

Project full title: From Biodiversity to Chemodiversity  
Novel Plant Produced Compounds with Agrochemical  
and Cosmetic interest

Project acronym: AgroCos

Grant agreement no.: 245336

Final report

SEPTEMBER 2014

Authored by the coordinator: National and Kapodistrian University of  
Athens

Grant Agreement number: 245336

Project acronym: AgroCos

Project title: **From Biodiversity to Chemodiversity Novel Plant Produced Compounds**

Name, title and organisation of the scientific representative of: **National and Kapodistrian University of Athens – Faculty of Pharmacy – Department of Pharmacognocny and Natural Products Chemistry.**

The project's coordinator: **Professor Mr. A. L. Skaltsounis**

Tel: 0030(0)2107274598

Fax: 0030(0)2107274594

E-mail: [skaltsounis@pharm.uoa.gr](mailto:skaltsounis@pharm.uoa.gr)

Project website address: <http://www.agrocos.eu/>

## **Executive summary**

The aim of the project was to discover and carry to the stage of development candidates; plant derived small molecules with potential as new cosmetic and agrochemical agents. These compounds have derive from plants originating from major biodiversity hotspots in Europe, Africa, Latin America, and the Asia-Pacific regions. The starting point of the project was a diversity-oriented natural product library of 484 compounds, from which promising scaffolds have been identified by a screening. Chemotaxonomy and chemodiversity oriented collection of plant material and 1810 plant species/organs, the generation of a library of 3620 extracts, and a state-of-the-art technology platform for miniaturized natural product discovery have generatet focused sub-libraries around these privileged scaffolds. A selection of 100 hit extracts was generated comprising of 60 active extracts with agrochemical properties and 40 active extracts with cosmetic potential. The hit extracts were microfractionated in order to focus the selected activity in active fractions, and the plant extracts were phytochemically investigated with state-of the-art techniques, for the isolation of the active constituents. All components (4000 microfractions and 100 isolated compounds) were examined for their biological properties: fungicidal, herbicidal, insecticidal (agrochemicals) and antioxidant, UV-B protective and antityrosinase (cosmetics). All data resulting from the extensive bioevaluation, spectral characterization and profiling of the active extracts and compounds were incorporated in the Agroc Database, implemented to foster this large number of data and provide solutions for easy dereplication of extracts. The evaluation of the existing results, in combination with advanced cellular biological evaluation and feasibility of commercialization, indicated five leads: three leads directed for cosmetic applications, and two leads for fungicidal agrochemical applications. A feasibility study was performed for the development of cosmetic agents from Greek biodiversity and several entries in the public media were published in order to communicate and support the important findings and the extent possibilities of development.

## **Description and Objectives**

The aim of the project was to discover and carry to the stage of development candidates, plant derived small molecules with potential as new cosmetic and agrochemical agents. These compounds have derived from plants originating from major biodiversity hotspots in Europe, Africa, Latin America, and the Asia- Pacific regions. The starting point of the project was a diversity-oriented natural product library of 484 compounds, from which promising scaffolds have been identified by a screening. Chemotaxonomy and chemodiversity oriented collection of plant material, the generation of a library of 3620 extracts, and a state-of-the-art technology platform for miniaturized natural product discovery have generated focused sub-libraries around these privileged scaffolds. Further evaluation of these sub-libraries led to the development of novel products in agrochemistry and/or cosmetics with new or improved properties over existing active ingredients. Through this rationale based process, five leads resulted from Agrococos research, three extracts with cosmetic potential and two candidates for agrochemical development. A feasibility study was conducted for the potential development of an extract enriched in compounds possessing cosmetic properties (antioxidant, UV-B protective and skin whitening), stemming from the Greek flora. The results were successfully disseminated via contributions at scientific congresses, workshops, research and by releases to the non-scientific community via the press, internet, press releases, newsletters, and video sources.

Through Agrococos, key-concepts were addressed with the establishment of

- a structurally diverse small molecule natural product library encompassing pure compounds from plants of geographically distant and megadiverse regions, to serve as a quick entry to scaffolds of potential interest for the cosmetic and agricultural applications sought
- a focused library of plant extracts assembled on the basis of chemodiversity and chemotaxonomy considerations, originating from plants from hotspots of biodiversity and endemism, to provide sub-libraries of compounds around the shortlisted scaffolds
- efficiency and traceability due to a high degree of standardization, automation and miniaturization in the generation and management of libraries, screening, and in profiling thanks to the development of a common platform for management and use of sample related spectral data and metadata.
- use of relevant in vitro assays for major areas of use in cosmetics and as agrochemicals like fungicidal, herbicidal and insecticidal evaluation for agrochemical use, and antioxidant, UV-B protective and anti-tyrosinase evaluation for cosmetic use
- complementary expertise available at the different partners of the consortium

Key objectives of Agrococos included:

- Discovery and characterization of novel plant derived small molecule-type natural products which qualify as development candidates for new cosmetic and agrochemical agents with innovative or improved properties
- Establishment of screening libraries with an unprecedented level of library-related spectroscopic information and metadata
- Development of new concepts for integrating information contents with screening libraries
- Enhancing the competitiveness of European cosmetic and agrochemical industry through discovery of novel compounds for development
- Opening new potentialities for the development of natural products derived from economically less favored but megadiverse regions, for sustainable production of products with high consumer acceptance

## **S/T Results and Foreground**

Agrocos project and its goals were implemented through seven Work Packages, namely:

**WP1. BIOPROSPECTING**

**WP2. CHEMODIVERSITY**

**WP3. BIOEVALUATION**

**WP4. BIOINFORMATICS/AUTOMATION**

**WP5. DEVELOPMENT**

**WP6. DISSEMINATION**

**WP7. PROJECT MANAGEMENT**

The main scientific and technological achievements of Agrocos were directly correlated with the work performed on the seven WPs and include:

- a. The targeted investigation of biodiversity hotspots through the screening of an existing library of 484 pure compounds originating from global biodiversity, and the compilation of a library of 3620 plant extracts
- b. The biological screening of an unprecedented number of extracts (3620), microfractions (4000) and pure compounds (600) for agrochemical (fungicidal, insecticidal, herbicidal) and cosmetic properties (anti-ageing and anti-hyperpigmentation), and advanced biological evaluation of potent candidates
- c. The fingerprinting of 100 hit plant extracts and 100 isolated compounds with state-of-the-art analytical techniques (MS/NMR) and full spectroscopic data of 30 lead compounds
- d. The identification of five lead entities (compounds/extracts) that can be further developed and exploited as agrochemical or cosmetic agents
- e. The implementation of the Agrocos database, incorporating all the spectral data and metadata resulting from the research activities and providing tools for the dereplication of extracts and 2D spectral matching
- f. The dissemination of Agrocos research activities to public media by entries in the internet and press
- g. The dissemination to the scientific community through publications to peer-review journals, participation in conferences and organization of workshops

### **Targeted investigation of biodiversity hotspots through the screening of an existing library of 484 pure compounds originating from global biodiversity, and the compilation of a library of 3620 plant extracts**

**A compound library of 484 pure small molecules has been generated from 5000 compounds available from existing collections** of the partners. Compounds were selected based on the following criteria:

- Compound should have a natural origin
- Known toxicological risks will be negative selection criteria
- Structural diversity and diversity of decoration of the scaffolds were preferred
- Physicochemical properties, such as solubility, were examined

The diversity of the selected compounds was analyzed for most of the compounds, according to classes of natural compounds. The distribution of molecular weights for the 484 small molecules is displayed in the diagram hereafter, while it was found that the average molecular weight is 378 and 71% of the molecular weights are within the range from 250 to 450.

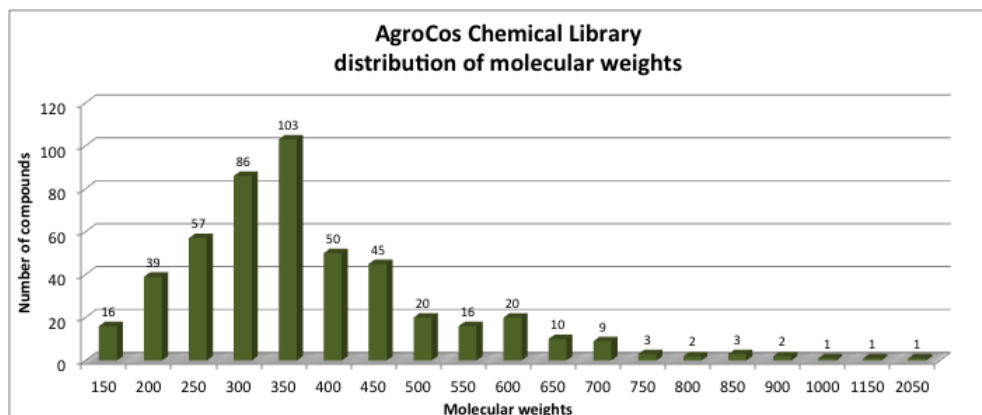


FIGURE 1 DISTRIBUTION OF MOLECULAR WEIGHTS IN THE 484-COMPOUND INITIAL LIBRARY FORWARDED FOR BIOLOGICAL EVALUATION

After analyzing the structural diversity of the compounds, the samples were formatted in 96well plates and forwarded for biological evaluation against a panel of targets associated to **agrochemical properties (fungicidal, insecticidal, herbicidal)** and **cosmetic properties (antioxidant, UV-B protective, tyrosinase inhibiting)**.

Based on the study on the chemodiversity of the initial 484-compound library and the biological screening, **a list of 1810 plant species/organs** was established by taking into account the presence of the desired structural diversity. The flora of source countries was analysed for plants containing the desired scaffolds and lists of 1810 plant part samples to be collected were established. More specifically:

- P1(NKUA) established a list of 450 plant species/organs
- P3(CNRS-ICSN) a list of 450 plant species/organs
- P3(CNRS-ECOFOG) a list of 300 plant species/organs
- P4(CIFLORPAN) a list of 300 plant species/organs
- P5(CSIR) a list of 300 plant species/organs

The diversity of the selected plant part species was analyzed, according to Family name. **147 plant families** are represented on this collection the largest are represented in the diagram hereafter.

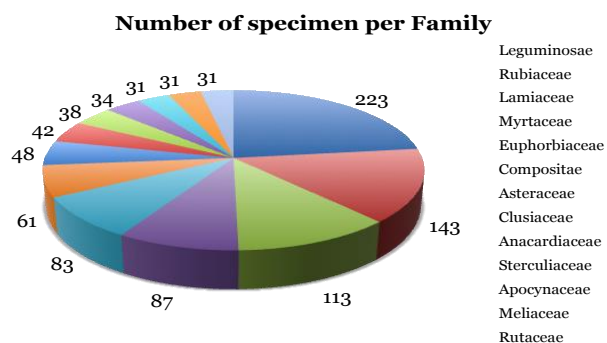


FIGURE 2 REPRESENTATION OF PLANT FAMILIES IN THE SELECTION OF 1810 PLANT SPECIES/ORGANS FOR THE COMPILATION OF THE 3620 EXTRACT LIBRARY

Below are some examples of how specific scaffolds with some promising biological activity led to the selection of the plant material:

Two funtumine type alkaloids (ICSN42-04-L-A11 & ICSN42-04-L-C02) were proved good fungicide agents. Funtumine is the main alkaloid isolated from several species belonging to Apocynaceae family, such as *Funtumia africana*, *Holarrhena febrifuga* and *H. flatifolia*. Thus, several Apocynaceae plants from two chemodiversity hotspots, French Guiana, South Africa and Panama were included in our plant list. Compound ICSN45-50-L-D03: Goniotalamin is a natural occurring styryl-lactone, with cytotoxic activity, isolated mainly from several *Goniotalamus* species (Annonaceae family), which also showed good fungicide activity. Thus, several species from Annonaceae family have been included in our plant list, from New Caledonia, Madagascar, French Guiana and Panama.

Compound ICSN45-50-L-D09 is a polygodial type sesquiterpene derivative that has shown potent antifungal activity against all three pathogens. Also, this compound has shown promising insecticidal activity. Such sesquiterpenes can be found in plants of Winteraceae, Asteraceae and Polygonaceae families. Selected species from Winteraceae family from New Caledonia, as well as several plants of Asteraceae (or Compositae) and Polygonaceae families, that contain several types of sesquiterpenes, from different hotspots are included in the plant list.

Concerning the good antifungal activity of tetrahydro usambarensine (ICSN74-45-L-C10), an alkaloid, derivative of usambarensine, isolated from the roots of *Strychnos* species, several plants of the Strychnaceae (or Loganiaceae) family from Madagascar, French Guiana and South Africa, that belong to *Strychnos*, as well as other genus, were selected in order to search for similar active structures.

Xanthyletin (NKUA-006) is a coumarin, characterized as phytoalexin and has been isolated from *Citrus* species. It showed potent herbicidal activity. As mentioned above, several Rutaceae species are included in our plant list. Also, several other potent herbicides, such quinoline alkaloids koniamborine (NKUA-009), Skimmianine (NKUA-018), haplopine (NKUA-012) and others, can be found in Rutaceae species. Another coumarin derivative, seselin (NKUA-007) showed also potent herbicidal, as well as insecticidal capacity and generally is a promising molecule. Seselin is found, apart from Rutaceae, in Apiaceae and Plumbaginaceae species. Plants from all three families are included in our list. Berberine (CR020), an isoquinoline alkaloid, also showing a good herbicidal capacity can be found in families such as Berberaceae, Rutaceae, Ranunculaceae and Papaveraceae. Plants from each one of the above families are included in the list.

Tryptanthrin (IC221), another promising herbicidal molecule was originally isolated from *Isatis tinctoria*, fam. Brassicaceae, thus several such species are included in our list.

For cosmetics:

The selection of the plant species by the partners was based on the possible cosmetic properties of the initially tested compounds.

More specifically, screening data deriving from the antioxidant activity results (cell-free system using the DPPH-method, reduction of the intracellular reactive oxygen species and UV-protection capacity assessment) indicated that sixteen compounds exhibit promising antioxidant properties and were used in the bibliographic search. Esculetin is a derivative of coumarin and its scaffold indicated Thymeleaceae, Ericaceae, Leguminosae, Euphorbiaceae, Convolvulaceae, Caprifoliaceae, Violaceae, Rutaceae, Loranthaceae, Bigoniaceae, Clusiaceae, Malvaceae, Oleaceae, Umbelliferae, Moraceae, Caprifoliaceae, Rubiaceae, Ranunculaceae, Compositae and Geraniaceae as candidate families. Likewise, the activity of the alkaloid boldine led us to select Annonaceae, Monimiaceae, Lauraceae, Papaveraceae, Berberidaceae and Euphorbiaceae families, of the phenolic ellagic acid Euphorbiaceae, Geraniaceae, Sapindaceae, Combretaceae, Ericaceae, Fagaceae, Rosaceae, Myrtaceae, Onagraceae and Guttiferae families and of curcumin Zingiberaceae family. The antioxidant activity of the flavonoids stimulated as to add to the final list families rich in such compounds such as Primulaceae, Compositae, Thymelaceae, Rosaceae, Guttiferae, Apocynaceae, Campalunaceae, Umbelliferae, Myrsinaceae, Annonaceae, Verbenaceae, Euphorbiaceae, Plantaginaceae, Leguminosae, Myrtaceae, Dioscoreaceae, Bignoniaceae, Burseraceae, Lamiaceae, Asclepiadaceae and Ericaceae.

Concerning the tyrosinase activity four compounds were found to be very active and the active scaffolds were determined. Esculetin was again found active and the selection of the previous mentioned families was reinforced.

Myrsinaceae, Leguminosae, Compositae, Lamiaceae, Dioscoreaceae and Pinaceae were selected based on the activity of olivetol, Myristicaceae, Combretaceae, Lauraceae and Myristicaceae based on the activity of nordihydroguaiaretic acid and Vitaceae, Leguminosae, Polygonaceae, Pinaceae, Myrtaceae, Moraceae and Dioscoreaceae were selected based on the activity of trans-e-viniferin.

The 1810 plant species/organs were represented by **3620 extracts**, resulting from the Pressurized Liquid Extraction of the raw material with two different solvents of increased polarity, namely **methanol and ethyl acetate**.

- P1(NKUA): 900 extracts (MeOH & EtOAc)
- P3(CNRS-ICSN ): 900 extracts (MeOH & EtOAc)
- P3(CNRS-ECOFOG): 600 extracts (MeOH & EtOAc)
- P4(CIFLORPAN): 600 extracts (MeOH & EtOAc)
- P5(CSIR): 600 extracts (MeOH & EtOAc)

**3620** extracts resulted from the extraction of 1810 plant species/organs were forwarded to BASF for fungicide, insecticide and herbicide screening and to KORRES & NCSR D for anti-aging and hyperpigmentation screening, correspondingly. Except the *per se* activity on the selected biological targets, other parameters were assessed in order to make a sustainable selection of **100 hit extracts directed for agrochemical (60 hits) and cosmetic (40 hits) used**. In particular, it was taken into account the availability of the plant material for future exploitation; rare species (red or endangered), plants difficult to collect (non accessible ecosystems e.g. jungle) were ranked in lower priority. In addition, it was very important to retain a balance between biodiversity hotspots and to this end the number of extracts per partner/source was kept proportional to the initial feeding.

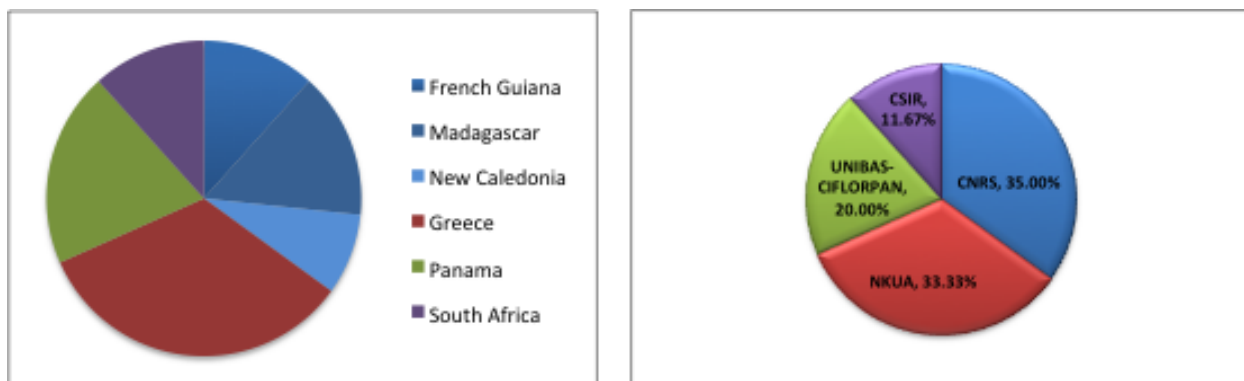


FIGURE 3 BIODIVERSITY INDEX AND REPRESENTATION OF PARTNERS IN THE SELECTION OF THE 60 HIT EXTRACTS FOR AGROCHEMICAL USE.



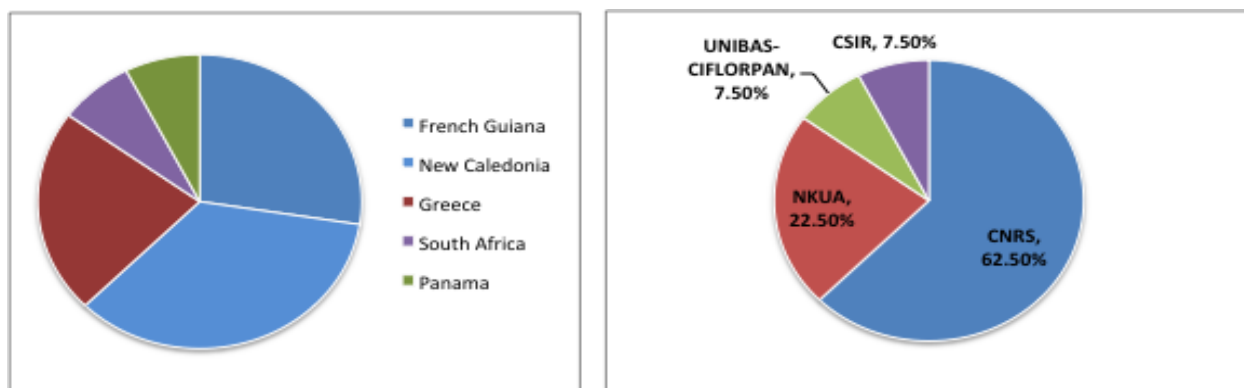


FIGURE 4 BIODIVERSITY INDEX AND REPRESENTATION OF PARTNERS IN THE SELECTION OF THE 60 HIT EXTRACTS FOR COSMETIC USE.

**Biological screening of an unprecedented number of extracts (3620), microfractions (4000) and pure compounds (600) for agrochemical (fungicidal, insecticidal, herbicidal) and cosmetic properties (anti-ageing and anti-hyperpigmentation)**

In the core of Agrococ project was the bioevaluation of all entities resulting from the extraction of plant material, microfractionation, library screening and compound isolation.

The public demand for safe agricultural products is continuously increasing. Agrochemicals derived from natural products may have better impact on clients and overall on the society's opinion on solutions for crop protection. The fungicidal activity was tested on **3 plant pathogenic fungus**: *Botritis*, *Phytophthora* and *Pyricularia*. The herbicidal activity was tested on **bentgrass** (agrostis) and commun **duckweed** (lemna minor) and in the insecticide assay, the compounds were tested against 5 species, representing **4 different classes of insects**: Lepidoptera, coleoptera, homoptera and diptera.



FIGURE 5 ALL THE EXTRACTS (3600), MICROFRACTIONS (4000) INITIAL PURE COMPOUNDS (484) AND ISOLATED COMPOUNDS (100) WERE FORMATTED IN 96-WELL PLATES FOR THE BIOEVALUATION ASSAYS

The assay panel used in the **cosmetics area** could enable the discovery of new agents for protection of the skin against UV irradiation, hyperpigmentation, and compounds with anti-aging properties. The first step for the bioevaluation of cosmetic properties was the determination of **antioxidant activity** in a cell-free system using the **DPPH-method** (antioxidant activity is an important prerequisite for a possible activity against skin photo-aging). All compounds were initially tested at a high concentration (75µg/ml final concentration). Those that inhibited more than 60% of DPPH were further tested at final concentrations 50 and 25 µg/ml. Those that showed over 60% inhibition of DPPH were considered as strong free radical scavengers and the IC50 for each one of these compounds was estimated. The compounds with high or moderate antioxidant activity in the cell-free DPPH assay were chosen to be tested for their capacity to reduce the **intracellular reactive oxygen species (ROS)**, i.e. to act as

antioxidants in the cellular context as well. The test compounds were applied overnight to human skin fibroblast cultures (see above) at the highest non-cytotoxic concentrations and their capacity to inhibit DCFH-DA fluorescence was measured. Then the ability of the above compounds to **protect human skin fibroblasts from UV-induced damage** was evaluated. Regarding **the skin whitening effects**, the ability of selected compounds to inhibit the oxidation of L-DOPA to dopaquinone and subsequently to dopachrome by the enzyme **tyrosinase** was investigated.

### *Screening of 484-compound library*

**Agrochemicals:** According to the prescreen fungicide assay provided 58 hits, (hit rate of 12%), herbicide assay provided 71 Hits (hit rate of 15 %) and in insecticide assay provided 29 hits (hit rate of 6%). Overall 73 compounds presented enough activity to be further evaluated in our screening. The global hit rate for the agrochemical evaluation of the initial 484-library was 15%.

**Cosmetics:** Five compounds were **very active**, i.e. they scavenged DPPH at the lowest concentration tested (5 µg/ml), **eleven compounds** showed a **moderate** activity (DPPH scavenging at 25 µg/ml), while **131 compounds were weak antioxidants** (DPPH scavenging at concentrations of 50 µg/ml or 75 µg/ml). The most promising compounds belong to the categories of flavonoids (Rutin - AG002/D10, Mearnsetin - AG004/G09, Taxifolin - AG006/B02, 3',4'-dihydroxy-5,6,7,8-tetramethoxyflavone - AG002/E03), phenylpropanoid glycosides (Myconoside - AG001/H04, Acteoside - AG006/G06, Forsythoside B - AG006/G10) and phenolics (Esculetin - AG001/A08, Nordihydroguaiaretic acid - AG001/E08, Ellagic acid - AG001/E06, Rosmarinic acid - AG006/C08, Salvianolic acid K- AG006/F08).

The **sixteen** compounds with high or moderate antioxidant activity in the cell-free DPPH assay were chosen to be tested for their capacity to reduce the **intracellular reactive oxygen species (ROS)**. Then the ability of the above 16 compounds to **protect human skin fibroblasts from UV-induced damage** was evaluated. Among these natural compounds are flavonoids (Rutin - AG002/D10, Mearnsetin - AG004/G09, Taxifolin - AG006/B02, 3',4'-dihydroxy-5,6,7,8-tetramethoxyflavone - AG002/E03, Sulfuretin - AG005/H11), lignans ((+)-Lariciresinol - AG006/C07), phenylpropanoid glycosides (Myconoside - AG001/H04, Acteoside - AG006/G06), coumarins (Esculetin - AG001/A08), alkaloids (Boldine - AG001/B11, ) and phenolics (Nordihydroguaiaretic acid - AG001/E08, Ellagic acid - AG001/E06, Rosmarinic acid - AG006/C08, Salvianolic acid K- AG006/F08, Curcumin - AG001/F07, Oleacein - AG006/B09).

Regarding **the skin whitening effects**, **four** compounds were found to be very active, i.e. they strongly inhibited tyrosinase activity ( $IC_{50} < 50 \mu\text{g/ml}$ ), 3 compounds showed a moderate activity ( $75 \mu\text{g/ml} > IC_{50} > 50 \mu\text{g/ml}$ ), while 10 compounds were found to be weak inhibitors ( $150 \mu\text{g/ml} > IC_{50} > 75 \mu\text{g/ml}$ ). The most active compounds belong to the class of coumarins (esculetin - AG001/A08), alkylphenols (olivetol - AG001/A10, Nordihydroguaiaretic acid - AG001/E08) and lignans (*trans-ε*-viniferin - AG006/E06, Gnetin H - AG006/G09).

### *Screening of 3620 extracts*

In Lead Finder **herbicide**, **365** hits were detected, meaning a hits rate of 15% (19 hits from priority 1, 16 hits from priority 2 and 77 hits from priority 3). In Lead Finder **insecticide**, **218** hits were detected, meaning a hits rate of 6% (27 hits from priority 1, 11 hits from priority 2 and 9 hits from priority 3). In Hit Finder **fungicide**, **124** hits were detected, meaning a hits rate of 3% (7 hits from priority 1, 18 hits from priority 2 and 4 hits from priority 3). From those extracts, the 60 Agrochemical Hit Extracts were selected and microfractionated.

The antioxidant activity was assessed in a cell-free system using the **DPPH-method**. All extracts were initially tested at a high concentration (200 µg/ml final concentration). Those that inhibited more than 80% of DPPH (**992 hits**, hit rate of 27%) were further tested at 100 µg/ml, then those that inhibited more than 70% of DPPH (**683 hits**, hit rate of 19%) were further tested at 50 µg/ml and finally those that inhibited more than 50% of DPPH (**537 hits**, hit rate of 15%) were further tested at 30 µg/ml. The most promising compounds (% inhibition of DPPH > 50 % at 30µg/ml, were identified as **256 hits**, representing a hit rate of 7%. The 29 most active extracts on the DPPH assays were also evaluated for their ability to protect human skin fibroblasts from **UV-induced damage**. A black box equipped with four Sankyo Denki UV-B lamps was used to irradiate cell cultures (3.07 mW/cm<sup>2</sup>). 2 out of the 29 extracts evaluated, were found more efficient than the positive control.

For the evaluation of hyperpigmentation activity and therefore, tyrosinase inhibition, extracts that suppressed enzyme activity under 70% were considered active (**157 hits**, hit rate of 4,4%) and were further tested at a final concentration of 150 µg/ml. Similarly, those that exhibited less than 60% inhibition of tyrosinase were considered as weak inhibitors, while compounds that showed over 60% inhibition (**140 hits**, hit rate of 3,9%), were further tested at 75 µg/ml. In the same way, those that exhibited less than 50% enzyme inhibition were considered as moderate inhibitors, while those that showed over 50% inhibition (**88 hits**, meaning a hit rate of 2,4%), were considered as **strong inhibitors**.

#### *Screening of 4000 microfractions*

The 100 hit extracts were subjected to microfractionation according to a standard protocol developed and commonly decided by the consortium. As described in Task 2.1., the microfractions were readily prepared in 96well plates in order to proceed for biological evaluation. The fractionation was performed by each partner providing the extract, and the protocol was followed with no alterations by all the partners.

**1040** microfractions corresponding to the **26** extracts selected for their **fungicidal** potential, were screened for fungicidal activity using four phytopathogenic fungal species and **193** fractions were found active. This corresponds to 18.56% of the total microfractions tested for fungicidal activity. The most sensitive strain was *Pyricularia oryzae*, against which 75 microfractions were active.

**600** microfractions corresponding to the **15** hit extracts for **herbicidal** activity were screened and **221** fractions out of 600 showed significant activity, corresponding to 36.83% of the total fractions tested for herbicidal activity. The most sensitive weed was the bentgrass, against which 58% of the active microfractions were active.

**760** microfractions corresponding to **19** hit extracts for **insecticidal** and nematocidal activity were screened against five different species and **114** microfractions were active corresponding to 15% of the total fractions tested for insecticidal activity.

For **antioxidant** activity, **1600** fractions were tested, and **198** microfractions were found to scavenge the DPPH radical by at least 70%. In addition with the initial screening, the microfractions that showed the most promising activity were further tested in a 5-time dilution, in order to assess their potential as DPPH scavengers.

Out of the **440** microfractions tested for **anti-hyperpigmentation** properties, **119** were found to be active (tyrosinase inhibition greater than 70%), which correspond to 27.05% of the fractions tested in this assay. The most promising microfractions were further tested in 5-times dilution in order to investigate their potential. Tyrosinase inhibition was very strong for the microfractions corresponding to *Morus alba* and *Castanea sativa*, where in the 5-times dilution assay the inhibition was still very strong (>85%).

### *Screening of 100 isolated compounds*

In the level of purified compounds, **100** compounds isolated from the 100 hit extracts were forwarded for biological evaluation on selected targets. A total of **66** compounds were tested for potential **agrochemical** use: **45** for **antifungal** activity, **8** for **herbicidal** activity and **13** for **insecticidal** activity. All compounds were tested in all agrochemical assays while those who showed sensitivity in one assay were hit compounds. The hit finder identified a total of **21 active** compounds with fungicidal activity, while no compound was found active on the insecticidal or herbicidal assay.

The pure compounds isolated from extracts with potential anti-ageing activity were screened for their **DPPH scavenging activity and UV-B protection activity**. For anti-hyperpigmentation, the corresponding compounds were tested for their inhibitory activity against **tyrosinase**. A total of **34** compounds were tested for their cosmetic properties: **22** for **anti-ageing** activity and **12** for **anti-hyperpigmentation** activity. Out of the compounds tested in the DPPH assay, **12** compounds were found to an IC<sub>50</sub> lower or comparable to that of the positive control (gallic acid), while **6** compounds showed an increased inhibition of cell death when cells were UV-B irradiated (>15%). Concerning the antihyperpigmentation properties, out of the **12** compounds tested, **6** compounds were found to significantly inhibit the activity of tyrosinase and for these compounds the IC<sub>50</sub> values were calculated.

### *Advanced biological evaluation*

The extensive biological evaluation of selected hits resulting from the biological screening was performed. A selection of active compounds has been evaluated for fungicidal and insecticidal use (agrochemicals) and antioxidant and UV-B protective activity in a cellular based assay (cosmetics). The activity of the compounds has been associated with the respective extracts that appeared to possess a significant interest for the further exploitation through Agrocos.

The extended assays for the evaluation of **fungicidal** activity was based on the testing of the compounds in an array of concentrations, ranging from 0.000118256 to 31 ppm. In order to calculate the activity of a compound on a given dose, the optical density values of each measurement of a compound is compared with those of the control and the reference, giving results from 0 to 1. The antifungal activity increases with increasing values. In total, **39** pure compounds were tested in a panel of concentrations for their antifungal properties. **9** of them were found completely inactive, while **30** of them were found to possess from good to moderate activity, especially when the fungal cultures were treated with higher concentrations. It is of interest that **seselin** and **xanthyletin** developed are both active in a concentration of 31 ppm.

For **insecticidal** activity, the evaluation of piercing/sucking insects through contact or systemic means the test unit consisted of 24-well-microtiter plates containing broad bean leaf disks. The compounds were formulated using a solution containing 75% v/v water and 25% v/v DMSO. For evaluating control of biting insects the test unit consisted of 24-well-microtiter plates containing an insect diet and 20-30 insect eggs. Different concentrations of formulated compounds were sprayed onto the leaf disks at 2.5 µl, using a custom built micro atomizer, at two replications. **13** compounds were tested in three different concentrations (2500, 1415, 800 ppm) for their insecticidal properties, while **7** of them were found to possess moderate activity.

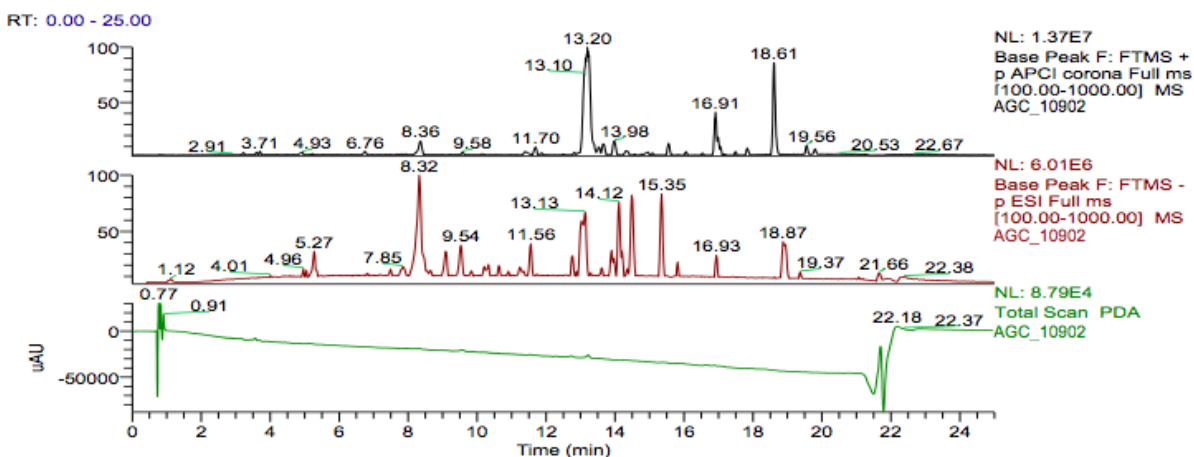
In the case of **cosmetics** the most promising compounds in terms of **antioxidant, DNA-protection hyperpigmentation** activities will be tested also in terms of **antiageing** capacity. The effect of the compounds on the extension of the cellular lifespan of skin fibroblasts *in vitro* was estimated. **Oxyresveratrol** and **resveratrol** showed maximal protective activities at 100 µM, being modestly active at 20 µM. **Glabridin** exhibited its best protective capacity at 4 µM, while at the highest concentration tested (20 µM) it was inactive.

## Fingerprinting of 100 hit plant extracts and 100 isolated compounds with state-of-the-art analytical techniques (MS/NMR) and full spectroscopic data of 30 lead compounds

The **profiles of 100 hit extracts**, showing the most promising biological activities, related to agrochemical and cosmetic properties are being generated using state-of-the-art techniques. Specifically, Liquid Chromatography-High Resolution Mass Spectrometry (**LC-HRMS**) and Nuclear Magnetic Resonance (**NMR**) spectroscopic techniques are being used for the initial fingerprinting. It must be noted that the procedure protocols used, are the ones established under the experimentation in WP4. Spectral data from LC-HRMS and NMR (1D- and 2D-spectra) are forwarded to the Agrocos database. The protocol of HPLC-DAD-based profiling of the extracts has already been circulated to all the partners involved and they have started the profiling procedure.

The **LC-MS** platform is a state-of-the-art equipment able to fulfil all the needs of metabolomics and fingerprinting. It is exceptional for the analysis of complex mixtures such as plant extracts, with the simultaneous acquisition of MS and UV spectra. It included an Orbitrap analyzer (ThermoScientific) with an Electron Spray Ionization (ESI) source operating both in positive and negative mode. MS/MS spectra were also recorded. For UV profiling, the observed wavelengths were set at 210, 254 and 365 nm. The samples were run in 23 successful sequences in duplicates (total of 46 sequences), programmed through the operating software of the LTQ Orbitrap platform (Xcalibur).

For the **HRMS/MS** acquisitions, a data dependent method was incorporated, including a full scan detection (1<sup>st</sup> FT) and a fragmentation of the most significant peaks per scan (2<sup>nd</sup> FT). The mass resolving power for both levels was 30.000 the mass range was 120-1200 m/z. The normalized collision energy was set to 35.0%. With this method, 100 precursor ions were selected per extract, and their HRMS/MS spectra were obtained. **Dynamic exclusion** (DE) is a very convenient and versatile tool to maximize the identification of secondary metabolites. It ensures the isolation of more ions in full scan mode and therefore it is possible to obtain the most of MS/MS data. While simple data dependent experiments work well with resolved components, when complex mixtures occur like in the case of total extracts, extended overlapping occurs. With dynamic exclusion it is possible to enter a list of ions to be excluded from the MS/MS throughout the duration of the experiment, and also temporarily put a mass into the exclusion list after the MS spectrum is obtained. In our case, one count and a 50-mass list was employed for dynamic exclusion, while with these conditions very good quality MS and MS/MS spectra of minor components are obtained.



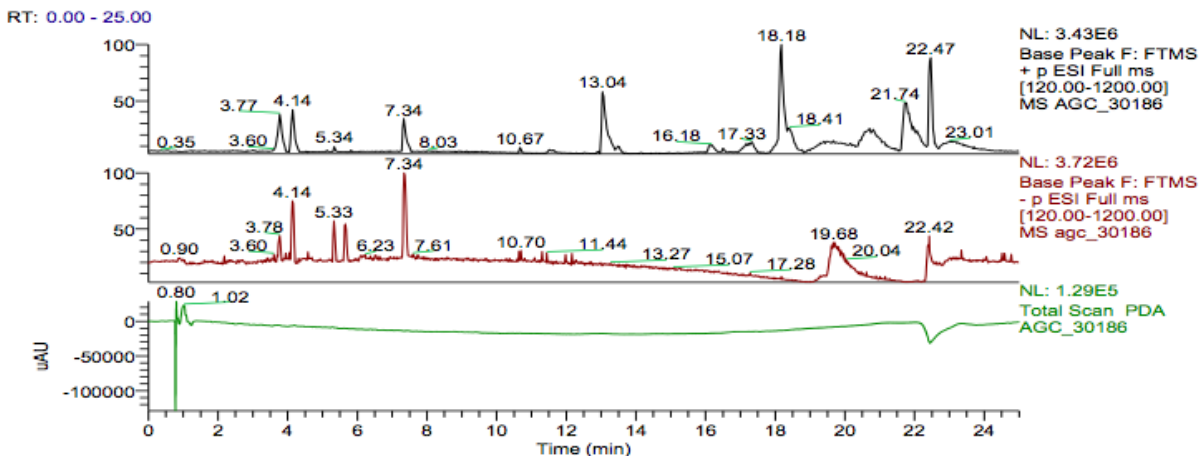


FIGURE 6 LC-HRMS/UV PROFILING OF SELECTED EXTRACTS

The value of mass spectrometry as a tool for structure elucidation was evident by the cases that the structure proposed by the providers of the compounds was not confirmed. For the 100 active compounds, 2 compounds were not verified, and 5 compounds were not ionizable in both ESI positive and negative mode. From the latter fact, it is evident that the ionization source plays a crucial part, although in general ESI is very versatile and succeeds in ionizing a large array of structures.

1D noesyppr1d spectra of active agrochemical extracts provided by NKUA

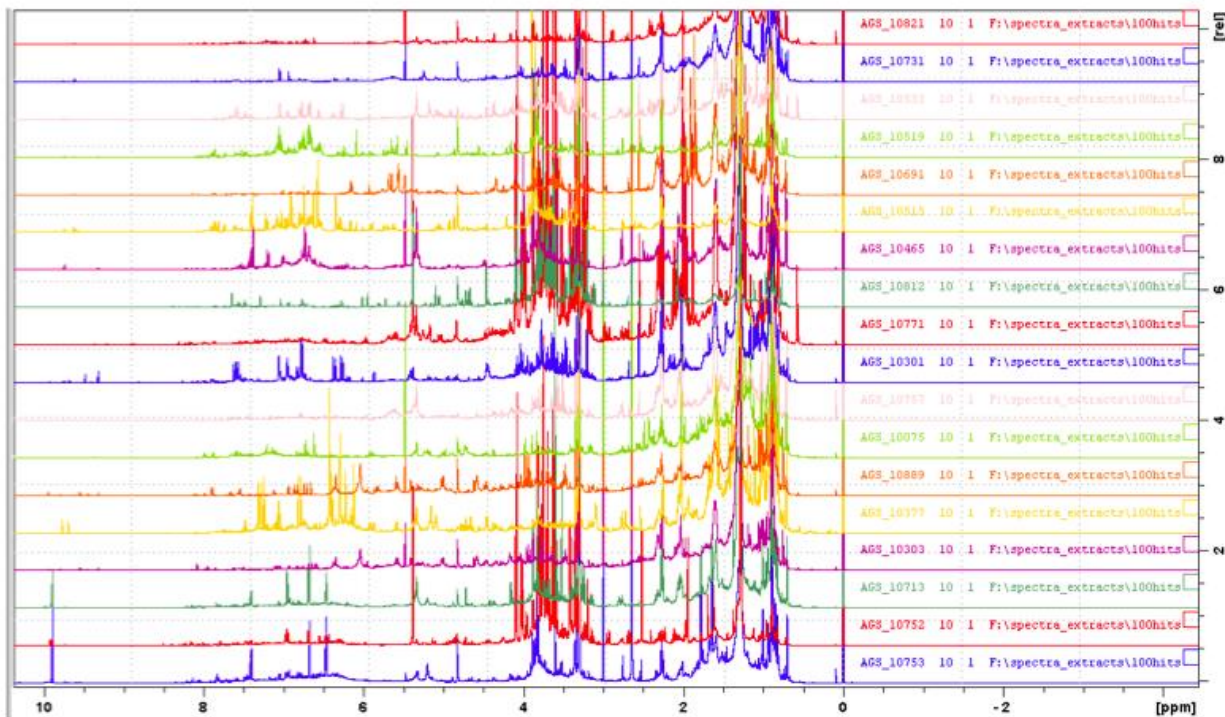


FIGURE 7 NOESYPPR1D PROFILING OF SELECTED AGROCHEMICAL EXTRACTS

The **NMR** experiments included in the profiling of 100 extracts and isolated compounds were **noesygppr1d** (1D H NMR) and **jresgpprqf** (2D homonuclear), while the sample preparation and the pulses used were specifically designed for the incorporation of the spectral data in the Agrocos database. For the full spectroscopic NMR data of the 30 lead compounds, additional experiments were acquired, namely **cosygpprqf** (2D homonuclear,  $^2J$  and  $^3J$  H correlations), **hmbcetgpl3nd** (2D heteronuclear,  $^2J$  and  $^3J$  correlations) and **hsqcetgpsisp2.2** (2D heteronuclear,  $^1J$  correlations, distinction between methyl/methine and methylene carbons). With this set of experiments and the combination with MS/MS data it was possible to obtain a full structural assignment for all compounds promoted as leads.

In total, **43 extracts** from the 100 hit extracts were submitted to phytochemical investigation, which correspond to **25 plants** of the global biodiversity. A total of **115** compounds was isolated, some of them being minor components, with quantities below 2 mg. Moreover, as explained in the profiling section, some of the compounds were of unacceptable purity in order to be inserted in the Agrocos database, or their structure was not verified after extensive spectroscopic analysis.

TABLE 1 STUDIED PLANTS AND EXTRACTS OF THE 100 EXTRACT HITS, SUBJECTED TO PHYTOCHEMICAL INVESTIGATION AND ISOLATION OF COMPOUNDS.

No. of extracts	Extract	Plant	Family	Part	Solvent	Partner	No. of plants
1	AGS35343	Andira coriacea	LEGUMINOSAE-FABACEAE	bark	EtOAc	CNRS-ECOF OG	1
2	AGS35344				MeOH		
3	AGS40445	Bocconia frutescens L.	PAPAVERACEAE	Stem	MeOH	UNIBAS-CIFLORPAN	2
4	AGS40021				EtOAc		
5	AGS10904	Castanea sativa	FAGACEAE	fruits	MeOH	NKUA	3
6	AGS10902			wood	MeOH		
7	AGS40378	Combretum racouia H. B. K. & Sandw.	COMBRETACEAE	Leaves	EtOAc	UNIBAS-CIFLORPAN	4
8	AGS40128				MeOH		
9	AGS40542	Combretum aff. maximum Jacq.	COMBRETACEAE	Leaves	EtOAc	UNIBAS-CIFLORPAN	5
10	AGS40153				MeOH		
11	AGS33552	Couroupita guianensis	LECYTHIDACEAE	bark	EtOAc	CNRS-ECOF OG	6
12	AGS33551				MeOH		
13	AGS30286	Eriosema sporaleoides	LEGUMINOSAE-PAPILIONOIDEAE	Aerial Part	EtOAc	CNRS-ICSN	7
14	AGS30520				MeOH		
15	AGS40377	Erythroxylum lucidum Kunth	ERYTHROXYLACEAE	Leaves	EtOAc	CIFLORPAN	8
16	AGS10066	Genista hassertiana	LEGUMINOSAE	aerial parts	MeOH	NKUA	9
17	AGS10227	Glycyrrhiza labra	LEGUMINOSAE	roots	EtOAc	NKUA	10
18	AGS10228				MeOH		
19	AGS30299	Macaranga ferruginea	EUPHORBIACEAE	Leave	EtOAc	CNRS-ICSN	11
20	AGS30533				MeOH		
21	AGS40223	Miconia affinis DC.	MELASTOMATACEAE	Stem	EtOAc	UNIBAS-CIFLORPAN	12
22	AGS30379	Monanthes pilosa	ANNONACEAE	Bark, Stem	EtOAc	CNRS-ICSN	13
23	AGS30559				MeOH		
24	AGS10377	Morus alba	MORACEAE	wood	EtOAc	NKUA	14
25	AGS10378				MeOH		
26	AGS40327	Mosquitoxylum jamaicense Krug. & Urb.	ANACARDIACEAE	Leaves	MeOH	UNIBAS-CIFLORPAN	15
27	AGS40045	Myrcia splendens (Sw.) DC.	MYRTACEAE	Leaves	EtOAc	UNIBAS-CIFLORPAN	16
28	AGS40331				MeOH		
29	AGS30345	Myrtopsis myrtoidea	RUTACEAE	Bark, Stem	EtOAc	CNRS-ICSN	17
30	AGS30678				MeOH		
31	AGS10753	Onobrychis alba	LEGUMINOSAE	Whole plant	EtOAc	NKUA	18
32	AGS30235	Operculicarya decaryi	ANACARDIACEAE	Leave	EtOAc	CNRS-ICSN	19
33	AGS30464				MeOH		
34	AGS35315	Ruizterania albiflora	VOCHYSIACEAE	bark	EtOAc	CNRS-ECOF OG	20
35	AGS35316				MeOH		
36	AGS50252	Sclerocarya birrea affra	ANACARDIACEAE	Twigs	EtOAc	CSIR	21
37	AGS50143				MeOH		
38	AGS35595	Swartzia panacoco	LEGUMINOSAE-CAESALPINOIDEAE	stem	EtOAc	CNRS-ECOF OG	22
39	AGS35596				MeOH		
40	AGS10691	Thapsia arganica	UMBELLIFERAE	aerial parts during flowering	EtOAc	NKUA	23
41	AGS30279	Uvaria nanjensis	ANNONACEAE	Bark, Stem	EtOAc	CNRS-ICSN	24
42	AGS30513				MeOH		
43	AGS10758	Veratrum album	LILIACEAE	flowering aerial parts and roots	MeOH	NKUA	25



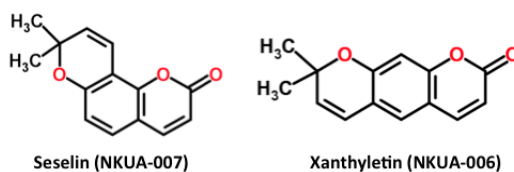
## Identification of five lead entities (compounds/extracts) that can be further developed and exploited as agrochemical or cosmetic agents

The **five confirmed leads** of Agrocos combine a series of properties associated to biological activity, raw material availability, chemical diversity and adaptability to the industrial practices of the agrochemical and cosmetic industry. The selection of verified leads for cosmetic use was made according to specific criteria that included increased bioactivity (antioxidant, UV protection, tyrosinase inhibition) of the selected extracts and the major compounds or category of compounds present in the extracts. It was of crucial importance that the term “lead” in cosmetics will be defined as an extract standardized in active constituents rather than a single compound. This rationale is in complete accordance with the needs of the cosmetic industry and consumers of cosmetics, that show higher appreciation in natural ingredients.

The research performed so far on the biological evaluation, phytochemical investigation and structural elucidation of plant constituents, extracts and enriched extracts, justifies the design of a novel cosmetic product based on the Agrocos findings. A **feasibility study** was conducted, based on these results and is focused on the hit extracts and active constituents of the Greek flora, with emphasis in enriched extracts and their preparation as cosmetic actives, as described below. In the feasibility study a successful case study is thoroughly discussed, giving the opportunity to associate success stories from the past launching of Korres natural ingredients (Black Pine extract in a series of cosmetic products) with a potential new success story based on the Agrocos findings for *Morus alba*, *Castanea sativa* and *Glycyrrhiza glabra*. The final product is expected to increase the gross income of the company within a year from its launching (expected approximately within a timeframe of 3 years from the end of the project). The most important aspect is the feasible supply of the plant material. In general, tropical plants are subjected to certain rules concerning their availability, as in many of the cases species are endangered or prone to endangerment if their collection is uncontrollable. Moreover, the procurement of plant material of high degree of endemism can be problematic in terms of import/export legislation and fees. This is a crucial reason why Korres focuses in **Greek Top Scorers**: the procurement of plant material is sustainable and feasible, while in many cases compounds present in the tropical plants can be also found in the form of chemical analogues in the Greek plants as well.

### **I. Purification of seselin from apolar crude extract obtained from Citrus roots – Proposal for the preparation of similar coumarins by sustainable synthetic routes**

The evaluation results of selected natural products with advanced agrochemical tests revealed that the coumarin **seselin** and its analogue **xanthyletin** are very promising agrochemical agents, possessing fungicidal activity. Seselein and xanthyletin are the only coumarins reported to be constituents of *Citrus* roots. The two coumarines were isolated in large scale by citrus roots, while a retrosynthetic scheme is proposed for the large scale synthesis of those natural compounds and their incorporation to the development plan of BASF.

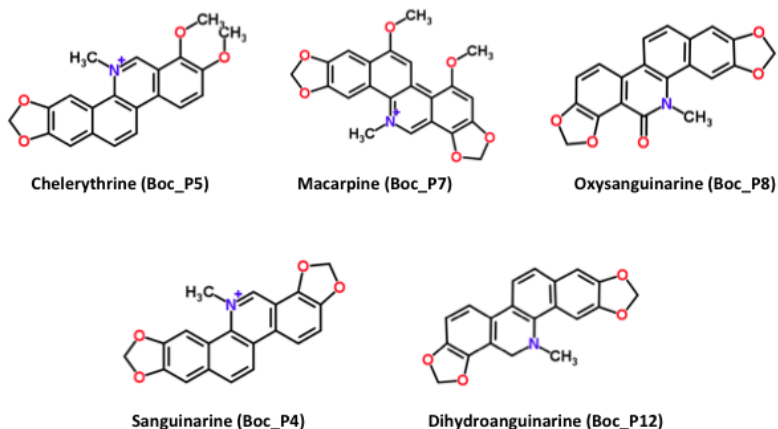


### **II. Benzylisoquinolines of *Bocconia frutescens* and fungicidal activity**

The screening of 3600 extracts and microfractions resulted in the identification of *Bocconia frutescens* as an effective fungicidal agent. Extensive phytochemical investigation of the species and evaluation of the isolated compounds for fungicidal activity revealed that benzylisoquinoline alkaloids isolated could be pursued as lead category of compounds

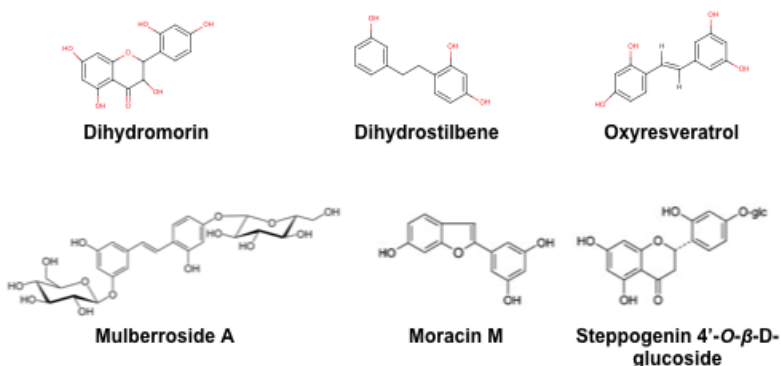
for agrochemical applications. The MeOH extract of the plant showed the best antifungal activity against *Pyricularia oryzae* and *Phytophthora infestas* while the activity of the microfractions was compared to the respective LC-MS profile.

Sanguinarine itself is a toxin, although it possesses no antifungal activity according to the assays performed. Chelerythrine was isolated from the plant in good yield, and along with macarpine possesses antifungal activity that is consistent with the literature available, reporting antibacterial activity for those compounds.



### III. Extract enriched in stilbenoids and other phenolics obtained from leaves and branches of *Morus alba* (Moraceae)

*Morus alba*, known as white mulberry, has been reported to exhibit a skin whitening effect. Extracts obtained from the leaves of *M. alba* possess low cytotoxicity, potent tyrosinase inhibitory activity and the ability to reduce cellular melanin content. Thus, the root bark of *M. alba* has been widely used as a raw material of skin whitening cosmetics. Our study has highlighted the oxyresveratrol, dihydrostilbenes and dihydromorin (phenolic compounds contained in the extracts of *Morus alba* bark and leaves) among the most promising tyrosinase inhibitors possessing also remarkable antioxidant properties.

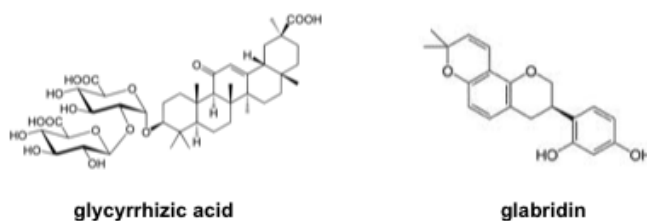


For the pilot scale isolation of stilbenoids of *M. alba*, the pulverized branches of *Morus alba* (20 kg) were extracted under stirring at 60 °C using a mixture of H<sub>2</sub>O:EtOH 50:50 (200 lt) as solvent (Extractor, 1000 lt) . After 6 hours, the extraction mixture was filtered and the filtrate obtained was concentrated to half the volume by removing the ethanol under vacuum (QVF evaporator, 200 lt). The resulting extract after dilution with water (100 lt) was passed

through the resin (Adsorption resin column, 450 lt, XAD-4) and the desorption of phenolic compounds was performed using ethanol (100 lt). Finally, 350 g of enriched extract was obtained by the evaporation of solvent after reduced pressure.

#### IV. Licorice root extract enriched in glabridin

Liquorice root (*Glycyrrhiza glabra*, European licorice, Fabaceae) is used worldwide as a natural sweetener and, in certain cases, as a flavour additive in the preparation of candies and speciality foods. The main constituent of liquorice root is glycyrrhizic acid which exhibits anti-inflammatory, anti-viral, anti-allergenic, anti-ulcer and anti-oxidative properties, and is also believed to have chemopreventive activity against cancer and AIDS. Glabridin, a prenylated isoflavonoid of *G. glabra* roots, has been associated with a wide range of biological properties such as antioxidant, anti-inflammatory, anti-atherogenic, regulation of energy metabolism, estrogenic, neuroprotective, anti-osteoporotic, and skin-whitening.



As part of Agrococ, glabridin has emerged as one of the most significant natural compound, among the 30 leads evaluated using the advanced test, to develop novel cosmeceuticals. Based on the fact that less polar components of licorice possess very significant bleaching and antioxidant properties, while several of these are sensitive to high temperature and prolonged heating, it was decided the preparation of extract by supercritical fluid extraction using CO<sub>2</sub> as solvent and ethanol as co-solvent. The chemical content of the fractions was evaluated using normal phase TLC and the results revealed that licorice roots should be extracted at 250 bar using EtOH (5 and 10 %) as co-solvent in order to produce fractions enriched in glabridin and other isoflavans. Indeed, when the extraction was performed in a pilot scale with the aforementioned conditions resulted in the production of extracts rich in the target substance as shown by HPLC and TLC chromatograms. Finally, 10 g of licorice roots resulted to 360 mg of enriched extracts, which possess higher antioxidant and whitening activities than the conventional extracts produced using EtOAc and MeOH as solvents.

#### V. Extract enriched in flavonoids obtained from leaves and branches of *Castanea sativa* (Fagaceae)

*Castanea sativa* Mill. is a species of the flowering plant family Fagaceae, mainly cultivated in temperate regions (Asia, Southern Europe and North of Africa). Chestnut processing generates several waste products, which are used as fuel in the food factory or remain in the woodland after the fruit harvesting and promotes the proliferation of certain insect larvae which cause damages to crops. These byproducts could be used as sources of natural antioxidants, since the beneficial effects derived from phenolic compounds, such as their anticarcinogenic, antimutagenic and cardioprotective activities, have been attributed to their antioxidant activity.

The aqueous extracts of *C. sativa* contain phenolic compounds (e.g. gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, vanillic acid, rutin, quercetin and apigenin) possess significant antioxidant activity. According to the results of the biological evaluation of 30 lead compounds quercetin glycosides and gallic acid derivatives are promising antioxidant and UV-protective agents. So, we decided to produce an extract enriched in phenolics and flavonoids using a similar procedure to that applied in the case of *M. alba* extraction. The evaluation of the total

phenolic (Folin-Ciocalteu assay, gallic acid equivalents) and total flavonoid (aluminium chloride colorimetric assay, catechin equivalents) content revealed that the final extract, which was obtained after the desorption of substances from the resin XAD-4, is rich in phenolic derivatives (289 mg GAE/g of extract, 96 mg CE/g of extract) and thus justifiably possess significant antioxidant and UV-protective activity.

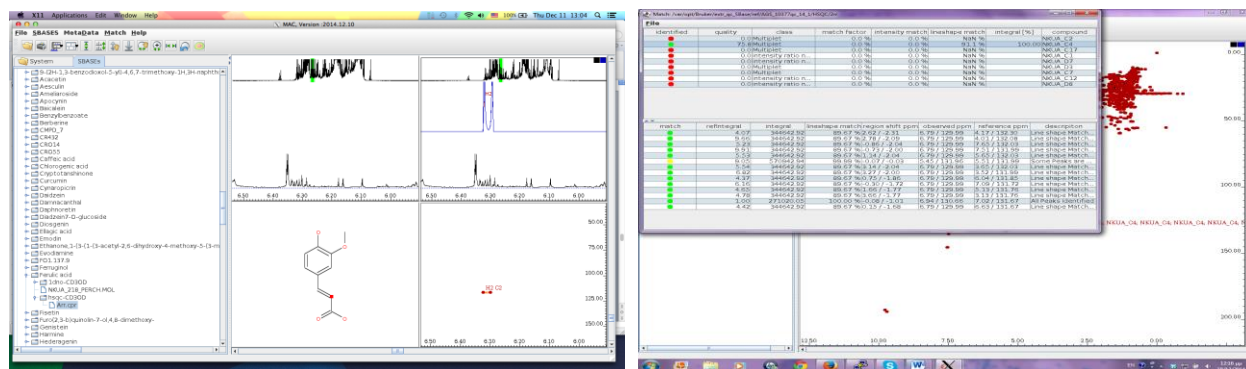
### **Implementation of the Agrocos database, incorporating all the spectral data and metadata resulting from the research activities and providing tools for the dereplication of extracts and 2D spectral matching**

A highly innovative aim of Agrocos was the generation of NMR and LC-MS data spectral libraries of extracts and pure compounds derived from plants originating from major biodiversity hotspots in Europe, Africa, Latin America, and the Asia-Pacific regions.

The Agrocos database is based on the integration of NMR/MS data, structural data and metadata for the pure compound and extracts, in one common platform. This platform is an essential part of the project and can be efficiently exploited after the end of the project, given the fact that it assembles in one comprehensive element all the research performed during the course of the project. The design of the spectral database was made on the basis of a common frame for all inputs for both plant extracts and pure compounds. The platform was developed based on already commercialized software. The dynamic interface allows for all partners to visualize the input files. It was decided that partners 1 and 8 have administrative permissions for adding and manipulating the acquired spectroscopic data as well as the corresponding metadata information. The software allows for multiple spectra upload for each compound/extract and related metadata linked with “keys” that enable the rapid search of entries for visualization and statistics.

The database comprises the cornerstone of Agrocos, where all the outcomes of the project concerning extract and compound data are input. Moreover, associated metadata with the extracts and the compounds in terms of biological activity are integrated, in order to have a comprehensive view of the project through the database. Metadata for collection and reporting of plant extracts include Agrocos-Code, IUPAC name, InCHIs, trivial name, biological evaluation on selected targets, complete NMR assignment with interactive view (on-structure clicking), genus, species, plant part, accession, collection-location, harvesting-date, harvesting-type, extraction-method, extraction-solvent, NMR-solvent, lab-code, taxonomy-info, botanical-authority, family, region, soil, eco-system and GPS coordinates. The corresponding metadata for the natural products of the compound database include CAS-number, molecular mass, Sum Formula, solvent, plant species, plant species (2<sup>nd</sup>), chemical taxonomy and biological properties. All partners collecting samples are now following the corresponding protocol.

The possibilities of the database extend beyond the scope of the project and beyond its duration. The database will be open to the addition of new elements and new spectra of extracts and compounds isolated or deposited for further exploitation. The database provides an easy way to browse through spectra of extracts and compounds, by simultaneously visualizing the molecular formula of the compounds of interest. A complete spectral assignment has been performed for all pure compounds inserted in the database, while the appropriate corrections have been made in cases where the structure proposed by the partners were not correct. In total more than **600** plant entities (**extracts** and **compounds**) are included in the Agrocos database, with full metadata.



In addition, statistical correlation tools have been developed that allow the dereplication and matching of compounds in the respective spectra. Standardized protocols (sample preparation, spectra acquisition procedures, data processing) were followed to allow comparison of spectra and common statistics and a state-of-the-art technology platform has been developed. Dereplication is performed by comparison of the 1D spectrum of the examined extract and the compounds of interest. Noise and shifting parameters can be adjusted in order to increase or decrease the level of uncertainty. Multiple compounds can be selected in order to examine their presence in a given extract.

Moreover, the Agrocos database has incorporated tools for the dereplication and 2D matching of spectra. In specific, it is possible to locate a specific compound in a given extract with the use of 1D NMR correlation, or even screen a set of compounds for their presence in a predefined extract. The compounds are categorized by the possibility to be present in the extract, with three degrees of uncertainty (high, medium and low possibility) 2D matching is based on the comparison of 2D NMR data (JRES and HSQC) of extracts or compounds, with the aim to identify similarities between unknown extracts or presence of compounds in extracts. 2D matching calculates similarities between a selected spectrum and one or more spectra, which are specified. The result is a sorted list of similarities and it will be recorded to a given output file.

### Dissemination of Agrocos research activities to public media by entries in the internet and press

The dissemination activities of Agrocos follow the axes of promoting and informing the general public about the activities and results of Agrocos, and publishing the scientific results in peer-review scientific journals.

Specifically, the Agrocos website (<http://www.agrocos.eu/>) provides an overview of the project and also includes a restricted area for Agrocos partners. Moreover, since the beginning of Agrocos implementation, partners have contributed to the dissemination of the project by several publications to the broad public. Many entries in the public media are constantly uploaded by the stakeholders interested in the project's outcomes. Since the beginning of AGROCOS implementation, partners have contributed to the dissemination of the project by several publications to the broad public.

In total, **more than 44** entries are launched for the moment in public media concerning Agrocos project and its outcomes, categorized in Newspaper entries, Internet sources, Video Presentations and Press Releases. The most important and efficient dissemination activity was "Flower Power": A detailed presentation of the Agrocos project covered by Euronews, with footage from the facilities of the University of Athens and Korres, interviews with researchers participating in the project and the coordinator of the project Prof. L. Skaltsounis.

(<http://www.euronews.com/2014/04/14/unearthing-the-hidden-healing-powers-of-flowers/>, <https://www.youtube.com/watch?v=J8um0H99bY4>). The video aired several times in the Science section of Euronews in television, and is a permanent upload in the official site and youtube. For the dissemination we have updated all dissemination activities in the DISSEMINATION section of ECAS and also inserted into the appropriate deliverable (DL6.2).

### **Dissemination to the scientific community through publications to peer-review journals, participation in conferences and organization of workshops**

In total, 14 scientific (9 NKUA, 4 CSIR, 1 PANAMA) posters and 4 scientific speeches in international conferences and/or workshop were presented in the framework of AGROCOS project. In addition four scientific papers in peer-review scientific journals have been published, where the scientific importance of AGROCOS project is mentioned.

- “A Quarter Century of Pharmacognostic Research on Panamanian Flora: A Review”, published on *Planta Medica*.
- “Screening of Panamanian plant extracts for pesticidal properties, and HPLC-based identification of active compounds”, published in *Scientia Pharmaceutica*
- “Screening of Panamanian plants for cosmetic properties, and HPLC-based identification of constituents with antioxidant and UV-B protecting activities”, published in *Scientia Pharmaceutica*.
- “Metabolomic studies on *Isatis tinctoria*– Comparison of different accessions, harvesting dates, and the effect of repeated harvesting”, submitted in *Journal of Natural Products*.

Moreover, exploitation of new knowledge to all scientists working in the field of natural products in Europe and internationally will be achieved through the organization of two workshops during the course of the project and a third at the end as a final event. Three workshops were organized in total, focusing to the main fields of applications of natural products. The title of the first workshop was “Enabling Technologies in Natural Product-based Lead Discovery” and was organised on 28th of June 2012 in Basel, Switzerland by P2(UNIBAS). The second one entitled “Holistic Analytical Technologies for BioMedical, Food and Plant Sciences” was organized on 9-11 of November 2012 in Athens, Greece by P1(NKUA). The 3<sup>rd</sup> workshop held was entitled “International Workshop on Biodiversity Research towards Cosmetic and Agrochemical applications”. The event took place as a side event of the 62nd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA), in Guimaraes, Portugal, organized by the University of Minho. The conference took place from August 31<sup>st</sup> to September 5<sup>th</sup>, while the Agrocos workshop was in the first day of the conference. The topic of the workshop was in complete relevance to the activities of Agrocos project, since it gave the opportunity to participants of the Agrocos consortium and independent researchers to provide an insight into the new methodologies applied in pharmacognosy for the discovery of new active agents with cosmetic and agrochemical applications. The presenters consisted a panel of renowned scientists in the field of pharmacognosy and biology, while specialized companies were also represented.

## Impact

Agrococos aimed to produce European added value by

- enhancing the pan-European public awareness of biodiversity of natural resources
- expanding the biochemical diversity of natural product libraries, generating novel lead compounds of plant origin
- improving the competitiveness of the European and non-European industry
- improving the sustainability and eco-efficiency of these products
- proposing novel uses of the target plant species, whose economic role may have **been so far very limited or non existent.**

The diversity of activities carefully planned in AgroCos project have contributed in:

- **Enhance the pan-European public awareness of biodiversity of natural resources.** The first objective of the **AgroCos** project was the consolidation of an international cooperative network of scientific institutions and private industries of Europe, Latin America, Africa, and the Asian Pacific devoted to the discovery of new natural products with useful properties as cosmetic or agricultural agents. Agrocos' research results have improve knowledge and public understanding of biodiversity, for managing biodiversity, and for developing novel products, processes and services derived from biodiversity. Through the dissemination activities e.g. organization of international workshop, web page construction, press releases to the mass media, scientific publications to specialized research journals and participation in international congresses our efforts we believe have enhance **the pan-European public awareness of biodiversity of natural resources.** Major aims of these activities included: understanding, documenting and conserving plant diversity; depict pathways for the sustainable use of this diversity; promoting education and awareness about plant diversity; and strengthening conservation networks at both regional and national levels.
- **Expand the biochemical diversity of natural product libraries, generating novel lead compounds of plant origin.** The core activities of **AgroCos** aimed to take advantage of cutting edge technologies in Natural Products extraction, profiling and activity determination in order to generate a **natural product library** originating from taxonomically and geographically highly diverse plants, and the identification of a set of compounds with **structurally diverse scaffolds.** The designed methodology had facilitate dereplication problems, and enable the discovery of new molecular scaffolds of potential interest for the cosmetic and agricultural applications. Given that the plant samples originate from geographically distant megadiverse regions and thus included mostly uninvestigated species, **AgroCos** had significantly **expand the biochemical diversity of natural product libraries and generate novel lead compounds of plant origin.** These efforts have taken us far beyond current state-of-the-art and have resulted in a unique, focused library of plant extracts assembled on the basis of chemodiversity and chemotaxonomy considerations.

- **Improve the competitiveness of the European and non-European industry.** **AgroCos** consortium focused its activities in these two areas of application (agrochemicals and cosmetics) to develop new knowledge concerning underexploited plant species and select new compounds with improved properties (potency, selectivity, physico-chemical characteristics) for cosmetic and agrochemical applications. The EU market is the biggest cosmetics market in the world. With an average growth of 3.8% annually between 2005 and 2007, the EU27 market amounted to € 67.8 billion in 2007, according to Colipa, the European Cosmetics, Toiletries and Perfumery Association. The natural cosmetics market is relatively small, but is growing faster than the overall market. Natural cosmetics continued to benefit from growing health concerns, a sense of well-being and looking good, as well as the influence of media attention. Within this overall picture, there is an increasing use of new, active natural ingredients with functional benefits, organic ingredients, exotic, African and Amazon ingredients and ingredients (saps and extracts, essential oils and vegetable oils) based on (exotic) fruits. On the other hand the increasing consumption of organic foods has created a pressing need for natural insecticides and herbicides that can be used on crops certified as "organic". Ideally, insecticides should reduce pest populations, be target-specific (kill the pest but not other organisms), break down quickly, and have low toxicity to humans and other mammals. As a result, there is increasing demand for alternatives to conventional synthetic insecticides. Many botanical insecticides have been known and used for hundreds of years, but were displaced from the marketplace by synthetic insecticides in the 1950s. These figures show that there is considerable market potential for future natural product-based agrochemical and personal care products. Hence, the economic impact for the European industry launching new commercial products based on natural sources will be accordingly. The exploitation of new plant-derived products will have significant economic impact in the source countries. **AgroCos** project was committed to an efficient study and testing of natural products as well as the development in scientific and technological capacity increasing thus access to new species and development of new commercial research. Moreover through the implementation of the project closer relationships with source countries and communities could build up in the future which allow the design of benefit-sharing packages to link not only the supply of raw material, but also local capacity- and institution-building.
  
- **Improving sustainability and eco-efficiency of natural products.** Through the **AgroCos** project activities possibilities for the utilization of natural resources in cosmetology and agriculture have been assessed and strategies for conservation and sustainable supply investigated. We believe that from the plant species that have been studied it is possible to identify endemic plants that are viable economic opportunities for farmers as high value alternative crops giving new opportunities for environmentally friendly sustainable development. These issues have been thoroughly considered in the feasibility study which will be carried out within the activities of WP5. The possibility of cultivation in the different regions represented in the **AgroCos** consortium has been examined in order to secure the **sustainability and eco-efficiency of the products that has to be considered for future development.** Moreover the development of environmental friendly industrial procedures within this project have given the opportunity to herbal extract producer to adopt technologies in line with EU policies for the protection of environment.