaves
45	VNB_45	*M. repandus*	April, 2010	MeLinh-Vinhphuc	leaves
46	VNB_46	*M. resinosus*	March,2010	TuyenHoa_QuangBinh	leaves
47	VNB_47	*M. sp*	Ferbuary, 2011	DeoNgang_HaTinh	leaves
48	VNB_48	*M. sp*	Ferbuary, 2011	DeoNgang_HaTinh	leaves
49	VNB_49	*P. acidus*	March,2010	KhanhHoa	leaves
50	VNB_50	*M. japonicus*	May,2010	Sapa_Laocai	leaves
51	VNB_51	*M. microcapus*	March,2010	TuyenHoa_QuangBinh	leaves

Figure 1. *Mallotus, Phyllanthus samples species, scientific name and pictures*

Mallotus macrostachyus

Mallotus japonicus

Phyllanthus emblica

Phyllanthus reticulatus

Phyllanthus urinaria

Mallotus barbatus

51 crude extracts of *Mallotus* and *Phyllanthus* species (Table 1) were prepare from those species which we brefly introduce here:

1. *Phyllanthus amarus*

Vietnamese Name: Diep ha chau, Cho de than xanh

Sciencetific Name: *Phyllanthus amarus*

Phyllanthus amarus

Phyllanthus amarus is widely used as a medicinal plant. An infusion is considered a good tonic, diuretic and antipyretic. A decoction of the aerial parts or only of the leaves is taken to treat gonorrhoea, diarrhoea, dysentery, stomach-ache, pain in the sides, haemorrhoids and absence of menstruation or female sterility. A suppository of the leaf paste is applied to the vagina to treat absence of menstruation and polyps. Leaf sap, mixed with palm oil or not, is applied as ear drops to treat otitis and applied to abscesses, sores and wounds..

Phyllanthus amarus aqueous extracts show potent anticarcinogenic activity against development of different tumour types. Administration of the extract after tumour development increased survival of rats and mice up to 1 year. An alcoholic extract was found to significantly reduce cytochrome P450 enzymes both in vitro as well as in vivo when orally administered to mice. A hexane extract, the lignans-rich fraction and the lignans nirtetralin, niranthin and phyllanthin exerted cytotoxic effects in 2 human leukaemia cell lines, as well as multidrug resistance reversing properties, mainly due to their ability to synergize with the action of conventional chemotherapeutics. An ethanolic extract showed significant preventive effect against benign prostatic hyperplasia in rats

2. *Phyllanthus reticulatus*

Vietnamese Name: Phen den

Sciencetific Name: *Phyllanthus reticulatus*

Phyllanthus reticulatus

Phyllanthus reticulatus is a many branched shrub, sometimes partially scrambling, usually 1-5 m high, or a small twiggy tree that grows up to 8 m in height. The bark is light reddish-brown or grey-brown with hairy stems when young, which become smooth with age.

The leaves alternate along slender branches. They are up to 25 cm long and appear as leaflets of large pinnate leaves. The leaves are thinly textured, usually hairless. They have a noticeable reddish net-veining which is more visible above than below

4. *Phyllanthus urinaria*

Vietnamese Name: Phen den
Sciencetific Name: *Phyllanthus urinaria*

Phyllanthus urinaria

The plant, reaching around 2 feet, has small alternate leaves resembling those of the mimosa tree, disposed in two ranges. The leaves are large at the tip and smaller towards the petiole. When touched, the leaves fold in automatically. Flowers are greenish white, minute and appear at axiles of the leaves, as well as the seed capsules. Numerous small green-red fruits, round and smooth, are found along the underside of the stems, which are erect and red.

5.*Mallotus barbatus*

Vietnamese Name: Bung buc
Sciencetific Name: *Mallotus barbatus*
Other names:

Bup bong gai, Bong bet, Bung buc gai, Ba bet long, Ruoi cau, Cam lon, Nhung dien rau.

Mallotus barbatus

Distribution

Vietnam

All over the mountainous areas from the North to the South of Vietnam at the altitude less than 1,100m.

The World

China, Laos, Cambodia, Thailand, Malaysia, Philippines, Indonesia, Myanmar and India.

Morphology and ecology

Shrubs or small trees, 6-10(15)m high, leaves and braches stellate puberulent, yellow. Leaves nearly peltate or ovate, entire or 3 lobed; apex oblong-acute; base rounded or slightly notched, main veins 7-9 spreaded from the apex of petiole; margin small serrate, scattered.

Inflorescence spike, up to 20cm long. Male flowers have many stamens (over 50 stamens). Female flowers ovary densely hairy.

Capsules, nearly globose, 1.3-1.5cm in diameter, bark spiny, densely hairy; yellow. Seed black.

Light-demanding plant, grows in ever-green forests, secondary forests, shrub-plots or forest-sides, on non-limestone soils.

Morphology and ecology

Shrubs or small trees, 2-10 m high, up to 15-20cm in diameter. Leaves alternate. Blades broadly-ovate, veins palmate; leaves thick, yellow hairy on both sides. Male flowers without staminodes. Female flower ovary 3 celled. Fruit big, about 1cm in diameter, yellow, glabrous.

Light-demanding plant, usually grows in evergreen forest-sides or shrub-plots, on soils weathered from limestone, at the altitude of 100-500m.

6. *Mallotus macrostachyus*

Vietnamese Name: **Ba bet chum to**
Sciencetific Name: *Mallotus macrostachyus*
Other names:

Bum bup bong to, Buc chum to, Ruoi duoi to, Ruoi trang, Nhung dien duoi to, Nhung dien trang.

Synonyms:

Rottlera macrostachya Miq., 1860; *Mallotus albus* Muell.-Arg., 1866; *Mallotus tetracoccus* (Roxb.) Kurz, 1873

Mallotus macrostachyus

Distribution

Vietnam

Lao Cai (Sa Pa), Lang Son, Hoa Binh (Lac Tho), Ninh Binh (Cuc Phuong), Nghe An (Co Ba), Ha Tinh, Quang Binh, Quang Tri (Lang Vieng Ap), Thua Thien - Hue (Lang Co), Dong Nai provinces.

The World

China, Thailand, Malaysia, Philippines, Indonesia, Singapore, India.

Morphology and ecology

Trees, 10-15m high, braches blonde hairy, thick. Leaves obtuse-oval, obtuse-ovate or nearly cuneate, 15-20cm long, 10-17cm wide; apex obtuse or slightly acute, base nearly rounded; margins entire; near base with 2 glands; underside yellowish hairy, thick; petioles pubescent.

Inflorescence umbellate. Male flower with 4 stamens. Female inflorescence less branched than the male. The female ovary truncated conical.

Capsules, about 1cm in diameter; bark sparsely pubescent and spiny.

The plant has large ecologic amplitude, light-demanding, grows in evergreen forests, on soils weathered from limestone, at the altitude of 100-500m.

ANNEX IMBC2- **Cuong Nguyen Xuan, Nam Nguyen Hoai, Huong Le Mai, Van Nguyen Thi Hong, Do Thi Thao**

A modified methodology to procedure Mallotus and Phyllanthus extracts and primary screening of some biological activities of the Mallotus and Phyllanthus extracts

Abstract

51 *Mallotus* and *Phyllanthus* samples, were collected in 16 different Vietnamese regions. For some species, samples were collected in different provinces of Vietnam and/or at different collection times. The samples were authenticated by Professor Nguyen Nghia Thin (Hanoi National University, Vietnam) and Dr Nguyen The Cuong (Institute of Ecology and Biological resources);

A methodology to produce *Mallotus* and *Phyllanthus* extract was modified based on the previous methodology in IMBC, other plant extracts were defined based on the experience from some Vietnamese national projects.

The primary screening of some biological activities of the Mallotus and Phyllanthus are carried out, the results show that some samples exhibited potential biological activities.

ANNEX IMBC3A*– Nguyen Thi Mai, Nguyen Xuan Cuong, Nguyen Phuong Thao, Nguyen Hoai Nam, Nguyen Huu Khoi, Chau Van Minh, Yvan Vander Heyden, Ngo Thi Thuan,Nguyen Van Tuyen, Joëlle Quetin-Leclerc and Phan Van Kiem, A New Lignan Dimer from *Mallotus philippensis
Natural Product Communications, Vol. 5(3), pp. 423-426 (2010).

Abstract

A new lignan dimer, bilariciresinol (**1**), was isolated from the leaves of *Mallotus philippensis*, along with platanoside (**2**), isovitexin (**3**), dihydromyricetin (**4**), bergenin (**5**), 4-*O*-galloylbergenin (**6**), and pachysandiol A (**7**). Their structures were elucidated by spectroscopic experiments including 1D and 2D NMR and FTICR-MS.

***ANNEX IMBC3B*– 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 QL, Do Thi Thao, Chau Van Minh, Chemical constituents of Mallotus macrostachyus growing in Vietnam and cytotoxic activity of some cycloartane derivatives**
Phytochemistry Letters, Vol.4(3), pp. 348-352 (2011).

Abstract

Two new cycloartane derivatives, macrostachyosides A (1) and B (2), and seventeen known compounds were isolated from the methanol extract of Mallotus macrostachyus leaves. Their structures were elucidated by NMR and MS data. Macrostachyosides A (1) and B (2) showed significant cytotoxic activities on KB (epidermoid carcinoma) and LU-1 (lung adenocarcinoma) human cancer cell lines with IC_{50} values ranging from 4.31 ± 0.09 to 7.12 ± 0.07 mg/mL.

ANNEX IMBC3C– **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

Abstract

11 known compounds as Lupeol (**1**), 24R*)-24-Methyldammara-25-ene-3-one (**2**), 6"-p-Coumaroylprunin (**3**), 2-[5-Hydroxy-2,3-bis-(4-hydroxy-3-methoxy-bezyl)-pentyloxy]-6-hydroxymethyl-tetrahydro-pyran-3,4,5-triol (**4**), Corchoionoside (**6**), (1R,2R)-methyl-5'-hydroxyjasmonate (**7**), 3,5-Dihydroxy-4-methoxy-benzoic acid (**8**), Multifidol glucoside 1-[(2methylbutyryl)phloroglucinyl]-β-D-glucopyranoside (**9**), 2"-O-Acetylquercitrin (**10**), (-)-isolariciresiol-4-O-β-D-glucopyranoside (**11**), respectively. Their structures were elucidated by their NMR and ESI-MS data and comparison of them with reported values

ANNEX IMBC3D– **Nguyen Hoai Nam, Phan Van Kiem, Chau Van Minh*, Ninh Khac Ban, Nguyen Phuong Thao, Nguyen Xuan Cuong, Nguyen Van Thanh, Bieke Dejaegher, Yvan Vander Heyden, Joëlle Quetin-Leclercq**
Chemical investigations and biological studies of genus Phyllanthus urinaria, in preparation

Abstract

Seven compounds as 5,8-Dimethoxy-7-hydroxy-2-(4-methoxyphenyl)-4-oxo-4*H*-chromene-6-sulfonic acid (1) as new compound, Dendranthemoside B (2), (1R,2R)-Methyl β-D-glucopyranosylepituberonate (3), 2 Heliobuphthalmin lactone (4), hypophyllanthin (5), Astragalin (6), nirtetralin (7), respectively. By detailed analyses of their NMR and ESI-MS data and comparison of them with reported values. Their structures were elucidated by their NMR and ESI-MS data and comparison of them with reported values

***ANNEX IMBC3E* – Nguyen Hoai Nam, Phan Van Kiem, Chau Van Minh*, Vu Anh Tu, Nguyen Phuong Thao, Nguyen Xuan Cuong, Nguyen Van Thanh, Bieke Dejaegher, Yvan Vander Heyden, Joëlle Quetin-Leclercq, Chemical investigations and biological studies of genus *Phyllanthus reticulatus*, in preparation**

Abstract

From this study 8 compounds as (3*S*,5*R*,6*S*,9*x*)-megastigman-7-ene-3,6,9,10-tetrol (1) as new compound, Methyl brevifolincarbonxylate (2), 3-*O*-methyl-4′-*O*-*a*-L-rhamnopyranosylellagic acid (3), Isolariciresinol (4), (+)-Pinoresinol-β-D-glucoside (5), Quercetine (6), Gingerglycolipid A (7), kaempferol 3-glucoside (8), respectively. By detailed analyses of their NMR and ESI-MS data and comparison of them with reported values. Their structures were elucidated by their NMR and ESI-MS data and comparison of them with reported values

***ANNEX IMBC3F*– Phan Thi Thanh Huong, Chau Ngoc Diep, Le Duc Dat, Vu Anh Tu, Ninh Thi Ngoc,Nguyen Phuong Thao, Nguyen Hoai Nam, Nguyen Xuan Cuong, Nguyen Thi KimThanh, Nguyen Nghia Thin, Phan Van Kiem and Chau Van Minh, Chemical constitutents of Mallotus japonicus, Vietnam Journal of Chemistry, Vol.50 (4A), 183-186.**

Abstract

Six compounds were isolated from a methanol extract of *Mallotus japonicus* leaves by various chromatographic methods. Comparison of the spectroscopic data (one dimensional nuclear magnetic resonance spectroscopy (1D-NMR):1H-NMR, 13C-NMR, and DEPT; 2D-NMR: HSQC and HMBC; and electrospray ionization mass spectrometry: ESIMS) with reported values, their chemical structures were elucidated to be 5,7-dihydroxy-4'-methoxy-6-(3-methylbut-2-enyl)flavanone (**1**), 29-norlupane-3,20-dione (**3**), lupeol (**4**), 25,26,27-trisnor-24-hydroxycycloartan-3-one(**5**), and 25,26,27-trisnor-3-ketocycloartan-24-oic acid (**6**). This is the first isolation of compounds **1**, **3**, **5**, and **6** from *M. japonicus* leaves.

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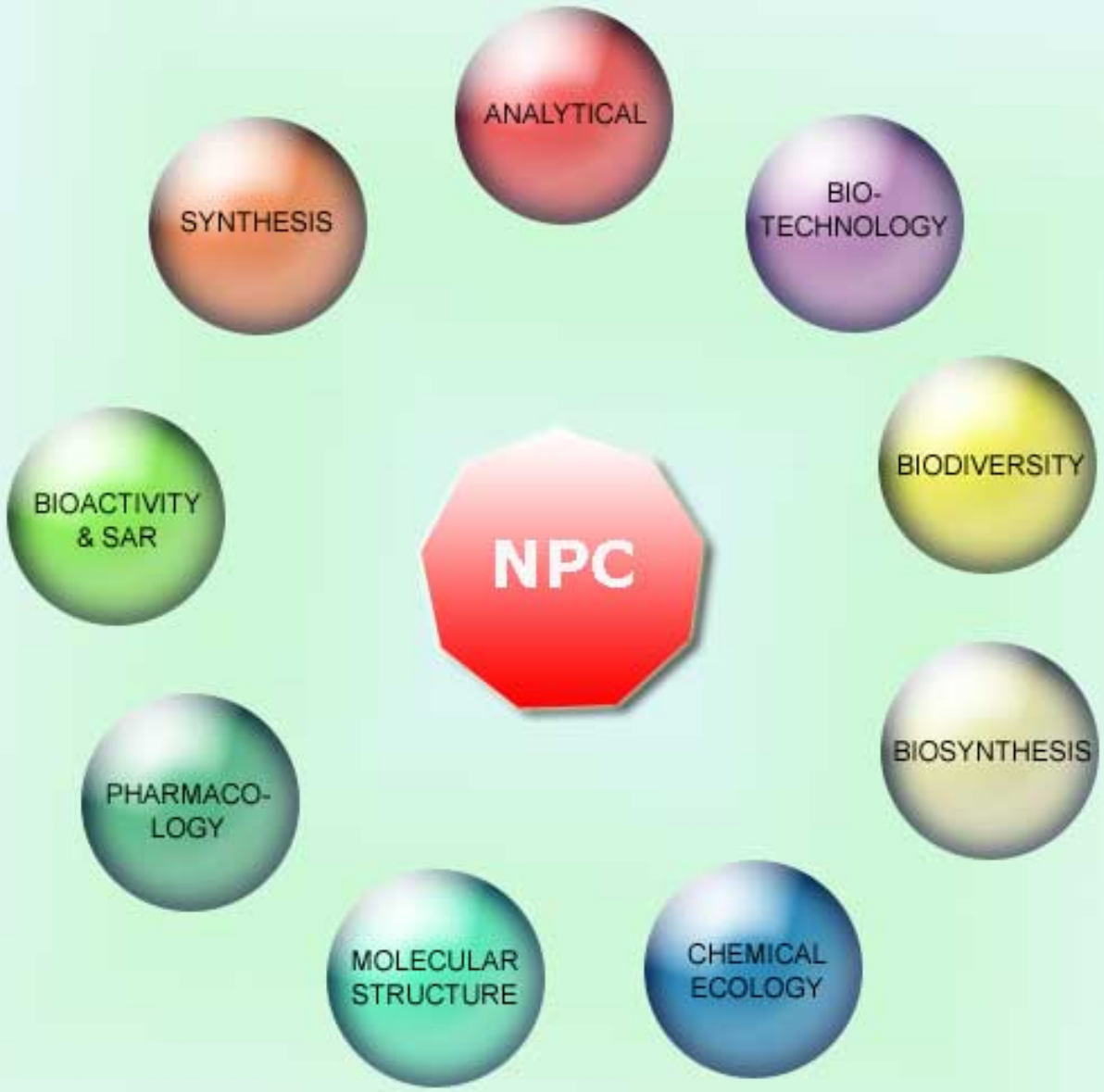

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A New Lignan Dimer from *Mallotus philippensis*

Nguyen Thi Mai[a,b], Nguyen Xuan Cuong[a], Nguyen Phuong Thao[a], Nguyen Hoai Nam[a], Nguyen Huu Khoi[a], Chau Van Minh[a], Yvan Vander Heyden[c], Ngo Thi Thuan[d], Nguyen Van Tuyen[e], Joëlle Quetin-Leclercq[f] and Phan Van Kiem[a,*]

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A new lignan dimer, bilariciresinol (**1**), was isolated from the leaves of *Mallotus philippensis*, along with platanoside (**2**), isovitexin (**3**), dihydromyricetin (**4**), bergenin (**5**), 4-*O*-galloylbergenin (**6**), and pachysandiol A (**7**). Their structures were elucidated by spectroscopic experiments including 1D and 2D NMR and FTICR-MS.

Keywords: *Mallotus philippensis*, Euphorbiaceae, lignan, bilariciresinol.

The *Mallotus* species are a rich source of biologically active compounds such as phloroglucinols, tannins, terpenoids, coumarins, benzopyrans, and chalcones [1]. In the course of our systematic phytochemical investigations of *Mallotus* species, we reported several flavonoids, triterpenes, benzopyrans, flavonolignans, and megastigmane derivatives possessing significant NF-κB inhibition, cytotoxic effects against several human cancer cell lines, and antiradical activity [2].

In line with this, we studied the chemical constituents of *Mallotus philippensis* (Lamk.) Muell.-Arg. (Euphorbiaceae, common name: kamala tree, Vietnamese name: Canh kien), which is abundant throughout Vietnam. The leaves and stem bark of this plant are traditionally used to treat acne and other cutaneous diseases. The fruit glands are used as medicine against syphilis, dropsy, and gastric diseases. Decoctions of the roots are employed to treat acute dysentery, swollen fauces and throat, epilepsy, and diarrhea. The seeds are also used in Thai folk medicine against dizziness and nausea [3]. In the present paper, we report the isolation and structural elucidation of a new lignan dimer, bilariciresinol (**1**), along with six

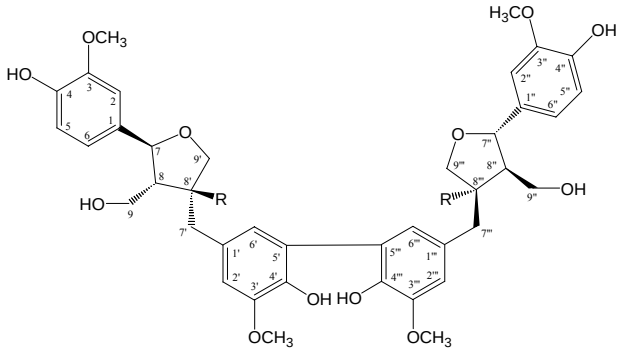

Figure 1: Structures of **1** (R = H) and **1a** (R = OH).

Known compounds (**2-7**) from the leaves of *M. philippensis*. Compound **1** was obtained as a white amorphous powder. The ^{1}H NMR spectrum showed signals of three ABX-type aromatic protons [δ_H 6.92 (1H, d, *J*= 2.0 Hz), 6.78 (1H, d, *J* = 8.0 Hz), and 6.79 (1H, dd, *J* = 8.0, 2.0 Hz)] and two *m*-coupled [δ 6.83 and 6.75 (each 1H, d, *J* = 2.0 Hz)], indicating one 1,3,4-trisubstituted and one 1,3,4,5-tetrasubstituted aromatic ring. Two methoxyl groups were identified by proton signals at δ 3.83 and 3.88 (each 3H, s). In addition, the presence of an oxymethine (δ 4.76, 1H, d, *J* = 6.5 Hz),

Table 1: The NMR spectral data of **1**[#].

N^0	δ_C [a, b]	DEPT	δ_H [a, c] mult. (*J* in Hz)
1 (1″)	135.77	C	-
2 (2″)	110.75	CH	6.92 d (2.0)
3 (3″)	148.96	C	-
4 (4″)	147.00	C	-
5 (5″)	116.00	CH	6.78 d (8.0)
6 (6″)	119.86	CH	6.79 dd (8.0, 2.0)
7 (7″)	84.02	CH	4.76 d (6.5)
8 (8″)	53.99	CH	2.40 m
9 (9″)	60.50	CH_2	3.82 dd (11.0, 7.0) 3.65 dd (11.0, 7.0)
1′ (1‴)	133.27	C	-
2′ (2‴)	112.28	CH	6.83 d (2.0)
3′ (3‴)	149.47	C	-
4′ (4‴)	142.79	C	-
5′ (5‴)	127.06	C	-
6′ (6‴)	124.68	CH	6.75 d (2.0)
7′ (7‴)	33.78	CH_2	2.53 dd (13.0, 12.0) 2.97 dd (13.0, 5.0)
8′ (8‴)	43.80	CH	2.77 m
9′ (9‴)	73.57	CH_2	4.03 dd (8.0, 7.0) 3.78 dd (8.0, 7.0)
3 (3″)-OCH_3	56.42	CH_3	3.83 s
3′ (3‴)-OCH_3	56.66	CH_3	3.88 s

[a]recorded in CD_3OD, [b]at 125 MHz, [c]at 500 Hz, [#]all the data were assigned by HSQC and HMBC experiments.

Two oxymethylene (δ 3.82/3.65, each 1H, dd, *J* = 11.0, 7.0 Hz and 4.03/3.78, each 1H, dd, *J* = 8.0, 7.0 Hz), and a methylene (δ 2.53, 1H, dd, *J* = 13.0, 12.0 Hz and 2.97, 1H, dd, *J* = 13.0, 5.0 Hz) suggested a 4,4',9-trihydroxy-3,3'-dimethoxy-7,9'-epoxylignan [4].

The ^{13}C NMR spectrum of **1** exhibited 20 carbon signals, in which the two methoxyl groups were indicated by signals at δ 56.42 and 56.66. Twelve carbon signals in range of δ 110.75 - 149.47 confirmed the two aromatic rings. In addition, one oxymethine and two oxymethylene groups were determined by signals at δ 84.02 (CH), 73.57 (CH_2), and 60.50 (CH_2), respectively. All carbons were assigned to relevant protons by an HSQC experiment and the results are summarized in Table 1. The NMR data of **1** were similar to those of (+)-lariciresinol [4]. The differences of the spectral data between the two compounds were only observed in ring B. The easily visible changes were the presence of a quaternary carbon C-5 (δ 127.06) in **1** instead of a methine (δ 116.5) in (+)-lariciresinol [4]. A strongly downfield-shifted (+10.56 ppm) C-5 (and C-5″) suggested that two lariciresinol units were linked in a magnetically symmetric mode between C-5 and C-5″ [5], which was confirmed by FTICR-MS peak at *m/z* 741.28712 $[M + Na]^+$ (calcd. for $C_{40}H_{46}O_{12}Na$, 741.28870) corresponding to a molecular formula of $C_{40}H_{46}O_{12}$ (M = 718).

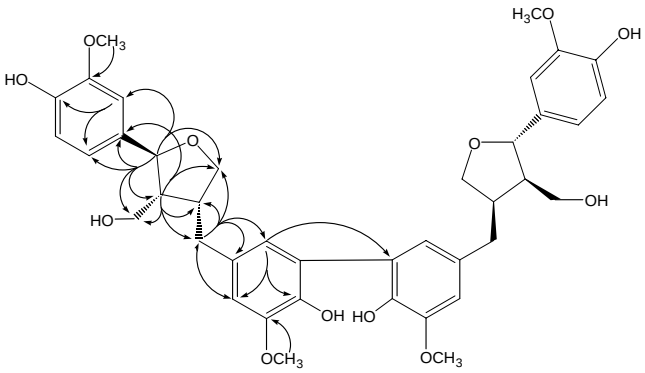

Figure 2: Key HMBC correlations of **1**.

The NMR data of **1** were first assigned by comparison with those of (+)-lariciresinol [4] and **1a** [5] and further confirmed by an HMBC experiment (Figure 2). The relative configuration of **1** was determined by the good agreement of its ^{13}C NMR data, as well as its ^{1}H NMR multiplicities and coupling constants with those of (+)-lariciresinol [4]. Thus, **1** was elucidated to be a new compound, bilariciresinol (Figure 1).

The known compounds **2**-**7** were characterized as platanoside [6], isovitexin [7], dihydromyricetin [8], bergenin [9], 4-*O*-galloylbergenin [9], and pachysandiol A [10], respectively, by detailed analyses of their NMR and ESI-MS data and comparison of them with reported values. Platanoside was isolated for the first time from a *Mallotus* species. This is the first report of these compounds from *M. philippensis*.

Experimental

General: Optical rotation was determined on a JASCO DIP-1000 KUY polarimeter. All NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C), and chemical shifts (δ) are reported in ppm using tetramethylsilane (TMS) as an internal standard. The ESI-MS was obtained on an AGILENT 1200 SERIES LC-MSD Trap spectrometer. The high resolution mass spectra were obtained using a Variant 910 FT-ICR mass spectrometer. Column chromatography (CC) was performed on silica gel 230 - 400 mesh (0.040 – 0.063 mm, Merck) or YMC RP-18 resins (30 - 50 μm, Fujisilisa Chemical Ltd.). Thin layer chromatography (TLC) was performed on DC-Alufolien 60 F_{254} (Merck 1.05715) or RP_{18} F_{254s} (Merck) plates. Compounds were visualized by spraying with 10% H_2SO_4 and heating for 5 minutes.

Plant materials: The leaves of *M. philippensis* were collected in Trang Dinh, Lang Son Province, Vietnam during February, 2009 and identified by Dr. Ninh Khac Ban, Insititute of Ecology and Biological Resources,

Vietnam Academy of Science and Technology. A voucher specimen (No TD34) was deposited at the Herbarium of the Institute of Natural Products Chemistry.

***Extraction and isolation*:** The air dried leaves of *M. philippensis* (5 kg) were exhaustively extracted (three times, each 60 min) with hot MeOH (40-50 ^{o}C) under ultrasonic conditions to obtain 180 g of MeOH residue. This was suspended in water and partitioned in turn with *n*-hexane, $CHCl_3$ and ethyl acetate giving 45, 35, and 25 g of the corresponding extracts. The $CHCl_3$ extract (35 g) was submitted to a silica gel CC using step wise elution of $CHCl_3$-MeOH (from 50/1 to 1/1, v/v) to give seven fractions, C1-C7. Fraction C3 (5 g) was further separated on a silica gel CC using $CHCl_3$-acetone 20/1 (v/v) to obtain compound **7** (23.5 mg). The new compound **1** (14 mg) was purified from fraction C5 (3.7 g) by using a silica gel CC with $CHCl_3$-MeOH-H_2O 8/1/0.1 (v/v/v) as eluent. The ethyl acetate extract (25 g) was separated into nine fractions, E1-E9, by a silica gel CC using step wise elution of $CHCl_3$-MeOH (from 10/1 to 1/1, v/v). Compound **4** (20 mg) was isolated from fraction E3 (2.1 g) after subjecting it to a silica gel CC eluting with $CHCl_3$-MeOH 8/1. Further separation of fraction E5 (5 g) by a silica gel CC using $CHCl_3$-acetone-H_2O 1/1/0.05 (v/v/v) as eluent, followed by a silica gel CC with $CHCl_3$-MeOH-H_2O 5/1/0.1 (v/v/v) to obtain compounds **5** (6.0 mg) and **6** (19.0 mg). Fraction E6 (3.7 g) afforded compounds **2** (12 mg) and **3** (9 mg) after using a silica gel CC eluting with $CHCl_3$-acetone-H_2O 1/2/0.1 (v/v/v), followed by an YMC RP-18 CC eluting with MeOH-H_2O 1.5/1 (v/v).

Bilariciresinol (**1**)

$[\alpha]_D$: +26 (*c* 0.50, MeOH).
Rf : 0.45 ($CHCl_3$-MeOH-H_2O, 3.5:1:0.1).
^{1}H (500 MHz, CD_3OD): Table 1.
^{13}C NMR (125 MHz, CD_3OD): Table 1.
ESIMS: *m/z* 741 $[M + Na]^+$ (positive).
FTICR-MS: *m/z* 741.28712 $[M + Na]^+$; calcd for $C_{40}H_{46}O_{12}Na$: 741.28870.
14 mg (2.8×10^{-4} % of dried weight).

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Chemical constituents of *Mallotus macrostachyus* growing in Vietnam and cytotoxic activity of some cycloartane derivatives

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ABSTRACT

Two new cycloartane derivatives, macrostachyosides A (**1**) and B (**2**), and seventeen known compounds were isolated from the methanol extract of *Mallotus macrostachyus* leaves. Their structures were elucidated by NMR and MS data. Macrostachyosides A (**1**) and B (**2**) showed significant cytotoxic activities on KB (epidermoid carcinoma) and LU-1 (lung adenocarcinoma) human cancer cell lines with IC_{50} values ranging from 4.31 ± 0.09 to 7.12 ± 0.07 μg/mL.

1. Introduction

Mallotus is a large genus of the spurge family Euphorbiaceae. To date, about 150 *Mallotus* species were recorded worldwide, and most of them are found in East and Southeast Asia and from Indomalaysia to New Caledonia and Fiji, northern and eastern Australia. There are about 40 *Mallotus* species in Vietnam, of which six species and one variety are endemic. *Mallotus macrostachyus* (Miq.) Muell.-Arg is a tree of about 10–15 m high. The plant has a wide ecological adaptation, is light-demanding, and grows in evergreen forests with altitudes from 100 to 1500 m. In Vietnam, this species is abundantly found in the provinces Lao Cai, Lang Son, Hoa Binh, Ninh Binh, Nghe An, Ha Tinh, Quang Binh, Quang Tri, Thua Thien-Hue, and Dong Nai (Céline et al., 2010; Chi, 1999; Thin, 2007). Until now, no investigations on the chemical constituents and biological activity of this plant have been reported. As a part of our systematic investigations on the *Mallotus* species growing in Vietnam, we reported herein the isolation, structure elucidation, and evaluation of cytotoxic activity of two new cycloartane derivatives, macrostachyosides A (**1**) and B (**2**), and seventeen known compounds from the methanolic extract of *M. macrostachyus* leaves (Fig. 1).

2. Results and discussion

Phytochemical study on the methanol extract of *M. macrostachyus* leaves led to the isolation of two new cycloartane derivatives, macrostachyosides A (**1**) and B (**2**), and seventeen known compounds. The known compounds were elucidated as 25,26,27-trisnor-24-hydroxycycloartan-3-one (**3**) (Cabrera et al., 1996), 25,26,27-trisnor-3-ketocycloartan-24-oic acid (**4**) (Anjaneyulu et al., 1985), ergosterol peroxide (**5**) (Kim et al., 2005), friedelin (**6**) (Hisham et al., 1995), epifriedelanol (**7**) (Kundu et al., 2000), taraxerol (**8**) (Chien et al., 2004; Sakurai et al., 1987), epitaraxerol (**9**) (Rahman et al., 1997), corchoionoside C (**10**) (Yamano and Ito, 2005; Yoshikawa et al., 1997), icariside B_5 (**11**) (Matsunami et al., 2010; Miyase et al., 1988), macarangioside F (**12**) (Matsunami et al., 2009) (+)-pinoresinol di-*O*-β-D-glucopyranoside (**13**) (Deyama, 1983), syringaresinol di-*O*-β-D-glucopyranoside (**14**) (Vermes et al., 1991), kaempferol 3-*O*-α-L-rhamnopyranosyl (1 → 2)-β-D-glucopyranoside (**15**) (Kazuma et al., 2003), quercetin 3-*O*-α-L-rhamnopyranosyl (1 → 2)-β-D-glucopyranoside (**16**) (Kazuma et al., 2003), benzyl-*O*-β-D-glucopyranoside (**17**) (Rosa et al., 1996), benzyl-*O*-[β-D-xylopyranosyl

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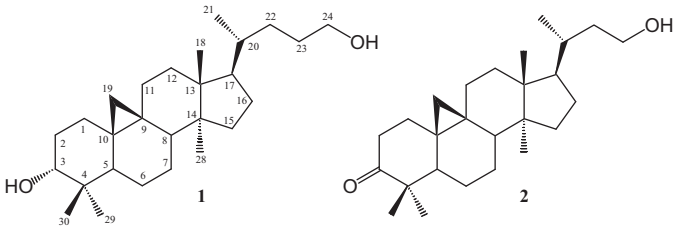

Fig. 1. Structures of **1** and **2**.

(1 → 6)-β-D-glucopyranoside] (**18**) (Matsumura et al., 1997), and N^1-methyl-2-pyridone-5-carboxamide (**19**) (Wong et al., 2002), by detailed analyses of their NMR and ESIMS data in comparison with literature values.

Compound **1** was isolated as an amorphous white powder. Its molecular formula was identified as $C_{27}H_{46}O_2$ by a pseudo-molecular ion peak at *m/z* 403.35938 $[M+H]^+$ (calcd. for $C_{27}H_{47}O_2$, 403.35760) in the Fourier transform ion cyclotron resonance mass spectrum (FTICRMS). Moreover, a fragment ion was observed at *m/z* 385.34897 $[M-H_2O+H]^+$ (calcd. for $C_{27}H_{45}O_1$, 385.34704) indicating the presence of one hydroxyl group in **1**. The ^{1}H NMR spectrum showed typical signals of four tertiary methyl groups (each 3H, s) at δ_H 0.88 (H-30), 0.90 (H-28), 0.95 (H-29), and 0.97 (H-18) and one secondary methyl group at δ_H 0.89 (3H, d, *J* = 7.0 Hz, H-21). The signals at δ_H 3.47 (1H, t, *J* = 2.5 Hz, H-3) and 3.62 (2H, m, H-24) suggested an oxymethine and an oxymethylene group, respectively. Moreover, the proton signals at δ_H 0.35 and 0.52 (each 1H, d, *J* = 4.0 Hz, H-19) are characteristic for non-equivalent protons of a cyclopropyl methylene group (Inada et al., 1997). The ^{13}C NMR spectrum of **1** revealed 27 carbon signals including 5 methyl, 12 methylene, 5 methine, and 5 quaternary carbons, detected by DEPT experiments. The presence of one oxymethine and one oxymethylene group were confirmed by carbon signals at δ_C 77.0 (CH, C-3) and 63.6 (CH_2, C-24). Five methyl groups were found at δ_C 18.0 (C-18), 18.3 (C-21), 19.3 (C-28), 25.8 (C-29), and 21.2 (C-30). All carbons were assigned to relevant protons by a heteronuclear single quantum coherence (HSQC) experiment and the results were summarized in Table 1. The ^{1}H and ^{13}C NMR data of **1** were identical to those of 25,26,27-trisnor-24-hydroxycycloartan-3-one (**3**) (Cabrera et al., 1996), except for the presence of an oxymethine group [δ_C 77.0 (CH, C-3)/δ_H 3.47 (1H, t, *J* = 2.5 Hz, H-3)] in **1** instead of a ketone group [δ_C 216.5 (C, C-3)] in **3** (Cabrera et al., 1996). Detailed analyses of the ^{1}H–^{1}H COSY correlations led to the assignment of connectivities C-1/C-2/C-3, C-5/C-6/C-7/C-8, C-11/C-12, and C-15/C-16/C-17/C-20/C-22/C-23/C-24. This evidence and the heteronuclear multiple bond coherence (HMBC) correlations between H_3-29 (δ_H 0.95)/H_3-30 (δ_H 0.88) and C-3 (δ_C 77.0)/C-4 (δ_C 39.6)/C-5 (δ_C 41.1) confirmed the placement of the oxymethine group at C-3 (Fig. 2). The α-orientation of the hydroxyl group at C-3 was assigned by the agreement of ^{13}C NMR data (in $CDCl_3$) for C-1, C-2, C-3, C-4, and C-5 of **1** at δ_C 27.5, 28.6, 77.0, 39.6, and 41.1, respectively, with those (in $CDCl_3$) of (25*R*)-cycloartane-3α,24,25-triol at δ_C 27.5 (C-1), 28.8 (C-2), 77.1 (C-3), 39.6 (C-4), and 41.1 (C-5) (Inada et al., 1997); but quite different from those (in $CDCl_3$) of (25*R*)-cycloartane-3β,24,25-triol at δ_C 32.0 (C-1), 30.4 (C-2), 78.9 (C-3), 40.5 (C-4), and 47.2 (C-5) (Inada et al., 1997). In addition, the proton signal of H-3 at δ_H 3.47 (1H, t, *J* = 2.5 Hz) is typical for H_β-3 of cycloartane triterpenoids (versus that at δ_H 3.28, 1H, dd, *J* = 10.1 and 4.4 Hz for H_α-3) (Inada et al., 1997). Thus, the structure of **1** was elucidated as 25,26,27-trisnor-3α,24-dihydroxycycloartane, named macrostachyoside A.

The molecular formula of **2** was identified as $C_{26}H_{42}O_2$ by a pseudo-molecular ion peak at *m/z* 387.32615 $[M+H]^+$ (calcd. for $C_{26}H_{43}O_2$, 387.32630) in FTICRMS. The ^{1}H and ^{13}C NMR data of **2** were close to those of 25,26,27-trisnor-24-hydroxycycloartan-3-one (**3**) (Cabrera et al., 1996), except for signals of the side-chain. The side-chain signals at δ_C 33.1 (CH, C-20), 18.5 (CH_3, C-21), 39.3 (CH_2, C-22), and 61.0 (CH_2, C-23) together with the presence of only 26 carbon signals in the ^{13}C NMR spectrum of **2** indicated the loss of one methylene group in the side-chain of **2** comparing to that of **3**. Moreover, the side-chain structure of **2** was confirmed by

Table 1
The NMR data ($CDCl_3$, 500 MHz) of **1** and **2**.

C	1		2	
	δ_C	δ_H mult. (*J* = Hz)	δ_C	δ_H mult. (*J* = Hz)
1	27.5	1.02 m, 1.87 m	33.4	1.52 m, 1.84 m
2	28.6	1.65 m, 1.93 m	37.5	2.30 ddd (2.5, 4.0, 14.0) 2.70 dt (6.5, 14.0)
3	77.0	3.47 t (2.5)	216.5	–
4	39.6	–	50.2	–
5	41.1	1.83 m	48.4	1.70 m
6	21.1	0.78 m, 1.49 m	21.5	0.95 m, 1.65 m
7	28.1	1.30 m, 1.90 m	25.9	1.14 m, 1.38 m
8	48.0	1.44 m	47.9	1.59 m
9	19.8	–	21.1	–
10	26.5	–	26.0	–
11	25.7	1.14 m, 1.31 m	26.7	1.17 m, 2.05 m
12	32.9	1.62 m	32.8	1.67 m
13	45.3	–	45.4	–
14	48.9	–	48.8	–
15	35.5	1.30 m	35.6	1.30 m
16	26.3	1.14 m, 2.00 m	28.3	1.31 m, 1.93 m
17	52.2	1.60 m	52.6	1.61 m
18	18.0	0.97 s	18.1	1.01 s
19	29.8	0.35 d (4.0), 0.52 d (4.0)	29.5	0.58 d (4.0), 0.79 d (4.0)
20	35.9	1.42 m	33.1	1.67 m
21	18.3	0.89 d (7.0)	18.5	0.93 d (7.0)
22	32.1	1.08 m, 1.38 m	39.3	1.25 m, 1.77 m
23	29.6	1.47 m, 1.66 m	61.0	3.65 m, 3.73 m
24	63.6	3.62 m		
28	19.3	0.90 s	19.3	0.92 s
29	25.8	0.95 s	22.2	1.05 s
30	21.2	0.88 s	20.8	1.10 s

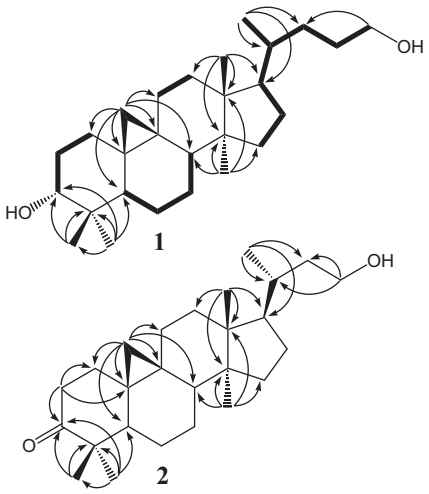

Fig. 2. Key HMBC (→) and ^{1}H–^{1}H COSY (–) correlations of **1** and **2**.

HMBC cross peaks (Fig. 2) between H_3-21 (δ_H 0.93) and C-17 (δ_C 52.6)/C-20 (δ_C 33.1)/C-22 (δ_C 39.3) and between H_2-23 (δ_H 3.65 and 3.73) and C-20 (δ_C 33.1)/C-22 (δ_C 39.3). Consequently, the structure 24,25,26,27-tetranor-23-hydroxycycloartan-3-one was assigned for **2**, named macrostachyoside B.

Since some cycloartane derivatives were reported to exhibit cytotoxic effects (Flores-Rosete and Martínez-Vázquez, 2008; Sashidhara et al., 2010), all isolated cycloartanes were evaluated for their cytotoxic activity against KB (epidermoid carcinoma) and LU-1 (lung adenocarcinoma) human cancer cells according to the method developed by Monks et al. (1991). From the initial screening step, all isolated cycloartanes exhibited more than 60% of inhibition on the growth of the KB cells at the screening concentration of 20 μg/mL. Thus, they were selected for further studies to determine the 50% inhibitory concentration (IC_{50}) against KB and LU-1 cells. Macrostachyosides A (**1**) and B (**2**) showed significant cytotoxic activities against the both KB and LU-1 cell lines with IC_{50} values ranging from 4.31 ± 0.09 to 7.12 ± 0.07 μg/mL (Table 2). Compound **3** exhibited moderate and weak cytotoxicities on LU-1 cells (IC_{50} = 9.45 ± 0.23 μg/mL) and KB cells (IC_{50} = 13.98 ± 0.22 μg/mL), respectively. However, compound **4** was weakly active on KB cells (IC_{50} = 15.86 ± 0.31 μg/mL) and inactive on LU-1 cells ($IC_{50} > 20$ μg/mL). The above results suggested that macrostachyosides A (**1**) and B (**2**), newly isolated from *M. macrostachyus* leaves may have some interest for further studies with regard to anticancer activity.

3. Experimental

3.1. General experimental procedures

Optical rotations were determined on a JASCO P-2000 polarimeter (Hachioji, Tokyo, Japan). The Fourier transform infrared (FTIR) spectra were obtained on a Bruker TENSOR 37 FTIR spectrometer (Bruker Optics, Ettlingen, Germany). Electrospray ionization mass spectra (ESIMS) were performed on an AGILENT 1200 Series LC-MSD Trap spectrometer (Agilent Technologies, Palo Alto, CA). The Fourier transform ion cyclotron resonance (FTICR) mass spectra were obtained using a Varian 910 FTICR mass spectrometer (Varian, CA, USA). The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker AM500 FTNMR spectrometer (Bruker, Billerica, MA, USA) and tetramethylsilane (TMS) was used as an internal standard. Column chromatography (CC) was performed using a silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck, Darmstadt, Germany) or YMC RP-18 resins (30–50 μm, Fujisilisa Chemical Ltd., Kasugai, Aichi, Japan). Thin layer chromatography (TLC) used pre-coated silica gel 60 F_{254} (1.05554.0001, Merck, Darmstadt, Germany) and RP-18 F_{254S} plates (1.15685.0001, Merck, Darmstadt, Germany) and compounds were visualized by spraying with aqueous 10% H_2SO_4 and heating for 5 min.

Table 2
Effects of **1**–**4** on the growth of human cancer cells.

Compounds	IC_{50} (μg/mL)[a]	
	KB (Epiderma)	LU-1 (Lung)
1	7.12 ± 0.07	6.40 ± 0.11
2	5.15 ± 0.12	4.31 ± 0.09
3	13.98 ± 0.22	9.45 ± 0.23
4	15.86 ± 0.31	>20
Ellipticine[b]	2.21 ± 0.15	1.08 ± 0.17

[a] Data are presented as the mean ± SD of experiments performed in triplicate.
[b] Ellipticine, an anticancer agent, was used as positive control.

3.2. Plant material

The leaves of *M. macrostachyus* (Miq.) Muell.-Arg. were collected in Van Ban, Lao Cai, Vietnam during August 2010 and identified by Prof. Nguyen Nghia Thin (College of Natural Science, Vietnam National University, Hanoi, Vietnam) and MSc Nguyen The Cuong (Institute of Ecology and Biological Resources, VAST, Hanoi, Vietnam). A voucher specimen (No. VNB-042) was deposited at the Herbarium of the College of Natural Science and Institute of Marine Biochemistry, VAST, Vietnam.

3.3. Extraction and isolation

The dried leaves of *M. macrostachyus* (2.5 kg) were powdered and extracted with methanol (MeOH, 3 L × 5 L) at 50 °C under ultrasonic condition. The resulting solutions were filtered, combined, and concentrated under low pressure to give 21.6 g residue. This extract was suspended in distilled water (2 L) and partitioned in turn with chloroform and ethyl acetate to obtain chloroform (MMc, 12.7 g), ethyl acetate (MMe, 6.1 g), and water (MMw, 2.2 g) partitions. MMc partition was crudely separated into three fractions, MMc1-MMc3, by silica gel CC (7 cm × 50 cm) using stepwise elution with *n*-hexane–ethyl acetate (50:1, 20:1, 10:1, 5:1, 2:1, and 1:1, v/v). Fraction MMc1 (7.3 g) was further separated on silica gel CC (4.5 cm × 50 cm) eluting with *n*-hexane–acetone (15:1, v/v) to obtain compounds **6** (40 mg), **7** (21 mg), **8** (17 mg), and **9** (8 mg). Compounds **1** (10 mg) and **3** (6 mg) were purified from fraction MMc2 (1.8 g) by silica gel CC (2.5 cm × 80 cm) using *n*-hexane–ethyl acetate (8:1, v/v) as eluent. Fraction MMc3 (2.6 g) afforded compounds **2** (13 mg), **4** (11 mg), and **5** (7 mg) after silica gel CC (3.0 cm × 60 cm) eluting with chloroform–*n*-hexane–MeOH (1:3:0.1, v/v/v). The MMe and MMw partitions were combined and separated into four fractions, MMw1–MMw4, by silica gel CC (4.5 cm × 50 cm) using stepwise elution with chloroform–MeOH (20:1, 10:1, 5:1, and 1:1, v/v). Purification of fraction MMw1 (0.9 g) with YMC RP-18 CC (2.0 cm × 80 cm) and acetone–water (1:5, v/v) as mobile phase furnished compounds **17** (17 mg) and **18** (13 mg). Fraction MMw2 (1.3 g) was further separated by silica gel CC (2.5 cm × 80 cm) eluting with chloroform–MeOH (7:1, v/v) to give compounds **11** (15 mg) and **12** (10 mg). Compounds **10** (7 mg), **15** (20 mg), and **16** (28 mg) were purified from fraction MMw3 (5.2 g) by silica gel CC (4.5 cm × 50 cm) using chloroform–MeOH (3:1, v/v) as eluent. Finally, fraction MMw4 (0.8 g) afforded compounds **13** (14 mg), **14** (21 mg), and **19**

(200 mg) after silica gel CC (2.0 cm × 80 cm) eluting with chloroform–MeOH (2.3:1, v/v).

3.3.1. Macrostachyoside A (1)

Amorphous white powder; $[\alpha]_D^{22} + 2.5$ (c 0.1, $CHCl_3$); ^1H and ^{13}C NMR are given in Table 1; FTICRMS m/z 403.35938 $[M+H]^+$ (calcd. for $C_{27}H_{47}O_2$, 403.35760).

3.3.2. Macrostachyoside B (2)

Amorphous white powder; $[\alpha]_D^{22} + 6.5$ (c 0.2, $CHCl_3$); ^1H and ^{13}C NMR are given in Table 1; FTICRMS m/z 387.32615 $[M+H]^+$ (calcd. for $C_{26}H_{43}O_2$ 387.32630).

3.4. Cell culture

The monolayer cancer cell lines KB (human epidermoid carcinoma, ATCC number: CCL-17) and LU-1 (human lung adenocarcinoma, ATCC number: HTB-57) were employed for the assays. Stock cultures were grown in T-75 flasks containing 50 mL of Dulbeco's modified Eagle medium (DMEM) with 2 mM L-glutamine, 1.5 g/L sodium bicarbonate and 10% fetal bovine serum (FBS). Media were changed at 48-h intervals. The cells were dissociated with 0.05% Trypsin–EDTA, subcultured every 3–5 days with the ratio of (1:3) and incubated at 37 °C under humidified 5% carbon dioxide.

3.5. Cytotoxicity assays

The cytotoxic potential was assessed by determining the amount of sulforhodamine B (SRB) bound to proteins and was performed in a standard microtiter plate. Test samples were examined over a concentration range of 0.03–20 μg/mL. DMSO (0.5% final) alone served as the negative control. Experimental cultures were plated in microtiter plates (Costar, USA), containing 10 μL of each test sample and 190 μL of growth medium (10% FBS) per well at density of 6000 cells/well. The duration assay was adopted as 3 days. One plate with no samples added served as a 0-day control. Test plates were incubated in a humidified atmosphere of 5% CO_2, 37 °C for 72 h, while the 0-day control was incubated for 1 h. After incubation, cells were fixed for 30 min to the plastic substratum by the addition of 100 μL of cold 20% aqueous trichloroacetic acid (TCA) for at least 1 h at 4 °C. Fixed cells were then stained with 0.4% SRB (w/v) dissolved in 1% acetic acid and washed four times with 1% acetic acid. The bound dye was then solubilized by the addition of 10 mmol unbuffered Tris base (Sigma), absorption was measured at 515 nm with a microplate reader (BioRad). All the experiments were performed three times with the mean absorbance values calculated.

Growth, expressed as a percentage of the negative control, was calculated with the equation (AB: absorbance):

$$\%\ \text{growth} = \frac{\text{AB (test substance)} - \text{AB (0 - day control)}}{\text{AB (negative control)} - \text{AB (0 - day control)}} \times 100.$$

The IC_{50} (50% inhibitory concentration) was determined by plotting concentrations against % growth using nonlinear regression analysis from TableCurve software.

4. Concluding remark

In conclusion, four cycloartane derivatives, including two new compounds macrostachyosides A (**1**) and B (**2**), were isolated and elucidated from the methanol extract of the *M. macrostachyus* leaves. Compounds **1** and **2** showed significant *in vitro* cytotoxicity on two human cancer cell lines. This is the first report of cycloartane derivatives from *Mallotus* species and these compounds can be used for chemical taxonomy of *M. macrostachyus* species.

Acknowledgements

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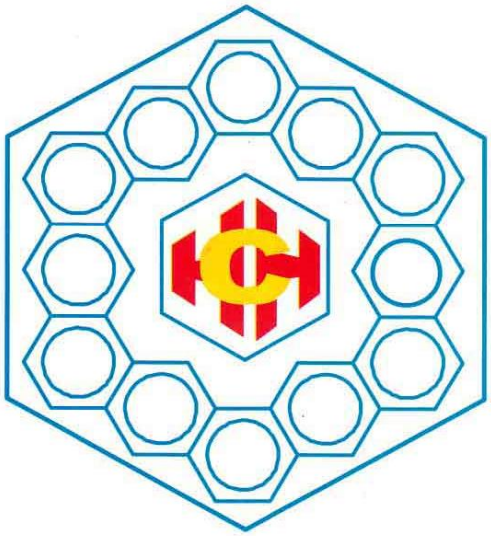

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CHEMICAL CONSTITUENTS OF *MALLOTUS JAPONICUS*

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Abstract

Six compounds were isolated from a methanol extract of *Mallotus japonicus* leaves by various chromatographic methods. Comparison of the spectroscopic data (one dimensional nuclear magnetic resonance spectroscopy (1D-NMR): ^{1}H-NMR, ^{13}C-NMR, and DEPT; 2D-NMR: HSQC and HMBC; and electrospray ionization mass spectrometry: ESI-MS) with reported values, their chemical structures were elucidated to be 5,7-dihydroxy-4'-methoxy-6-(3-methylbut-2-enyl)flavanone (**1**), 29-norlupane-3,20-dione (**3**), lupeol (**4**), 25,26,27-trisnor-24-hydroxycycloartan-3-one(**5**), and 25,26,27-trisnor-3-ketocycloartan-24-oic acid (**6**). This is the first isolation of compounds **1**, **3**, **5**, and **6** from *M. japonicus* leaves.

Keyword: *Mallotus japonicus*.

1. INTRODUCTION

Mallotus is a genus of the spurge family Euphorbiaceae. Spread throughout South-East and North Asia, the genus comprises over 140 species, of which many *Mallotus* species have been used in traditional medicine to treat various diseases. For example, *Mallotus apelta* has been used to treat chronic hepatitis, hepatalgia, enteritis, diarrhea, and lymphopathy; *Mallotus repandus* has been used to treat influenza and fever; *Mallotus barbatus* has been used in both Vietnamese and Chinese folk medicine for treating antipyretic, diuretic, anticholeraic, relieving pain, and cholera; *Mallotus macrostachyus* has been used to treat wounds and pimple; *Mallotus paniculatus* has been used to treat traumatic injuries and swelling; *Mallotus japonicus* has been used in Chinese folk medicine for treating stomach disorders and gastric ulcers while the leaves have been used to reduce swelling [1, 2].

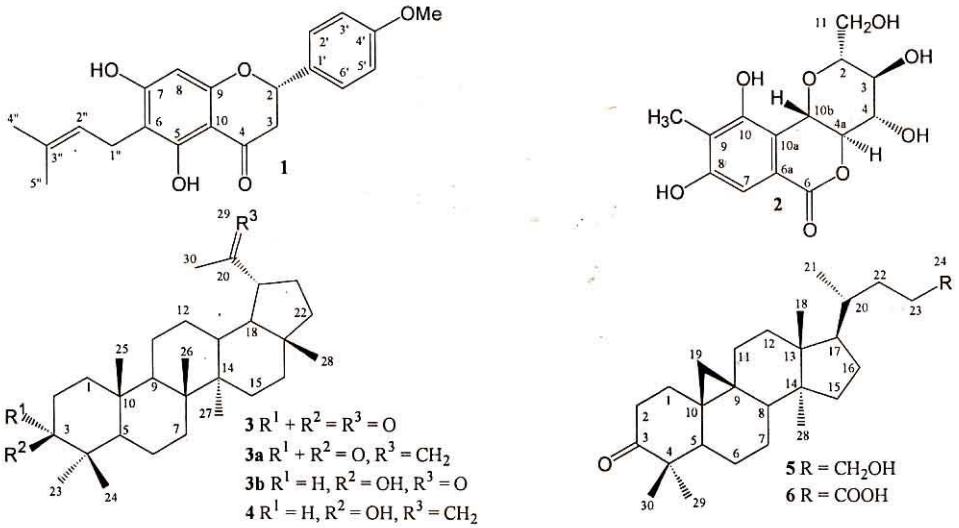

Figure 1: Structures of compounds **1–6**

The roots, stem barks, leaves, and fruits provide researchers with a broad basis in their search for new pharmaceutical active components. Over the years, several studies on *Mallotus* species have been

published and a number of pharmacologically active components were isolated and determined. The reported activities include anti-inflammatory, antioxidant, hepatoprotective, cytotoxic, and antimicrobial effects [3,4]. As a part of our systematic investigations on the *Mallotus* species growing in Vietnam, we reported herein the isolation and structure elucidation of six known compounds from the methanol extract of *Mallotus japonicus* leaves (Fig. 1).

2. MATERIAL AND METHODS

2.1. Plant materials

The samples of *M. japonicus* were collected in Sapa, Lao Cai, Vietnam during May 2010 and identified by Prof. Nguyen Nghia Thin and MSc Nguyen Thi Kim Thanh (College of Natural Science, Vietnam National University, Hanoi, Vietnam). A voucher specimen (No VNB-050) was deposited at the Herbarium of the College of Natural Science and Institute of Marine Biochemistry, Vietnam.

2.2. General experimental procedures

Electrospray ionization mass spectra (ESI-MS) were performed on an AGILENT 1200 Series LC-MSD Trap spectrometer. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer and tetramethylsilane (TMS) was used as an internal standard. Column chromatography (CC) was performed using a silica gel or YMC RP-18 resins. Thin layer chromatography (TLC) used pre-coated silica gel 60 F_{254} and RP-18 F_{254S} plates and compounds were visualized by spraying with aqueous 10% H_2SO_4 and heating for 3-5 minutes.

2.3. Isolation

The dried leaves of *M. japonicus* (3.5 kg) were powdered and extracted with methanol (MeOH, 3 × 5 L) at 50°C with ultrasonic condition. The MeOH extract were filtered, combined, and concentrated under low pressure to give 50.3 g residue. This was suspended in distilled water (2 L) and partitioned in turn with chloroform and ethyl acetate to obtain chloroform (MJc, 22.5 g), ethyl acetate (MJe, 9.3 g), and water (MJw) extracts. The MJc extract was separated into five fractions, MJc1-MJc5, by silica gel CC (7 × 50 cm) using stepwise elution with *n*-hexane/ethyl acetate (50:1-1:1, v/v). Fraction MJc2 (2.8 g) was further separated on silica gel CC (4.5 × 50 cm) eluting with *n*-hexane/ethyl acetate (8:1, v/v) to obtain **3** (8 mg), **4** (250 mg), **5** (7 mg), and **6** (11 mg). Fraction MJc4 (3.5 g) was further separated by silica gel CC (2.5 × 80 cm) eluting with chloroform/*n*-hexane/MeOH (1:3:0,1, v/v) to give **1** (10 mg) and **2** (17 mg).

5,7-Dihydroxy-4'-methoxy-6-(3-methylbut-2-enyl)flavanone (**1**): a light yellow amorphous solid, ESI-MS *m/z* 355 $[M+H]^+$, molecular formula $C_{21}H_{22}O_5$, M = 354; ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6) see table 1.

29-Norlupane-3,20-dione (3): a white powder; ESI-MS *m/z* 483 $[M+H]^+$, molecular formula $C_{29}H_{46}O_2$, M= 426; ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see table 1.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a light yellow amorphous solid. Positive-ion ESI-MS analysis gave a pseudo-molecular ion at *m/z* 355, which tentatively suggested a formula of $C_{21}H_{23}O_5$ ($[M+H]^+$). The ^{1}H-NMR spectrum of **1** showed the presence of one chelated hydroxyl group (δ_H 12.39, s, 5-OH) due to a hydrogen bond between the proton at 5-OH and a carbonyl group at C-4 is formed, one methylene α to the carbonyl (δ_H 3.21, dd, J = 12.5, 17.0 Hz, H_{ax}-3 and δ_H 2.70, dd, J = 4.0, 17.0 Hz, H_{eq}-3), and one oxymethine (δ_H 5.45, dd, J = 4.0, 12.5 Hz, H-2). These data suggested that **1** possesses a flavanone skeleton. The basic flavanone skeleton was also approved to be present in **1** as specified in the ^{1}H-NMR spectrum by a downfield singlet (H-8, δ_H 5.67 s) and a pair of downfield doublets characteristic of *para*-substitution (δ_H 6.95, d, J = 8.5 Hz, H-3′, 5′ and δ_H 7.41, d, J = 8.5 Hz, H-2′, 6′), a 3H singlet (δ_H 3.75, s, 4′-OMe). The ^{1}H NMR spectrum of **1** exhibited the typical signal of protons at [δ_H 3.10 (2H, br d, J = 7.0 Hz, H-1"), 5.11 (1H, dt, J = 1.0, 7.0 Hz, H-2"), 1.60 (3H, s, H-4") and 1.68 (3H, s, H-5")] for the prenyl group. The ^{13}C NMR spectrum of **1** showed signals of 21 carbons including fifteen signals of flavanone skeleton, five signals of prenyl and one methoxy signal. All protons were assigned from an HSQC experiment (table 1). The ^{1}H and ^{13}C NMR data of **1** were identical to those of 5,7-dihydroxy-4'-methoxy-8-(3-methylbut-2-enyl)flavanone [3], except for the different from C-6 and C-8 positions (see table 1). The NMR data of **1** also were matching with 4'-*O*-methylbonannione A [4], exclusive of the different from the prenyl group and the genanyl group (see table 1). The HMBC spectrum of **1** revealed correlations of the olefinic prenyl proton H-2" (δ_H 5,11, dt) with C-6 (δ_C 107.59), while H-4" (δ_H 1,60, s) and H-5" (δ_H 1,68, s) correlated with C-2" (δ_C 122.61) and C-3" (δ_C 130.22). The chelated hydroxyl group (δ_H 12.39 s, 5-OH) correlated with three quaternary carbons (C-5, C-6, and C-10 at δ_C 160.54, 107.59, and

Table 1: NMR spectral data (500 MHz) of **1** and **3** with the literature values

	1[a]					**3**[b]			
C	[c]δ_C	[d]δ_C	δ_C	δ_H mult. (J = Hz)	C	[e]δ_C	[f]δ_C	δ_C	δ_H mult. (J = Hz)
2	78.7	79.0	78.05	5.45 dd (4.0, 12.5)	1	39.6		39.55	1.39 m/1.88 m
3	43.0	43.4	42.05	3.21 dd (12.5, 17.0) 2.70 dd (4.0, 17.0)	2	33.6		34.09	1.43 m/2.46 m
4	196.5	196.3	196.25	-	3	217.7		218.02	-
5	162.1	161.3	160.54	-	4	47.3		47.28	-
6	96.7	106.9	107.59	-	5	54.9		54.84	1.35 m
7	163.7	164.2	164.22	-	6	19.7		19.60	1.47 m
8	106.4	95.8	94.37	5.67 s	7	34.1		33.48	1.43 m/2.43 m
9	159.8	161.3	160.38	-	8	40.8	40.9	40.71	-
10	103.1	103.1	101.59	-	9	49.4	50.0	49.63	1.40 m
1'	130.7	130.7	130.73	-	10	36.8	37.3	36.89	-
2', 6'	127.5	127.9	128.13	7.41 d (8.5)	11	21.5	20.8	21.47	1.30 m/1.44 m
3',5'	114.1	114.4	113.86	6.95 d (8.5)	12	26.8	27.1	27.21	1.13 m/1.68 m
4'	159.8	160.2	159.40	-	13		37.5	37.18	1.61 m
1"	21.7	29.8	20.60	3.10 br d (7.0)	14		42.7	42.77	-
2"	121.6	121.4	122.61	5.11 dt (1.0, 7.0)	15		27.4	27.33	1.05 m/1.47 m
3"	134.6	139.8	130.22	-	16		35.5	34.93	1.46 m/1.53 m
4"	25.6	16.5	25.43	1.60 s	17		43.1	43.05	-
5"	17.8	39.9	17.59	1.68 s	18		49.0	49.52	1.84 t (11.5)
5-OH			-	12.39 s	19		52.8	52.57	2.59 m
OMe	55.3	55.5	55.15	3.75 s	20		213.0	212.67	-
					21		27.6	27.65	1.48 m/2.04 m
					22		39.9	39.84	1.35 m/1.49 m
					23	21.0		21.03	1.02 s
					24	26.7		26.75	1.07 s
					25	15.9		15.96	0.92 s
					26	15.8	15.9	15.71	1.06 s
					27	14.4	14.6	14.42	0.98 s
					28	18.1	18.0	18.01	0.78 s
					29		29.0	29.18	2.15 s

[a]measured in DMSO-d_6, [b]measured in $CDCl_3$, [c]δ_C of 5,7-dihydroxy-4'-methoxy-8-(3-methylbut-2-enyl)flavanone [3], [d]δ_C of 4'-*O*-methylbonannione A [4], [e]δ_C of lupane-3-one (**3a**) [5], [f]δ_C of olibanumol (**3b**) [6].

101.59). The A ring proton H-8 (δ_H 5.67, s) showed correlations with four of the six aromatic A ring carbons (C-7, C-9, C-6 and C-10 at δ_C 164.22, 160.38, 107.59, and 101.59). The correlations confirmed the proposed structure of **1** as 5,7-dihydroxy-4'-methoxy-6-(3-methylbut-2-enyl)-flavanone. The configuration at C-2 of **1** was proposed to be 2*S* by comparison with the literature values [3, 4].

Compound **3** was isolated as a white powder. Its molecular formula $C_{29}H_{46}O_2$ was determined from the positive-ion ESI-MS with a pseudo-molecular ion at *m/z* 427 $[M+H]^+$. The ^1H- and ^{13}C-NMR (Table 1) spectra of **3** showed the signals assignable to seven methyls [δ_C 0.78, 0.92, 0.98, 1,02, 1,06, 1.07, 2.15 (3H each, s, H-28, 25, 27, 23, 26, 24, 29)], ten methylenes, five methines, and five quaternary carbons, two carbonyl carbon [δ_C 212.67 (C-20) and 218.02 (C-3)]. All carbons were assigned to relevant protons by an HSQC experiment (Table 1). The HMBC spectrum of **3** revealed correlations between proton H-19 (δ_H 2.59) and carbon C-20, while H_3-29 (δ_H 2.15) correlated with C-19 (δ_C 52.57) and C-20; H_3-23 (δ_H 1.02) correlated with C-3 and C5; H_3-24 (δ_H 1.07) correlated with C-3 (δ 218.02)/C-4 (δ 47.28)/C-5 (δ 54.84). Thus, the

signals of carbonyl carbons C-3 and C-20 in **3** were clarified, so that the 29-norlupane-type triterpene structure was elucidated. The ^{1}H- and ^{13}C-NMR spectra of **3** were similar to those of olibanumol [6], except for some signals around the C-3 position. While the signals around the C-3 position and the signal of C-3 position of **3** were similar to those of lupane-3-one (**3a**) [5]. The proposed structure of **3** was confirmed to be 29-norlupane-3,20-dione by comparison with the literature values [5,6].

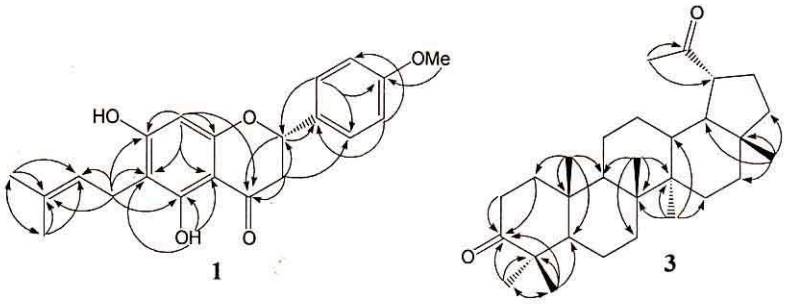

Figure 2: Key HMBC correlations of **1** and **3**

The other known compounds were elucidated as bergenin (**2**) [7], lupeol (**4**) [8], 25,26,27-trisnor-24-hydroxycycloartan-3-one (**5**) [9], and 25,26,27-trisnor-3-ketocycloartan-24-oic acid (**6**) [10] by detailed analyses of their NMR and ESI-MS data in comparison with literature values. The compounds **1**, **3**, **5**, and **6** are reported for the first time from *M. japonicus*.

Acknowledgements: *This work was financially supported by Vietnam-Belgium bilateral cooperation project.*

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VIỆN KHOA HỌC VÀ CÔNG NGHỆ VIỆT NAM
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Abstract

The genus *Mallotus* belongs to Malphighiales order and Euphorbiaceae family. Mallotus, commonly known as ''Ba bet'' in Vietnam, is one of the most diverse and richest genera of the Euphorbiaceae family in Vietnam, where about 40 *Mallotus* species may be found. Some *Mallotus* species are used in traditional medicine in Vietnam for different indications. They are concentrated in mountainous areas with an altitude below 1,000 m, but some species can grow at an altitude of 2,000 m, such as *Mallotus oreophilus* Muëll. Arg. Some *Mallotus* species are known to contain different natural compounds, mainly diterpenoids, triterpenoids, steroids, flavonoids, coumarinolignoids, phloroglucinol derivatives or benzopyrans, and to exhibit interesting biological activities such as antimicrobial, antioxidant, antiviral, or cytototoxic ones. Some of these properties may be explained by their chemical composition as, for example, benzopyrans accounting for the cytotoxicity of *Mallotus apelta* extracts. However, although these species seem to have a great medicinal potential, the existing knowledge about most *Mallotus* species is still in most cases very limited. This review underlines the interest to continue the study of this genus of the Euphorbiaceae.

ANNEX UCL 3 – **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**

Abstract

Several *Mallotus* species (Euphorbiaceae) are used in the traditional medicine in Vietnam for different indications, some related with the treatment of inflammatory diseases. Many of these Vietnamese species are edible and consumed instead of tea. This study investigated the antioxidant activities of 33 samples of *Mallotus* belonging to 17 species and collected in Vietnam. We also evaluated safety aspects by determining the cytotoxic effects against human cervix carcinoma HeLa and human lung fibroblast WI-38 cells. Our aim is to develop safe dietary supplements with a protective effect on various diseases caused by tissue damage and acceleration of the aging process linked to reactive oxygen species. These tests allowed identifying non cytotoxic plants exhibiting significant antiradical properties. Some of these properties may be explained by their chemical composition as, for example, hydrolyzable tannins accounting for the antioxidant properties of *Mallotus japonicus* extracts. The antioxidant activity of the most active *Mallotus* species was further analysed and we evaluated the effect of removing tannins. We also identified by LC-ESI-MS some flavonoids responsible for a part of this activity.

ANNEX UCL 4 **– 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, Biochemical Systematics and Ecology 44 (2012) 212–215**

Abstract

Some *Mallotus* species are known to contain different natural compounds, mainly diterpenoids, triterpenoids, steroids, flavonoids, coumarinolignoids, phloroglucinol derivatives or benzopyrans, and to exhibit interesting biological activities such as antimicrobial, antioxidant, antiviral, or cytototoxic ones (Rivière et al., 2010). In the course of our ongoing project to investigate the biologically active chemical constituents from *Mallotus* growing in Vietnam (Chau et al., 2004, 2005, 2009; Rivière et al., 2009, 2010; Hoai et al., 2009), we reported herein the identification of some compounds from *M. barbatus* leaves.

An earlier phytochemical study revealed that the leaves contained polyprenols (Sasak and Chonjnacki, 1973). However, to our knowledge, no other reports on the chemical composition of *M. barbatus* have been made in the literature so far.

ANNEX UCL 5 **– A. Gordien, N Xuan Cuong, V. Nguyen Thi Hong, B. Dejaegher, N. Hoai Nam, C. Van Minh, P. Buc Calderon, Y. Vander Heyden, J. Quetin-Leclercq Variations of Hepatoprotective, Antioxidant, and Cytotoxic Activity within Vietnamese *Phyllanthus* species - submitted**

Abstract

Several *Phyllanthus* species have traditionally been used for hepatoprotection. They enter the preparation of herbal remedies prescribed in Vietnam and throughout Asia against liver ailments. The hepatoprotective effect of 26 Phyllanthus leaves extracts from *P. amarus*, *P. emblica*, *P. reticulatus* and *P. urinaria* was tested on paracetamol injured precision cut rat liver slices. Antioxidant activity and cytotoxicity to CHO cells were also evaluated. Results were only homogenous within *P. emblica* extracts for which no influence of collection time or place was observed. *P. emblica* extracts showed moderate hepatoprotective activity, good antioxidant activity and no toxicity. More variations of activity were observed within extracts from the other species. The results confirmed *in vitro* the validity of some traditional use of *Phyllanthus* species. However fluctuation of activity with collection time and place within a same species was observed for 3 out of 4 species which emphasises the necessity of quality control and standardisation of extracts prior to their incorporation into herbal preparation.

ANNEX UCL 1

Table 1: Antioxidant activity of *Mallotus* extracts.

Code	Species	Collection time	Collection place	IC_{50}	95% confidence interval	
VNB 01	*Mallotus apelta*	August 2009	VanVan-LaoCai	**72.66**	51.36	101.2
VNB 04	*Mallotus apelta*	November 2009	TamDao-Vinhphuc	**74.59**	54.91	102.4
VNB 05	*Mallotus apelta*	November 2009	DongDang-Langson	**98.42**	65.89	137.6
VNB 08	*Mallotus apelta*	December 2009	HamYen-TuyenQuang	**>500**		
VNB 34	*Mallotus apelta*	June 2010	PaCo-HoaBinh	**105.56**	70.08	151.7
VNB 37	*Mallotus barbatus*	May 2010	Dackrong-Quangtri	**17.89**	15.4	20.52
VNB 38	*Mallotus barbatus*	May 2010	KyAnh-Hatinh	**12.91**	10.42	15.22
VNB 39	*Mallotus barbatus*	December 2009	HamYen-TuyenQuang	**39.47**	31.7	48.67
VNB 50	*Mallotus japonicus*	May 2010	Sapa_Laocai	**15.26**	12.95	17.37
VNB 42	*Mallotus macrostachyus*	Agust 2010	VanBan-LaoCai	**>500**		
VNB 43	*Mallotus macrostachyus*	November 2009	DongDang-Langson	**117.05**	97.02	141.6
VNB 51	*Mallotus microcapus*	March 2010	TuyenHoa_QuangBinh	**5.31**	4.58	6.29
VNB 06	*Mallotus paniculatus*	November 2009	DongVan-Hagiang	**37.31**	27.84	55.08
VNB 13	*Mallotus paniculatus*	March 2010	HuongHoa-Quangtri	**38.87**	31.07	52.68
VNB 17	*Mallotus paniculatus*	April 2010	MeLinh-Vinhphuc	**35.76**	27.3	48.59
VNB 35	*Mallotus paniculatus*	June 2010	VQG-Pimat	**31.47**	23.72	42.63
VNB 36	*Mallotus paniculatus*	July 2010	Cucphuong-NinhBinh	**36.70**	28.85	49.01
VNB 44	*Mallotus repandus*	June 2010	PaCo-HoaBinh	**14.49**	12.37	17.08
VNB 45	*Mallotus repandus*	April 2010	MeLinh-Vinhphuc	**75.25**	52.86	100.8
VNB 46	*Mallotus resinosus*	March 2010	TuyenHoa_QuangBinh	**25.76**	22.54	30.74
VNB 41	*Mallotus* sp	February 2011	KyAnh_HaTinh	**20.60**	16.92	25.36
VNB 47	*Mallotus* sp	February 2011	DeoNgang_HaTinh	**51.00**	40.72	61.4
VNB 48	*Mallotus* sp	February 2011	DeoNgang_HaTinh	**31.06**	26.19	36.69

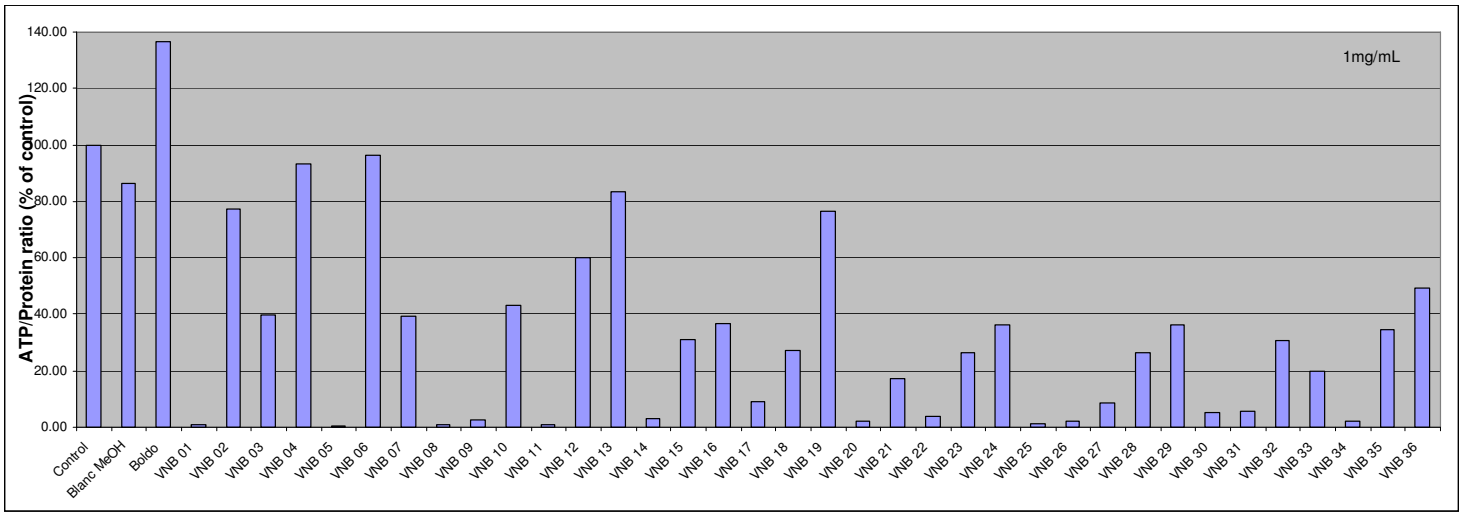
Table 2: Antioxidant activity of *Phyllanthus* extracts.

Code	Species	Collection time	Collection place	IC_{50}	95% confidence interval	
VNB 40	*Phyllanthus acidus*	March 2010	PhanRang-NinhThuan	>500		
VNB 49	*Phyllanthus acidus*	March 2010	KhanhHoa	7.66	6.72	8.69
VNB 11	*Phyllanthus amarus*	February 2010	NghiaTrai-HungYen	99.32	29.23	142.5
VNB 12	*Phyllanthus amarus*	March 2010	VanDien-Hanoi	13.74	9.638	19.08
VNB 19	*Phyllanthus amarus*	April 2010	MeLinh-Vinhphuc	13.12	10.77	15.87
VNB 22	*Phyllanthus amarus*	April 2010	LanOng-Hanoi	14.33	9.904	20.69
VNB 27	*Phyllanthus amarus*	May 2010	QueVo-Bacninh	15.51	11.14	21.22
VNB 29	*Phyllanthus amarus*	May 2010	DongAnh-Hanoi	10.15	6.909	14.95
VNB 32	*Phyllanthus amarus*	May 2010	NinhHiep-Hanoi	16.12	11.62	21.39
VNB 02	*Phyllanthus emblica*	September 2009	VanVan-LaoCai	9.90	7.61	13.89
VNB 03	*Phyllanthus emblica*	November 2009	DongDang-Langson	9.55	7.543	12.58
VNB 07	*Phyllanthus emblica*	December 2009	DongVan-Hagiang	8.57	6.952	10.57
VNB 15	*Phyllanthus emblica*	March 2010	HuongHoa-Quangtri	10.92	7.609	14.79
VNB 21	*Phyllanthus emblica*	April 2010	MeLinh-Vinhphuc	8.75	6.994	10.85
VNB 28	*Phyllanthus emblica*	May 2010	DongAnh-Hanoi	10.26	8.324	12.73
VNB 09	*Phyllanthus reticulatus*	February 2010	NghiaTrai-HungYen	28.06	19.25	38.18
VNB 14	*Phyllanthus reticulatus*	March 2010	VanDien-Hanoi	29.70	21.74	45.66
VNB 18	*Phyllanthus reticulatus*	April 2010	MeLinh-Vinhphuc	13.42	10.51	17.28
VNB 20	*Phyllanthus reticulatus*	April 2010	LanOng-Hanoi	22.18	13.47	33.6
VNB 25	*Phyllanthus reticulatus*	May 2010	NinhHiep-Hanoi	23.49	15.23	33.48
VNB 26	*Phyllanthus reticulatus*	May 2010	DongAnh-Hanoi	27.31	13.42	42.45
VNB 10	*Phyllanthus urinaria*	February 2010	NghiaTrai-HungYen	9.75	7.355	12.43
VNB 16	*Phyllanthus urinaria*	March 2010	VanDien-Hanoi	8.45	6.033	11.46
VNB 23	*Phyllanthus urinaria*	April 2010	MeLinh-Vinhphuc	8.83	7.248	10.7
VNB 24	*Phyllanthus urinaria*	April 2010	LanOng-Hanoi	8.56	6.949	10.49
VNB 30	*Phyllanthus urinaria*	May 2010	QueVo-Bacninh	17.99	11.95	19.88
VNB 31	*Phyllanthus urinaria*	May 2010	NinhHiep-Hanoi	21.13	13.71	28.78
VNB 33	*Phyllanthus urinaria*	May 2010	DongAnh-Hanoi	13.60	8.348	19.86

Table 3: Cytotoxic activities of VNB 1-36 extracts.

Code	Species	Collection time	Collection place	IC50 (µg/mL) CHO	IC50 (µg/mL) J774
VNB 01	*Mallotus apelta*	August 2009	VanVan-LaoCai	**56.93** (38.88-84.37)	**94.51** (85.08-105)
VNB 04	*Mallotus apelta*	November 2009	TamDao-Vinhphuc	**71.05** (29.58-190.9)	**>100**
VNB 05	*Mallotus apelta*	November 2009	DongDang-Langson	**52.605** (39.97-72.2)	**>100**
VNB 08	*Mallotus apelta*	December 2009	HamYen-TuyenQuang	**97.62** (59.4-170.1)	**>100**
VNB 34	*Mallotus apelta*	June 2010	PaCo-HoaBinh	**55.18** (36.22-77.95)	**>100**
VNB 06	*Mallotus paniculatus*	November 2009	DongVan-Hagiang	**>200**	**>200**
VNB 13	*Mallotus paniculatus*	March 2010	HuongHoa-Quangtri	**>200**	**>200**
VNB 17	*Mallotus paniculatus*	April 2010	MeLinh-Vinhphuc	**ND**	**>200**
VNB 35	*Mallotus paniculatus*	June 2010	VQG-Pimat	**95.53** (ND)	**>200**
VNB 36	*Mallotus paniculatus*	July 2010	Cucphuong-NinhBinh	**79.83** (68.86-92.53)	**>100**
VNB 11	*Phyllanthus amarus*	February 2010	NghiaTrai-HungYen	**>100**	**>100**
VNB 12	*Phyllanthus amarus*	March 2010	VanDien-Hanoi	**>100**	**>200**
VNB 19	*Phyllanthus amarus*	April 2010	MeLinh-Vinhphuc	**>50**	**>200**
VNB 22	*Phyllanthus amarus*	April 2010	LanOng-Hanoi	**43.7** (36.3-51.4)	**>100**
VNB 27	*Phyllanthus amarus*	May 2010	QueVo-Bacninh	**>100**	**>100**
VNB 29	*Phyllanthus amarus*	May 2010	DongAnh-Hanoi	**>100**	**>100**
VNB 32	*Phyllanthus amarus*	May 2010	NinhHiep-Hanoi	**>100**	**>100**
VNB 02	*Phyllanthus emblica*	September 2009	VanVan-LaoCai	**>100**	**>200**
VNB 03	*Phyllanthus emblica*	November 2009	DongDang-Langson	**>100**	**>200**
VNB 07	*Phyllanthus emblica*	December 2009	DongVan-Hagiang	**>100**	**>200**
VNB 15	*Phyllanthus emblica*	March 2010	HuongHoa-Quangtri	**>100**	**>200**
VNB 21	*Phyllanthus emblica*	April 2010	MeLinh-Vinhphuc	**>100**	**>100**
VNB 28	*Phyllanthus emblica*	May 2010	DongAnh-Hanoi	**>100**	**>100**
VNB 09	*Phyllanthus reticulatus*	February 2010	NghiaTrai-HungYen	**11.5** (10.3-14.5)	**>100**
VNB 14	*Phyllanthus reticulatus*	March 2010	VanDien-Hanoi	**23.3** (20.0-27.2)	**>100**
VNB 18	*Phyllanthus reticulatus*	April 2010	MeLinh-Vinhphuc	**>100**	**>200**
VNB 20	*Phyllanthus reticulatus*	April 2010	LanOng-Hanoi	**>100**	**>100**
VNB 25	*Phyllanthus reticulatus*	May 2010	NinhHiep-Hanoi	**10.4** (9.1-17.6)	**>100**
VNB 26	*Phyllanthus reticulatus*	May 2010	DongAnh-Hanoi	**>100**	**>100**
VNB 10	*Phyllanthus urinaria*	February 2010	NghiaTrai-HungYen	**>100**	**>100**
VNB 16	*Phyllanthus urinaria*	March 2010	VanDien-Hanoi	**>100**	**>100**
VNB 23	*Phyllanthus urinaria*	April 2010	MeLinh-Vinhphuc	**>100**	**>100**
VNB 24	*Phyllanthus urinaria*	April 2010	LanOng-Hanoi	**>100**	**>100**
VNB 30	*Phyllanthus urinaria*	May 2010	QueVo-Bacninh	**>200**	**>100**
VNB 31	*Phyllanthus urinaria*	May 2010	NinhHiep-Hanoi	**>100**	**>100**
VNB 33	*Phyllanthus urinaria*	May 2010	DongAnh-Hanoi	**88.6** (61.0-115.7)	**>100**
cycloheximide				**1.45** (0.14-8.21)	**0.03698** (0.03532-0.03871)

Fig 1: Hepatotoxicity of VNB 1-36 extracts at 1 and 0.5 mg/ml.



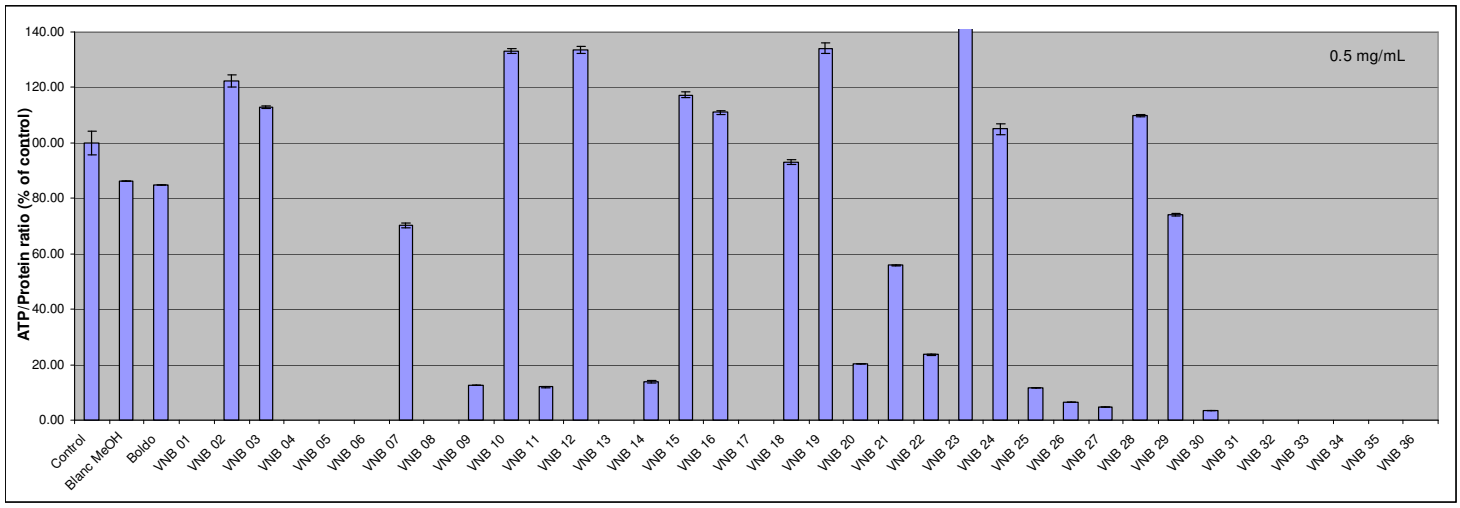


Table 4: Hepatoprotective activity of VNB1-36 extracts.

		ATP/Prot (nmol/mg)		% controle							
Control		**2.669**	0.118	**100.00**	4.44						
Blanc MeOH		**2.302**	0.003	**86.24**	0.13						
Paracetamol (APAP)		**0.141**	0.001	**5.28**	0.02						
APAP+N-acetylcysteine (NAC)		**1.135**	0.008	**42.51**	0.32						
		1mg/mL ATP/Prot (nmol/mg)		**% controle**			**0.5mg/mL ATP/Prot (nmol/mg)**		**% control**		
Boldo	*Peumus boldus*	**2.2450**	0.0380	**98.67**	1.67		**1.1672**	0.0012	**84.75**	0.09	
Boldo+APAP		**0.4928**	0.0063	**35.78**	0.46		**0.4606**	0.0433	**33.44**	3.15	
VNB 01	*Mallotus apelta*	**0.0160**	0.0121	0.66	0.50						
VNB 01+APAP		**0.0218**	0.4257	0.90	17.51						
VNB 02	*Phyllanthus emblica*	**1.8786**	0.0037	**77.28**	0.15		**1.6853**	0.0285	**122.38**	2.07	
VNB 02+APAP		**0.0628**	0.0093	2.58	0.38		**0.2757**	0.0010	**20.02**	0.07	
VNB 03	*Phyllanthus emblica*	**0.9652**	0.0197	**39.70**	0.81		**1.5537**	0.0059	**112.82**	0.43	
VNB 03+APAP		**0.0855**	0.0950	3.52	3.91		**0.1642**	0.0007	**11.93**	0.05	
VNB 04	*Mallotus apelta*	**2.2698**	0.0081	**93.37**	0.33						
VNB 04+APAP		**0.0581**	0.0010	2.39	0.04						
VNB 05	*Mallotus apelta*	**0.0066**	0.0029	0.27	0.12						
VNB 05+APAP		**0.0228**	0.0180	0.94	0.74						
VNB 06	*Mallotus paniculatus*	**2.3410**	0.0001	**96.30**	0.01						
VNB 06+APAP		**0.0467**	0.0013	1.92	0.05						
VNB 07	*Phyllanthus emblica*	**0.9576**	0.0000	**39.39**	0.00		**0.9652**	0.0114	**70.09**	0.82	
VNB 07+APAP		**0.0862**	0.1439	3.54	5.92		**0.1497**	0.0002	**10.87**	0.02	
VNB 08	*Mallotus apelta*	**0.0246**	0.0108	1.01	0.45						
VNB 08+APAP		**0.0114**	7.3712	0.47	303.22						
VNB 09	*Phyllanthus reticulatus*	**0.0580**	0.0165	2.39	0.68		**0.1733**	0.0002	**12.58**	0.01	
VNB 09+APAP		**0.0118**	0.0012	0.49	0.05		**0.0113**	0.0000	0.82	0.00	
VNB 10	*Phyllanthus urinaria*	**1.0458**	0.0061	**43.02**	0.25		**1.8348**	0.0121	**133.23**	0.88	
VNB 10+APAP		**0.0910**	0.0130	3.74	0.53		**0.2461**	0.0084	**10.82**	0.37	
VNB 11	*Phyllanthus amarus*	**0.0171**	0.0152	0.70	0.63		**0.1641**	0.0023	**11.92**	0.17	
VNB 11+APAP		**0.0081**	0.0132	0.33	0.54		**0.0158**	0.0000	1.14	0.00	
VNB 12	*Phyllanthus amarus*	**1.4549**	0.0015	**59.85**	0.06		**2.2269**	0.2983	**97.88**	13.11	
VNB 12+APAP		**0.0182**	0.3903	0.75	16.06		**0.0693**	0.0014	3.05	0.06	
VNB 13	*Mallotus paniculatus*	**2.2313**	0.0250	**83.59**	0.93						
VNB 13+APAP		**0.0576**	0.0003	2.16	0.01						

		1mg/mL ATP/Prot (nmol/mg)		% control		0.5mg/mL ATP/Prot (nmol/mg)		% control	
VNB 14	*Phyllanthus reticulatus*	**0.0837**	0.0008	3.14	0.03	**0.1888**	0.0059	**13.71**	0.43
VNB 14+APAP		**0.0161**	0.0001	0.60	0.00	**0.0295**	0.0001	2.15	0.01
VNB 15	*Phyllanthus emblica*	**0.8266**	0.0054	**30.97**	0.20	**1.6152**	0.0142	**117.28**	1.03
VNB 15+APAP		**0.0680**	0.0003	2.55	0.01	**0.1600**	0.0002	**11.62**	0.01
VNB 16	*Phyllanthus urinaria*	**0.9829**	0.0098	**36.82**	0.37	**1.5289**	0.0085	**111.02**	0.62
VNB 16+APAP		**0.0410**	0.0002	1.54	0.01	**0.2219**	0.0003	**16.12**	0.02
VNB 17	*Mallotus paniculatus*	**0.2419**	0.0079	**9.06**	0.29				
VNB 17+APAP		**0.0550**	0.0001	2.06	0.00				
VNB 18	*Phyllanthus reticulatus*	**0.7219**	0.0859	**27.05**	3.22	**1.2824**	0.0099	**93.12**	0.72
VNB 18+APAP		**0.0248**	0.0003	0.93	0.01	**0.0333**	0.0001	2.42	0.01
VNB 19	*Phyllanthus amarus*	**2.0447**	0.0056	**76.60**	0.21	**2.2544**	0.0992	**99.08**	4.36
VNB 19+APAP		**0.3741**	0.1032	**14.02**	3.87	**0.3611**	0.0021	**26.22**	0.15
VNB 20	*Phyllanthus reticulatus*	**0.0528**	0.0008	1.98	0.03	**0.2771**	0.0010	**20.12**	0.07
VNB 20+APAP		**0.0110**	0.0000	0.41	0.00	**0.0106**	0.0000	0.77	0.00
VNB 21	*Phyllanthus emblica*	**0.4556**	0.0542	**17.07**	2.03	**0.7709**	0.0031	55.98	0.23
VNB 21+APAP		**0.0472**	0.0000	1.77	0.00	**0.7247**	0.0091	52.62	0.66
VNB 22	*Phyllanthus amarus*	**0.0984**	0.0007	3.69	0.03	**0.3242**	0.0024	**23.54**	0.18
VNB 22+APAP		**0.0149**	0.0000	0.56	0.00	**0.0256**	0.0001	1.86	0.00
VNB 23	*Phyllanthus urinaria*	**0.7091**	0.0136	**26.57**	0.51	**1.7312**	0.0614	**76.09**	2.70
VNB 23+APAP		**0.0592**	0.0529	2.22	1.98	**0.3670**	0.0567	**16.13**	2.49
VNB 24	*Phyllanthus urinaria*	**0.9712**	0.0075	**36.38**	0.28	**1.4460**	0.0254	**105.00**	1.84
VNB 24+APAP		**0.0562**	0.0000	2.11	0.00	**0.2545**	0.0005	**18.48**	0.04
VNB 25	*Phyllanthus reticulatus*	**0.0361**	0.0003	1.35	0.01	**0.1600**	0.0028	**11.62**	0.20
VNB 25+APAP		**0.0123**	0.0000	0.46	0.00	**0.0175**	0.0001	1.27	0.01
VNB 26	*Phyllanthus reticulatus*	**0.0560**	0.0021	2.10	0.08	**0.0873**	0.0006	**6.34**	0.04
VNB 26+APAP		**0.0112**	0.0000	0.42	0.00	**0.0124**	0.0001	0.90	0.01
VNB 27	*Phyllanthus amarus*	**0.2353**	0.0055	**8.81**	0.21	**0.0668**	0.0001	4.85	0.01
VNB 27+APAP		**0.0215**	0.0000	0.81	0.00	**0.0297**	0.0001	2.16	0.00
VNB 28	*Phyllanthus emblica*	**0.2786**	0.0146	**12.25**	0.64	**1.5126**	0.0071	**109.84**	0.51
VNB 28+APAP		**0.0916**	0.0061	4.03	0.27	**0.1098**	0.0256	**7.97**	1.86
VNB 29	*Phyllanthus amarus*	**0.9740**	0.0153	**36.49**	0.57	**1.0200**	0.0036	**74.07**	0.26
VNB 29+APAP		**0.0574**	0.0001	2.15	0.00	**0.0827**	0.0004	6.01	0.03
VNB 30	*Phyllanthus urinaria*	**0.1411**	0.0011	**5.29**	0.04	**2.0389**	0.8618	**89.61**	37.87
VNB 30+APAP		**0.0167**	0.0000	0.62	0.00	**0.0630**	0.0032	2.77	0.14

		1mg/mL ATP/Prot (nmol/mg)		% control		0.5mg/mL ATP/Prot (nmol/mg)		% control	
VNB 31	*Phyllanthus urinaria*	**0.1475**	0.0008	**5.53**	0.03	**2.2925**	0.4174	**100.76**	18.35
VNB 31+APAP		**0.0195**	0.0001	0.73	0.00	**0.0480**	0.0004	2.11	0.02
VNB 32	*Phyllanthus amarus*	**0.8200**	0.0132	**30.72**	0.50	**2.1600**	0.0735	**94.94**	3.23
VNB 32+APAP		**0.0917**	0.0002	3.44	0.01	**0.1323**	0.0009	**5.81**	0.04
VNB 33	*Phyllanthus urinaria*	**0.5327**	0.0005	**19.96**	0.02	**2.0886**	0.5051	**91.80**	22.20
VNB 33+APAP		**0.0225**	0.0006	0.84	0.02	**0.0920**	0.0007	4.04	0.03
VNB 34	*Mallotus apelta*	**0.0594**	0.0001	2.23	0.00				
VNB 34+APAP		**0.0108**	0.0001	0.40	0.00				
VNB 35	*Mallotus paniculatus*	**0.9264**	0.0084	**34.71**	0.31				
VNB 35+APAP		**0.0425**	0.0001	1.59	0.00				
VNB 36	*Mallotus paniculatus*	**1.3108**	0.0227	**49.11**	0.85				
VNB 36+APAP		**0.0260**	0.0013	0.97	0.05				

Fig 2: Hepatoprotective activity of *Phyllanthus amarus* extracts.

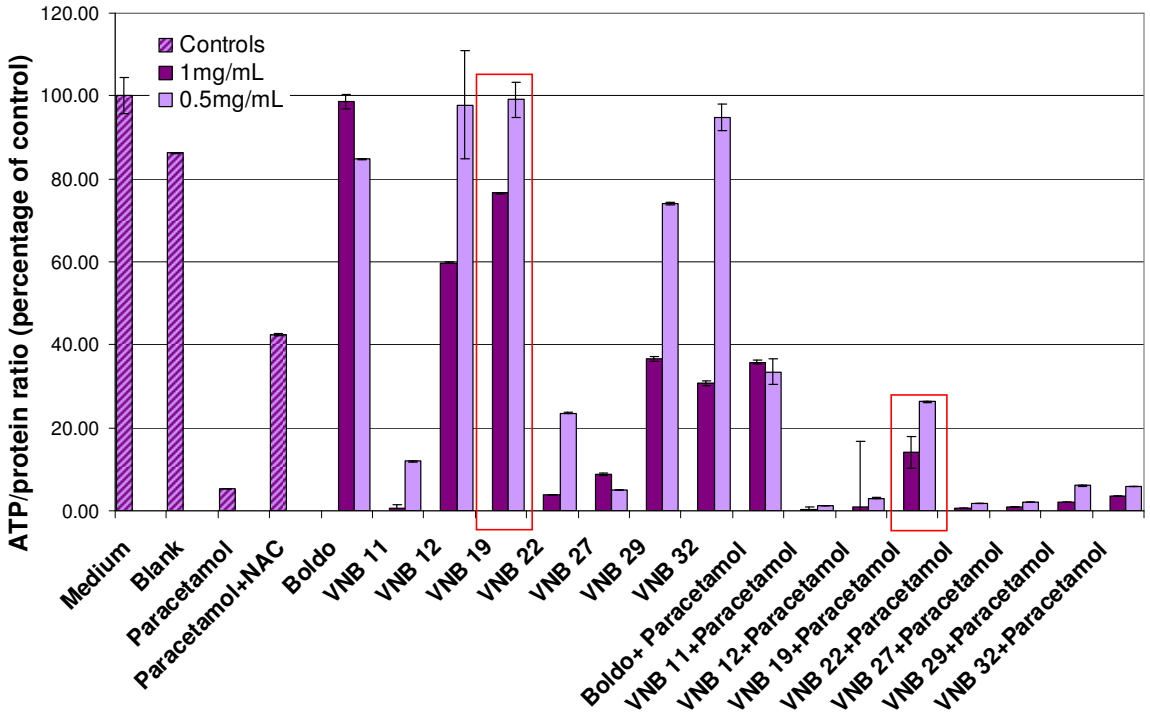


Table 5: Cytotoxic activity of the pure compounds isolated from *Phyllanthus urinaria* and *Mallotus japonicus*.

N0	Compound	IC_{50} (µg/ml)			
		J774		CHO	
		1fst time	2sd time	1fst time	2sd time
1	PUW1	>50		>50	
2	PU1	>50	>50	>50	>50
3	PU2	>50	>50	>50	>50
4	PU3	NT	NT	NT	NT
5	PU4	NT	NT	NT	NT
6	PU5	NT	NT	NT	NT
7	PU6	>50	>50	>50??	>50
8	PU7	3.659	10.98	4.956	9.565
9	PU8	1.672	1.816	3.760	3.196
10	PU9	>50	>50	>50	>50
11	PU10	3.731	3.740	16.90	12.35
12	PU11	>50	>50	>50	>50
13	PU12	47.05	>50	>50	>50
14	MJ1	>50		>50	
15	MJ2	37.12		47.31	

NT: Not tested because these compounds were not pure.

Among 12 compounds tested, three compounds PU08, PU09 and PU11 have cytotoxic activity on the two cell lines CHO and J774.

Table 6: Cytotoxicity of *Phyllanthus* extracts and fractions (IC_{50} in µg/mL).

	J774 cells			CHO cells		
	Day 1	Day 2	**Day 1+Day 2**	Day 1	Day 2	**Day 1+Day 2**
PAM	56.36±7.38	69.16±3.31	**62.76±8.65**	68.38±4.04	90.63±2.36	**79.50±12.54**
PRM	60.93±6.84	55.39±4.78	**58.16±6.09**	70.02±42.58	128.66±21.14	**99.34±44.00**
PRC	14.98±0.82	14.32±0.20	**14.70±0.70**	4.39±0.41	15.85±12.80	**10.12±10.25**
PRE	29.02±15.33	37.23±2.68	**33.12±10.82**	116.10±9.43	179.55±4.24	**147.83±35.36**
PRW1	109.93±5.38	98.11±8.84	**104.02±9.21**	112.06±5.70	155.66±10.10	**133.86±24.98**
PRW2	39.88±0.32	75.55±3.85	**57.72±19.69**	131.48±9.67	>200	**ND**
PRW3	>200	>200	**>200**	>200	>200	**>200**
PRW4	48.15±2.68	76.71±0.55	**62.43±15.75**	120±38.40	106.37±5.54	**113.62±25.79**
PRW5	57.14±6.67	67.98±9.88	**62.56±9.60**	92.71±22.00	117.53±8.65	**105.12±20.21**
PRW6	78.80±2.00	80.52±2.71	**79.66±2.33**	50.16±1.62	39.29±0.17	**44.72±6.04**
Camptothecine	0.0221±0.0028	0.0098±0.0006	**0.0151±0.0068**	0.1407±0.0386	0.0565±0.0233	**0.0986±0.0542**
Cycloheximide	1.47E-4±2.52E-5	3.89E-4±3.41E-5	**2.68E-4±1.35E-4**	4.83E-4±3.42E-5	1.41E-4±4.81E-5	**3.13E-4±1.91E-4**

PAM: *P. amarus* methanol extract
PRM: *P. reticulatus* methanol extract
PRC: *P. reticulatus* chloroform fraction
PRE: *P. reticulatus* ethyl acetate fraction
PRW1-6: Subfractions from *P. reticulatus* water fraction

Table 7: DPPH antiradical activity of PRC, PRC1-13, W2E1A, W2E5 and W2G1.

	IC50	95% confidence interval	
PRC	31.84	23.30	56.14
PRC1	>1000		
PRC2	>1000		
PRC3	>1000		
PRC4	>1000		
PRC5	58.95	48.39	72.99
PRC6	145.00	120.30	181.00
PRC7	>900		
PRC8	>1000		
PRC9	>1000		
PRC10	>700		
PRC11	>200		
PRC12	>400		
PRC13	178.35	110.80	295.20
W2E1A	0.88	0.46	1.84
W2E5	>400		
W2G1	5.77	1.97	10.48
Tocopherol	9.33	5.217	15.46
Gallic acid	0.89	0.79	1.04

Table 8: Cytotoxic activity of P. *reticulatus extracts*

N 0	Fraction		J774 IC50 (µg/ml)			CHO IC50 (µg/ml)		
1	**PR/$CHCl_3$** CHO: IC50>32 µg/ml J774: IC50 = 9.3 µg/ml	Non-chlorophyll	5.35	83.96	51.2		49.14	>50
		Chlorophyll	39.34	12.32	6.43	7.313	20.71	10.41
2	**PR/C/1-3** CHO: IC50>32 µg/ml	Non-chlorophyll	>50	>50		32.91	>50	
		Chlorophyll	32.16	34.03		>50	17.98	31.68
3	**PR/C/4** CHO: IC50>32 µg/ml	Non-chlorophyll	>50	>50		>50	47.26	
		Chlorophyll	20.99	26.52		9.804	9.183	
4	**PR/C/5** CHO: IC50 > 32 and 15.96 µg/ml	Non-chlorophyll	33.35	42.98		45.13	21.20	
		Chlorophyll	13.12	16.24		1.582	3.516	
5	**PR/C/6** CHO: IC50 > 32 and 14.68 µg/ml	Non-chlorophyll	11.79	>50	5.11	49.06	38.94	
		Chlorophyll	>50	15.67		>50	1.668	
6	**PR/C/7** CHO: IC50 >32 µg/ml	Non-chlorophyll	>50	40.19				
		Chlorophyll	16.29	2.598		34.39	3.578	
7	**PR/C/8** CHO: IC50 = 15.8 and 16.97 µg/ml J774: IC50 = 20.62 µg/ml	Non-chlorophyll	0.9409	15.2	9.624	0 ???	61.34	40.91
		Chlorophyll	9.086	1.917	3.105	2.598	21.12	0.3869
8	**PR/C/9** CHO: IC50 = 2.02 and	Non-chlorophyll	1.422	36.35	35.82	>50	>50	50.34

	2.94 µg/ml J774: IC50 = 5.081 µg/ml	Chlorophyll	21.68	4.52	4.419	2.79	2.959	
9	**PR/C/10** CHO: IC50 = 3.29 and 3.46 µg/ml J774: IC50 = 10.32 µg/ml	Non-chlorophyll	49.54			57.56	69.31	
		Chlorophyll	12.89	3.604		8.583	2.647	
10	**PR/C/11** CHO: IC50 = 2.17 and 1.99 µg/ml J774: IC50 = 0.001125 µg/ml	Non-chlorophyll	47.36			52.92	271.6	
		Chlorophyll	21.12	3.453		7.637	3.693	
11	**PR/C/12** CHO: IC50 = 2.375 µg/ml J774: IC50 = 2.474 µg/ml							
12	**PR/C/13** CHO: IC50 = 3.44 and 4.94 µg/ml							

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Mallotus species from Vietnamese mountainous areas: phytochemistry and pharmacological activities

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Abstract The genus *Mallotus* belongs to Malphighiales order and Euphorbiaceae family. *Mallotus*, commonly known as "Ba bet" in Vietnam, is one of the most diverse and richest genera of the Euphorbiaceae family in Vietnam, where about 40 *Mallotus* species may be found. Some *Mallotus* species are used in traditional medicine in Vietnam for different indications. They are concentrated in mountainous areas with an altitude below 1,000 m, but some species can grow at an altitude of 2,000 m, such as *Mallotus oreophilus* Müll. Arg. Some *Mallotus* species are known to contain different natural compounds, mainly diterpenoids, triterpenoids, steroids, flavonoids, coumarinolignoids, phloroglucinol derivatives or benzopyrans, and to exhibit interesting biological activities such as antimicrobial, antioxidant, antiviral, or cytototoxic ones. Some of these properties may be explained by their chemical composition as, for example, benzopyrans accounting for the cytotoxicity of *Mallotus apelta* extracts. However, although these species seem to have a great medicinal potential, the existing knowledge about most *Mallotus* species is still in most cases very limited. This review underlines the interest to continue the study of this genus of the Euphorbiaceae.

Keywords Mallotus · Euphorbiaceae · Vietnam · Natural compounds · Biological activities

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Introduction

The genus *Mallotus*, commonly known as "Ba bet" in Vietnam, is one of the most diverse and richest genera of the Euphorbiaceae family in Vietnam where about 40 *Mallotus* species may be found among which six species and one variety are endemic. These endemic species, *Mallotus canii* Thin, *Mallotus chuyenii* Thin, *Mallotus eberhardtii* Gagnep., *Mallotus hanheoensis* Thin, *Mallotus poilanei* Gagnep., *Mallotus*

sathavensis Thin, *Mallotus cuneatus* Ridl. var. *glabratus* Thin, have been recently found, distributed in several regions in Vietnam and there is still a lack of information about them. Species belonging to the *Mallotus* genus are usually shrubs or small trees and grow in rainy, ever green primary or secondary forests. They can be also found in deciduous forests. Some species are considered as "first-coming plants" of forests recycling. Naturally, species are chiefly propagated from seeds. They are concentrated in mountainous areas with an altitude below 1,000 m, but some species can grow at an altitude of 2,000 m, such as *Mallotus oreophilus* Müll. Arg. (Thin 2003).

The genus *Mallotus* belongs to the Malphighiales order, the Euphorbiaceae family, Acalyphoideae subfamily, Acalypheae pro parte, Rottlerinae subtribe (Nowicke and Takahashi 2002). This genus includes approximately 150 species distributed in tropical and sub-tropical regions in Asia (Cambodia, China, India, Laos, Malaysia, Sri Lanka, Thailand, Vietnam). A few species are found in the North and East of Australia and the Pacific-Ocean Archipelago (the East of Fiji). Only two species are found in Africa and Madagascar (Schatz 2001). *M. oppositifolius* (Geiseler) Müll. Arg. is distributed in different African countries (Central Africa, Ghana, Nigeria, Tanzania) and Madagascar. *M. baillonianus* Müll. Arg. is endemic to Madagascar. The genus *Mallotus* is richer in Vietnam than in China, where 28 species are described of which seven are endemic. Sixteen species are common in Vietnam and China. In general, these species are distributed in higher altitude in China than Vietnam (Qiu and Gilbert 2008). Some species of the genus *Mallotus* (*M. apelta*, *M. barbatus*, *M. floribundus*, *M. glabriusculus*, *M. macrostachyus*, *M. oblongifolius*, *M. paniculatus*, *M. philippinensis*, *M. poilanei*) are used as medicinal plants in the traditional medicine in Vietnam and the South-East Asian countries for the treatment of various ailments ranging from minor infections such as gastrointestinal disorders to dysentery, hepatic diseases, cutaneous diseases, fever and malaria, and a series of other indications. The researched parts of the *Mallotus* species include aerials parts, bark, heartwood, leaves, roots, seeds, stem bark and whole plants. Some *Mallotus* species are known to contain different natural compounds, mainly terpenoids, polyphenols and benzopyrans. The compounds isolated from the *Mallotus* genus and extracts show many different biological activities including antioxidant, antiviral, antimicrobial, anti-inflammatory or cytotoxic. Some of these properties are attributed to the presence of specific classes of natural compounds, for example, benzopyrans accounting for the cytotoxicity of *Mallotus apelta* extracts (Van Chau et al. 2005a; Van Kiem et al. 2004) or polyphenols accounting for the antiradical activity of *Mallotus metcalfianus* extracts (Rivière et al. 2009).

In this review paper, we will summarize the data of the literature concerning the phytochemistry and the pharmacological activities of *Mallotus* species, described over the past few decades (Table 1; Fig. 1).

Phytochemistry

For some *Mallotus* species, studies were published on their chemical composition, especially for *M. apelta*, *M. metcalfianus*, *M. philippinensis*, *M. paniculatus*, *M. repandus*. These *Mallotus* species are known to contain different natural compounds, mainly diterpenoids, triterpenoids, steroids, benzopyranes, flavonoids, coumarinolignoids or phloroglucinol derivatives. The existing knowledge about the other investigated plants is in most cases very limited. However, some data underline the isolation of a novel furanocarboxamide from *M. cuneatus* (Groweiss et al. 1994), scopoletin from *M. resinosus* (Ma et al. 2004), phloroglucinol derivatives from *M. pallidus* (Supudompol et al. 2004; Likhitwitayawuid and Supudompol 2005) or triterpenoids and casbane-type diterpenoid lactones from *M. hookerianus* (Hui and Li 1976; Bai et al. 2006).

Terpenoids and steroids

Diterpenoids and diterpenic lactones (Table 2)

Cheng et al. (1999) and Cheng and Chen (1999) isolated five new diterpenoids (**1–5**) from the petroleum ether fraction of the ethanolic extract of *M. apelta*. Three highly oxidized casbane-type diterpenoids with unique α,β-unsatured γ-lactones, named hookerianolides A, B, and C (**6–8**), were isolated from the methylene chloride extract of *M. hookerianus* (Bai

Table 1 Vietnamese *Mallotus* species

Botanical name	Synonyms[a]	Vietnamese vernacular names	Plant[b]	Distribution[c]	Altitude Vietnam (m)	Altitude China (m)	Traditonal uses[d,e]	Phytochemistry[f,g]	Pharmacological activities[g]
Mallotus anisopodus Airy Shaw	ND	Ruoikhe	T	V (South province AG), Ca, L	100–500	ND	ND	ND	ND
Mallotus apelta (Lour.) Müll. Arg.	*Ricinus apelta* Lour.	Babet trang, Buc trang, Bui bui, Bai bai, Bum bup, Bang bac, Cay ruong	Sm T, Sh	V (N to S), Ch	100–700	100–1,000	B: dh, ga, gy, hem, hep, oe L: co, cu, dh, gy, hep, oe, ot R: ai, dh, ga, gy, hep, oe	L: benzopyrans[1,2,3,4], pentacyclic triterpenoids and steroids[5,6,7,8], flavonoids[6,9,10], carotenoids[11], anthraquinone, coumarin and nicotinic acid[12] R: pentacyclic triterpenoids and steroids[13], pyridine type alkaloid and ellagic acid derivative[14] S: pentacyclic triterpenoids and steroids[15], iridoid (mussaenoside)[15], coumarinolignoids[16,17] Wp: diterpenoids[18,19]	Antiviral D-HBV[20] Bacteriostatic: triterpenoids and benzopyrans[1,13] Cytotoxic: benzopyrans[4] Hepatoprotective: coumarinolignoids[17,21] Inhibitory effect of reverse transcriptase and cellular DNA polymerase[22] Inhibitory effect of NFAT transcription and NF-κB activation[11]
Mallotus barbatus (Wall.) Müll. Arg.	*Rottlera barbata* Wall.	Bung buc, Bup bong gai, Bong bet, Bung buc gai, Ba bet long, Ruoi cau, Cam lon, Nhung dien rau	Sm T, Sh	V (N to S), Ch, Ind, L, Mal, Mya, Th	100–1,100	200–1,300	B: ga L: ac, hem, oe, sc R: an, fe, diu, cho, hea	L: polyprenols[23]	ND
Mallotus canii Thin	ND	Babet gialai	T	Endemic V (South province GL)	100–500	ND	ND	ND	ND
Mallotus chrysocarpus Pamp.	*M. contubernalis* var. *chrysocarpus* (Pamp.) Hand.-Mazz., *M. repandus* var. *chrysocarpus* (Pamp.) S.M. Hwang	Babet qua vang, Ruoi trai vang	Sm Sh	V (North province HT) Ch	100–500	500–1,000	ND	ND	Antiviral HIV[24]

Table 1 continued

Botanical name	Synonyms[a]	Vietnamese vernacular names	Plant[b]	Distribution[c]	Altitude Vietnam (m)	Altitude China (m)	Traditonal uses[d,e]	Phytochemistry[f,g]	Pharmacological activities[g]
Mallotus chuyenii Thin	ND	Babet hoabinh	T	Endemic V (North province HB)	100–500	ND	ND	ND	ND
Mallotus clellandii Hook.f.	ND	Ruoi clelland, Ruoi tron, Ruoi khong long, Nhung dien clelland, Nhung dien khong long	Sm T, Sh	V (South provinces), Ca, L, Mya, Th	100–500	ND	ND	ND	ND
Mallotus contubernalis Hance	*M. repandus* var. *repandus*, *M. repandus* var. *scabrifolius* (A. Juss.) Müll. Arg.	Babet, Don xuong, Canhkien la bac, Buctruong ba ngan, Rem ban day	Sm T, Sh	V (North provinces), Ch, L	100–500	100–600	ND	ND	ND
Mallotus cuneatus Ridl.	*M. resinosus* var. *cuneatus* (Ridl.) N.P. Balakr. & Chakrab.	Duoi rung, Ruoi rung	Sm T, Sh	V (N to S), Ca, Ind, Mal, Phi, Th	100–500	ND	ND	L, T: furanocarboxamide[25]	ND
Mallotus cuneatus Ridl. var. *glabratus* Thin	ND	Babet nhan	T	Endemic V (North provinces)	100–500	ND	ND	ND	ND
Mallotus dispar (Blume) Müll. Arg.	*Rottlera dispar* Blume	Ruoikhong deu, Nhung dien khong deu	Sm T, Sh	V, Indo, Mal, Phi, Th	ND	ND	ND	ND	ND
Mallotus eberhardtii Gagnep	ND	Dodot	Sm T, Sh	Endemic V (Central and South provinces, TTH and KG)	100–500	ND	ND	ND	ND
Mallotus esquirolii H. Lév.	*M. grossedentatus* Merr. & Chun	Babet esquirol	T	V (North provinces LS and HB) Ch	100–500	300–1,500	ND	ND	ND

Table 1 continued

Botanical name	Synonyms[a]	Vietnamese vernacular names	Plant[b]	Distribution[c]	Altitude Vietnam (m)	Altitude China (m)	Traditonal uses[d,e]	Phytochemistry[f,g]	Pharmacological activities[g]
Mallotus floribundus (Blume) Müll. Arg.	*Adisca floribunda* Blume	Babet nhieu hao, Bach dan, Ruoi trung bo, Ba bet hoa nhieu	Sm T, Sh	V (N to S), Austr, Ca, L, Indo, Mal, NG, Phi, Th	100–500	ND	R: dh, fe, gy Wp: sc	ND	ND
Mallotus glabriusculus (Kurz) Pax & K. Hoffm.	*Coelodiscus glabriusculus* Kurz	Babet nhan, Chiet canh, Kien canh, Nhung dien coudere, Ruoi khong long	Sm T, Sh	V (South provinces), Ca, L, Mya	100–500	ND	R: cou	ND	ND
Mallotus hanheoensis Thin	ND	Babe thon heo	T	Endemic V (South province KH)	100–500	ND	ND	ND	ND
Mallotus hookerianus (Seem.) Müll. Arg.	*Hancea hookeriana* Seem.	Babet cuong long, Ba bet long dung, Nhot vang, Chua nga, Choi moi nep, Nhung dien hooker, Ruoi hooker	T	V (N to S), Ch, NG	100–500	100–900	ND	L, S: pentacyclic triterpenoids[26], casbane-type diterpenoid lactones[27]	ND
Mallotus lanceolatus (Gagnep) Airy Shaw	*Coelodiscuslanceolatus* Gagnep	Babet thon, Ruoi thon, Nhung dien thon	Sm T, Sh	V (N to S), Ca, L, Th	100–500	ND	ND	ND	ND
Mallotus luchenensis F.P. Metcalf.	*M. barbatus* var. *barbatus*, *M. barbatus* var. *wui* H.S. Kiu	Camlon, Bum bup, Ruoi luchen	Sm T, Sh	V(N), Ch	100–800	200–1,300	ND	ND	ND
Mallotus macrostachyus (Miq.) Müll. Arg.	*Rottlera macrostachya* Miq.	Babet chum to, Bum bup bong to, Buc chum to, Ruoi duoi to, Ruoi trang, Nhung dien duoi to, Nhung dien trang	T	V (N to S), Ind, Indo, Mal, Phi, Th	100–500	ND	L: ac, hem, wo	ND	ND

Table 1 continued

Botanical name	Synonyms[a]	Vietnamese vernacular names	Plant[b]	Distribution[c]	Altitude Vietnam (m)	Altitude China (m)	Traditonal uses[d,e]	Phytochemistry[f,g]	Pharmacological activities[g]
Mallotus metcalfianus Croizat	ND	Babet do, Ba bet mecalf, Ruoi mecalf	Sm T	V (N to S), Ch	100–1,000	100–1,900	ND	Wp: flavonoids, policosanol, flavonolignanes, pentacyclic triterpenoids, phenolic acids, megastigmane[28]	ND
Mallotus microcarpus Pax & K. Hoffm.	ND	Babet qua nho, Ruoi trai nho	Sm T, Sh	V (N to C), Ch	100–500	200–1,000	ND	ND	ND
Mallotus mollissimus (Vahl ex Geiseler) Airy Shaw	*Croton mollissimus* Vahl ex Geiseler	Bucnau, Babet nau, Ruoi mem, Buc qua thau dau	Sm T	V (N to S), Austr, Ca, Indo, L, Mal, NG, Phi	100–500	ND	ND	ND	ND
Mallotus nanus Airy Shaw	ND	Ba bet lun, Ruoi thorel	Sm T, Sh	V(C), Ca, L	100–500	ND	ND	ND	ND
Mallotus oblongifolius (Miq.) Müll. Arg.	*Rottlera oblongifolia* Miq., *Hancea muricata* Benth., *M. alternifolius* Merr., *M. columnaris* Warb., *M. furetianus* Müll. Arg., *M. helferi* Müll. Arg., *M. maclurei* Merr., *M. oblongifolius* var. *helferi* (Müll. Arg.) Pax & K. Hoffm., *M. odoratus* Elmer, *M. proterianus* Müll. Arg., *M. puberulus* Hook. f.	Choc mon, Choc moc, Choc mot, Cam heo, Ruoi tron dai	Sm T, Sh	V (N to S) Austr, Ca, Ind, Indo, L, Mal, Mya, NG, Phi, Th	100–800	ND	R: ma Wp: dh, ga	ND	ND
Mallotus oreophilus Müll. Arg.	*M. japonicuss* var. *oreophilus* (Müll. Arg.) S.M. Hwang	Babet nui cao	Sm T, Sh	V (Lao Cai province), Ch, Ind	700–2,000	600–2,000	ND	ND	ND
Mallotus pallidus (Airy Shaw) Airy Shaw	*M. philippensis* var. *menglianensis* C.Y. Wu ex S.M. Hwang, *M. philippinensis* var. *pallidus* Airy Shaw	Babet tai	Sm T, Sh	V (N to C), Ch, Th	100–500	1,200–1,400	ND	L: phloroglucinol derivatives[29,30,31]	Antiviral HIV-1, HSV-1, HSV-2: phloroglucinol derivatives[31]

Table 1 continued

Botanical name	Synonyms[a]	Vietnamese vernacular names	Plant[b]	Distribution[c]	Altitude Vietnam (m)	Altitude China (m)	Traditonal uses[d,e]	Phytochemistry[f,g]	Pharmacological activities[g]
Mallotus paniculatus (Lam.) Müll. Arg.	*Croton paniculatus* Lam., *Echinus trisulcus* Lour., *M. chinensis* Müll. Arg., *M. cochinchinensis* Lour., *M. formosanus* Hayata, *M. paniculatus* var. *formosanus* (Hayata) Hurus., *Rottlera paniculata* (Lam.) A Juss.	Buc bac, Bong bet, Bai dai, Bum bup nau, Bung buc nau, Ba bet nam do, Bach thu	Sm T, T	V (N to S), NE Austr, Ca, Ch, Ind, Indo, L, Mal, Mya, NG, Phi, Th	100–1,650	100–1,300	F: co, oe R: gy Wp: fe, hea, wo	L: pentacyclic triterpenoids and steroids[32] SE: cardenolides[33,34], fatty acids[35] S: pentacyclic triterpenoids and steroids[26,32]	ND
Mallotus peltatus (Geiseler) Müll. Arg.	*Aleurites peltata* Geiseler, *Rottlera oblongifolia* Miq., *Hancea muricata* Benth., *M. furetianus* Müll. Arg., *M. maclurei* Merr., *M. oblongifolius* (Miq.) Müll. Arg.	Babet long, Ruoi long	Sm T	V (LC and South provinces), Ch, Ind, Indo, Mal, Mya, NG, Phi, Th	1,000–1,500	200–1,000	ND	L: tannins, triterpenoids and steroids, saponins, reducing sugars[36,37]	Antibacterial[36,38] Anti-inflammatory[36,38] Antipyretic[39] Neuropharmacological[37]
Mallotus philippinensis (Lamk.) Müll. Arg.	ND	Canhkien, Mot, Rum nao, Ba chia, Thuoc san, Tho khang sai, Rum hao	Sm T, Med T	V (N to S), Austr, Ca, Ind, Indo, L, Mal, Mya, Phi, Th, SL	100–500	ND	B: an, antis, cu, fe, hem, wo F: antis, cu, fe, ga, hem, oe, pa, sy L: cu, dh, diu, dy, wo R: antic, antis, dh, dy, fe, hem SE: diz, ve	B: phenolic compounds, condensed tannins[40,41], triterpenoids[42] F: dimeric chalcones derivatives[43,44], phenolic compounds[40] HW: triterpenoids[42], isocoumarin (bergenin)[42] L: tannins[45], bergenin[42] SB: pentacyclic triterpenoids and steroids[46]	Antioxidant[40,41] Antibacterial and antifungal[51,52] Bactericidal (*Helicobacter pylori*): rottlerin[53] Anti-inflammatory, immunoregulatory: chalcones[44] Antifertility[54]: rottlerin[55] Proteine kinase inhibitor PKCδ: rottlerin[50,56] Anthelminthic in ruminants[57] Anticestodal in beetal goats[58]

Table 1 continued

Botanical name	Synonyms[a]	Vietnamese vernacular names	Plant[b]	Distribution[c]	Altitude Vietnam (m)	Altitude China (m)	Traditonal uses[d,e]	Phytochemistry[f,g]	Pharmacological activities[g]
								SE: cardenolides[47], kamala oil (kamlolenic acid and hydroxy acids)[48]	
								Wp: flavonoids, phloroglucinol derivatives (rottlerin)[49,50]	
Mallotus pierrei (Gagnep) Airy Shaw	*Coelodiscus pierrei* Gagnep	Nhungdien pierre, Ruoi pierre	Sm T	V (South provinces DN, BRVT), Th	100–600	ND	ND	ND	ND
Mallotus poilanei Gagnep.	ND	Sita, Sito	Sm Sh	V (Central provinces)	100–500	ND	L: hea	ND	ND
Mallotus repandus (Rottler) Müll. Arg.	*Croton repandus* Willd.	Buc buc truon, Buc buc leo, Bum bup truon, Bum bup leo, Ruoi tran, Nhung dien bai	Sm T, Sh	V (N to S), N Austr, Ca, Ch, Ind, Indo, L, Mal, Mya, NG, Phi, SL, Th	100–500	100–1,000	L: sc, pimp R: fe, infl Wp: co, se	AP: iso-coumarin (bergenin)[59], cyano-γ-pyridone (mallorepine)[59] L: hydrolyzable tannins[60] S: triterpenoids[61], bergenin[61,62], D: A friedo-oleanane lactones[63] RB: triterpenoids, bergenin[61] Wp: diterpenic lactones[64,65], triterpenoids[66]	Antiradical[67] Antiviral HIV-1: hydrolyzable tannins[68]Antiulcerogenic: bergenin[59] Antitumor[69] Uterus muscle stimulant[70] Antihepatotoxic[71]
Mallotus resinosus (Blanco) Merr.	*Adelia resinosa* Blanco	Nhungdien mut, Ruoi resin	T	V (N to S), Ca, Ind, Indo, Mal, NG, Phi, SL	100–500	ND	ND	R: coumarins (scopoletin)[72]	DNA cleavage[72]
Mallotus sathayensis Thin	ND	Babet sa thay	T	Endemic V (Central province KT)	100–500	ND	ND	ND	ND

Table 1 continued

Botanical name	Synonyms[a]	Vietnamese vernacular names	Plant[b]	Distribution[c]	Altitude Vietnam (m)	Altitude China (m)	Traditonal uses[d,e]	Phytochemistry[f,g]	Pharmacological activities[g]
Mallotus spodocarpus Airy Shaw	ND	Babet set, Ruoi trai set kem	Sm Sh	V (South province NT), Th	100–500	ND	ND	ND	Anti-inflammatory, analgesic[73]
Mallotus thorelii Gagnep.	ND	Nhungdien thorel, Ruoi thorel	Sh, Sm T	V (South province KG), Ca, Ch, L, Th	100–500	1,200–1,300	ND	ND	ND
Mallotus tsiangii Merr. & Chun	*Macaranga lowii* King ex Hook. f.	Ruoi tsiang	Sm Sh	V (North province VP), Ch	500–1,000	100–500	ND	ND	ND
Mallotus ustulatus (Gagnep.) Airy Shaw	*Coelodiscus ustulatus* Gagnep.	Babet lua, Ruoi cui	Sm Sh	V (C to S), Ca	100–500	ND	ND	ND	ND
Mallotus yunnanensis Pax & K. Hoffm.	*Mallotus hainanensis* S.M. Hwang	Babet van nam, Ruoi van nam	Sm Sh	V(N), Ch	100–500	100–1,400	ND	ND	ND

ND no data

[a] Synonyms: Missouri Botanical Garden website: http://www.tropicos.org/ (basionyms, synonyms or accepted names)

[b] Plant: *T*, tree; *Sm T*, small tree; *Med T*, medium tree; *Sh*, shrubs, *Sm Sh*, small shrubs

[c] Distribution: *AG*, An Giang province; *Ba*, Ria-Vung Tau province; *Aust*, Australia; *C*, Center; *Ca*, Cambodia; *Ch*, China; *DN*, Dong Nai province; *GL*, Gia Lai province; *HB*, Hoa Binh; *HT*, Ha Tai province; *Ind*, India; *Indo*, Indonesia; *KG*, Kien Giang province; *KH*, Khanh Hoa province; *KT*, Kon Tum; *L*, Laos; *LC*, Lao Cai province; *Mal*, Malaysia; *Mya*, Myamar; *N*, North; *NG*, New Guinea; *NT*, Ninh Thuan; *Phi*, Philippines; *S*, South; *SE A*, South-East Asia; *SL*, Sri Lanka; *Th*, Thailand; *TTH*, Thua Thien Hue province; *V*, Vietnam; *VP*, Vinh Phuc

[d] Parts used: *B*, bark; *C*, branches; *F*, fruits; *L*, leaves; *R*, roots; *SE*, seeds; *Wp*, whole plant

[e] Vietnamese traditional usage: *ac*, acne; *ai*, antiinflammatory; *an*, analgesic; *antic*, anticonvulsivant; *antis*, antiseptic; *cho*, cholera; *co*, contusions and traumatic injuries; *cou*, cough; *cu*, cutaneous diseases; *dh*, diarrhea; *diu*, diuretic; *diz*, dizziness; *dy*, dysentery; *fe*, fever; *ga*, gastrointesinal disorders; *gy*, gynecological infection; *hea*, headache; *hem*, hemostatic; *hep*, hepatic diseases; *infl*, influenza; *ma*, malaria; *oe*, oedema; *ot*, otitis; *pa*, parasiticid; *pimp*, pimple; *sc*, scabies; *se*, sedative; *sy*, syphilis; *ve*, vertigo; *wo*, wounds

[f] Parts studied: *A*, aerial parts; *B*, bark; *C*, branches; *F*, fruits; *HW*, heartwood; *L*, leaves; *R*, roots; *RB*, root bark; *S*, stems; *SB*, stem bark; *SE*, seeds; *T*, twigs; *Wp*, whole plant

[g] Sources: [1] An et al. (2001), [2] An et al. (2003), [3] Van Chau et al. (2005a), [4] Van Kiem et al. (2005), [5] Van Kiem et al. (2004), [6] Van Chau et al. (2004), [7] Van Chau et al. (2005b), [8] Van Chau et al. (2005c), [9] Wu et al. (2006), [10] Zhu et al. (2007), [11] Van Chau et al. (2005d), [12] Kang and Lu (2007), [13] Shan et al. (1985), [14] Cheng et al. (1998), [15] Qi et al. (2005), [16] Cheng and Chen (2000), [17] Xu et al. (2008), [18] Cheng et al. (1999), [19] Cheng and Chen (1999), [20] Xu et al. (2006), [21] Zhao et al. (2002), [22] Ono et al. (1989), [23] Sasak and Chonjnacki (1973), [24] Nguyen et al. (1997), [25] Groweiss et al. (1994), [26] Hui and Li (1976), [27] Bai et al. (2006), [28] Rivière et al. (2009), [29] Supudompol et al. (2004), [30] Likhitwitayawuid et al. (2005), [31] Likhitwitayawuid and Supudompol (2005), [32] Hui et al. (1969), [33] Roberts et al. (1966), [34] Roberts et al. (1967), [35] Yu et al. (1991), [36] Chattopadhyay et al. (2002a), [37] Chattopadhyay et al. (2003), [38] Chattopadhyay et al. (2006), [39] Chattopadhyay et al. (2002b), [40] Arfan et al. (2007), [41] Arfan et al. (2009), [42] Bandopadhyay et al. (1972), [43] Tanaka et al. (1998), [44] Daikonya et al. (2004), [45] Saijo et al. (1989b), [46] Nair and Rao (1993), [47] Roberts et al. (1963), [48] Gupta et al. (1953), [49] Lounasmaa et al. (1975), [50] Gschwendt et al. (1994), [51] Kumar et al. (2006), [52] Moorthy et al. (2007), [53] Zaidi et al. (2009), [54] Thakur et al. (2005), [55] Gujral et al. (1960), [56] Liao et al. (2005), [57] Jabbar et al. (2006), [58] Akhtar and Ahmad (1992), [59] Hikino et al. (1978), [60] Saijo et al. (1989a), [61] Huang et al. (1999), [62] Tomizawa et al. (1976), [63] Sutthivaiyakit et al. (2001), [64] Nakatsu et al. (1981), [65] Kawashima et al. (1976a), [66] Hui and Li (1977), [67] Lin et al. (1995), [68] Ogata et al. (1992), [69] Kawashima et al. (1976b), [70] Kawashima et al. (1975), [71] Yang et al. (1987), [72] Ma et al. (2004), [73] Intahphuak et al. (2004)

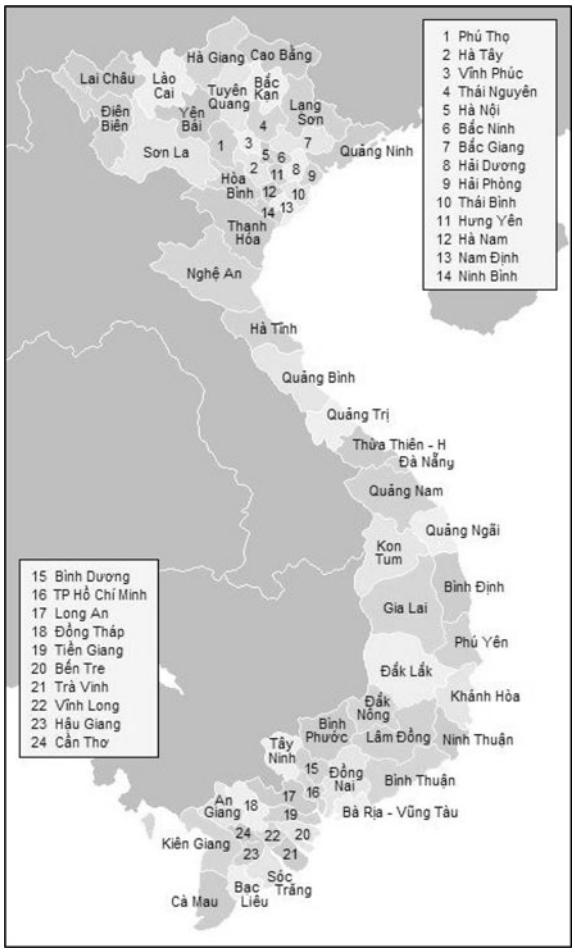


Fig. 1 Vietnamese provinces. http://commons.wikimedia.org/wiki/Image:VietnameseProvincesMapTiengViet.png(GNU_Free_Documentation_License)

et al. 2006). In 1976, two diterpenic lactones named mallotucin A and B (**9–10**) were obtained from *M. repandus* (Kawashima et al. 1976a). In 1981, Nakatsu et al. reported the isolation of three diterpenic lactones of which two were new from *M. repandus*: mallotucin B, C, and D (**10–12**).

Cardenolides (Table 3)

The seeds of *M. paniculatus* and *M. philippinensis* contain cardenolides. From the seeds of *M. paniculatus*, after fermentation, seven cardenolides were isolated, of which four were genins: two known (**18–19**), two new (**13–14**), and three were glycosides (**15–17**) (Roberts et al. 1966, 1967).

The seeds of *M. philippinensis* were found to contain after fermentation four cardenolides (**19–22**), of which two were new: corotoxigenin L-rhamnoside and coroglaucigenin L-rhamnoside (Roberts et al. 1963).

Carotenoids (Table 4)

β-Carotene and lutein (**23–24**) were isolated from the methanolic extract of the dried leaves of *M. apelta* (Van Chau et al. 2005b).

Iridoids (Table 5)

An iridoid, mussaenoside (**25**), was obtained from the ethyl acetate extract of the stems of *M. apelta* (Qi et al. 2005).

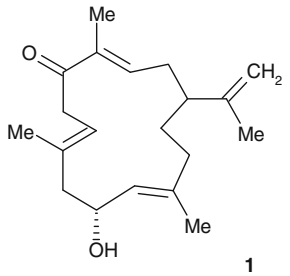
Polyprenols

In 1973, polyprenols were isolated from the leaves of *M. barbatus* (Sasak and Chonjnacki 1973). They were of 14–20 isoprene residues chain-length and they occurred in the form of acetic acid esters. The presence of long-chain polyprenols is frequent in leaves. It has been observed that the content of polyprenols in leaves increases with the age of the leaf and that in some species the age-dependent accumulation of polyprenols may attain extremely high values (Ranjan et al. 2001). In 2005, Van Chau et al. (2005d) reported the isolation of betulaprenol from *M. apelta*.

Triterpenoids (Tables 6, 7, 8)

Some pentacyclic triterpenoids with a 6/6/6/6/5 ring system (Table 6) were reported in some *Mallotus* species. A known triterpenoid, hennadiol (**26**) and a new, malloapelta A (**28**), were isolated from the methanolic extract of the dried leaves of *M. apelta* (Van Kiem et al. 2004; Van Chau et al. 2005d), whereas 3β,29-dihydroxylupane (**27**) was obtained from the roots of *M. apelta* (Shan et al. 1985). In 1976, Hui and Li reported the isolation of 29-nor-21αH-hopane-3,22-dione (**29**) from the stems of *M. paniculatus*. The petroleum ether extract of the heartwood of *M. philippinensis* yielded triterpenoids: betulin-3-acetate (**30**) as a major compound, lupeol acetate (**31**) and lupeol (**32**) (Bandopadhyay et al.

Table 2 Diterpenoids and diterpenic lactones



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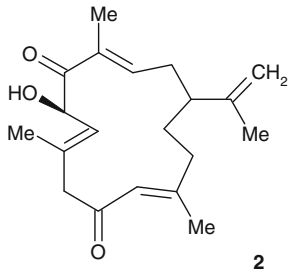

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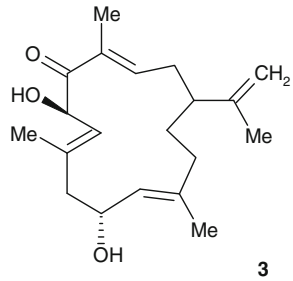

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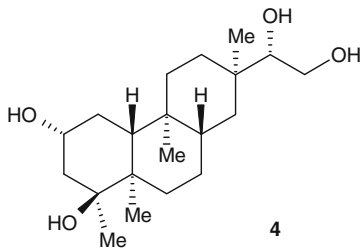

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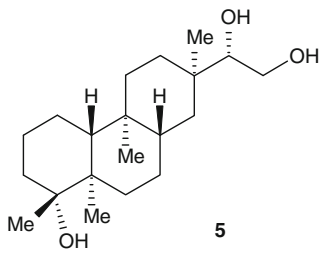

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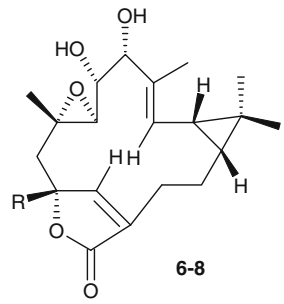

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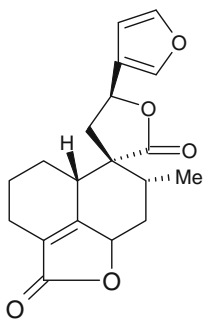

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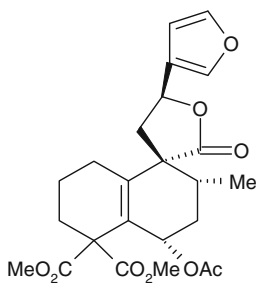

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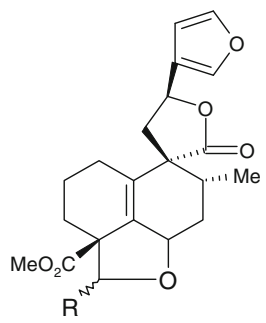
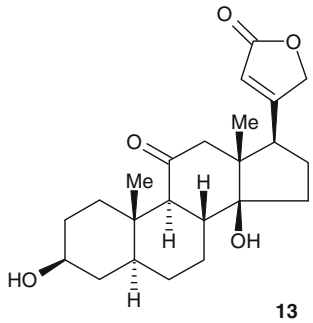


11-12

No.	Name	R	Plant	Ref.
1	10-Hydroxycembren-5-one		*M. apelta*	Cheng et al. (1999) and Cheng and Chen 1999)
2	6-Hydroxycembrene-5,10-dione		*M. apelta*	Cheng et al. (1999) and Cheng and Chen 1999
3	6,10-Dihydroxycembrene-5-one=malloapeltene		*M. apelta*	Cheng et al. (1999) and Cheng and Chen 1999
4	2α,4β,15,16-Tetrahydroxydolabradane		*M. apelta*	Cheng et al. (1999) and Cheng and Chen 1999
5	4α,15,16-Tetrahydroxydolabradane=malloapeltin		*M. apelta*	Cheng et al. (1999) and Cheng and Chen 1999
6	Hookerianolide A	OH	*M. hookerianus*	Bai et al. (2006)
7	Hookerianolide B	H	*M. hookerianus*	Bai et al. (2006)
8	Hookerianolide C	OC_2H_5	*M. hookerianus*	Bai et al. (2006)
9	Mallotucin A		*M. repandus*	Kawashima et al. (1976a)
10	Mallotucin B		*M. repandus*	Kawashima et al. (1976a)
				Nakatsu et al. (1981)
11	Mallotucin C	β-OH	*M. repandus*	Nakatsu et al. (1981)
12	Mallotucin D	α-OH	*M. repandus*	Nakatsu et al. (1981)

Table 3 Cardenolides



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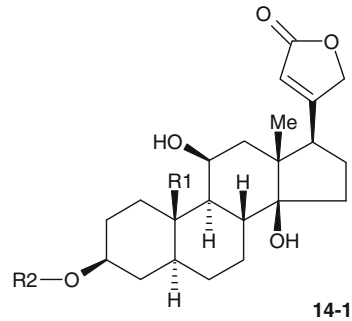
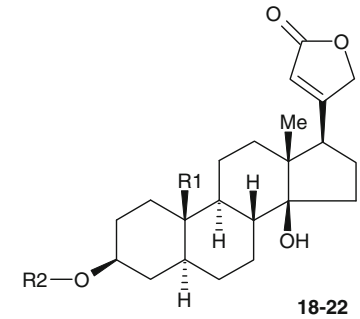


14-17



18-22

No.	Name	R1	R2	Plant	Ref.
13	5-Desarogenin			*M. paniculatus*	Roberts et al. (1966, 1967)
14	Mallogenin	CH_3	H	*M. paniculatus*	Roberts et al. (1966, 1967)
15	Malloside	CH_3	L-rham	*M. paniculatus*	Roberts et al. (1966, 1967)
16	Panoside	CH_2OH	L-rham	*M. paniculatus*	Roberts et al. (1966, 1967)
17	Glucopanoside	CH_2OH	Glc	*M. paniculatus*	Roberts et al. (1966, 1967)
18	Uzarigenin	CH_3	H	*M. paniculatus*	Roberts et al. (1966, 1967)
19	Coroglaucigenin	CH_2OH	H	*M. paniculatus*	Roberts et al. (1966, 1967)
				M. philippinensis	Roberts et al. (1963)
20	Coroglaucigenin L-rhamnoside	CH_2OH	L-rham	*M. philippinensis*	Roberts et al. (1963)
21	Corotoxigenin	CHO	H	*M. philippinensis*	Roberts et al. (1963)
22	Corotoxigenin L-rhamnoside	CHO	L-rham	*M. philippinensis*	Roberts et al. (1963)

1972). Lupeol was also obtained from *M. repandus* (Hui and Li 1977).

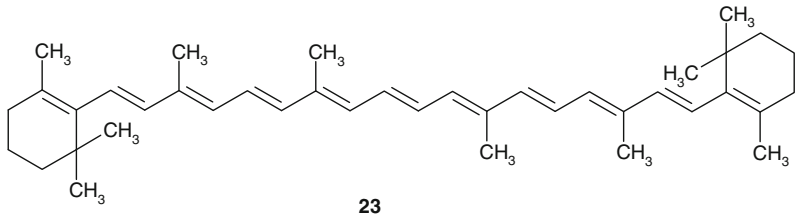
Pentacyclic triterpenoids with a 6/6/6/6/6 ring system are more often mentioned (Tables 7, 8). Friedelane-type triterpenoids are common in *Mallotus* species. Friedelin (**33**) was obtained from several *Mallotus* species: from the leaves of *M. apelta* (Van Kiem et al. 2004; Van Chau et al. 2005d), from the leaves and stems of *M. hookerianus* (Hui and Li 1976), from the leaves of *M. paniculatus* (Hui et al. 1969), from the stem bark of *M. philippinensis* (Nair and Rao 1993) and from *M. repandus* (Hui and Li 1977). Friedelin is common to many genera of Euphorbiaceae such as *Drypetes* (Wansi et al. 2006) or *Celaenodendron* (Castenada et al. 1993) and is also found in plants from other orders.

Friedelinol (**34**) was isolated from the leaves of *M. apelta* (Van Kiem et al. 2004; Van Chau et al. 2005d) and from *M. metcalfianus* (Rivière et al. 2009), whereas epifriedelanol (**35**) was isolated from the leaves of *M. apelta* (Van Kiem et al. 2004; Van Chau et al. 2005d), from the leaves and stems of *M. hookerianus* (Hui and Li 1976) and from the leaves of *M. paniculatus* (Hui et al. 1969). Three new D:A-friedo-oleanane lactones (**36–38**) were isolated from the stems of *M. repandus* (Sutthivaiyakit et al. 2001).

Other known pentacyclic terpenoids were detected in different *Mallotus* species: taraxerone (**39**), taraxerol (**40**) and epitaraxerol (**41**) in the leaves of *M. apelta* (Van Kiem et al. 2004; Wu et al. 2006; Van Chau et al. 2005d), erythrodiol-3-acetate (**42**) in the roots of *M. apelta* (Shan et al. 1985), acetylaleuritolic acid (**43**) in the stems of *M. apelta* (Qi et al. 2005) and in the petroleum ether and ether extracts of bark of *M. philippinensis* (Bandopadhyay et al. 1972). The first olean-18-ene triterpene oxidized at C-22 (**44**) was isolated from the stem bark of *M. philippinensis* (Nair and Rao 1993).

Several ursane-type triterpenoids were also isolated from *Mallotus* species: α-amyrin (**45**) from the petroleum ether and ether extracts of bark of

Table 4 Carotenoids



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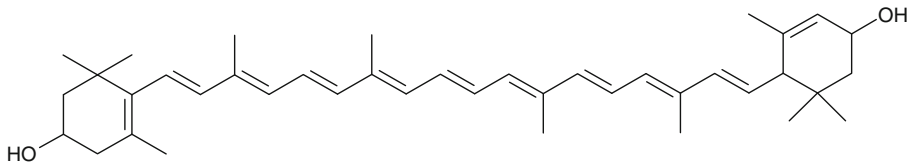
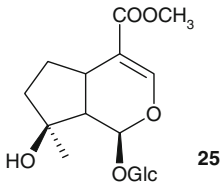


24

No.	Name	Plant	Ref.
23	*β*-Carotene	*M. apelta*	Van Chau et al. (2005b)
24	Lutein	*M. apelta*	Van Chau et al. (2005b)

Table 5 Iridoids



25

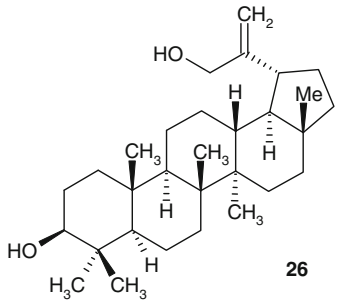
No.	Name	Plant	Ref.
25	Mussaenoide	*M. apelta*	Qi et al. (2005)

M. philippinensis (Bandopadhyay et al. 1972) and from *M. repandus* (Hui and Li 1977), ursolic acid (**46**) from the ethyl acetate extract of the stems of *M. apelta* (Qi et al. 2005), from *M. peltatus* (Chattopadhyay et al. 2002a, 2003) and from the stems and root bark of *M. repandus* (Hui and Li 1977; Huang et al. 1999), ursolic acid acetate (**47**) from the roots of *M. apelta* (Shan et al. 1985), 12-ursen-3-one (**48**) and 3-hydroxy-12-ursen (**49**) from the ethyl acetate extract of the stems of *M. apelta* (Qi et al. 2005). In 1976, Hui and Li reported the isolation of two new triterpene acids (**50–51**) from the ethanolic extract of the leaves of *M. hookerianus* (Hui and Li 1976). In 1977, the new triterpenes 3α-hydroxy-13α-ursan-28,12*β*-olide (**52**), 3*β*-hydroxy-13α-ursan-28, 12*β*-olide (**54**) and its benzoate (**55**) were isolated from *M. repandus* (Hui and Li 1977). In 1999, Huang et al. reported the isolation of three new triterpenoids, 3α-hydroxy-13α-ursan-28,12*β*-olide 3-benzoate (**53**), 3α-hydroxy-13α-ursan-28-oic acid (**56**) and 3α-hydroxy-28*β*-methoxy-13α-ursan-28,12*β*-epoxide 3-benzoate (**57**) from the stems and root bark of *M. repandus* (Huang et al. 1999).

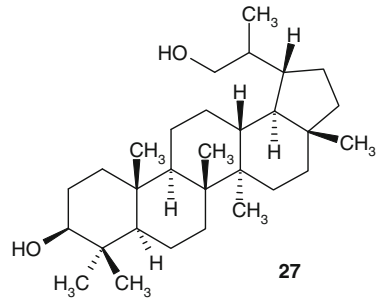
Steroids (Table 9)

Common steroids are mentioned in *Mallotus* species. *β*-Sitosterol (**58**) was isolated from the roots, the stems and the leaves of *M. apelta* (Shan et al. 1985; Qi et al. 2005; Wu et al. 2006), from the leaves and stems of *M. hookerianus* (Hui and Li 1976), from the stems of *M. paniculatus* (Hui et al. 1969), from *M. peltatus* (Chattopadhyay et al. 2002a, 2003, 2006), from the petroleum ether extracts of the heartwood and bark of *M. philippinensis* (Bandopadhyay et al. 1972). Daucosterol (**59**) was obtained from the leaves and the stems of *M. apelta* (Van Chau et al. 2004; Qi et al. 2005) and from the ether extract of the bark of *M. philippinensis* (Bandopadhyay et al. 1972).

Table 6 6/6/6/6/5-Ring triterpenoids



26



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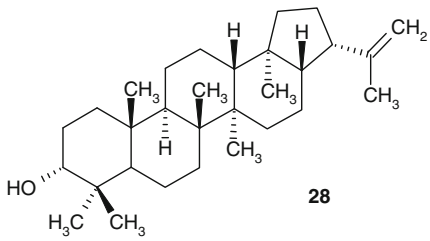
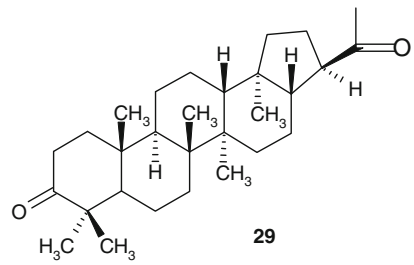


28



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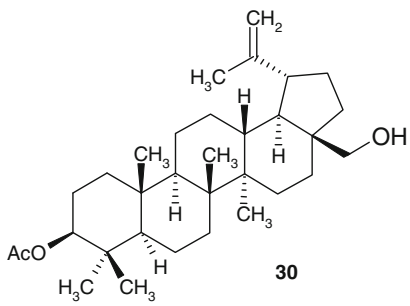

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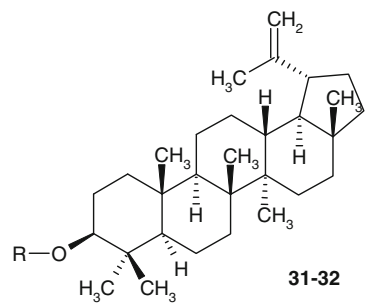
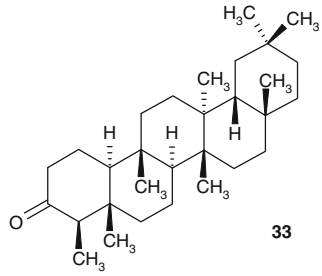
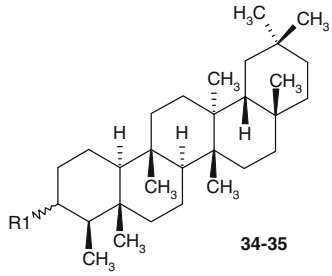


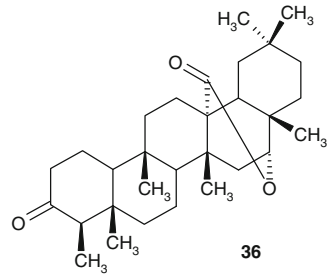
31-32

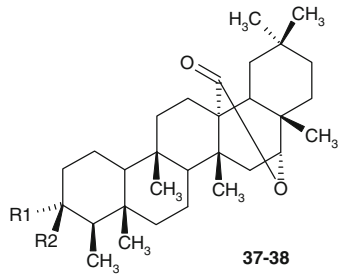
No.	Name	R	Plant	Ref.
26	Hennadiol		*M. apelta*	Van Kiem et al. (2004)
				Van Chau et al. (2005d)
27	3β,29-Dihydroxylupane		*M. apelta*	Shan et al. (1985)
28	3α,Hydroxyhop-22(29)-ene or malloapeltaA		*M. apelta*	Van Kiem et al. (2004)
29	29-Nor-21αH-hopan-3,22-dione		*M. paniculatus*	Hui and Li (1976)
30	Betulin-3-acetate		*M. philippinensis*	Bandopadhyay et al. (1972)
31	Lupeol-3-acetate	Ac	*M. philippinensis*	Bandopadhyay et al. (1972)
32	Lupeol	H	*M. philippinensis*	Bandopadhyay et al. (1972)
			M. repandus	Hui and Li (1977)

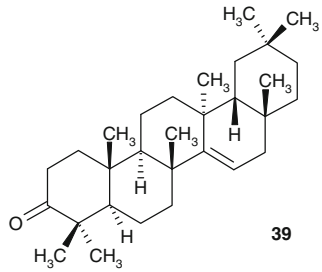
Table 7 6/6/6/6/6-Ring triterpenoids (1)

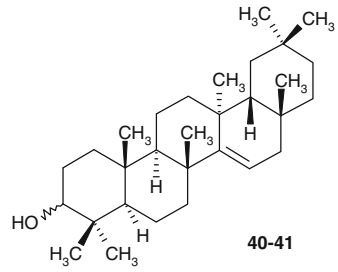


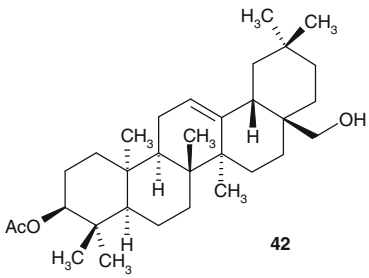


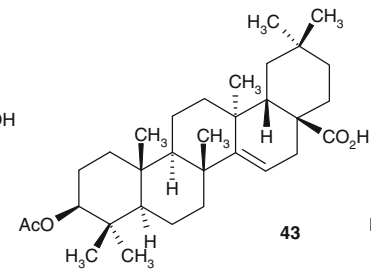


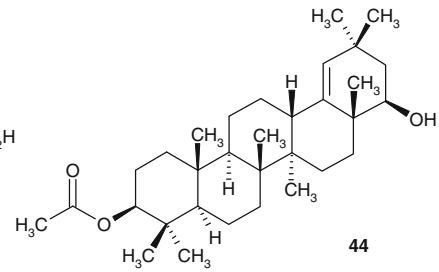












No.	Name	R1	R2	Plant	Ref.
33	Friedelin			*M. apelta*	Van Kiem et al. (2004) and Van Chau et al. (2005d)
				M. hookeriauns	Hui and Li (1976)
				M. paniculatus	Hui et al. (1969)
				M. philippinensis	Nair and Rao (1993)
				M. repandus	Hui and Li (1977)
34	Friedelinol or friedelin-3α-ol or friedelanol	α-OH		*M. apelta*	Van Kiem et al. (2004) and Van Chau et al. (2005d)
				M. metcalfianus	Rivière et al. (2009)
35	Epifriedelinol or friedelin-3β-ol or epifriedelanol	β-OH		*M. apelta*	Van Kiem et al. (2004) Van Chau et al. (2005d)
				M. hookerianus	Hui and Li (1976)
				M. paniculatus	Hui et al. (1969)
36	3-Oxo-D:A-friedo-oleanan-27,16α-lactone			*M. repandus*	Sutthivaiyakit et al. (2001)

Table 7 continued

No.	Name	R1	R2	Plant	Ref.
37	3α-Benzoyloxy-D:A-friedo-oleanan-27,16α-lactone	O–C(=O)Ph	H	*M. repandus*	Sutthivaiyakit et al. (2001)
38	3β-Hydroxy-D:A-friedo-oleanan-27,16α-lactone	H	OH	*M. repandus*	Sutthivaiyakit et al. (2001)
39	Taraxerone			*M. apelta*	Van Kiem et al. (2004)
					Van Chau et al. (2005d)
40	Taraxerol	β-OH		*M. apelta*	Wu et al. (2006)
41	Epitaraxerol	α-OH		*M. apelta*	Van Kiem et al. (2004)
					Van Chau et al. (2005d)
42	Erythrodiol-3-acetate			*M. apelta*	Shan et al. (1985)
43	Acetylaleuritolic acid or aleuritolic acid acetate			*M. apelta*	Qi et al. (2005)
				M. philippinensis	Bandopadhyay et al. (1972)
44	3β-Acetoxy-22β-hydroxyolean-18-ene			*M. philippinensis*	Nair and Rao (1993)

Ergosterol (**60**) was reported in the leaves of *M. apelta* (Van Chau et al. 2004), as well as stigmasterol (**61**). This last compound was also mentioned in the stems of *M. paniculatus* (Hui et al. 1969).

Other terpenoids (Table 10)

Squalene (**62**) and *trans*-phytol (**63**) were isolated from the methanolic extract of the leaves of *M. apelta* (Van Chau et al. 2004).

Phenolic compounds

Coumarins, isocoumarins and coumarinolignoids (Table 11)

Scopoletin (**64**), a simple coumarin, was detected in *M. resinosus* (Ma et al. 2004). Isoscopoletin (**65**) was obtained from the leaves of *M. apelta* (Kang and Lu 2007). Isopimpinellin (**66**), a furanocoumarin, was reported in the leaves of *M. apelta* (Van Chau et al. 2005d). Bergenin (**67**), an isocoumarin, was isolated in 1972, from the heartwood of *M. philippinensis*. This compound was also obtained from the bark and the leaves of *M. philippinensis* (Bandopadhyay et al. 1972). In 1976, Tomizawa et al. reported also the isolation of this same isocoumarin from *M. repandus*. Bergenin was also isolated in 1999 by Huang et al. (1999) from the stems and root bark of *M. repandus*.

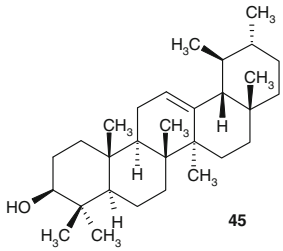
In 2000, three coumarino-lignoids, aquillochin (**74**), cleomiscosin A (**69**) and 5′-demethylaquillochin (**73**) were isolated from *M. apelta* (Cheng and Chen 2000).

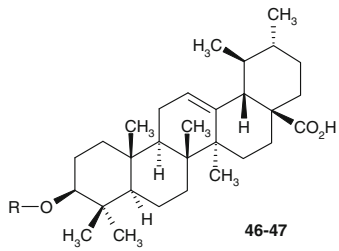
In 2008, three new coumarinolignoids, malloapelins A–C (**68**, **71**, **72**), together with three known coumarinolignoids (**69–71**), were isolated from the roots of *Mallotus apelta*. These compounds are three pairs of regioisomeric coumarinolignoids (Xu et al. 2008).

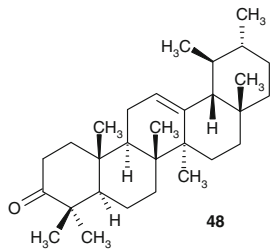
Flavonoids: flavonols, flavones, chalcones, flavonolignanes (Tables 12, 13)

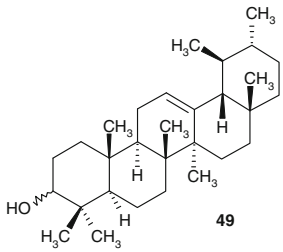
Flavonols glycosides such as quercitrin (**75**), were isolated from several *Mallotus* species: *M. apelta* (Van Chau et al. 2004), *M. metcalfianus* (Rivière et al. 2009), or identified, in a recent study conducted in our laboratory, in *M. nanus*, *M. cuneatus*, *M. paniculatus* (unpublished). Quercitrin was also obtained from other Euphorbiaceae genera: *Alchornea* (Manga et al. 2004), *Euphorbia* (Liu et al. 2007), *Phyllanthus* (Fang et al. 2008) and *Pedilanthus* (Abreu et al. 2008) but also in many plants from other families. Similarly, kaempferol glycosides have been described in some species of the Euphorbiaceae, for example in the genera *Euphorbia* (Saleh 1985) and *Acalypha* (Nahrstedt et al. 2006) but also in other families. Kaempferol 3-*O*-α-L-rhamnoside (**76**) was isolated from *M. metcalfianus* (Rivière et al. 2009) and identified in our laboratory in *M. barbatus* and several samples of *M. nanus* (unpublished). Glycoside dihydroflavonols such as astilbin (**79**) was isolated from *M. apelta* (Van Chau et al. 2004) and from *M. metcalfianus* (Rivière et al. 2009). To our knowledge, astilbin has not been described in other Euphorbiaceae, thus may have some

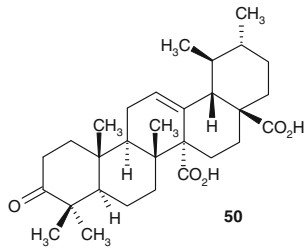
Table 8 6/6/6/6/6-Ring triterpenoids (2)

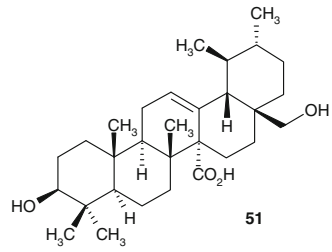


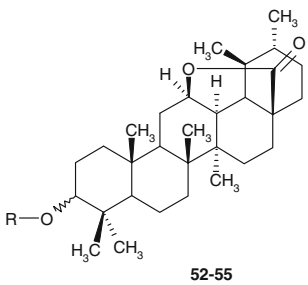


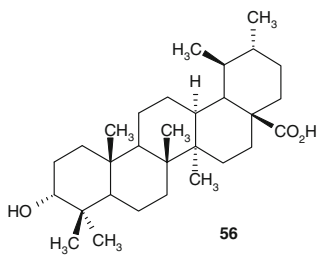


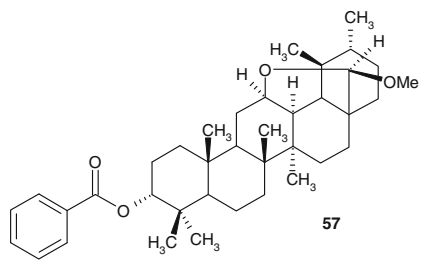










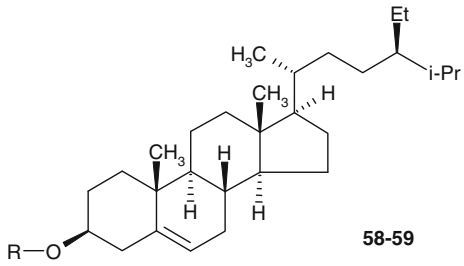


No.	Name	R	Plant	Ref.
45	α-Amyrine		*M. philippinensis*	Bandopadhyay et al. (1972)
			M. repandus	Hui and Li (1977)
46	Ursolic acid	H	*M. apelta*	Qi et al. (2005)
			M. peltatus	Chattopadhyay et al. (2002a, 2003)
			M. repandus	Hui and Li (1977) and Huang et al. (1999)
47	Ursolic acid acetate	Ac	*M. apelta*	Shan et al. (1985)
48	12-Ursen-3-one		*M. apelta*	Qi et al. (2005)
49	3-Hydroxy-12-ursen		*M. apelta*	Qi et al. (2005)
50	3-Oxours-12-ene-27,28-dioic acid		*M. hookerianus*	Hui and Li (1976)
51	3*β*,28-Dihydroxyurs-12-en-27-oic acid		*M. hookerianus*	Hui and Li (1976)
52	3α-Hydroxy-13α-ursan-28,12*β*-olide	α H	*M. repandus*	Hui and Li (1977)
53	3α-Hydroxy-13α-ursan-28,12*β*-olide 3-benzoate	α (C=O)Ph	*M. repandus*	Huang et al. (1999)
54	3*β*-Hydroxy-13α-ursan-28,12*β*-olide	*β* H	*M. repandus*	Hui and Li (1977)

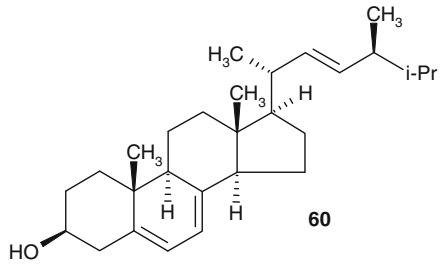
Table 8 continued

No.	Name	R	Plant	Ref.
55	3β-Hydroxy-13α-ursan-28,12β-olide 3-benzoate	β (C=O)Ph	*M. repandus*	Hui and Li (1977)
56	3α-Hydroxy-13α-ursan-28-oic acid		*M. repandus*	Huang et al. (1999)
57	3α-Hydroxy-28β-methoxy-13α-ursan-28,12β-epoxide 3 benzoate		*M. repandus*	Huang et al. (1999)

Table 9 Steroids



58-59



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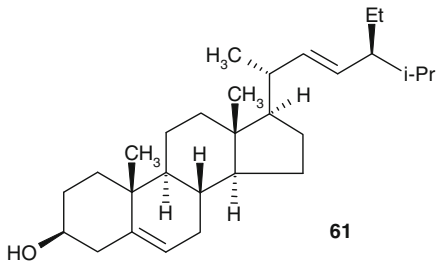
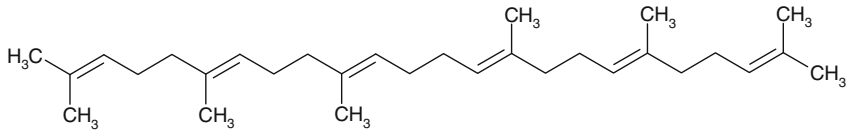


61

No.	Name	R	Plant	Ref.
58	β-Sitosterol	H	*M. apelta*	Shan et al. (1985), Qi et al. (2005) and Wu et al. (2006)
			M. hookerianus	Hui and Li (1976)
			M. paniculatus	Hui et al. (1969)
			M. peltatus	Chattopadhyay et al. (2002a, 2003, 2006)
			M. philippinensis	Bandopadhyay et al. (1972)
59	Sitosteryl β-D-glucose or daucosterol	Glc	*M. apelta*	Van Chau et al. (2004) and Qi et al. (2005)
			M. philippinensis	Bandopadhyay et al. (1972)
60	Ergosterol		*M. apelta*	Van Chau et al. (2004)
61	Stigmasterol		*M. apelta*	Van Chau et al. (2004)
			M. paniculatus	Hui et al. (1969)

chemotaxonomical interest. In a previous study, from *M. metcalfianus*, we isolated two other glycoside flavonols, quercetin 3-*O*-β-neohesperidoside (**77**) and kaempferol 3-*O*-β-neohesperidoside (**78**), but also a mixture of two pairs of new diastereoisomeric flavonolignans (±)-hydnocarpin 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside)/(±)-hydnocarpin-D 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside) with a 2:1 ratio (**86**) (Rivière et al. 2009). The isolation of these products seems to have a chemotaxonomic interest as it is the first report of a flavonolignan in this family. Hydnocarpin not substituted by a

Table 10 Other terpenoids



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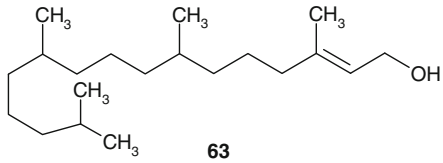


63

No.	Name	Plant	Ref.
62	Squalene	*M. apelta*	Van Chau et al. (2004)
63	*Trans*-phytol	*M. apelta*	Van Chau et al. (2005d)

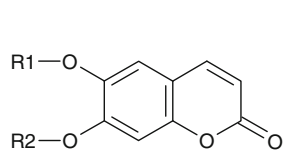
coumaric acid was also isolated from other families of the same order Malphigiales such as *Hydnocarpus wightiana* Blume (Flacourtiaceae) (Guz and Stermitz 2000) and from other families of different orders such as Ranunculales with *Meconopsis*, Papaveraceae or Sapindales with *Brucea*, Simaroubaceae (Shang et al. 2002; Pan et al. 2009). Lignans and neo-lignans are more common in Euphorbiaceae, such as in the genera *Phyllanthus* and *Trewia* (Bagalkotkar et al. 2006; Zhao and Shen 2004). We also isolated from *M. metcalfianus* two new flavones, luteolin 7-*O*-(4″-*O*-(*E*)-coumaroyl)-*β*-glucopyranoside (**80**) and chrysoeriol 7-*O*-(4″-*O*-(*E*)-coumaroyl)-*β*-glucopyranoside) (**81**). Flavonoid *p*-coumaroylglucosides are commonly found in some genera of the Lamiaceae and they are generally considered as valuable markers in this family from a chemotaxonomic point of view (Sahpaz et al. 2002). The position of the coumaroyl substitution on the glucose is often described in position 3″ or 6″ but not in position 4″ (Sahpaz et al. 2002; Karioti et al. 2003). The substitution in position 4″ is more unusual. Apigenin 7-*O*-(4″-*O*-(*E*)-coumaroyl)-*β*-glucopyranoside was described recently in the genus *Turnera* belonging to the family Turneraceae. This family belongs to the same order, Malphighiales, as the Euphorbiaceae family (Zhao et al. 2007). A few flavonoid *p*-coumaroylglucosides have been described in the Euphorbiaceae family (Zhang et al. 2002; Yuan et al. 2007). The substitution in position 4″ on glucose was also described in the genus *Cnidoscolus* with naringenin 7-*O*-(4″-*O*-(*Z*)-coumaroyl)-*β*-glucopyranoside or aromadendrin 7-*O*-(4″-*O*-(*E*)-coumaroyl)-*β*-glucopyranoside (Yuan et al. 2007). From the leaves of *M. apelta*, apigenin (**82**), apigenin-7-*O*-*β*-D-glucoside (**83**), mallotusin (**84**) were isolated (Xu et al. 2006). Vicenin II (**85**) was obtained from the butanolic extract of *M. apelta* (Zhu et al. 2007).

Two new chalcone derivatives, kamalachalcone A and B (**87–88**) with a unique ring system caused by dimerization between a dimethylchromene ring and a phenoxyl group, were isolated from kamala (*Mallotus philippinensis*) (Tanaka et al. 1998). Three other novel chalcone derivatives, mallotophilippens C, D, and E (**89–91**), were isolated from the fruits of *M. philippinensis* (Daikonya et al. 2004; Li et al. 2006).

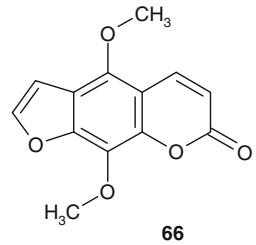
Phloroglucinol derivatives (Table 14)

Five phloroglucinol derivatives (**92–96**) were isolated from the leaves of *M. pallidus* (Supudompol et al. 2004). In 2005, a phytochemical investigation of an ethyl acetate extract of the leaves of the same species led to the isolation of a new phloroglucinol dimer, mallopallidusol (**97**) (Likhitwitayawuid and Supudompol 2005).

Table 11 Coumarins, isocoumarins and coumarinolignoids



64-65



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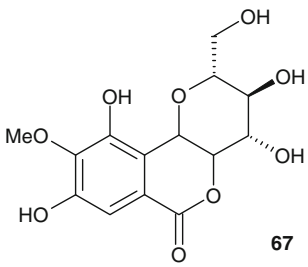
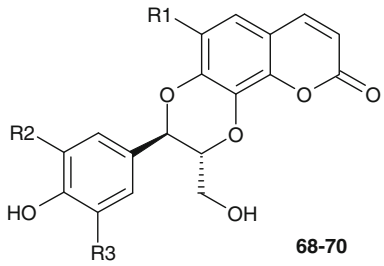
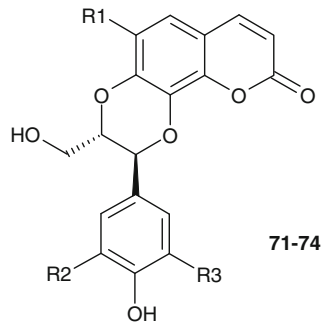


67



68-70



71-74

No.	Name	R1	R2	R3	Plant	Ref.
64	Scopoletin	CH_3	H		*M. resinosus*	Ma et al. (2004)
65	Isoscopoletin	H	CH_3		*M. apelta*	Kang and Lu (2007)
66	Isopimpinellin				*M. apelta*	Van Chau et al. (2005d)
67	Bergenin				*M. philippinensis*	Bandopadhyay et al. (1972)
					M. repandus	Tomizawa et al. (1976) and Huang et al. (1999)
68	Malloapelin A	OH	OH	OCH_3	*M. apelta*	Xu et al. (2008)
69	Cleomiscosin A	OCH_3	OH	OCH_3	*M. apelta*	Cheng and Chen (2000) and Xu et al. (2008)
70	Cleomiscosin B	OCH_3	H	OCH_3	*M. apelta*	Xu et al. (2008)
71	Malloapelin B	OH	OH	OCH_3	*M. apelta*	Xu et al. (2008)
72	Malloapelin C	OCH_3	OH	OCH_3	*M. apelta*	Xu et al. (2008)
73	5′-demethylaquillochin	OCH_3	H	OCH_3	*M. apelta*	Cheng and Chen (2000) and Xu et al. (2008)
74	Aquillochin	OCH_3	OCH_3	OCH_3	*M. apelta*	Cheng and Chen (2000)

Four phloroglucinol derivatives (kamalins) were isolated from *M. philippinensis*, including rottlerin (**98**) and isoallorottlerin (**99**) (Lounasmaa et al. 1975). Isorottlerin (**100**) was also mentioned in *M. philippinensis* (Zaidi et al. 2009).

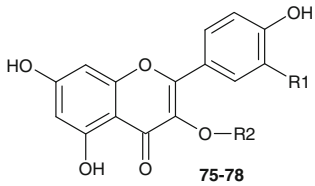
Quinones and phenolic acids (Table 15)

Chrysophanol (**101**), an anthraquinone, was isolated from the leaves of *M. apelta* (Kang and Lu 2007). Ferulic acid (**102**), a phenolic acid, was reported in *M. metcalfianus* (Rivière et al. 2009).

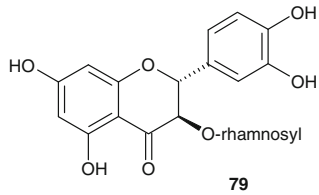
Tannins (Table 16)

A phytochemical examination of the leaves of *M. repandus* led to the isolation of four new hydrolyzable tannins, named repandusinin (**105**), repandusinic acids A and B (**106–107**) and mallotinin (**109**) together with eight other hydrolyzable tannins (**103, 108, 110–115**) and a phenolcarboxylic acid named brevifolin carboxylic acid (**104**) (Saijo et al. 1989a). 4,6,4′-Trimethyl-ellagic acid (**116**) was reported in the roots of *M. apelta* (Cheng et al. 1998).

Table 12 Flavonoids (flavonols, flavones, flavonolignans)



75-78



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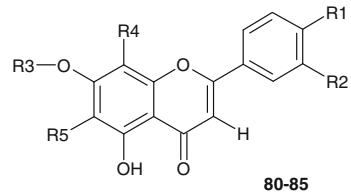
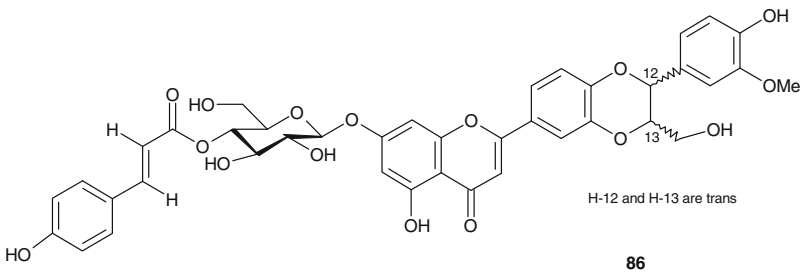
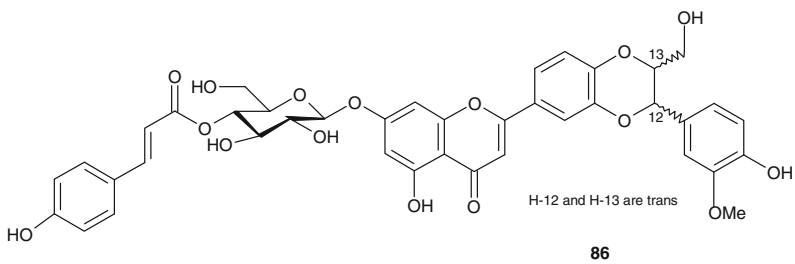


80-85



86



86

No.	Name	R1	R2	R3	R4	R5	Plant	Ref.
75	Quercitrin or quercetin 3-*O*-α-L-rhamnoside	OH	Rham				*M. apelta* *M. metcalfianus*	Van Chau et al. (2004) Rivière et al. (2009)
76	Kaempferol 3-*O*-α-L-rhamnose	H	Rham				*M. metcalfianus*	Rivière et al. (2009)
77	Quercetin 3-*O*-β-neohesperoside or quercetin 3-*O*-(2″-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside	OH	Glc-Rham				*M. metcalfianus*	Rivière et al. (2009)
78	Kaempferol 3-*O*-β-neohesperoside or kaempferol 3-*O*-(2″-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside	H	Glc-Rham				*M. metcalfianus*	Rivière et al. (2009)
79	Astilbin or dihydroquercetin 3-*O*-α-L-rhamnoside						*M. apelta* *M. metcalfianus*	Van Chau et al. (2004) Rivière et al. (2009)
80	Luteolin 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside	OH	OH	Glc-coumaroyl	H	H	*M. metcalfianus*	Rivière et al. (2009)
81	Chrysoeriol 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside	OH	OCH_3	Glc-coumaroyl	H	H	*M. metcalfianus*	Rivière et al. (2009)

Table 12 continued

No.	Name	R1	R2	R3	R4	R5	Plant	Ref.
82	Apigenin	OH	H	H	H	H	*M. apelta*	Wu et al. (2006)
83	Apigenin-7-*O*-*β*-D-glucoside	OH	H	Glc	H	H	*M. apelta*	Wu et al. (2006)
84	Mallotusin or 5,7-dihydroxy-6-isopentenyl-4′-methoxy-flavanone	H	OCH_3	H	H	CH_2–CH=C(Me)$_2$	*M. apelta*	Wu et al. (2006)
85	Vicenin II	OH	H	H	C Glc	C Glc	*M. apelta*	Zhu et al. (2007)
86	Mixture of (±)-hydnocarpin 7-*O*-(4″-*O*-(*E*)-coumaroyl) *β*-glucopyranoside/(±)-hydnocarpin-D 7-*O*-(4″-*O*-(*E*)-coumaroyl) *β*-glucopyranoside						*M. metcalfianus*	Rivière et al. (2009)

Phytochemical study of the crude methanolic extract of *M. peltatus* leaves revealed the presence of tannins along with saponins, terpenoids, steroids and reducing sugars (Chattopadhyay et al. 2002a, 2003). Tannins were highlighted in the polar fractions of *M. metcalfianus*, partly responsible for the anti-radical activity of these fractions (Rivière et al. 2009). Phenolic compounds of which condensed tannins, responsible for the antioxidant activity, were quantified in several extracts of *M. philippinensis* fruits and bark and in the fractions obtained after separation from the methanolic extract of *M. philippinensis* bark on a Sephadex LH-20 column using ethanol and acetone–water as the mobile phases. The content of total phenolics in the bark extract was 541 mg/g. The content of total phenolics in the fractions ranged from 54 mg/g (fraction I) to 927 mg/g (fraction VI) and condensed tannins were detected in fractions II-VI (Arfan et al. 2007, 2009). In 1989, known tannins and related compounds were isolated from the leaves of *M. philippinensis* (Saijo et al. 1989b).

Other compounds

Unsatured fatty acids (Table 17)

Octadeca-9,12,15-trienoic acid (**117**) and octadeca-9,12,15-trienoic acid 1-*β*-D-glucopyranosyl ester (**118**) were isolated from the methanolic extract of the leaves of *M. apelta* (Van Chau et al. 2004). The seed oil of *M. paniculatus* contains long-chain fatty acids (Yu et al. 1991). Kamala (*M. philippinensis*) seed oil has been shown to contain the triply-unsatured hydroxy acid kamlolenic acid (**119**), different fatty acids and glyceride (Gupta et al. 1953).

Benzopyrans (Table 18)

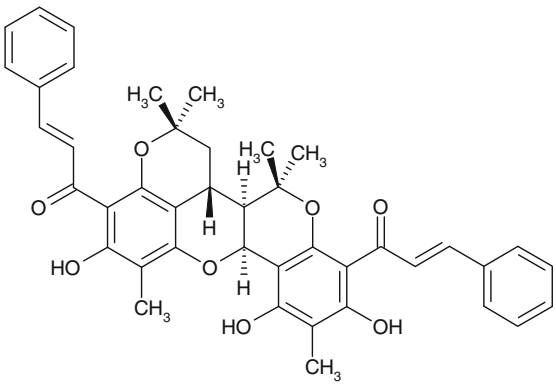
From the leaves of *M. apelta*, seven benzopyran compounds (**120–126**) were obtained in 2001 (An et al. 2001) and two new (**127–128**) in 2003 (An et al. 2003).

In 2005, two new benzopyrans (**133–134**) were isolated from the leaves of *M. apelta* by Van Kiem et al. 2005, as well as four other benzopyrans (**129–132**) by Van Chau et al. 2005a. The compound (**135**), 6-methoxy-benzopyran-4-one, was obtained from the ethyl acetate extract of the stems of *M. apelta* (Qi et al. 2005).

Various compounds (Table 19)

α-Tocopherol (**136**) was isolated from the leaves of *M. apelta* (Van Chau et al. 2005d). In 2007, nicotinic acid (**140**) was isolated from the leaves of *M. apelta* (Kang and Lu 2007). From *M. apelta*, one new pyridine type alkaloid, named malloapeltine (**142**) was isolated and structurally elucidated (Cheng et al. 1998). The methanolic extract of the aerial parts of *M. repandus* was fractionated monitored by the antiulcerogenic activity to give mallorepine (**143**), a cyano-*γ*-pyridone, together with bergenin as one of the active principles. Mallorepine may be an

Table 13 Flavonoids (chalcones)



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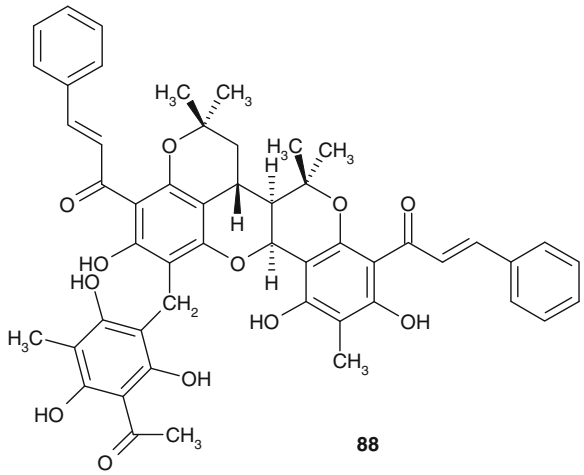

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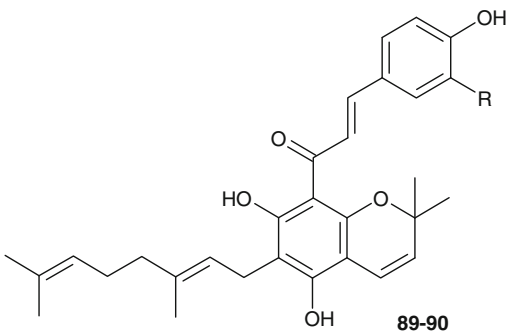
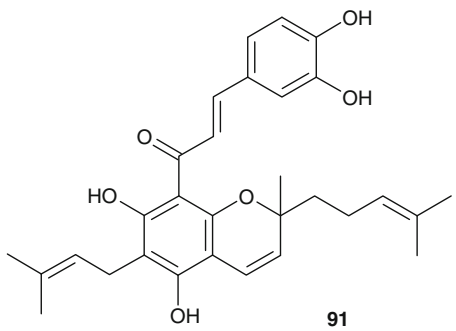


89-90

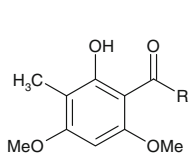


91

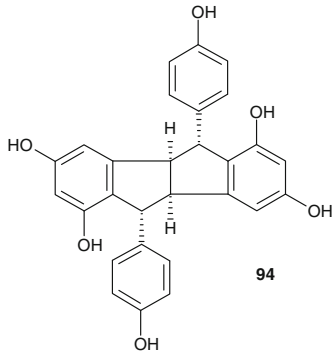
No.	Name	R	Plant	Ref.
87	Kamalachalcone A		*M. philippinensis*	Tanaka et al. (1998)
88	Kamalachalcone B		*M. philippinensis*	Tanaka et al. (1998)
89	1-[6-(3,7-Dimethyl-octa-2,6-dienyl)-5,7-dihydroxy-2, 2-dimoethyl-2H-chromen-8-yl]-3-(4-hydroxy-phenyl)-propenone or Mallotophilippen C	H	*M. philippinensis*	Daikonya et al. (2004), Li et al. (2006)
90	3-(3,4-Dihydroxy-phenyl)-1-[6-(3,7-dimethyl-octa-2,6-dienyl)-5, 7-dihydroxy-2,2-dimethyl-2H-chromen-8-yl]-propenone or Mallotophilippen D	OH	*M. philippinensis*	Daikonya et al. (2004), Li et al. (2006)
91	1-[5,7-Dihydroxy-2-methyl-6-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-2H-chromen-8-yl]-3-(3,4-dihydroxy-phenyl)-propenone or Mallotophilippen E		*M. philippinensis*	Daikonya et al. (2004), Li et al. (2006)

intermediate in the biosynthetic pathway from nicotinamide to ricinine (Hikino et al. 1978). Moreover, *trans*-2-carboxy-4-hydroxytetrahydrofuran-*N*,*N*-dimethylamide (**141**), a novel furanocarboxamide, was reported in *M. cuneatus* (Groweiss et al. 1994). In 2009, we reported the isolation of a fattyl alcohol named *n*-hexacosanol (**137**), a megastigmane named blumenol-*C*-glucoside (**138**) and methyl-2-*O*-*β*-D-glucopyranosylbenzoate (**139**) from *M. metcalfianus* (Rivière et al. 2009).

Table 14 Phloroglucinol derivatives



92-93



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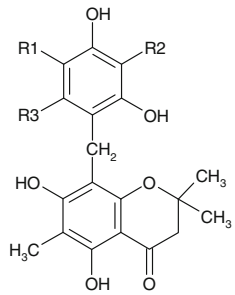

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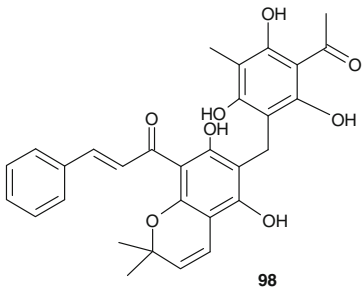

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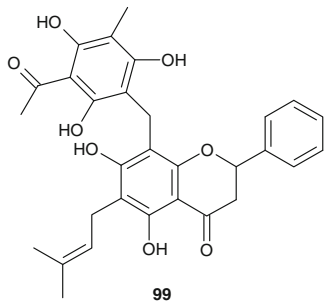

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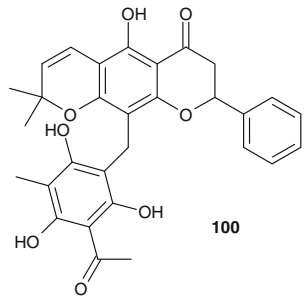


100

No.	Name	R1	R2	R3	Plant	Ref.
92	Pallidusol	Bu-i			*M. pallidus*	Supudompol et al. (2004)
93	Dehydropallidusol	$CH{=}C(Me)_2$			*M. pallidus*	Supudompol et al. (2004)
94	Pallidol				*M. pallidus*	Supudompol et al. (2004)
95	Mallopallidol	CH_3	C(=O)–Pr-i	OCH_3	*M. pallidus*	Supudompol et al. (2004)
96	Homomallopallidol	CH_3	C(=O)–CH(Me)Et	OCH_3	*M. pallidus*	Supudompol et al. (2004)
97	Mallopallidusol	C(=O)–Pr-i	CH_3	OH	*M. pallidus*	Likhitwitayawuid et al. (2005)
98	Rottlerin				*M. philippinensis*	Lounasmaa et al. (1975)
99	Isoallorottlerin				*M. philippinensis*	Lounasmaa et al. (1975)
100	Isorottlerin				*M. philippinensis*	Zaidi et al. (2009)

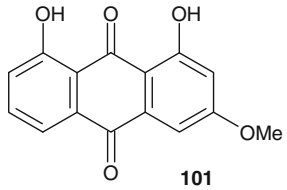
Pharmacological activities

Anti-inflammatory and immunoregulatory activities

The anti-inflammatory activity of the methanolic extract of *M. peltatus* leaves against carrageenan (acute model) and dextran-induced (subacute model) rat paw oedema and cotton pellet-induced granuloma (chronic model) in rats were studied using indomethacin as standard. The methanolic extract of this species at 200 and 400 mg/kg, and two *n*-butanolic fractions (A and B) at 25 mg/kg, exhibited significant anti-inflammatory activity in Albino rats, compared with indomethacin. Further study with fractions showed that the anti-inflammatory activity is due to either fraction A, ursolic acid (**46**), alone or the combination of fractions A and B, *β*-sitosterol (**58**) and fatty acids (Chattopadhyay et al. 2002a). The methanolic extract of *M. peltatus* showed also a significant dose-dependent anti-inflammatory and antioxidant activity at nontoxic concentrations (Chattopadhyay et al. 2006).

The chalcones isolated from the fruits of *M. philippinensis*, mallotophilippens C, D and E (**89–91**)

Table 15 Quinones and phenolic acids



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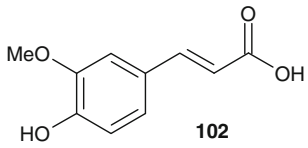


102

No.	Name	Plant	Ref.
101	Chrysophanol	*M. apelta*	Kang and Lu (2007)
102	Ferulic acid	*M. metcalfianus*	Rivière et al. (2009)

inhibited nitric oxide (NO) production and inducible NO synthase (iNOS) gene expression by a murine macrophage-like cell line (RAW 264.7), which was activated by lipopolysaccharide and recombinant mouse interferon-γ (IFN-γ). Furthermore, they down-regulated cyclooxygenase-2 gene, interleukin-6 gene and interleukin-1β gene expression. These results suggest that these chalcones have anti-inflammatory and immunoregulatory effects (Daikonya et al. 2004).

The chloroform extract from the roots of *M. spodocarpus* was investigated for anti-inflammatory and analgesic activities in animal models. The results obtained suggest marked anti-inflammatory and analgesic activity of the extract. In acute inflammatory models, the extract significantly inhibited ethyl phenylpropiolate-induced ear oedema and carrageenin- and arachidonic acid-induced hind paw oedema in rats. In the chronic inflammatory model using the cotton pellet-induced granuloma in rats, the extract exhibited inhibitory activity on the formation of granuloma. The extract also elicited pronounced inhibitory effect on acetic acid-induced writing response in mice in the analgesic test (Intahphuak et al. 2004).

Antifertility activity

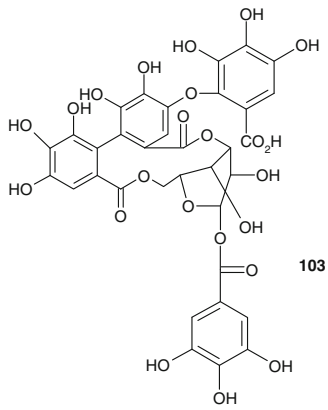
The Kamala (*M. philippinensis*) seeds extract presents adverse effects on various reproductive parameters of female rats. The data indicate that Kamala reduced serum FSH and LH levels probably by affecting hypothalamic/pituitary axis in treated animals. Thus, reduced levels of FSH and LH and estradiol might have affected the follicular development, quality of ovulated eggs, corpora lutea formation, estrus cycle, establishment and maintenance of pregnancy in treated rats (Thakur et al. 2005). The antifertility effect of this species seems to be caused by rottlerin (**98**); a phloroglucinol derivative. Acetylrottlerin is also active, but isorottlerin (**100**) is either inactive or only slightly active (Gujral et al. 1960).

Antimicrobial activity

Among seven benzopyrans obtained from the leaves of *M. apelta*, one compound (**120**) showed moderate antibiotic activity against *Micrococcus lutens* (An et al. 2001). Moreover, erythrodiol-3-acetate (**42**), β-sitosterol (**58**), 3β,29-dihydroxylupane (**27**) and ursolic acid acetate (**47**) isolated from the roots of *M. apelta* possess some bacteriostatic activities on *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Bacillus pyocyaneum* (Shan et al. 1985).

The antimicrobial activity of several fractions of *M. metcalfianus* was evaluated on 20 strains. This activity was moderate: fractions were not active on some Gram negative bacteria at the highest concentrations tested (1,000 μg/ml) but were effective on at least eight strains at 500 μg/ml (MAC, minimal active concentration, the minimal concentration reducing the growth of the microorganism as compared to controls), i.e., on Gram positive bacteria (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212), on Gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Morganella morganii* 180, *Yersinia enterocolitica* E 170/98, *Yersinia enterocolitica* E 169/98) and on Saccharomycetes fungi (*Candida albicans*). Some MAC were as low as to

Table 16 Tannins



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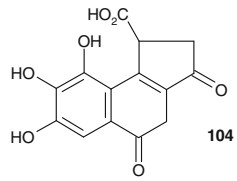

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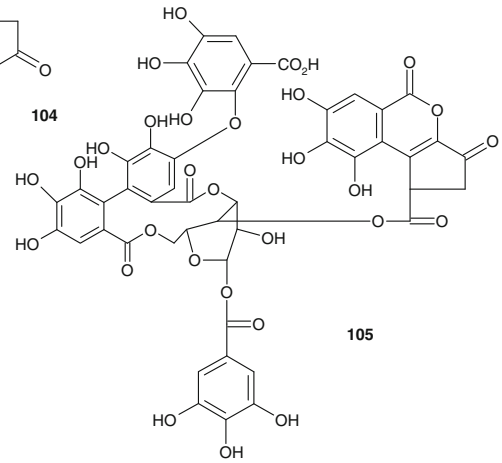

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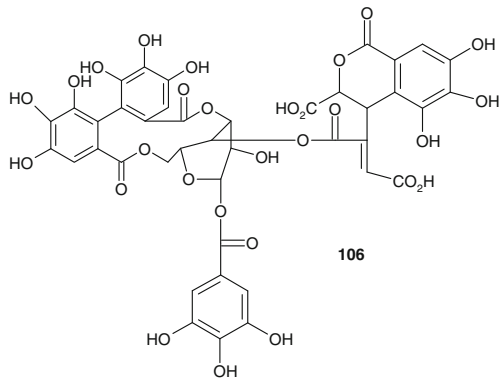

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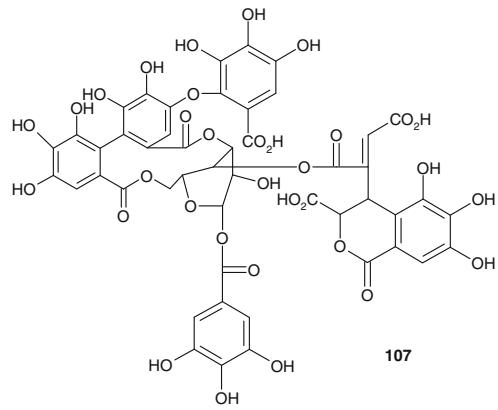

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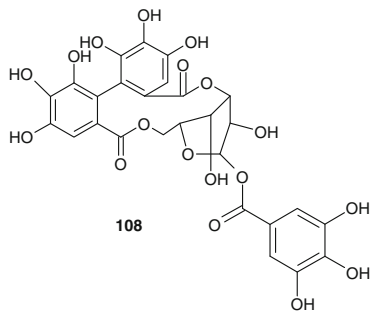

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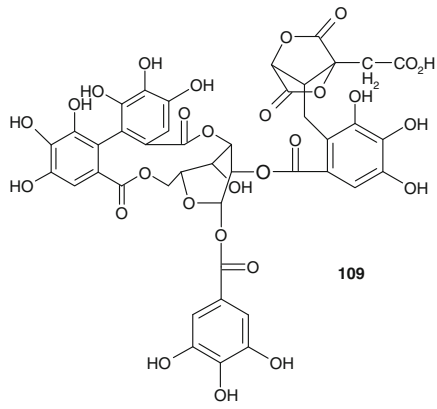

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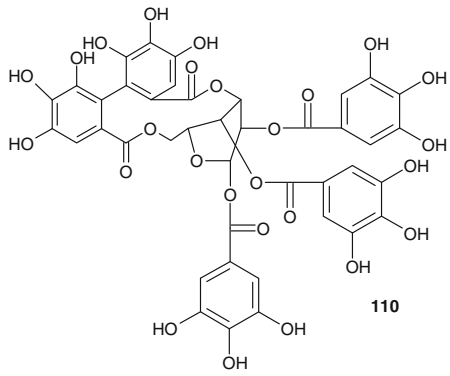

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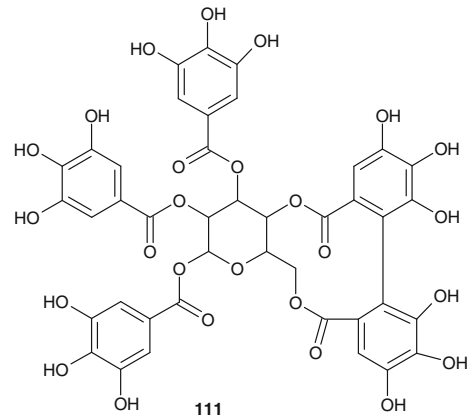

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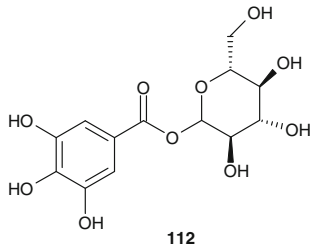

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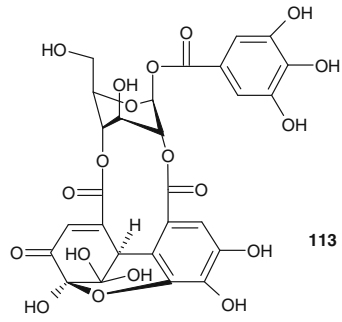

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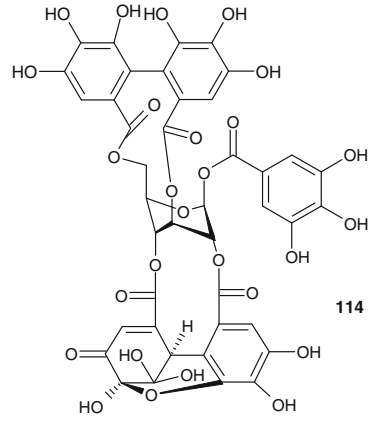

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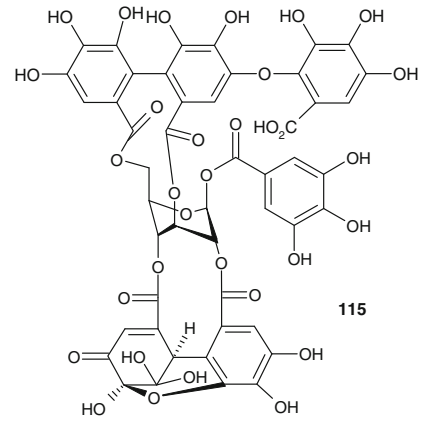

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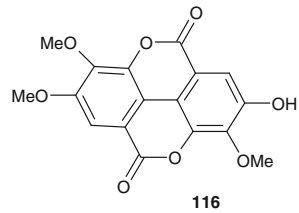


116

Table 16 continued

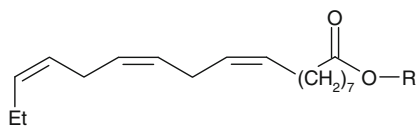
No.	Name	Plant	Ref.
103	Mallotinic acid	*M. repandus*	Saijo et al. (1989a)
104	Brevifolin carboxylic acid	*M. repandus*	Saijo et al. (1989a)
105	Repandusinin	*M. repandus*	Saijo et al. (1989a)
106	Repandusinic acid A	*M. repandus*	Saijo et al. (1989a)
107	Repandusinic acid B	*M. repandus*	Saijo et al. (1989a)
108	Corilagin	*M. repandus*	Saijo et al. (1989a)
109	Mallotinin	*M. repandus*	Saijo et al. (1989a)
110	Punicafolin	*M. repandus*	Saijo et al. (1989a)
111	Eugeniin	*M. repandus*	Saijo et al. (1989a)
112	Glucogallin	*M. repandus*	Saijo et al. (1989a)
113	Furosin	*M. repandus*	Saijo et al. (1989a)
114	Geraniin	*M. repandus*	Saijo et al. (1989a)
115	Mallotusinic acid	*M. repandus*	Saijo et al. (1989a)
116	4,5,4′-Trimethyl-ellagic acid	*M. apelta*	Cheng et al. (1998)

200 μg/ml. This activity in most cases (polar extracts) may be explained at least partly by the presence of tannins as minimal inhibitory concentration (MIC) increases after their removal. Hexanic and some chloroformic fractions show also an interesting activity. Pure isolated major flavonoids, quercitrin (**75**), kaempferol 3-*O*-α-L-rhamnoside (**76**) and astilbin (**79**), have a moderate activity (MIC = 128 μg/ml on some strains) (Rivière et al. 2009).

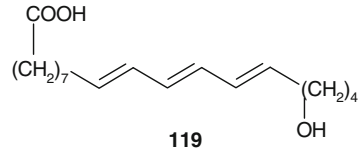
The crude methanolic extract of *M. peltatus* leaves was found to be active against *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Streptococcus faecalis*, *Bacillus subtilis*, *Escherichia coli*, and *Proteus mirabilis* and the dermatophytic fungi *Microsporum gypseum*. The minimum inhibitory concentration (MIC) ranges from 128 to 2,000 μg/ml for bacteria and 128 mg/ml for fungi, while the minimum bactericidal concentration (MBC) was twofold to fourfold higher than MIC. The methanol–water fraction of the extract showed similar activity against *Staphylococcus*, *Streptococcus*, *Bacillus*, and *Proteus* isolates. The fraction A, ursolic acid (**46**), alone or the combination of fractions A and B, β-sitosterol (**58**) and fatty acids, are responsible for the antimicrobial and anti-inflammatory activities (Chattopadhyay et al. 2002a). The methanolic extract of *M. peltatus* showed also an antibacterial activity at 64–1,000 μg/ml (Chattopadhyay et al. 2006).

A series of 61 Indian medicinal plants belonging to 33 different families used in various infectious disorders, were screened for their antimicrobial properties. On the basis of the results obtained, the crude extract of *M. philippinensis* exhibited significant antimicrobial activity (Kumar et al. 2006). *M. philippinensis* var. *tomentosus* was tested against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Bacillus subtilis*. From the results obtained, the chloroformic fractions and the methanolic extract showed zones of inhibition comparable to the standard drug used. However, the hexanic extract did not show any appreciable activity. The results of the study may justify the use of the plant against bacterial pathogens (Moorthy et al. 2007). Moreover, in the quest for potent anti-*Helicobacter pylori* agents, ethanolic extract of *M. philippinensis* showed a strong bactericidal activity at the concentration of 15.6–31.2 mg/l against eight *H. pylori* strains. Further fractionation and purification of this extract led to the isolation of five compounds. Among the isolated compounds, rottlerin (**98**), exhibited the most potent bactericidal activity with a minimal bactericidal concentration (MBC) value of 3.12–6.25 mg/l against several clinical *H. pylori* isolates including Japanese and Pakistani strains, nine clarithromycin resistant (CR), and seven metronidazole resistant (MR) strains. This

Table 17 Unsatured fatty acids



117-118



119

No.	Name	R	Plant	Ref.
117	9,12,15-Octadecatrienoic acid or linolenic acid	H	*M. apelta*	Van Chau et al. (2004)
118	9,12,15-Octadecatrienoic acid 1β-D-glucopyranosyl ester	Glc	*M. apelta*	Van Chau et al. (2004)
119	Kamlolenic acid		*M. repandus*	Gupta et al. (1953)

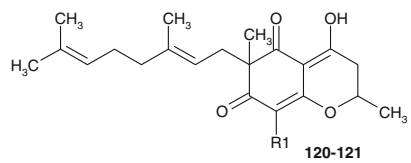
study thus revealed the potent in vitro anti-*H. pylori* activity of the ethanolic extract and of rottlerin, specially against CR and MR strains, which could be gainfully utilized for the development of novel antimicrobials to prevent *H. pylori* related disorders (Zaidi et al. 2009).

Antioxidant, antiradical activity

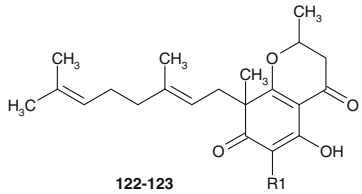
From our phytochemical results, *M. metcalfianus* is rich in flavonoids and phenolic compounds. These flavonoids were mainly present in the ethyl acetate extract while the aqueous fraction and the residue were richer in tannins. Concerning the antiradical activity, for the ethyl acetate fraction which was the most active, we observed that tannins were only responsible for a small part of the activity which seems to be mainly due to flavonoids. In fact, the elimination of tannins in this fraction only slightly decreased the antiradical properties. On the contrary, tannins seem to be responsible for a large part of the antioxidant activities of the residue and the aqueous fraction: their elimination greatly decreased the activity. We tested the different pure compounds isolated from *M. metcalfianus* and different reference samples (flavonoids and cinnamic acid derivatives) for their antiradical activities in order to discuss about structure–activity relationships of these products. We observed that quercetin 3-*O*-β-neohesperidoside (**77**) shows about 50% of the activity of rutin. This decrease in activity can be due to the different position of rhamnose on glucose. Kaempferol 3-*O*-β-neohesperidoside (**78**), having an OH less on the B ring, shows a very moderate activity. The new flavonolignans (**86**) were not very active. This lack of activity could be explained by the cyclization of the catechol group of the B ring of the flavone. Indeed, by comparison with luteolin, luteolin 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside) (**80**) was found to be moderately active in the DPPH assay. The substitution of the flavone by a coumaric acid could explain this decrease of activity, as coumaric acid does not show a real antioxidant activity unlike caffeic acid. Chrysoeriol 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside) (**81**) is less active than luteolin 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside), probably because of the loss of phenol function in position 3′, replaced by a methoxy group. *n*-Hexacosanol (**137**), blumenol C glucoside (**138**), methyl 2-*O*-β-D-glucopyranosylbenzoate (**139**) and friedelinol (**34**) were found to have only a low activity. Friedelin (**33**) (hydroxyl function of friedelinol in position three replaced by a ketone) was not more active than friedelinol (Rivière et al. 2009).

The total antioxidant activity (TAA), antiradical activity against DPPH and reducing power of several extracts of *M. philippinensis* fruits and bark and of the fractions, obtained after separation of the methanolic extract bark on a Sephadex LH-20 column using ethanol and acetone–water as the mobile phases, were evaluated. The extract of the bark showed the strongest antiradical activity and reducing power; its TAA was 5.27 mmol Trolox equiv./g. The TAA of other extracts ranged from 0.05 to 1.79 mmol Trolox equiv./g. The TAA of phenolic fractions of *M. philippinensis* bark extract ranged from 0.58 mmol Trolox/g (fraction I) to 6.82 mmol Trolox/g (fraction IV). Fraction IV also showed the strongest antiradical activity against DPPH and reducing power (Arfan et al. 2007, 2009).

Table 18 Benzopyrans



120-121



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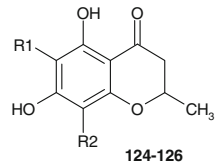

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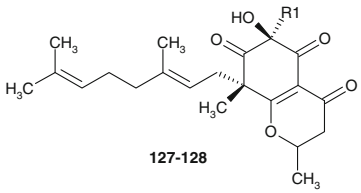

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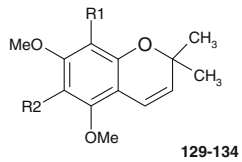
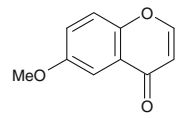


129-134



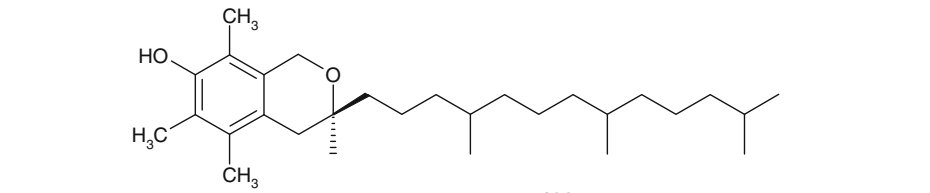
135

No.	Name	R1	R2	Plant	Ref.
120	4-Hydroxy-2,6-dimethyl-6-(3,7-dimethyl-2,6-octadienyl)-8-(3-methyl-2-butenyl)-2H-1-benzopyran-5,7(3H,6H)-dione	CH_2–CH=C(Me)$_2$		*M. apelta*	An et al. (2001)
121	4-Hydroxy-2,6,8-trimethyl-6-(3,7-dimethyl-2,6-octadienyl)-2H-1-benzopyran-5,7(3H,6H)-dione	CH_3		*M. apelta*	An et al. (2001)
122	5-Hydroxy-2,8-dimethyl-6-(3-methyl-2-butenyl)-8-(3,7-dimethyl-2,6-octadienyl)-2H-1-benzopyran-4,7(3H,8H)-dione	CH_2–CH=C(Me)$_2$		*M. apelta*	An et al. (2001)
123	5-Hydroxy-2,6,8-trimethyl-8-(3,7-dimethyl-2,6-octadienyl)-2H-1-benzopyran-4,7(3H,8H)-dione	CH_3		*M. apelta*	An et al. (2001)
124	2,3-Dihydro-5,7-dihydroxy-2,6-dimethyl-8-(3-methyl-2-butenyl)-4H-1-benzopyran-4-one	CH_3	CH_2–CH=C(Me)$_2$	*M. apelta*	An et al. (2001)
125	2,3-Dihydro-5,7-dihydroxy-2,8-dimethyl-6-(3-methyl-2-butenyl)-4H-1-benzopyran-4-one	CH_2–CH=C(Me)$_2$	CH_3	*M. apelta*	An et al. (2001)
126	2,3-Dihydro-5,7-dihydroxy-2,6,8-trimethyl-4H-1-benzopyran-4-one	CH_3	CH_3	*M. apelta*	An et al. (2001)
127	6-Hydroxy-2,6,8-trimethyl-8-(3,7-dimethyl-2,6-octadienyl)-2H-1-benzopyran-4,5,7(3H,6H,8H)-trione	CH_3		*M. apelta*	An et al. (2003)
128	6-Hydroxy-2,8-dimethyl-6-(3-methyl-2-butenyl)-8-(3,7-dimethyl-2,6-octadienyl)-2H-1-benzopyran-4,5,7(3H,6H,8H)-trione	CH_2–CH=C(Me)$_2$		*M. apelta*	An et al. (2003)
129	8-(1′-Oxo-2′-en-butyl)-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran or malloapelta B	CO–CH=CH–CH_3	H	*M. apelta*	Van Chau et al. (2005a)
130	8-(1′-Oxo-3′(*R*)-hydroxy-butyl)-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran	CO–$CH_2CH(CH_3)OH$	H	*M. apelta*	Van Chau et al. (2005a)
131	8-(Acetic acid 1′-oxo-3′(*R*)-hydroxy-butyl ester)-5,7-dimethoxy-2,2-dimethyl-2H-1- benzopyran	CO–$CH_2CH(CH_3)OAc$	H	*M. apelta*	Van Chau et al. (2005a)
132	6-(1′-Oxo-2′-en-butyl)-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran	H	CO–CH=CH–CH_3	*M. apelta*	Van Chau et al. (2005a)
133	6-(1′-Oxo-3′(*R*)-hydroxy-butyl)-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran	H	CO–$CH_2CH(CH_3)OH$	*M. apelta*	Van Kiem et al. (2005)

Table 18 continued

No.	Name	R1	R2	Plant	Ref.
134	6-(1′-Oxo-3′(*R*)-methoxy-butyl)-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran	H	CO–$CH_2CH(CH_3)OCH_3$	*M. apelta*	Van Kiem et al. (2005)
135	6-Methoxy-benzopyran-4-one			*M. apelta*	Qi et al. (2005)

Table 19 Various compounds



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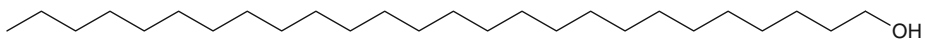

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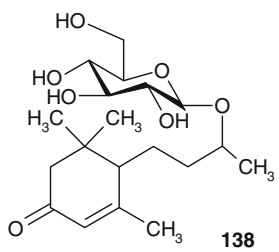

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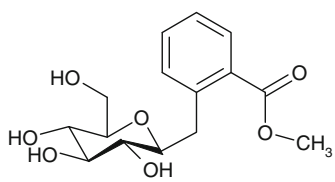

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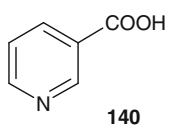

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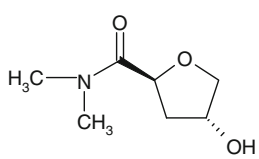

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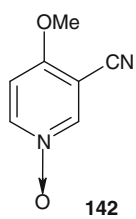

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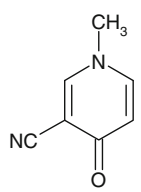

143

No.	Name	Plant	Ref.
136	α-Tocopherol	*M. apelta*	Van Chau et al. (2005d)
137	*n*-Hexacosanol	*M. metcalfianus*	Rivière et al. (2009)
138	Blumenol C glucoside	*M. metcalfianus*	Rivière et al. (2009)
139	Methyl-2-*O*-*β*-D-glucopyranosylbenzoate	*M. metcalfianus*	Rivière et al. (2009)
140	Nicotinic acid	*M. apelta*	Kang and Lu (2007)
141	Trans-2-carboxy-4-hydroxytetrahydro furan-*N*,*N*-dimethylamide	*M. cuneatus*	Groweiss et al. (1994)
142	4-Methoxy-3-cyano-pyridine 1-oxide or malloapeltine	*M. apelta*	Cheng et al. (1998)
143	Mallorepine	*M. repandus*	Hikino et al. (1978)

The ethyl acetate fraction of *M. repandus* stems showed the greatest superoxide-scavenging activity, and the hexanic extract of stems and roots had the greatest hydroxyl-scavenging activity (Lin et al. 1995).

As phenolic compounds have been identified in several *Mallotus* species and because of the health interest of antioxidant extracts or compounds (prevention of cancers, anti-inflammatory properties) (Tapiero et al. 2002; Soobrattee et al. 2006), we determined the antiradical activity of 33 samples (methanolic extracts) of seventeen *Mallotus* species from Vietnam by the DPPH assay. Some species were collected in different provinces. For some species, different parts of the plant were studied. The most effective methanolic extracts come from *Mallotus barbatus* MA29, *Mallotus cuneatus* MA17, *Mallotus floribundus* MA15, *Mallotus hookerianus* MA22, *Mallotus nanus* MN37R, MN37L, and MN39C, *Mallotus oblongifolius* MA14, *Mallotus paniculatus* MP35R, and *Mallotus philippinensis* MA28. According to the literature and what is known about their chemical compositions, antioxidant activities of *Mallotus nanus*, *M. paniculatus*, *M. philippinensis* could be explained by the presence of flavonoids and tannins. We noted that some extracts have an antiradical activity similar to tocopherol. They thus represent valid alternative sources of antioxidant agents, as we also showed that they did not show cytotoxicity on cultured cells. Combining fingerprint technology with data-handling techniques allows indicating the peaks potentially responsible for given activities. We indicated from chromatographic fingerprints the peaks potentially responsible for the antioxidant activity of these *Mallotus* species. Relevant information was extracted using linear multivariate calibration techniques (Nguyen Hoai et al. 2009; Tistaert et al. 2009).

Antipyretic activity

The leaf extract of *M. peltatus* showed a potential anti-pyretic effect in rats. At oral doses of 100, 200, and 300 mg/kg, the extract showed significant reduction in normal body temperature and yeast-provoked elevated temperature in a dose-dependent manner and the anti-pyretic effect was comparable to that of standard anti-pyretic agent paracetamol (150 mg/kg). The effect also extended up to 5 h after the drug administration (Chattopadhyay et al. 2002b).

Antiulcerogenic activity

The methanolic extract of the aerial parts of *M. repandus* was fractionated monitored by the antiulcerogenic activity to give mallorepine (**143**), together with bergenin (**67**) as one of the active principles. Mallorepine was shown to be inactive in inhibiting the formation of the stress-induced gastric ulcers (Hikino et al. 1978).

Antiviral activity

In 1989, 40 preparations of extracts from 28 kinds of Asian herbs were tested for their ability to inhibit the activities of murine retroviral reverse transcriptase and human DNA polymerases. Among the 40 extracts, very strong inhibitions were observed with the extract from *M. apelta* as shown by its low IC_{50} values for reverse transcriptase (0.4–0.5 μg/ml) and DNA polymerase-α (0.9–1.4 μg/ml). The mode of inhibition of reverse transcriptase by this extract was competitive with respect to the template-primer [poly(rA)-oligo(dT)] and noncompetitive with respect to dTTP substrate. Besides reverse transcriptase and DNA polymerase-α, DNA polymerase I and RNA polymerase from *Escherichia coli* were inhibited by this extract (Ono et al. 1989). In 2002, a massive screening of natural products showed also that *M. apelta* has significant anti-HIV activity. From this species, thirty compounds have been isolated and structurally elucidated. The most interesting and promising compounds for further study were terpenoids, pyridine type alkaloids; cerebrosides and three coumarinolignoid compounds. The coumarinolignoids have been proved to be the most active compounds against HIV (Cheng and Chen 2002). The root of *M. apelta* has therapeutic effect on duck hepatitis B virus (D-HBV). It can restrain the duplication of D-HBV in vivo. Although this effect is weaker than that of lamivudine, it lasts longer (Xu et al. 2006).

M. chrysocarpus is reported to have potential anti-HIV activity (Nguyen et al. 1997).

Five phloroglucinol derivatives isolated from *M. pallidus* were studied for their inhibitory effects against herpes simplex virus HSV-1, HSV-2, and human immunodeficiency virus HIV-1. The data obtained in this study suggest the bis-hydroxyphenyl structure as a potential lead for anti-HSV

and anti-HIV drugs development (Likhitwitayawuid et al. 2005).

The inhibitor of human immunodeficiency virus type-1 reverse transcriptase (HIV-1-RT) isolated from an aqueous extract of *Phyllanthus niruri* was purified and identified as repandusinic acid A monosodium salt (**106**), an hydrolyzable tannin, which was originally isolated from *Mallotus repandus*. The 50% inhibitory doses (ID_{50}) of this compound on HIV-1-RT and DNA polymerase-α (from HeLa cells) were 0.05 and 0.6 μM, respectively, representing approximatively a ten-fold higher sensitivity for HIV-1-RT compared to DNA polymerase α. This tannin was shown to be a competitive inhibitor with respect to the template-primer while it was a noncompetitive inhibitor with respect to the substrate (Ogata et al. 1992).

Cytotoxic and antitumor activities

In 2005, two benzopyrans isolated from the leaves of *M. apelta* showed a cytotoxic activity. The benzopyran, 6-[1′-oxo-3′(*R*)-hydroxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran (**133**), was found to have a strong cytotoxic effect against two human cancer cell lines, human hepatocellular carcinoma (Hep-2, $IC_{50} = 0.49$ μg/ml) and rhabdosarcoma (RD, $IC_{50} = 0.54$ μg/ml), while the benzopyran, 6-[1′-oxo-3′(*R*)-methoxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran (**134**), showed moderate activity against the Hep-2 cell line ($IC_{50} = 4.22$ μg/ml) by in vitro assay (Van Kiem et al. 2004). In searching for bioactive compounds from natural products by analyzing their cytotoxic effects against various cancer cell lines, 22 compounds isolated from *M. apelta* were tested for their cytotoxic effects against various cancer cell lines, such as KB (human epidermoid carcinoma), FL (fibrillary sarcoma of the uterus), and Hep-2 (human hepatocellular carcinoma) cells in an in vitro assay system. Malloapelta B (**129**), a benzopyran, showed strong cytotoxic effect against the three cancer cell lines, while the other compounds did not show inhibitory activities and had IC_{50} values over 50 μM (Van Chau et al. 2005a).

Two antitumor agents, AK-3A [62534-39-8] and AK-3B [62534-40-1] were isolated from the leaves, bark and xylem of *M. repandus* (Kawashima et al. 1976b).

DNA cleavage activity

A crude extract prepared from roots of *M. resinosus* exhibited significant Cu^{2+}-dependent DNA strand scission activity and was thus selected for bioassay-guided fractionation. Scopoletin (**64**), a simple coumarin, was identified as the active principle responsible for the DNA cleavage activity of the crude extract (Ma et al. 2004).

Hepatoprotective activity

An extract from the roots of *M. apelta* could reduce the progression of liver fibrosis, having a capacity of anti-oxidation (Zhao et al. 2002). Malloapelin C (**72**), a coumarinolignoid isolated from *M. apelta*, showed promising hepatoprotective activity against D-galactosamine-induced toxicity in WB-F344 rat hepatic epithelial stem-like cells (Xu et al. 2008).

One hundred twenty-nine samples of Taiwanese plants were screened for antihepatotoxic activity in primary cultured hepatocytes, against cytotoxicity produced by carbon tetrachloride and D-galactosamine. *M. repandus* belongs to the plants which disclosed significant antihepatotoxic activity in both methods (Yang et al. 1987).

Inhibition of proteins implicated in cancer process

In searching for inhibitory components from natural products on NFAT transcription factor and NF-κB activation, the methanolic extract from the leaves of *M. apelta* has been investigated. Fourteen compounds were isolated. Of these compounds, malloapelta B (**129**) exhibited also a strong activity against the NFAT transcription factor and inhibition of NF-κB activation (Van Chau et al. 2005d).

Rottlerin (**98**), a compound isolated from *M. philippinensis*, is shown to inhibit protein kinases with some specificity for PKC. To some extent, the novel inhibitor is able to differentiate between PKC isoenzymes, with IC_{50} values for PKC δ of 3–6 μM, PKC α,β,γ of 30–42 μM and PKC υ,η,ζ of 80–100 μM. Inhibition of PKC appears, at least in part, to be due to a competition between rottlerin and ATP. Among the protein kinases tested, only CaM-kinase III is suppressed by rottlerin as effectively as PKC δ. The chemical structure of rottlerin might serve as a

basis for the development of novel inhibitors with improved selectivity for a distinct PKC isoenzyme, such as PKC δ, or for CaM-kinase III (Gschwendt et al. 1994; Liao et al. 2005).

Neuropharmacological activity

The methanolic extract and different fractions of *M. peltatus* leaves showed several neuropharmacological effects in rats and mice. The results revealed that the crude extract at 200–300 mg/kg and its fractions A and B at 50 mg/kg caused a significant reduction in spontaneous activity, remarkable decrease in exploratory behavioral pattern, a reduction in muscle relaxant activity and also a significantly potentiated phenobarbitone sodium-induced sleeping time. Further fractionation and purification yielded two major fractions A, ursolic acid (**46**), and B, β-sitosterol (**58**) with some fatty acids, as major compounds. The psychopharmacological activity of the crude leaf extracts appeared to be either due to fraction A (50 mg/kg) or a combination of fractions A and B (50 mg/kg) along with some fatty acids present in the *n*-butanolic part of methanolic extract of *M. peltatus* leaf (Chattopadhyay et al. 2003).

Uterus muscle stimulant

A compound stimulating the uterus muscles was isolated from the methanolic fraction of *M. repandus* (Kawashima et al. 1975).

Veterinary applications

A survey was conducted in southern Punjab, Pakistan, in order to document existing ethnobotanical knowledge by the herdsmen/key respondents about anthelmintics in ruminants. *M. philippinensis* is one of the main plants used (Jabbar et al. 2006). The fruits of *M. philippinensis* showed a gastrointestinal anticestodal activity in Beetal goats (Akhtar and Ahmad 1992).

Conclusions

The results of this review confirm the great potential of *Mallotus* species. For many of them still only very limited information is available. It leads us to continue studies on certain *Mallotus* species which showed interesting pharmacological properties, to identify the compounds responsible for these activities.

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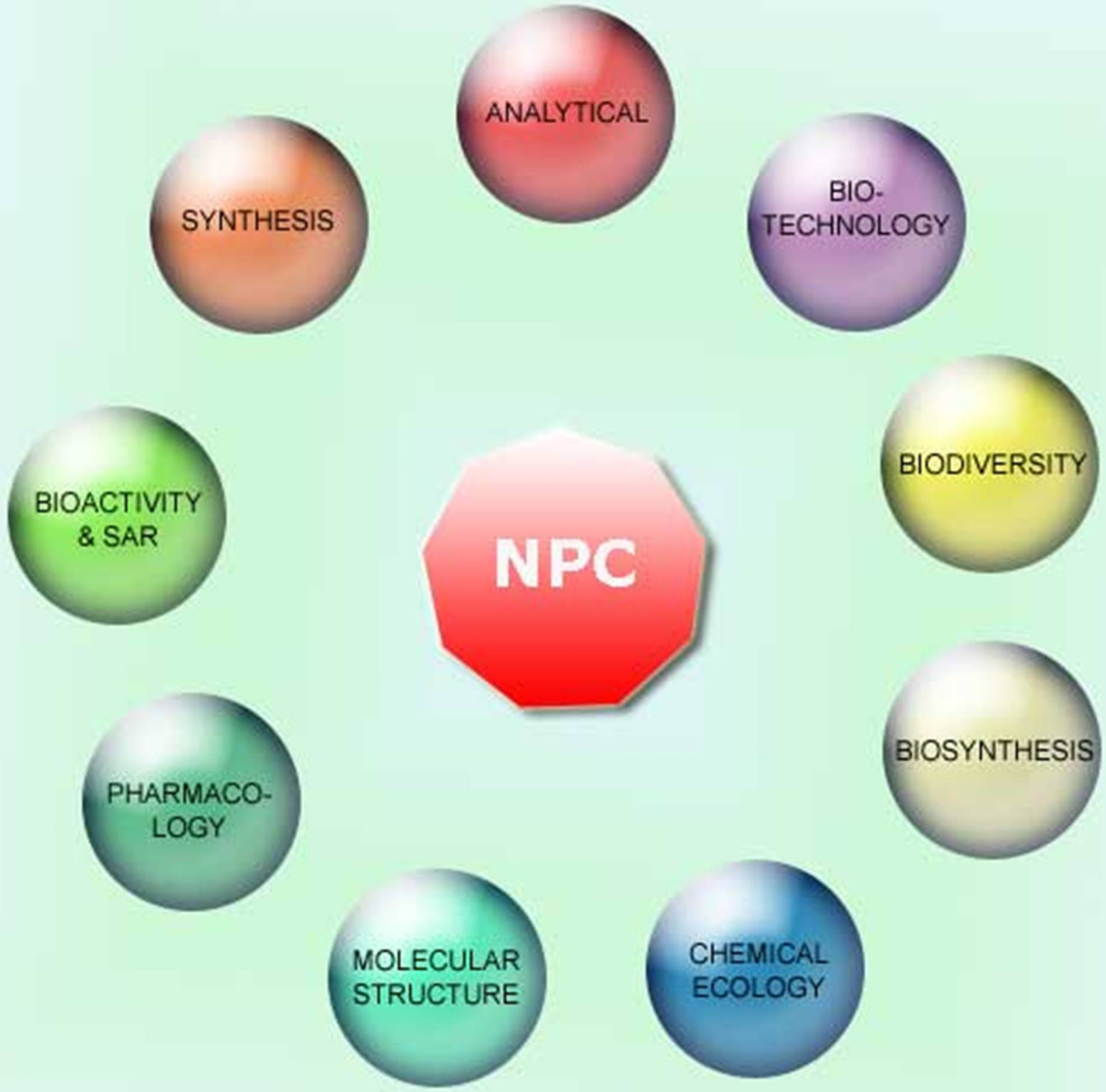

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Identification by LC-ESI-MS of Flavonoids Responsible for the Antioxidant Properties of *Mallotus* Species from Vietnam

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Several *Mallotus* species (Euphorbiaceae) are used in Vietnam as edible plants or as traditional medicines for different indications, some related to the treatment of inflammatory diseases. This study investigated the antioxidant activities of 33 samples from 17 Vietnamese *Mallotus* species. We also evaluated potential cytotoxic activity against human cervix carcinoma HeLa and human lung fibroblast WI-38 cells. Our aim is to develop safe dietary supplements with a protective effect against various diseases caused by tissue damage and the acceleration of the aging process linked to reactive oxygen species. These tests allowed the identification of non-cytotoxic plant species exhibiting significant antiradical properties. These antioxidant properties may be explained by their polyphenol composition. The antioxidant activity of the most active *Mallotus* species was further analyzed with and without tannins removal. We also identified by LC-ESI-MS some flavonoids responsible for a part of this activity.

Keywords: *Mallotus*, Euphorbiaceae, Vietnam, Cytotoxic activities, Antioxidant activities, Flavonoids.

The genus *Mallotus* belongs to the Euphorbiaceae family, within the Acalyphoideae sub-family, Acalypheae pro parte and Rottlerinae subtribe [1a]. *Mallotus*, commonly known as *ba bet* in Vietnam, is represented by approximately 40 species, among which, 6 species and 1 variety are endemic [1b]. Some Vietnamese *Mallotus* species are known to contain different natural compounds, mainly diterpenoids, triterpenoids, steroids, flavonoids, coumarinolignoids, phloroglucinol derivatives and benzopyrans [2a]. More recently, a new flavonolignan and flavones from *M. metcalfianus* [2b], two new megastigmane sulphonoglucosides from *M. anisopodus* [2c] and a new lignan dimer from *M. philippensis* [2d] were identified. For some species, promising biological activities have been determined, particularly for *M. apelta* [2e], and for *M. philippinensis* [2f,2g,3]. In addition, *M. pallidus* has antiviral activity attributable to its phloroglucinol derivatives [4]. Some species (*M. apelta*, *M. barbatus*, *M. floribundus*, *M. glabriusculus*, *M. macrostachyus*, *M. oblongifolius*, *M. paniculatus*, *M. philippinensis*, *M. poilanei*) are used as medicinal plants in Vietnam and in other countries in Southeast Asia for the treatment of various ailments ranging from minor infections such as gastrointestinal disorders to dysentery, hepatic diseases, cutaneous diseases, fever and malaria, cancer and infectious or inflammatory diseases [2a]. Different parts of the plants are used medicinally (Table 1). Many of these Vietnamese species are edible and the leaves are boiled and consumed instead of tea (*Camellia sinensis* (L.) Kuntze). Several authors have underlined the possible application of some *Mallotus* species as natural antioxidants [2b,3,5a]. Different polyphenol-rich fractions of *M. philippinensis* possess strong antioxidant and antiradical properties [3]. The leaves of *M. japonicus*, containing hydrolysable tannins, provide protective activity in the human body against oxidative stress and various related diseases [5a]. The potential of antioxidants, and particularly polyphenols, for preventing oxidation in human tissue and reducing the risk of different illnesses, such as cardiovascular diseases or cancers [5b], on the one hand, and the sparse

Table 1: Vietnamese traditional use of selected *Mallotus* species and percentage yield of 33 methanolic extracts.

Botanical name	Vernacular names	Uses[a, b]	Voucher number	Source[c]	Location[d]	Part used[a]	Percentage yield (% w/w) dry wt
***Mallotus apelta* (Lour.) Müll. Arg.**	*babet trang, buc trang, bui bui, bai bai, bum bup, bang bac, cay ruong*	B: dh, ga, gy, hem, hep, oe L: co, cu, dh, gy, hep, oe, ot R: ai, dh, ga, gy, hep, oe	MA01	NNT	VP	L	6.5%
			MA02	NNT	VP	L	6.8%
***Mallotus barbatus* (Wall.) Müll. Arg.**	*bung buc, bup bong gai, bong bet, bung buc gai, ba bet long, ruoi cau, cam lon*	B: ga L: ac, hem, oe, sc R: an, fe, diu, cho, hea	03	THT	SL	L	10.0%
			MA29	NNT	NB	L	8.4%
			NT01	NNT	HG	L	10.5%
***Mallotus cuneatus* Ridl.**	*duoi rung, ruoi rung*		MA16	NNT	LS	L	6.5%
			MA17	NNT	QN	L	6.8%
***Mallotus floribundus* (Blume) Müll.Arg.**	*babet nhieu hao, bach dan, ruoi trung bo, ba bet hoa nhieu*	R: dh, fe, gy Wp: sc	MA15	NNT	LS	L	6.1%
***Mallotus hookerianus* (Seem.) Müll. Arg.**	*babet cuong long, ba bet long duong, nhot vang, chua nga, choi moi nep, nhung dien hooker, ruoi hooker*		MA22	NNT	QT	L	5.3%
***Mallotus luchenensis* F.P. Metcalf.**	*camlon, bum bup, ruoi luchen*		01	THT	SL	L	7.1%
***Mallotus macrostachyus* (Miq.) Müll. Arg.**	*babet chum to, bum bup bong to, buc chum to, ruoi trang, nhung dien duoi to*	L: ac, hem, wo	MA11	NNT	LS	L	6.5%
***Mallotus metcalfianus* Croizat**	*babet do, ba bet mecalf, ruoi mecalf*		NT03	NNT	HG	L	9.7%
***Mallotus microcarpus* Pax & K. Hoffm.**	*babet qua nho, ruoi trai nho*		02	THT	SL	L	9.2%
			MA12	THT	SL	L	8.0%
***Mallotus nanus* Airy Shaw**	*ba bet lun, ruoi thorel*		MA23	THT	D	L	7.8%
			MN37R	NNT	BMNP	R	7.5%
			MN37L	NNT	BMNP	L	5.7%
			MN39C	NNT	BMNP	C	7.0%
***Mallotus oblongifolius* (Miq.) Müll. Arg.**	*choc mon, choc moc, choc mot, cam heo, ruoi tron dai*	R: ma Wp: dh, ga	MA14	NNT	QT	L	6.2%
***Mallotus oreophilus* Müll. Arg.**	*babet nui cao*		M25	LDM	LC	L	5.0%
***Mallotus pallidus* Airy Shaw**	*babet tai*		MA13	NNT	PNKBNP	L	5.8%
***Mallotus paniculatus* (Lam.) Müll. Arg.**	*buc bac, bong bet, bai dai, bum bup nau, bung buc nau, ba bet nam do, bach thu*	F : co, oe R: gy Wp: fe, hea, wo	NT02	NNT	HG	L	6.2%
			MA03	NNT	VP	L	6.0%
			MP31L	NNT	PNP	L	5.3%
			MP32R	NNT	PNP	R	6.5%
			MP33L	NNT	BMNP	L	5.6%
			MP34R	NNT	BMNP	R	6.2%
			MP35R	NNT	CPNP	R	6.0%
			MP36L	NNT	CPNP	L	5.1%
***Mallotus philippinensis* Müll. Arg.**	*canhkien, mot, rum nao, ba chia, thuoc san, tho khang sai, rum hao*	B: an, antis, cu, fe, hem, wo F: antis, cu, fe, ga, hem, oe, pa, sy L: cu, dh, diu, dy, wo R: antic, antis, dh, dy, fe, hem S: diz, ve	SP5	NNT	LS	L	5.7%
			MA28	NNT	CPNP	L	6.7%
***Mallotus poilanei* Gagnep.**		L : hea	MA20	NNT	PNKBNP	L	6.5%
***Mallotus yunnanensis* Pax & K. Hoffm.**	*babet van nam, ruoi van nam*		MA19	NNT	LS	L	6.0%

[a] Parts used : B, Bark; C, Branches; F; Fruits; L, Leaves, R: Roots; S, Seeds; Wp, Whole plant

[b] Vietnamese traditional usage : ac, acne; ai, antiinflammatory; an, analgesic; antic, anticonvulsivant; antis, antiseptic; cho, cholera; co, contusions and traumatic injuries; cu, cutaneous diseases; dh, diarrhea; diu, diuretic; diz, dizziness; dy, dysentery; fe, fever; ga, gastrointesinal disorders; gy, gynecological infection; hea, headache; hem, hemostatic; hep, hepatic diseases; ma, malaria; oe, oedema; ot, otitis; pa, parasiticide; sc, scabies; sy, syphilis; ve, vertigo; wo, wounds

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[d] Location (Vietnamese provinces) : BMNP, Bach Ma National Park (Thua Thien Huê province, Central province); CPNP, Cuc Phuong National Park (Ninh Binh province, Northern province); D, Dak Lak (Central province); HG, Ha Giang (Northern province); LC, Lao Cai (Northern province); LS, Lang Son (Northern province); NB, Ninh Binh (Northern province); PNKBNP, Phong Nha-Ke Bang National Park (Quang Binh province, Central province); PNP, Pumat National Park (Nghê An province, Central province); QN, Quang Ninh (Northern province); QT, Quang Tri (Central province, Da Krong district); SL, Son La (Northern province); VP, Vinh Phuc (Northern province, Tam Dao district)

information available concerning pharmacological activities of Vietnamese *Mallotus* species on the other hand, motivated us to analyze the antiradical properties of 33 *Mallotus* methanolic extracts from 17 species from Vietnam (Table 2). For some species, several samples were collected from different provinces, in order to compare the differences in chemical compositions and activities (Tables 1 and 2).

In a previous study, combining chemical fingerprint technology with data-analysis allowed us to obtain information on peaks potentially responsible for the antioxidant activity of these *Mallotus* species. Relevant information was extracted using linear multivariate calibration techniques, both before and after alignment of the fingerprints with correlation optimized warping

Table 2: Cytotoxicity on WI-38 and HeLa cells and antiradical activities of 33 *Mallotus* methanolic extracts.

Botanical name	Voucher number	Viability % 50 µg/mL[a, b, c] WI-38 cells	Viability % 50 µg/mL[a, d] HeLa Cells	Percentage of remaining DPPH° ($DPPH°_{REM}$)[a, e] 20 µg/mL
Mallotus apelta	MA01	**16.0% ± 1.6** (4)*	**43.0% ± 1.0** (3)*	94.5% ± 0.4 (3)*
	MA02	**13.0% ± 1.0** (3)*	**50.2% ± 4.9** (6)*	92.5% ± 3.3 (3)*
Mallotus barbatus	03	64.5% ± 2.0 (4)*	84.0% ± 2.9 (3)*	79.4% ± 9.7 (5)*
	MA29	86.5% ± 2.6 (3)*	86.3% ± 9.0 (3)	**11.3% ± 4.8** (3)*
	NT01	114.0% ± 2.9 (3)*	61.1% ± 1.0 (6)*	77.2% ± 10.4 (3)*
Mallotus cuneatus	MA16	**13.5% ± 2.0** (4)*	**46.7% ± 2.6** (3)*	86.9% ± 3.2 (3)*
	MA17	61.0% ± 12.0 (4)*	**48.3% ± 2.0** (3)*	**10.3% ± 4.1** (3)*
Mallotus floribundus	MA15	74.3 ± 2.6 (3)*	73.8% ± 6.8 (6)*	**6.4± 0.2** (3)*
Mallotus hookerianus	MA22	98.7% ± 5.0 (3)	79.3% ± 5.6 (6)*	**50.0% ± 4.6** (3)*
Mallotus luchenensis	01	118.0% ± 3.5 (3)*	62.3% ± 3.4 (6)*	82.0% ± 12.1 (3)
Mallotus macrostachyus	MA11	**13.3% ± 3.5** (3)*	**41.3% ± 0.5** (3)*	75.7% ± 2.2 (3)*
Mallotus metcalfianus	NT03	59.3% ± 5.5 (3)*	82.3% ± 2.1 (3)*	51.1% ± 14.6 (5)*
Mallotus microcarpus	02	61.0% ± 6.9 (3)*	45.3% ± 4.5 (3)*	63.6% ± 13.0 (5)*
	MA12	**24.3% ± 4.5** (3)*	52.7% ± 2.4 (6)*	83.1% ± 2.0 (3)*
Mallotus nanus	MA23	101.0% ± 4.0 (3)	67.0% ± 4.5 (3)*	78.4% ± 9.5 (3)*
	MN37R	93.0% ± 4.0 (3)*	66.9% ± 0.7 (6)*	**12.2% ± 1.7** (3)*
	MN37L	81.0% ± 1.0 (3)*	74.8% ± 4.9 (6)*	**4.5% ± 1.0** (3)*
	MN39C	87.0% ± 4.5 (3)*	68.6% ± 2.2 (6)*	**27.1% ± 4.7** (3)*
Mallotus oblongifolius	MA14	96.0% ± 4.0 (3)	72.0% ± 2.4 (6)*	**6.7% ± 0.3** (3)*
Mallotus oreophilus	M25	94.0% ± 5.0 (3)	82.7% ± 4.0 (3)*	88.8% ± 10.5 (3)
Mallotus pallidus	MA13	110.0% ± 3.6 (3)*	77.7% ± 1.5 (3)*	65.3% ± 1.9 (3)*
Mallotus paniculatus	NT02	109.3% ± 14.7 (3)	61.0% ± 3.4 (6)*	82.2% ± 5.5 (3)*
	MA03	99.3% ± 4.9 (3)	89.0% ± 7.6 (3)	58.4% ± 5.4 (3)*
	MP31L	79.3% ± 3.5 (3)*	76.1% ± 5.4 (6)*	73.5% ± 8.5 (3)*
	MP32R	76.8% ± 5.6 (4)*	73.9% ± 2.7 (3)*	91.5% ± 5.7 (3)
	MP33L	84.7% ± 7.4 (3)*	82.7% ± 1.5 (3)*	81.5% ± 3.8 (3)*
	MP34R	96.0% ± 3.6 (3)	91.3% ± 1.5 (3)*	83.5% ± 6.6 (3)*
	MP35R	77.8% ± 5.8 (4)*	77.3% ± 5.1 (6)*	**27.9% ± 11.3** (3)*
	MP36L	103.7% ± 1.2 (3)*	85.3% ± 3.8 (3)*	75.3% ± 8.8 (3)*
Mallotus philippinensis	SP5	97.3% ± 3.5 (3)	71.1% ± 6.8 (6)*	98.9% ± 12.6 (3)
	MA28	103.3% ± 7.4 (3)	86.0% ± 5.0 (3)*	**22.3% ±10.0** (3)*
Mallotus poilanei	MA20	61.3% ± 2.6 (3)*	56.8% ± 3.7 (6)*	90.5% ± 7.0 (3)
Mallotus yunnanensis	MA19	**15.3%± 1.5** (3)*	61.3% ± 4.4 (6)*	91.6% ± 6.6 (3)

[a] Each value represents the mean ± SD of (n) determinations, *Significantly different from the background control (DMSO for cytotoxic tests and MeOH for antioxidant tests): $p < 0.05$

[b] Campthotecin: IC_{50} = 0.23 µg/mL or 0.7 µM, [c] Malloapelta B: IC_{50} = 3.8 µg/mL or 13 µM; [d] Campthotecin: IC_{50} = 0.17 µg/mL or 0.5 µM; [e] Tocopherol: 4.1% ± 0.2 (5)*

(COW) [6a,6b]. Some active compounds were subsequently identified [6c]. Of the 33 methanolic extracts, the most active were from *M. barbatus* MA29, *M. cuneatus* MA17, *M. floribundus* MA15, *M. hookerianus* MA22, *M. oblongifolius* MA14, *M. paniculatus* MP35R and *M. philippinensis* MA28, and three from *M. nanus* : MN37, MN37L and MN39C. While the results obtained for the multiple samples of *M. barbatus* or *M. philippinensis* are different, depending on their place of collection, they are similar for the different samples of *M. paniculatus*. Some species of *M. nanus* and *M. paniculatus* were collected in the same location, but the parts collected were different. Indeed, the antioxidant activity of the roots of *M. paniculatus* (MP35R) is higher than that of the leaves (MP36L).

Considering the cytotoxic potential of *M. apelta*, we also analyzed the antiproliferative activity of the *Mallotus* extracts on a non cancerous cell line (WI-38) and on a cancer cell line (HeLa) (Table 2). WI38 cells seem to be more sensitive than HeLa. Six *Mallotus* methanolic extracts seem promising in the search for cytotoxic compounds: *M. apelta* MA01 and MA02, *M. cuneatus* MA16, *M. macrostachyus* MA11, *M. microcarpus* MA12, *M. yunnanensis* MA19. Our results also confirm the activity of *M. apelta* containing cytotoxic benzopyrans [2e] such as malloapeltaB which showed an IC_{50} = 13 µM on WI38 cells (Table 2).

According to the literature, the antioxidant activities of *M. nanus* and *M. philippinensis* could be explained by the presence of flavonoids and tannins [7a,7b]. Additionally, several extracts had an antiradical activity similar to tocopherol. They thus represent valid alternative sources of antioxidant agents, as we also showed that they did not show cytotoxicity on cultured cells. Consequently, we studied the antiradical activities of the crude methanolic extracts of the ten most active species (set stored 6 months at 3-5°C) and of their ethyl acetate and aqueous partitions before and after tannins removal (Table 3). In general, we found approximately the same antiradical activities for the crude methanolic extracts of all plants tested, except for *M. paniculatus* MP35R and *M. nanus* MN39C, which were less active. This loss of activity could perhaps be explained by the degradation of active secondary metabolites. To verify this hypothesis, we also tested the remaining methanolic crude extracts of the first series stored 6 months at -18°C in Belgium. Their activity also decreased, but less than when extracts are conserved at 3-5°C. The percentage of remaining DDPH° at 20 µg/mL for *M. nanus* MN39C were 27.1% ± 4.7 (3) (first set), 47.9% ± 2.1 (3) (second set stored 6 months at -18°C), 76.8% ± 2.9 (3) (second set stored 6 months at 3-5°C), giving IC_{50} of 16.0 ± 1.8 µg/mL (3) and 59.3 ±: 10.0 µg/mL (3) respectively for these two last samples. For *M. paniculatus* MP35R, the percentage of remaining DDPH° at 20 µg/mL was 27.9% ± 11.2 (3) (first set), 47.5 % ± 3.1 (3) (second set stored at 6 months -18°C), 79.8 % ± 1.4 (3) (second set stored 6 months at 3-5°C), giving IC_{50} of 19.2 ± 2.0 µg/mL (3) and 79.7 ± 9.2 (3) µg/mL (3), respectively, for these two 6 months old samples. To determine if tannins were responsible for the observed activities, we analyzed their antiradical effects after tannins removal and showed that for *M. cuneatus* MA17, the activity of the MeOH extract is due to the ethyl acetate soluble tannins, while for *M. hookerianus* MA22 and *M. nanus* MN39C, it is due to the tannins from the aqueous partition. For other species, both the ethyl acetate and aqueous partitions are active. For *M. barbatus* MA29, *M. floribundus* MA15, *M. oblongifolius* MA14, *M. paniculatus* MP35R and *M. philippinensis* MA28, this activity seems to be primarily due to the presence of tannins. The ethyl acetate and aqueous partitions of *M. nanus*, MN37L and MN37R, are the only extracts with activity in non-tannin containing samples (Table 3).

Table 3: Antiradical activities of selected *Mallotus* species (crude methanolic extract, ethyl acetate and aqueous partitions with and without tannins).

Botanical name	Compound, extract or partitions[a]	EtOAc partition yield (%)	Percentage of removed tannins (on 21 mg extract)	Percentage of remaining DPPH° ($DPPH°_{REM}$)[b] 20 µg/mL	IC_{50} (µg/mL)
Tocopherol				**4.7% ± 0.6 (3)***	**5.3**
Mallotus barbatus MA29	CME			**6.6% ± 0.7 (3)***	**6.0**
	EtOAcP	9.6%		**7.1% ± 0.5 (3)***	**5.0**
	EtOAc P WT		21.9%	97.1% ± 0.4 (3)*	> 40
	H_2OP			**10.3% ± 6.9 (3)***	**9.7**
	H_2OP WT		7.3%	99.0 ± 0.8 (3)	> 40
Mallotus cuneatus MA17	CME			**22.4% ± 2.6 (3)***	**10.1**
	EtOAcP	29.8%		**7.8% ± 0.7 (3)***	**4.3**
	EtOAc P WT		6.7%	96.5 ± 1.0 (3)*	> 40
	H_2OP			79.0% ± 1.0 (3)*	61.5
	H_2OP WT		32%	97.7% ± 2.1 (3)	> 40
Mallotus floribundus MA15	CME			**6.5 ± 0.6% (3)***	**5.4**
	EtOAcP	38%		**7.3 ± 0.7% (3)***	**4.5**
	EtOAc P WT		42%	100.1% ± 0.3 (3)	> 40
	H_2OP			**6.7 ± 2.9% (3)***	**7.5**
	H_2OP WT		48.6%	95.5% ± 1.1 (3)*	> 40
Mallotus hookerianus MA22	CME			55.6% ± 4.4 (3)*	**20.7**
	EtOAcP	37.7%		74.5% ± 6.4 (3)*	**42.4**
	EtOAc P WT		10.7%	96.6% ± 1.7 (3)*	> 40
	H_2OP			**48.4% ± 3.4 (3)***	**17.0**
	H_2OP WT		26%	95.9% ± 2.8 (3)	> 40
Mallotus nanus MN37R	CME			**5.0% ± 0.4 (3)***	**3.4**
	EtOAcP	15.2%		**6.3% ± 0.9 (3)***	**5.5**
	EtOAc P WT		22%	**49.7% ± 10.8 (3)***	**23.8**
	H_2OP			**5.8% ± 0.8 (3)***	**3.4**
	H_2OP WT		18.6%	**7.4% ± 2.8 (3)***	**8.8**
Mallotus nanus MN37L	CME			**4.7% ± 0.5 (3)***	**3.6**
	EtOAcP	16.5%		**5.9% ± 0.4 (3)***	**5.3**
	EtOAc P WT		34.7%	**41.7% ± 10.6 (3)***	**18.4**
	H_2OP			**4.7% ± 0.7 (3)***	**3.9**
	H_2OP WT		25.3%	**20.0% ± 4.6 (3)***	**9.7**
Mallotus nanus MN39C	CME			**76.8% ± 2.9 (3)***	59.3
	EtOAcP	68.3%		87.9% ± 6.9 (3)*	85.1
	EtOAc P WT		3.3%	97.1% ± 1.5 (3)*	> 40
	H_2OP			55.6 ± 6.1 % (3)*	**24.0**
	H_2OP WT		22.8%	84.1 ± 1.5 % (3)*	> 40
Mallotus oblongifolius MA14	CME			**4.9% ± 0.5 (3)***	**4.0**
	EtOAcP	29.1%		**5.9% ± 0.1 (3)***	**2.9**
	EtOAc P WT		20.7%	97.2% ± 1.2 (3)*	> 40
	H_2OP			**4.2% ± 0.9 (3)***	**5.6**
	H_2OP WT		38.1%	97.6% ± 1.1 (3)*	> 40
Mallotus paniculatus MP35R	CME			**79.8% ± 1.4 (3)***	**79.7**
	EtOAcP	19.1%		**30.6 % ± 11.0 (3)***	**12.4**
	EtOAc P WT		18%	95.5% ± 0.3 (3)*	> 40
	H_2OP			**34.2% ± 11.4 (3)***	**15.9**
	H_2OP WT		30.9%	97.3% ± 1.0 (3)*	> 40
Mallotus philippinensis MA28	CME			**24.3% ± 4.7 (3)***	**10.3**
	EtOAcP	23%		**7.4% ± 1.0 (3)***	**6.4**
	EtOAc P WT		6.7%	93.7% ± 3.7 (3)*	> 40
	H_2OP			**30.9% ± 9.5 (3)***	**14.1**
	H_2OP WT		48.6%	85.5 ± 1.3% (3)*	> 40

[a] CME: Crude Methanolic Extract, EtOAcP: Ethyl Acetate Partition, EtOAcP WT: Ethyl Acetate Partition Without Tannins, H_2OP: Aqueous Partition, H_2OP WT: Aqueous Partition Without Tannins;[b] Each value represents the mean ± SD of (n) determinations; * Significantly different from the background control (MeOH): $p < 0.05$

In a previous study on *M. metcalfianus* NT03 [2b], we showed that the ethyl acetate partition had the most potent antioxidant activity of the different extracts tested. We developed an HPLC method to separate compounds from this fraction, without removing tannins. Quercitrin, astilbin and kaempferol 3-*O*-α-L-rhamnoside were the major compounds from this partition identified by LC-ESI-MS. Of thirteen compounds isolated from *M. metcalfianus* and tested for their antiradical activities, quercitrin and kaempferol 3-*O*-*α*-L-rhamnoside showed the strongest antiradical activity with quercetin 3-*O*-*β*-neohesperoside [2b]. We further applied this chromatographic method for the antioxidant rich ethyl acetate *Mallotus* partitions. We analyzed *M. nanus* MN37L and *M. nanus* MN37R whose activity was not mainly due to tannins and identified kaempferol 3-*O*-*α*-L-rhamnoside in both species as major constituent and quercitrin in *M. nanus* MN37L. This last extract was found to be particularly rich in kaempferol 3-*O*-*α*-L-rhamnoside and quercitrin. The chromatographic profiles of *M. nanus* MN37L and R also show the presence of another compound at 16.2 min. Its molecular formula $C_{21}H_{20}O_{12}$ was determined by LC/HRESIMS, which showed an ion $[M-H]^-$ at *m/z* 463.0858 with the negative ion mode, compatible with the molecular formula of different flavonoids. This compound is present in trace amounts in only three other antiradical containing species (*M. metcalfianus* NT03, *M. cuneatus* MA17 and *M. oblongifolius* MA14). *M. nanus* MN39C, which does not contain this compound, is less active. *M. nanus* MN37R also contains a compound, different from quercitrin, at 18.1 min giving a molecular ion $[M-H]^-$ at *m/z* 530.8030 by LC/HRESIMS. We also analyzed other antioxidant active ethyl acetate partitions and found that three of them contained quercitrin and/or kaempferol 3-*O*-α-L-rhamnoside: *M. nanus* MN39C (kaempferol 3-*O*-α-L-rhamnoside and quercitrin), *M. paniculatus* MP35R (quercitrin), *M. cuneatus* MA17 (quercitrin). Quercitrin has also been isolated from *M. apelta* [7c] and from other genera of Euphorbiaceae: *Alchornea* [7d], *Euphorbia* [7e], *Phyllanthus* [7f] or *Pedilanthus* [7g]. Kaempferol glycosides have been described in some species of Euphorbiaceae, for example in the genus *Euphorbia* [8a]. To our knowledge, astilbin was not yet described in other Euphorbiaceae species.

The results of our screening justify the traditional uses of some investigated plants in the Vietnamese medicine. This work is the first report of biological tests of *M. barbatus*, *M. cuneatus*, *M. floribundus*, *M. hookerianus*, *M. luchenensis*, *M. macrostachyus*, *M. metcalfianus*, *M. microcarpus*, *M. nanus*, *M. oblongifolius*, *M. oreophilus*, *M. paniculatus*, *M. poilanei* and *M. yunnanensis*. The antioxidant or antiproliferative activities of some species are particularly interesting and could lead to the use of some of these species, for example, as antioxidant rich nutrients, particularly *M. nanus*, *M. floribundus* and *M. oblongifolius*. For some species, this study also shows important differences in the activities of different samples of the same species, depending on location. This effect might be explained by the ecological and environmental parameters (soil, climate…) that can vary from one Vietnamese province to another and may affect the production of secondary metabolites. As we saw for *M. nanus* MN39C and *M. paniculatus* MP35R, the active secondary metabolites can also degrade with time and storage conditions. The results of these screening

investigations confirm the potential of *Mallotus* species as rich sources of potent antioxidant compounds.

Experimental

Chemicals: All solvents, MeOH, EtOAc, DMSO as well as tetracycline, tocopherol, 1,1-diphenyl-2-picrylhydrazyl, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and camptothecin were purchased from Sigma, Belgium. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco® BRL, Invitrogen, Merelbeke, Belgium.

Samples: Plant material (leaves, roots and branches) were collected from various provinces of Vietnam. These were identified by Dr. Nguyen Nghia Thin (Department Biology, National University Hanoi, Hanoi, Vietnam), Dr. Tran Huy Thai and Dr. La Dinh Moi (Institute of Natural Products Chemistry (VAST), Hanoi). Voucher specimens (Table 1) were deposited at VAST, Hanoi, Vietnam.

Preparation of plant extracts: In Vietnam, leaves, roots and/or branches of 33 *Mallotus* samples were air-dried at room temperature and powderized. 2.5 g of powdered material from each sample was extracted three times with MeOH (25 mL) in an ultrasonic instrument (Ultrasonic 2010, 950W, 40°C, 60 min, Branson Ultrasonic Corporation, Connecticut, USA), then filtered by filter-paper. The filtrates were combined and concentrated to dryness under reduced pressure with a rotatory evaporator at a temperature of 50°C. These crude extracts were stored in Vietnam at 3-5°C and aliquots were sent to Belgium for cytotoxic and antiradical tests. Percentage yields (% w/w) of each methanolic extract are given in Table 1. For the second series of analysis (determination of antiradical activities of the ten most active extracts, fractionation and LC-MS analysis), a separate aliquot of the same crude methanolic extracts, stored at 3-5°C for 6 months, were sent to Belgium. These extracts were suspended in water (60 mL) and extracted using ethyl acetate (3 x 60 mL). The yields of the ethyl acetate partitions are given in Table 3.

Analysis of samples by LC-ESI-MS: Mass spectra in positive or negative-ion modes were acquired with a Thermo Scientific LTQ Orbitrap XL mass spectrometer, equipped with an electrospray (ESI) source. Chromato-graphic separations were achieved on a 250 x 4 mm I.D. C-18 Merck Lichrospher® 100 analytical column 5 µm particle size. The mobile phase consisted of a mixture H_2O/MeOH using a gradient (90:10 to 0:100) in 30 min.

Elimination of tannins: 21 mg of both ethyl acetate and aqueous partitions were dissolved in water and applied onto a 5 g polyamide column in order to eliminate the tannins. Elution was performed with water (10 mL), water/MeOH (10 mL), and MeOH (40 mL) until the eluate was clear. The eluates were combined and then evaporated under reduced pressure.

Antioxidant activity: The DPPH assay was performed as described by Aquino *et al*. [8b]. An aliquot (50 µL) of the MeOH solution containing an extract or tocopherol (positive control) was added to 2.5 mL of freshly prepared DPPH° solution (25 µg/mL in methanol). An equal volume (50 µL) of the vehicle alone (MeOH) was added to control tubes (DPPH°0). Absorbance at 515 nm was measured on a Uvikon 933 spectrophotometer (Kontron Instruments, Muenchen, Germany) 20 min after starting the reaction. Crude extracts and tocopherol were tested at a concentration of 20 µg/mL. For the second part of the study, samples were tested at several doses, ranging from 40 µg/mL to 0.4 µg/mL, in order to determine the IC_{50}. The DPPH° concentration in the reaction medium was calculated from a calibration curve obtained after linear regression of the absorbance at 515 nm, at concentrations ranging from 1 to 50 µg/mL of DPPH°. The percentage of remaining DPPH° (DPPH°REM) was calculated as follows:
% DPPH°REM = [[DPPH° 20min] / [DPPH° 0]] x 100

All experiments were carried out at least in triplicate. For the ten most antiradical methanolic extracts, two series of three replicates were tested at 6 month intervals. Ethyl acetate and aqueous partitions of these crude methanolic extracts were tested after the second series of tests of methanolic extracts, before and after the elimination of tannins.

Cytotoxic assay: The cytotoxicity of the extracts on HeLa (Human cervix carcinoma) and WI-38 (Human lung fibroblast) cells was evaluated as described by Block *et al.* [8c], using the tetrazolium salt MTT colorimetric method. HeLa and WI-38 cells were grown in DMEM containing L-glutamine, D-Glucose, sodium pyruvate and supplemented with 10% FBS and antibiotics (100 IU penicillin/mL, 100 µg streptomycin/mL). Cells were incubated in a humidified atmosphere containing 5% CO_2. Stock solutions of extracts were prepared at 5 mg/mL in DMSO and stored at -4°C. Briefly, 5000 HeLa or WI-38 cells per well were seeded in 100 µL of DMEM with FBS 10% in 96-well microculture plates for 24 h. After 24 h, the medium was removed and 200 µL of fresh medium containing 50 µg/mL extract were added to each well, while negative control cells received fresh medium containing analogous DMSO concentration. Each extract was tested in 6 wells. After 72 h incubation, the medium was replaced by 100 µL DMEM (without serum) containing 10 µL of MTT solution (3 mg/mL in PBS). After 45 min, the medium was removed and 100 µL of DMSO were added to each well. The plates were shaked and absorbances were recorded at two wavelenghts (570 nm and 620 nm), against a background control as blank (100 µL of pure DMSO) on a microplate reader (Spectra Max 190, Sopachem). In each case, camptothecin was used as positive control (from 0.00025 to 25 µg/mL). The viability percentage was expressed relative to the control cells which were considered as: 100% viability % = [AT/ANT] x 100
with: A: absorbance, NT: control cells, T: treated cells

All experiments were made in triplicate.

Statistical analysis: Statistical calculations were carried out with GraphPad Prism 4. Results are expressed as the mean ± SD (Standard Deviation) of (n) independent experiments with individual values. Unpaired student's t-test was used for statistical comparison; P values < 0.05 were considered as significantly different from the control.

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N-methyl-5-carboxamide-2-pyridone from *Mallotus barbatus*: A chemosystematic marker of the Euphorbiaceae genus *Mallotus*

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1. Subject and source

Mallotus Lour., in the Malpighiales order and in the Euphorbiaceae family, consists of a large paleo(sub)tropical genus of shrubs, trees and rarely climbers with a wide distribution in different habitats of Southeast Asian forests, whether in the primary forest understories or in the more disturbed secondary forests (Kulju et al., 2007a, 2007b; Kulju and van Welzen, 2008). Thus some species are used as indicators of forest disturbance (Slik et al., 2003). *Mallotus* is placed in the tribe Acalypheae pro-parte of the uniovulate sub-family Acalyphoideae and has been classified in subtribe Rottlerinae with seven small genera (*Coccoceras*, *Cordemoya*, *Deuteromallotus*, *Neotrewia*, *Octospermum*, *Rockinghamia* and *Trewia*) according to the Euphorbiaceae classifications of Webster and Radcliff-Smith (Webster, 1994; Radcliffe-Smith, 2001; Nowicke and Takahashi, 2002). *Mallotus* is morphologically close to the large monophyletic genus *Macaranga*, classified in the same tribe Acalypheae but in a separate monogeneric subtribe Macaranginae (Kulju et al., 2007a). A recent molecular phylogenetic study on *Mallotus* and its eight related genera underlined the paraphyly of *Mallotus* (Kulju et al., 2007a). This genus, commonly known as *Ba bet*

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in Vietnam, is one of the most diverse and richest genera of the Euphorbiaceae family in this country, where about 40 *Mallotus* species may be found among which six species and one variety are endemic (Thin, 2003).

Mallotus barbatus (Wall.) Müll. Arg., referred as *Bung buc* in Vietnam, is a small tree growing particularly in the evergreen forests in all over the mountainous areas from the North to the South of Vietnam at an altitude inferior to 1100 m. *M. barbatus* is also distributed in other countries (China, Laos, Cambodia, Thailand, Malaysia, Philippines, Indonesia, Myanmar and India). Different parts of this plant (roots, stem bark, leaves) are used in traditional medicine for treating gastrointestinal disorders, oedema and headache (Thin, 2003). The leaves of *M. barbatus* were collected in May 2006 at the SonLa mountains (Northern Province) in Vietnam and identified by Dr. Tran Huy Thai, Institute of Ecology and Biological Resources, VAST, Vietnam. A voucher specimen has been deposited at the Institute of Natural Products Chemistry, VAST, Vietnam (N° M.b. 03).

2. Previous works

Some *Mallotus* species are known to contain different natural compounds, mainly diterpenoids, triterpenoids, steroids, flavonoids, coumarinolignoids, phloroglucinol derivatives or benzopyrans, and to exhibit interesting biological activities such as antimicrobial, antioxidant, antiviral, or cytototoxic ones (Rivière et al., 2010). In the course of our ongoing project to investigate the biologically active chemical constituents from *Mallotus* growing in Vietnam (Chau et al., 2004, 2005, 2009; Rivière et al., 2009, 2010; Hoai et al., 2009), we reported herein the identification of some compounds from *M. barbatus* leaves. An earlier phytochemical study revealed that the leaves contained polyprenols (Sasak and Chonjnacki, 1973). However, to our knowledge, no other reports on the chemical composition of *M. barbatus* have been made in the literature so far.

3. Present study

The dried and powdered leaves of *M. barbatus* (840 g) were extracted three times with MeOH (3 × 2 L) to give 80 g residue. This crude extract was suspended in water (2 L) and sequentially extracted using n-hexane (2 L), chloroform (2 L) and ethyl acetate (2 L).

Quercitrin **1** and 3-*O*-α-L-rhamnosyl kaempferol **2** were identified in the ethyl acetate partition of *M. barbatus* by LC-MS using comparison with reference samples isolated from another *Mallotus* species, *Mallotus metcalfianus* Croizat (Rivière et al., 2009). Mass spectra in positive or negative-ion modes were acquired with a Thermo Scientific LTQ orbitrap XL mass spectrometer equipped with an electrospray (ESI) source. Chromatographic separations were achieved on a 250 × 4 mm I.D. C-18 Merck Lichrospher® 100 analytical column, 5 μm particle size. The mobile phase consisted of a mixture H_2O/MeOH using a gradient (90/10 to 0/100%/%) in 30 min. Data acquisition and processing were performed with Xcalibur software.

N-methyl-2-pyridone-5-carboxamide **3** was obtained from ethyl acetate partition by crystallization in CH_2Cl_2–MeOH and identified by IR, LC-MS and extensive NMR studies. These data correlate with those of the literature (Wong et al., 2002). IR spectra (KBr) were measured on a Perkin–Elmer FTIR 286 spectrometer. NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer in CD_3OD at 400 MHz (^1H) and 100 MHz (13C), at 30 °C. A combination of COSY, HMQC and HMBC experiments was used when necessary for the assignment of ^1H and ^{13}C chemical shifts. Mass spectra in positive-ion mode were acquired with a Thermofinnigan LCQ Advantage ion trap mass spectrometer, equipped with an APCI source.

N-methyl-2-pyridone-5-carboxamide. White powder (15 mg), IR (KBr) υ_{max} 3402 (N–H amide), 1660 (C═O amides), 1602 (aromatic rings) cm^{-1}; APCI-MS m/z 153 $[MH]^+$ ($C_7H_8N_2O_2$ requires 152); ^1H NMR (400 MHz, MeOD): δ 8.35 (1H, *d*, $J = 2.5$ Hz, H-6), 7.95 (1H, *dd*, $J = 9.5$, 2.5 Hz, H-4), 6.52 (1H, *d*, $J = 9.5$ Hz, H-3), 3.62 (3H, *s*, CH_3) and ^{13}C NMR (100 MHz, MeOD): 166.7 (C-8), 163.5 (C-2), 143.5 (C-6), 138.5 (C-4), 119.3 (C-3), 113.2 (C-5), 36.6 (C-7).

The chloroformic partition was subjected to a vacuum liquid chromatography (VLC) on silica gel normal phase with a gradient solvent system of CH_2Cl_2–AcOEt to give 10 fractions (A–I). Friedelin **4** was obtained in fraction A by crystallization in CH_2Cl_2–MeOH and identified by GC–MS and comparison with reference sample. The gas chromatograph was a TRACE GC 2000 series, equipped with an autosampler AS2000 Thermo-Quest. The GC system was interfaced to a Trace MS mass spectrometer operating in the electron-impact mode. Chromatographic separations were performed on a capillary nonpolar column (DX-XLB; column length 15 m × 0.25 mm with 0.25 μm film thickness) from Agilent Technologies (Rivière et al., 2009) (Fig. 1).

4. Chemotaxonomic significance

N-methyl-2-pyridone-5-carboxamide **3** is a metabolite of nicotinic acid in mammals. Nicotinic acid, also known as niacin, is a member of the B-vitamin family and has been identified in *Mallotus apelta* (Kang and Lu, 2007). N-methyl-2-pyridone-5-carboxamide has been already isolated from Euphorbiaceae, from *Trewia nudiflora* L. (Sastry and Waller, 1972) and more recently from *Mallotus anisopodus* Airy Shaw (Chau et al., 2009). In the Euphorbiaceae, *Mallotus* and *Trewia* are placed in the tribe Acalypheae pro parte in the subtribe Rottlerinae (Webster, 1994; Radcliffe-Smith, 2001). *Trewia* is an Asiatic ditypic genus (*T. nudiflora* L. present from India to the Philippines and *T. polycarpa* Benth. & Hook. f., an Indian endemic species) morphologically very close to *Mallotus*. They share the pollen type, the extrafloral nectaries on the upper leaf surface and a similar type of glandular hairs, a character which is also typical for most species of *Macaranga* but rare for species in other sub-families within Euphorbiaceae (Kulju et al., 2007a, 2007b). *Trewia* differs from *Mallotus* only in the fruit type. The fruits in *T. nudiflora* L. are indehiscent and drupaceous instead of generally dehiscent in *Mallotus* (Kulju and van Welzen, 2008).

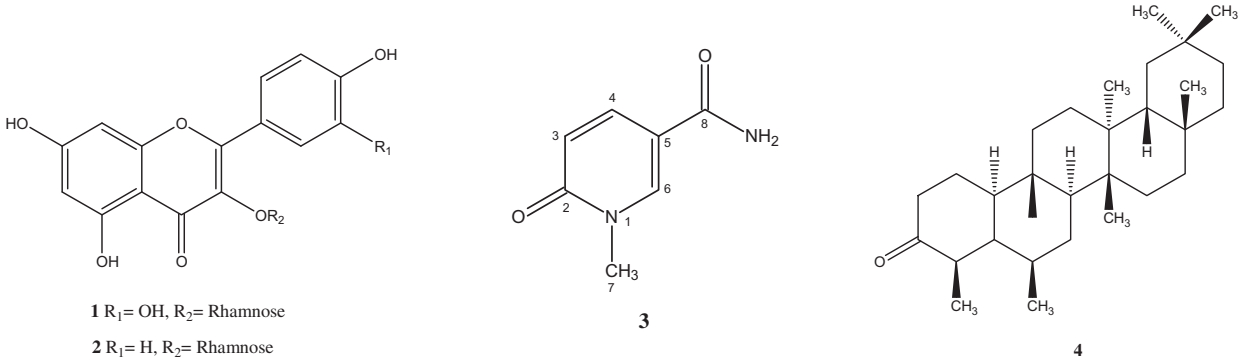

Fig. 1. Structure of compounds 1–4.

A recent molecular phylogenetic analysis of *Mallotus* and related genera, including *Trewia* and *Macaranga*, demonstrated the paraphyly of *Mallotus*. *Cordemoya* and *Deuteromallotus* and the Asian *Mallotus* sections *Hancea* and *Oliganthae* form a basal *Cordemoya* s.l. clade; whereas, four Rottlerinae genera, including *Coccoceras*, *Neotrewia*, *Octospermum* and *Trewia*, were found to be part of the main *Mallotus* clade named *Mallotus* s.s. clade, sister group with the *Macaranga* clade (Kulju et al., 2007a). Although the fruit type is a noticeable morphological difference, this character is not a sufficient justification to maintain a separate generic status. *Trewia*, along with the other three Rottlerinae genera *Coccoceras*, *Neotrewia* and *Octospermum*, were thus merged recently with *Mallotus* (Kulju et al., 2007b). *T. nudiflora* L. is now called *Mallotus nudiflorus* (L.) Kulju & Welzen (Kulju and van Welzen, 2008). To our knowledge, no other reports on the isolation of N-methyl-2-pyridone-5-carboxamide from other genera have been reported. Thus, the isolation of this compound from *T. nudiflora* L., as from some species of *Mallotus*, could justify the molecular phylogeny data leading to the merger of the genus *Trewia* with the genus *Mallotus*. N-methyl-2-pyridone-5-carboxamide could be a chemotaxonomical indicator of the genus *Mallotus*.

Friedelin **4**, a pentacyclic triterpene, has been isolated from other species of *Mallotus*: *M. apelta* (Chau et al., 2005), *M. hookerianus* (Hui and Li, 1976), *M. paniculatus* (Hui et al., 1969), *M. philippinensis* (Nair and Rao, 1993) and *M. repandus* (Hui and Li, 1976). The flavonoid, quercitrin **1**, has been isolated from *M. apelta* (Chau et al., 2004) and from *M. metcalfianus* (Rivière et al., 2009), and more recently identified by LC-ESI-MS in *M. nanus*, *M. cuneatus*, *M. paniculatus* growing in Vietnam (Nguyen et al., 2011). 3-*O*-α-L-rhamnosyl kaempferol **2** has been also isolated from *M. metcalfianus* and identified in *M. nanus* (Rivière et al., 2009; Nguyen et al., 2011).

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