Wood Bark and Peat Based Bioactive Compounds, Speciality Chemicals, and Remediation Materials: from Innovations to Applications

Reporting

Project Information

FORESTSPECS

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Executive Summary:

Qualitative and quantitative analysis of extractives from the bark of industrially important tree species Pinus sylvestris, Picea abies, P. ajanensis, Abies nephrolepis, Populus tremula, Larix sibirica, L. decidua, L. gmelinii, L. sukaczewii, Pinus pumila, and knotwood from P. abies were carried out; analysis of bark from Betula pubescens has been carried out by the consortium earlier. Additionally, humic substances from peat were analyzed and studied.

- in total 152 compounds were isolated and identified from the bark samples (7-20 compounds/tree species); five of them were new compounds
- over 70 compounds were identified from the humic acid samples, and over 20 volatile substances from peat distillates
- some 130 compound-derivatives were additionally synthesized for further study

All extracts and the isolated and synthesized compounds were subjected to screening of bioactivity for medical, cosmetic, and plant protection purposes, for industrial applications, and the use of the total or remaining biomass in production of growth substrates, organic fertilizers, and bioremediation materials was investigated.

The ForestSpeCs consortium found, among other results:
- an improved method of extracting bioactive compounds from bark applying microwave assisted extraction techniques requiring less solvents, less energy and less time than with conventional extraction techniques
- excellent opportunities for using bark-derived compounds for medical applications, including antibacterial, antifungal, and antileishmanial activities, and easily obtainable, high-quality compounds for replacing current and/or developing new treatments e.g. for type 2 diabetes, menopausal hormone treatments, allergies, and Alzheimer’s disease
- superb sunscreen activity for both UV-A and UV-B, and birch bark molecules capable of apoptosis induction in melanoma cells to stop and even cure skin cancer, for cosmetic use
- about 10-15 extracts/compounds with highly promising fungicidal or antifeedant properties for applications in plant protection; e.g. compounds equivalent or better than current toxic copper compounds used for plant disease control, particularly on grapevine
- many excellent compounds for technical uses, e.g. suberin derivatives for binder, surfactant, lubricant, and plasticizer applications, and humic acids for use as chelating agents and partial replacement for phenol-formaldehyde type resins in adhesives
- high potential to use bark in composting to produce growth substrates to replace peat, and as soil conditioners and fertilizer in poor and arid soils; if inoculated with the beneficial fungus Trichoderma, these composts can remediate polluted soils (e.g. toxic soils suffering from heavy metal pollution, such as created by mining industry)

The ForestSpeCs project has demonstrated beyond doubt that forestry side-products such as abundant
bark materials, as well as humic substances easily obtained from peat, possess properties, which make them a valuable resource for exploitation. Simply burning these materials as a low-value energy source is wasting opportunities for wealth creation: while the energy value of bark is in the range of 10-20 € per ton, its value as growth substrate is more than tenfold, and as a source of bioactive molecules for high value-added products in medicine, cosmetics, industrial chemicals, or plant protection products, the potential value is several orders of magnitude greater. Bark represents an excellent opportunity to help in improving the rural economies and employment all over Europe, and in increasing the sustainability of agriculture and forestry by replacing fossil-oil based processes and products by rapidly renewable natural materials, and by contributing to slowing down climate change. However, if these promising leads are not actively followed and backed up by further R&D and fostered, they all may remain latent, and their potential will not be materialized.

Project Context and Objectives:

Context and objectives

Background
The development of modern, environmentally friendly technologies, processes, materials, and products utilizing the abundant sources of wood waste and humic substances as raw material for value-added products has a high business potential. The European forest industry produces annually about 25-30 Mt of bark as a by-product, but the possibilities of using bark as a raw material for bioactive compounds, specialty chemicals, or for remediation, have gained only moderate attention in comparison with using bark as a marginal and small-scale energy source. The production of fine and specialty chemicals that are based on rapidly renewable, naturally occurring raw materials has recently become a very exciting research topic worldwide. The majority of this research has focused on studying relatively low-volume products, such as β-sitosterol and lignan derivatives for medicinal uses, and as ingredients in various functional foods. So far only few studies on producing high-volume chemicals and materials with substantial markets from the side products of forest, wood-processing and peat industry have been reported.

The overall aim of the ForestSpeCs-project was to utilize different types of wood residues from the wood processing industry, such as bark of various species, especially from Betula, Pinus, Picea, Larix, Abies and Populus, as well as peat, as raw material to produce bioactive compounds and environmentally benign industrial chemicals and polymers, as well as remediation materials. This work consisted of innovative studies to find feasible ways to replace petrochemical-based raw materials not only in high value-added products, such as pharmaceuticals, fungicides and plant protection agents, but also in technical products such as adhesives, coatings, surfactants and chelating agents. Furthermore, one of the main aims of this project was to create options for total usage of processed wood and peat residues, so that the overall operations suggested are economically feasible.

All the raw materials used in the project are available in high volumes, and are currently used as energy. However, they represent a remarkably rich source of bioactive compounds and key intermediates, which can be upgraded to value-added specialty chemicals and materials, opening an opportunity for creation of new types of forest-based value chains. They are promising sources for innovative applications in sectors such as agriculture, environmental remediation and health.
Bark compounds

The content and spectrum of extractives in bark varies between different tree species, but may be even as high as 30-40% of bark dry weight. In white birches (Betula spp) the bark contains large quantities of the triterpenoid betulin. The chemical properties of betulin make it an attractive starting material e.g. for various pharmaceutical products. The predominant type of components in lipophilic bark extractives are various fatty acids, terpenes (mono-, sesqui-, di-, sester- and triterpenes) and sterols, while the hydrophilic fraction generally consists of large quantities of aromatic phenolic compounds including e.g. tannins, tannin-like oligomeric phenols and flavonoids, as well as some other classes of polyphenols. An interesting group of phenolic extractives are the stilbene glucosides found in minor amounts in the bark of Norway spruce (Picea abies).

Presently, the outer bark of birch is a low-value waste product in the forest industry. The extraction of betulin on an industrial scale from birch bark waste is a large and potentially low-cost source of raw material available for utilization. The white-barked birches are widespread in the northern latitudes of the world, and currently there is no economically significant use for this abundant, easily isolable compound. It has been estimated that a pulp mill with an annual production capacity of 200 000 tonnes of birch kraft pulp produces bark waste permitting isolation of ca. 4000 tonnes of betulin of ca. 95% purity per annum. The major interest in betulin lies in the fact that it is a synthetic precursor of various triterpenoid compounds having important pharmacological, physiological or biological properties. The chemical constituents of birch bark are useful in pharmaceutical and other industrial applications. Moreover, there are very interesting application possibilities for betulin in cosmetics and in agriculture.

The same birch bark processing unit (vide supra) could additionally produce ca. 4000 tonnes of suberin polymer, another main component of birch outer bark, per annum. Suberin can be further hydrolyzed by base to the corresponding suberin acids. Suberin acids can be used to produce new industrially potential value-added products, for example binders for coatings and composite materials, biodegradable lubricants, and surface-active agents. Finally, the hydrolyzed phenolic fraction and its constituents obtained from the raw suberin have, in turn, potential uses as antioxidants, antibacterial agents and pesticides.

In the ForestSpeCs-project bark from industrially important tree species (Betula, Pinus, Picea, Larix, Abies, Populus), and other wood residues, has been studied. The focus was on natural and modified chemicals based on isolated components from wood residues, such as betulin and suberin. Our work included innovative natural products chemistry, extraction and process technology; as well as basic research on mode of action and structure-function/structure-activity relationships within the application areas e.g. in plant protection products (‘biological plant protection products, BPPP’): insect/pest antifeedants, insecticides, herbicides, antifungal and antibiotic products; in pharmaceutical/medical applications, cosmetics, and bioremediation. Furthermore, the possibility to use other types of large-volume wood waste, e.g. sawdust and chipped wood from the pulp and paper industry, as organic fertilizers and growth substrates in the cultivation of vegetables and in reforestation were studied in the ForestSpeCs-project.

Peat compounds

Peat, another large-volume raw material in the ForestSpeCs-project, contains small amounts of low-boiling bioactive compounds with potential use in pharmaceuticals and cosmetics, and 10-20% of humic substances, mostly humic and fulvic acids. Humic acids (HA) and fulvic acids (FA) are the most widespread natural polyelectrolytes in all terrestrial and aquatic environments. Humic acids have an
amphiphilic nature because of the presence of both hydrophilic and hydrophobic moieties in their structure. Fulvic acids are water-soluble under all pH conditions. HAs can be isolated quite straightforwardly from peat by using alkaline extraction methods.

HAs have been shown to reduce surface tension very effectively in aqueous solutions. Critical micelle concentration (cmc) of the compost humic acid is 2.6×10⁻⁵ M compared to that of synthetic surfactant, sodium dodecyl sulfate (8.2×10⁻³ M). Moreover, the molecular weight of HA and FA fractions (3-15 kDa) enables these products to be used directly as raw materials in industrial applications such as resins. Also the highly functional molecular structures of HAs and FAs give various possibilities for chemical tailoring especially via hydroxy and carboxy groups. For example, HAs can be sulfated/sulfonated to produce even more effective surfactants, and FAs can be modified with fatty alcohols to give proprietary surfactants and modifiers. Humic acid derivatives have potential use in various industrial applications, such as wood gluing e.g. in particle board and plywood applications, as flotation agents in the mining industry, as chelating agents in water purification and as additives (super plasticizers) in cement and concrete.

Until now, the medical uses of peat derivatives have been very limited. One medicinal product made from peat is Torfot, a Soviet Union-time product, primarily used for ophthalmic diseases, as a stimulator for general immunity of human body and regeneration processes, as well as non-specific immunomodulator. The product is made by steam distillation of cotton grass-sedge peat. It is a mixture of variety of volatile compounds including phenols, amines, and saturated carboxylic acids.

The ForestSpeCs-approach

The real challenge and the novelty of the ForestSpeCs-project was in utilizing readily available large-volume, low-price wood residues and humic-origin substances to produce value-added chemicals and materials leading to new environmentally benign pharmaceuticals and BPPP, as well as to replace various commodity chemicals of petrochemical origin. To achieve this, innovative ways to combine multidisciplinary and complementary skills of the project group were needed. These included knowledge of separation technologies, chemical synthesis, biochemical modification, characterization of molecular structures; understanding of agricultural, biological and pharmaceutical sciences, and a wide range of application-specific knowledge, and the availability of testing facilities (Fig. 1).

Figure 1. The strategy and value chain for upgrading of wood residues and substances of humic origin to value-added chemicals and materials in ForestSpeCs. The chart describes the two main types of basic raw materials as the starting points (bark on the left and peat on the right hand sides of the chart), the value-added secondary raw materials (towards the middle), and their possible application areas (middle of the chart) as bioactive substances (upper part, light blue), speciality chemicals (middle part, turquoise), as core substances in remediation materials (lower part, dark blue), or as sources of energy (bottom; not a topic for ForestSpeCs).

S&T Objectives

The overall objective of the ForestSpeCs-project was to develop and encourage sustainable, environmentally responsible, and economically attractive management of natural resources based on side products from the forest and forest-based industries. The ultimate target of the project was - and is - to replace certain large-volume, oil-based chemical materials with bio-renewable and innovative products based on wood-related residues and humic substances. The aim was to find feasible ways to produce high value added, bioactive compounds such as pharmaceuticals and biological plant protection products.
(BPPP), as well as to develop new environmentally benign industrial chemicals and polymers. Furthermore, one of the main targets was to create economically attractive options for the total usage of processed wood and peat residues either as a whole, or after extraction of the main bioactive fractions, for example in soil remediation.

The scientific objectives were:
1. to develop new synthetic methods to modify compounds isolated from wood-related residues (e.g. betulin and suberin) as well as humic acids to obtain new bioactive compounds and environmentally benign industrial chemicals and polymers;
2. to determine the biological activities, properties, and potential uses in various applications (e.g. in health, cosmetics, and plant protection) of the extractive fractions and purified compounds based on wood residues and peat, as well as synthesized derivatives of the compounds isolated from the processed wood and wood bark;
3. to analyse the relationship between the chemical structure of these compounds and their biological activity, in order to facilitate a more efficient design of bioactive molecules in the future;
4. to establish the potential of wood bark as raw material for products needed in remediation of poor and/or arid soils, in order to facilitate agricultural production, and ultimately, to combat climate change and desertification;
5. to examine the suitability of wood bark and peat as components in biotechnological processes aimed at remediation of contaminated soils;

The technological objectives were:
6. to develop sustainable processes to separate and modify bioactive compounds from wood residues and peat as raw materials for value-added products such as pharmaceuticals and biological plant protection products;
7. to develop transformation routes needed to produce new type of adhesives, coatings, lubricants, surfactants and chelating agents from wood residues and peat;
8. to develop reliable methods for screening extractive fractions and isolated, identified substances from the wood and wood-related residues, for their efficacy as BPPP;
9. to evaluate the technical properties and business potential of the processes and end-products, as well as the overall feasibility of the operations suggested.

Project Results:

3. Main S&T results
3.1. Raw materials and processes
3.1.1. Extraction, purification, characterization
Qualitative and quantitative analysis of extractives from the bark of Pinus sylvestris, Picea abies, P. ajanensis, Abies nephrolepis, Populus tremula, Larix sibirica, L. decidua, L. gmelinii, L. sukaczewii, Pinus pumila, and knotwood from P. abies were carried out (analysis of bark from Betula pubescens has been carried out by the consortium in earlier projects).
Experiments on the optimum extraction technique led to the conclusion that microwave assisted extraction was the ideal technique to be used in extracting the plant materials. This technique gave higher yields of extracts in a relatively short period of time, and with less amount of solvents. Using microwave instead of
the traditional extraction by agitation and/or Soxhlet extraction enabled a switch to green chemistry. This is because of extraction with low energy, reduced solvent levels, lower capital investment, faster processing time and repeatability in terms of output.

Qualitative analysis yielded a large number of compounds from the bark: Pinus sylvestris gave 14 compounds, P. pumila gave 19 compounds, Larix gmelinii gave 15 compounds, L. sibirica gave 14 compounds, L. sukaczewii gave 20 compounds, L. decidua gave 20 compounds, Picea abies (bark) gave 4 compounds, P. abies (knotwood) gave 12 compounds, P. ajanensis gave 16 compounds, Abies nephrolepis gave 7 compounds and Populus tremula gave 11 compounds. In total 152 compounds were identified (about 30 compounds were isolated from more than one source).

Five compounds were identified for the first time (Fig. 3.1.1):

Fig. 3.1.1. New compounds identified from the barks of ForestSpeCs trees.

Single compound yields typically were less than 0.1% of the bark dry mass, but several of the main compounds (and many of those of further interest) occurred at levels of up to 2% of the bark dry mass, making their commercial extraction feasible in many cases.

Volatile components of the essential oils of Pinus sylvestris, Larix gmelinii, L. sibirica, L. sukaczewii, L. decidua, and P. abies were also analyzed. On average 12 compounds were identified per species (range 8-15); their percentage yields ranged from 1.5% to 2%.

Separation of volatile and humic substances from peat

Three samples of peat (several kilograms) were collect from different locations in Finland (1 sample) and Russia (2 samples) for separation of humic and volatile substances. Humic substances were separated by alkaline extraction, and volatile substances by steam distillation. Distillation of 10 g of peat macerated with 100 mL of water yielded 50 mL of clear, colourless distillate.

<table>
<thead>
<tr>
<th>Table Yields of humic acids and humin from alkaline extraction of peat samples (50g)</th>
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Humic acids were characterized by NMR and pyrolysis-GC-MS spectroscopy and by acid-alkaline titration. Volatile substances were characterized by GC finger print analysis. Over 70 compounds of compound groups were identified from humic acid samples, and over 20 volatile substances from the distillates (Table ).

<table>
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<th>Table Characterization of humic acids</th>
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Table Distribution of different degradation products groups of HA and FA samples. [Russia A (HA) = RUSS HA A; Russia B (HA) = RUSS HA B; Finland (HA) = VAPO HA; Finland (HA-salt) = VAPO HA, H2O; Finland (HA-salt-bitumen) = VAPO HA, H2O + Et2]  

Table Example of qualitative characterization GC/MS of volatile compounds from peat

3.1.2. Modification of the identified compounds

Synthesis of new betulin and abietane derivatives

Synthesis of betulin and abietane derivatives having potential UV-protection, antibacterial, antifungal, antifeedant or other antimicrobial activity identified and assayed in WP3 and WP4 were carried out. In total almost 100 di- or triterpene derivatives were synthetized and characterised. These compounds include
around 50 betulin derivatives, including novel derivatives having additional heterocyclic ring fused into betulin ring A. Also few novel betulin ester derivatives were synthetized. In addition to betulin derivatives almost 50 abietane derivatives were synthetized. These derivatives include mostly novel urea and amide derivatives. Over 350 test samples were supplied to the partners in the WP3 and WP4 and for collaborating parties for bioactivity assays.

In addition, a total of 33 à la carte” derivatives were synthesized for the needs of the consortium groups, as reported in Deliverable D2.5.

Suberin derivatives

Alkali hydrolysis is proved to be simple and industrially attractive method to obtain suberin acids from birch outer bark. In spite of highly functional chemical structure of suberin acids it is quite unstudied material. In ForestSpecs project, suberin acids as sodium salts were isolated by extractive hydrolysis from birch bark and further processed to suberin acids, methoxy methyl esters, maleates and sulphate esters derivatives. Study for suitability of suberin fatty acids as aid for manufacturing of medical tablets is on-going. The three types of suberin derivatives were further prepared for surfactant, lubricant and coating applications.

Modification of humic substances

Predominant functional group in humic acids is carboxylic group. Thereby humic acids bind efficiently cations and their water-binding power is excellent. The ion exchange capacity of peat humic acids can be used for the manufacture of cation-exchange resin and for water purification applications. Humic acids are excellent products for chelating metals, and can be used in water purification to remove hazardous metals e.g. Fe, Cu, Mn, Zn, Pb and Cr and also radioactive metals such as Cs and U. Humic acids also have potential use as adhesives in wood and modified wood products, as binders in composites. Chemical modifications can be performed to humic acids to improve their properties. The chemical modification methods used for humic acids include polymerization, esterification, sulfonation and cross-linking. Thus, several chemically modified humic acid samples were synthetized and characterized in the ForestSpeCs project, for testing of their properties in specific applications.

3.2. Applications for health, cosmetics and specialty chemicals

3.2.1. Health

Antimicrobial screening

The primary antimicrobial screening against six different microbes, E. aerogenes, E. coli, E. faecalis, P. aeruginosa, S. aureus and C. albicans yielded altogether 30 hits (3% of all the results) by using inhibition of >70% as the threshold for considering a sample active. Some of the samples were active against several strains, so 21 samples showed promising antimicrobial properties (hit rate 14%). This set included six betulin derivatives, two abietane derivatives, three natural compounds /derivatives /mixtures, eight bark extracts and two pure compounds isolated from bark. Interestingly, no hits were found against any of the Gram-negative bacteria included in the study whereas there were several samples that displayed significant activities against Gram-positive E. faecalis (12 hits) and S. aureus (7 hits) and fungi C. albicans (11 hits).

Further dose-response studies revealed that the betulin derivative SC047 was the most active, showing MIC of 6.25 μM against two Gram-positive bacteria, E. faecalis and S. aureus. In addition, MIC of 25 μM was demonstrated for SC311, DRA18 and TCI-D1588 against C. albicans. However, follow-up studies
carried out on mammalian cells, such as evaluation of cytotoxicity, showed that SC047 was also cytotoxic to hepatocytes based on ATP measurement of the cells after 24 h exposure to the compound. The activity of this compound is also clearly affected by albumin binding, which was demonstrated by loss of activity in the host-pathogen co-culture assay as well as in the antibacterial assay in the presence of increased concentration of albumin. Altogether, the results show the importance of taking a multidimensional approach to study biological activities of novel compounds. Excluding the follow-up studies carried out on mammalian cells from this study would have given very partial view on the real therapeutic potential of the hits identified in the primary antimicrobial screening.

Structure-activity relationship analyses of the most interesting betulin derivatives

The structure-activity relationship of betulin derivatives was evaluated using their activity against the parasitic disease leishmaniasis, which affects millions of people in developing countries and has been designated as a neglected tropical disease by the World Health Organization. It is caused by the protozoan parasite Leishmania, and is transmitted by sand flies. First line drugs for treatment include pentavalent antimony compounds, pentamidine or amphotericin B. All these drugs are administered by intravenous injection and require clinical supervision or hospitalization due the possibility of severe side effects.

We have shown that by simple chemical modification, anti-leishmanial activity of betulin can be improved considerably. It is possible to derive relatively potent anti-leishmanial compounds with low micromolar GI50 values. In general, carbonyl or carboxyl groups at C-3 or C-28 have beneficial effect in anti-leishmanial inhibition activity, and these compounds can be regarded as significant lead molecules for further improvement and optimization. In addition a series of new heterocycles derived from betulin was synthesized. We found that small R1 substituents at the nitrogen atom of the triazolo moiety as well as the least sterically hindered acyl groups at the R2 positions in the betulin skeleton promoted anti-leishmanial activity. Also triazolo ring had beneficial effect on anti-leishmanial activity, as it was found out in our previous work that 3,28-di-O-acetylbetulin was totally inactive. The most effective derivative against Leishmania donovani amastigotes was a heterocycloadduct with a GI50 = 8.9 μM, however this compound showed some toxicity (39%) for the macrophage cell line at approximately the same concentration (12.5 μM). Several additional derivatives with slightly higher GI50, ~25 μM, showed none or only limited toxicity for macrophages at this concentration, and demonstrated good activity against the intracellular parasites.

New therapeutic agents for the leishmaniases are urgently needed. Most existing drugs are toxic or expensive and drug resistance by parasites causing fatal visceral disease has led to discontinued use of first-line drugs in some highly endemic regions of India. By simple chemical modification anti-leishmanial activity betulin can be improved considerably. In addition heterocyclic betulin derivatives show promising activity against Leishmania donovani. Further studies to develop more potent betulin derivatives with leismanicidal properties but no toxicity for the human host macrophages are underway.

Antioxidant activity

Antioxidants are recognized as having an important role in maintaining human and animal health and are considered a useful adjunct in pharmaceutical approaches against various indications. Thus, there is a real interest in the discovery of new and novel sources of antioxidants and, moreover, from natural sources. In this study, a number of extracts from bark were assessed for their total phenolic content, iron(III) reducing, and free radical scavenging activity. With few exceptions, the extracts demonstrated
varying degrees of activity with some demonstrating relatively strong effects. The activity data suggests that these extracts possess antioxidant activity and would merit further investigation. The analysis of the pure compounds indicated that these compounds possessed poor activity in the two assays used to assess them.

Table Total phenolic content expressed as mg gallic acid equivalents/g (dry wt.) extract

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total Phenolic Content</th>
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<tbody>
<tr>
<td>Larix gmelinii dichloromethane (LGD)</td>
<td>10 mg/g</td>
</tr>
<tr>
<td>Pinus sylvestris dichloromethane (PSD)</td>
<td>15 mg/g</td>
</tr>
<tr>
<td>Ethyl acetate (PSE)</td>
<td>20 mg/g</td>
</tr>
<tr>
<td>Methanol extract (PSM)</td>
<td>25 mg/g</td>
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Apoptotic/antiapoptotic effects

Apoptosis is one form of programmed cell death occurring in healthy organism to remove unwanted and/or harmful cells. It is also needed for the maintenance and function e.g. of homeostasis, differentiation and embryonic development. The molecular mechanisms of apoptosis are closely regulated and the impairments of function associated with several diseases, e.g. cancer and Alzheimer disease. One of the key regulators of apoptosis is a protein p53 which is involved in numerous cell signaling pathways related to apoptotic cell death. Caspases are central regulators of cell death.

In this study, the cytotoxic effects of selected Larix- and Pinus -extracts were defined in both normal fibroblast CV1-P and cancerous melanoma A375 cell lines. In addition, the possible apoptotic mechanisms of selected extracts were defined in A375 cells by caspase 3 activity assay and preliminary Western blot analysis of protein p53.

Larix gmelinii dichloromethane (LGD) and Pinus sylvestris dichloromethane (PSD), ethyl acetate (PSE) and methanol extracts (PSM) showed selective cytotoxic effects in cancerous melanoma cells. Larix gmelinii ethyl acetate (LGE) extract was toxic to both cancerous and normal cells. Additional tests revealed intracellular mechanisms behind cytotoxic effects. Cytotoxicity of LGD, LGE and PSD was not related to caspase 3 activity supporting the cytotoxic effects were not related to caspase-dependent apoptosis. Cytotoxicity may be explained by other cell death mechanisms than apoptosis. The results support a potential role of natural products altering the cell signaling mechanisms of cell death.

Evaluation of acute and chronic toxicity

The toxicity of betulin and suberin acids, and the irritation activity of tall oil rosin, and the irritating and allergenic activity tall oil fatty acids were evaluated. All studies were based on the ICH Harmonised tripartite guidelines "Note for guidance on toxicokinetics: The assessment of systemic exposure in toxicity studies" and Guidance for Industry Single Dose Acute Toxicity Testing for Pharmaceuticals Center for Drug Evaluation and Research (CDER), August, 1996. Male and female outbreed rats, and outbreed male and female mice, were used for the experiments. For the irritation experiments, male and female guinea pigs, and outbred male mice were used.

The results of single intragastric administration of betulin in doses of 1000-16000 mg/kg in rats and mice, of single intraperenial injection of betulin in doses 250-4000 mg/kg in rats, and of single intragastric administration of suberin acids in dose of 15000 mg/kg, all show that no toxic effects could be observed. Results of 14 days of observation of animals, and data of necropsy after single dose administration of betulin and suberin acids evidenced the safety of these substances. Betulin and suberin acids are thus non-toxic, and may be classified as substances of VI class toxicity according to Gosselin et al.

In contrast, TOR and TOFA appear to be dubious substances with high allergenic activity after multiple sensibilisation. Severe anaphylactic reaction (anaphylaxis shock, type I hypersensitivity) was observed after multiple sensibilisation of animals with TOR in doses of 0.00255 g/kg and 0.255 g/kg. Fatal anaphylactic reaction (anaphylaxis shock, type I hypersensitivity) was observed after single and multiple sensibilisation of animals with TOFA in doses of 0.0925 g/kg and 9.25 g/kg.
TOR and TOFA are able to show allergic reaction (type I hypersensitivity) after multiple sensibilisation of organism and skin contact. The allergic conjunctivite (immediate hypersensitivity, type I hypersensitivity) could be developed after multiple sensibilisation and contact of TOR and TOFA with conjunctive. However, inflammation reaction is not observed 24 hours after contact. ROS in doses of 0.0046 g/kg and 0.46 g/kg and TOFA in doses of 0.0925 g/kg and 9.25 mg/kg were able to stimulate allergic reactions of delayed-type hypersensitivity. Allergic reactions (type III hypersensitivity) were observed for TR and TOFA in immune complex hypersensitivity model. Application of TOR in dose of 0.0046 g/kg was not resulted in cutaneous irritation effect. Other samples in other doses were able to stimulate irritation effects.

Evaluation of anti-inflammatory, antidiabetic, and hepatoprotective effects
In ForestSpeCs, the first evaluation of anti-inflammatory effects of distillates of peat has been carried out. Peat distillates exhibited significant anti-inflammatory and analgesic activity in rats in the model of carrageenin induced paw edema, and carrageenin air pouch in the rat. Statistically significant differences between distillates of peat collected in Russia and in Finland were not observed. In the model of adjuvant-induced arthritis in the rat the antipyretic effect was observed for PD 1 in dose of 6 ml/kg, and PD 2 in dose of 12 ml/kg. Anti-inflammatory effect was observed for PD 1 and PD 2 at the doses of 12 ml/kg and 12 ml/kg respectively. PD 2 in doses of 12 ml/kg and 18 ml/kg showed chondroprotective and osteoprotective activity.

Betulin in all doses did not affect glucose, TG, and Chol levels. However, diabetic rats showed reduced islet cells, which were restored to near normal upon treatment with betulin dose of 1.5 mg/kg, and suberin acids in all tested doses.

This study provides evidence that betulin and suberin acids are potent candidates for treatment of hepatocellular damage of liver. Betulin (10 mg/kg) and suberin acids (5 mg/kg and 15 mg/kg) contributed to the evacuation of bile by reducing the tone of the bile ducts, stimulation of the contractile function of the gall bladder, and reducing the pressure in the biliary system. Administration of suberin acid (15 mg/kg) decreases the cytolysis by decrease of ALT. Betulin and suberin acids in doses of 5 mg/kg and 10 mg/kg have potent antioxidant activity, and protect the liver.

3.2.2. Cosmetics
Betulin and abietane derivatives in cosmetics: evaluation of UV-A and UV-B properties
Betulin and abietane derivatives were screened for their UV-protection properties for their potential use in cosmetics (sunscreens). In total, 18 betulin and 12 abietane derivatives, synthesized in ForestSpeCs were measured, and compared with several commercial references. Additionally, suitable formulations were made together with Granula Oy for performance testing. UV absorption was measured for UV-A (320 – 400 nm) and UV-B (280 – 320 nm) ranges.

Fig. The best commercial reference for joint UV-B and U-A absorption.

It was concluded from the screening studies that conjugated double bonds or aromatic rings are necessary for UV-B activity. Nitro, hydroxyl, methoxy or amine moiety at aromatic ring seems to result compounds having UV-A and UV-B activity in a single molecule.

Evaluation of safety of cosmetic products
The safety of cosmetic products for topical application using pine spruce knot extract (PSKE), natural
cream scrub with Pinus sibirica crushed cones (PSC), and cream-gel with betulin were evaluated for irritation and allergic effects using guinea pigs as test animals. The irritant standard was sodium dodecyl sulfate (SDS) 5%. Skin irritation was assessed according to a standard scale daily, and an end-of-treatment assessment was made on Day 6. The antiallergic effects after topical application were assessed with 150 mg of cream scrub, which was applied to the 2x2 cm treatment areas 20 times. Skin reaction was scored according to the standard colour scale.

Twenty-four hour application of SDS 5% resulted in an increase in the median clinical score of skin irritation from 0 to 6.5. There was a difference in response pattern over time between the pine spruce knot extract treatment and the glycerol, as compared with ‘No treatment’, resulting in significantly quicker healing. Six days transdermal application of pine spruce knot extract (120 mg) significantly reduced erythema induced with SDS 5% irritation, and hydration of skin was decreased to one-sixth, as compared with the control (Table).

Table Assessment of skin hydration after application of test samples

After application of cream scrub with PSC, cream gel with betulin, and bases of creams, no erythema or edema were observed after 4 - 72 h of topical application. Application of cream scrub with PSC and cream gel with betulin during 20 days was safe: no erythema was observed.

3.2.3. Specialty chemicals and polymers
Applicability of suberin derivatives as lubricants, surfactants, plasticizers and binders
Suberin derivatives prepared in ForestSpeCs are potential for lubricant, surfactant and binder applications. The potential use of the prepared suberin derivatives as lubricants and surfactants can be evaluated by determining the key properties important in these applications; viscosity in different temperatures and surface tension in different concentrations.

To evaluate the potential of suberin derivatives for surfactant applications, the surface tensions of suberin sodium salts, suberin sulphate esters and SDS (sodium dodecyl sulphate, commercial surfactant) were measured.

Figure Surface tension measurements for Na-suberin, suberin sulphate ester and SDS.
Both suberin sodium salt and suberin sulfate ester reduce surface tension of water even better than commercial SDS. This study shows that suberin sodium salt and suberin sulfate ester are very potential compounds for surfactant applications.

Lubricant properties of suberin methoxy-methyl ester, and the transesterification product, were determined by measuring their dynamic viscosities. The graphs from viscosity measurements of suberin methoxy methyl ester and methoxy tert-butyl ester resemble the typical viscosity-temperature curves of paraffinic and naphthenic oils. The most important temperature range for lubricants is 40-100 °C, and in this range it is very important that the temperature doesn’t impact the viscosity properties too much. In this temperature range the viscosity of suberin derivatives doesn’t change much, and this indicates that these two suberin derivatives have a good potential for lubricant applications. The graphs also show that replacing the methoxy group with a t-butoxy group slightly increases the viscosity. This indicates that viscosity properties can be easily modified by derivatizing the product. These suberin derivatives are thus suitable for e.g. clockwork and instrument oils, or motor and engine oils, or as industrial lubricants.

Studies concerning the potential of suberin derivatives for binder applications show that suberin maleates
are applicable as binders in coatings and composites, and that they can be used as cross-linkers since they contain a lot of double bonds.

Overall, suberin is very interesting and valuable raw material, which will via hydrolysis produce a mixture of fatty acids with rich functionalities, e.g. epoxides and diols. Based on the studies in ForestSpeCs suberin fatty acids and their sodium salts can be easily modified for use in several applications such as lubricants, binders and surfactants.

Applicability of humic acid derivatives as chelating agents and wood adhesives

In this work the approach taken for both adhesives and adsorbent materials was to incorporate native humic acid (HA) into a traditional phenol-formaldehyde (PF) resol resin material (abbreviated here as HA-PF resins) with different humic acid contents. So far, various type of lignin has been used particularly in phenol-formaldehyde (PF) resins for wood adhesive applications to replace expensive and harmful phenol. Humic acid, which has structural similarities with lignin, could replace phenol as well. Humic acids' capability to bind metal ions from aqueous solutions is well known, but their high solubility in water is a drawback for efficient practical utilisation for instance in water purification as their recovery is difficult.

HA-PF adhesives were tested both qualitatively (paper and veneer) and quantitatively (veneer) and for their capacity to bind Ca\textsuperscript{2+} ions from aqueous solutions. A couple native-like (non-immobilised) HA’s were also tested for their Cu\textsuperscript{2+} and Cd\textsuperscript{2+} ion adsorption capacity.

Adhesive tests on veneer and paper indicate that humic acid content can be as high as ca. ~44 % of all solidifiable/ polymerisable matter (HA, phenol, formaldehyde, and NaOH) to still possess adhesive strength. Ways should be found to get a higher solid content in its entirety as they were found to be less than that to be expected. From some samples the humic acid is leached out, but there is no consistency for this in terms of HA content. It is possible that reaction parameters have not been suitable for all samples and this may have nothing to do with HA content itself. There are certain parameters (temperature, reaction time, choice of catalyst, catalyst loading, choice of hardener, curing time, usage of formaldehyde pre-treated HA, for instance and other practical matters that can be screened. Fixing and optimising these would probably result in a stronger mechanical strength of HA-PF resins. For now, mechanical strength of the products in wet conditions is rather poor but aforementioned things should apply to these as well.

These same HA-FP’s showed rather good resistance toward leaching in water and were thus successfully immobilised. As to the practical yields of these immobilised HAs, from experience, it can be said that they may be improved since significant amounts of matter was lost during decantation. Also, with larger batches the yield percentages presumably are better.

Ca\textsuperscript{2+} ion adsorption tests indicate increasing adsorption capacities with higher HA contents: samples 167 and 171, which contain HA the most, bound Ca\textsuperscript{2+} ions the most. Compared to the native Vapo HA at pH 5.1 sample 171 adsorbed ca. 79 % of Vapo Ha’s capacity. At higher pH of 9.0 the samples adsorbed even more. It must be noted that straight comparison of immobilised and native-like HAs is not fully possible because ion sorption capacity can be affected by different Ca\textsuperscript{2+} concentrations (24-100 mg/ L; 0.6-2.5 mmol/ L) used in these experiments. For now, there are no reference values for HA-PF resins in the chemical literature, with which to compare these materials in Ca\textsuperscript{2+} binding in dynamic conditions. On the other hand, Ca\textsuperscript{2+} does not seem to be most investigated ion, in general. Nevertheless, the values obtained in ForestSpeCs are a lot better than those reported in the literature. It is evident that more experimental data from HA-PF adsorbents for their adsorption capacity as a function of metal ion concentrations is needed. In that way their adsorption behaviour in terms of isotherms can be obtained.

To conclude, HA-PF resins seem to be a promising research line with lots of prospects, and should be
3.3. Applications for pest management

3.3.1. Pest antifeedants/insecticides

Extracts from all the tree bark species studied in ForestSpeCs were tested for their antifeedant properties in insect control. Altogether, close to 100 different extracts or their mixtures were tested. Six different insect species were used in the standard experiments. Two of them were Lepidopteran species: the Cabbage white butterfly (Pieris brassicae) larvae [46 different extracts tested], and the African cotton leaf worm (Spodoptera littoralis) larvae [56 extracts tested], and four were beetles (Coleoptera): the Alder leaf beetle (Agelastica alni) larvae and adults [8 selected extracts tested], Cloudberry leaf beetle Galeruclla sagittariae larvae and adults [2 selected extracts in several types of tests], the Colorado potato beetle (Leptinotarsa decemlineata) larvae and adults [3 selected extracts], and the Mustard leaf beetle (Phaedon cochleariae) adults [3 selected extracts tested]. Additional tests were conducted in the laboratory with the Siberian silk moth Dendrolimus sibiricus at FEFRI (Khabarovsk) [9 selected extracts tested], and with the great web-spinning sawfly Acantholyda posticalis in the field in Finland [betulonic acid tested]. The extracts included total ethanol extracts from all bark species, total water extracts obtained at 60 °C and at 30 °C, single compounds obtained at UNIS as explained in chapter 3.1 (above) using three different solvents, and some selected compound mixtures.

The basic initial antifeedant testing was performed using the standard dual-choice bioassay method based on treated and control (solvent only) leaf discs on a petri-dish, using the two standard butterfly test species. Additional species and different types of tests were carried out with those substances showing the best results in the initial screenings. After the experiments were done with single components, those showing best feeding deterrence on Spodoptera were taken into new experiments as mixtures. Additionally, dual-choice, and no-choice experiments with newly hatched larvae of Galeruclla sarittariae were carried out. Also, behavioural cage experiments with G. sagittariae were conducted. The tested compounds were betulonic acid and abietic acid. The aim was to test if the selected compounds deter egg laying and/or feeding by the beetles. Tests with Dendrolimus sibiricus were carried out in the laboratory as dual-choice experiments in petri dishes (similar to our standard tests), using conifer needles treated with specific strength of the test substances, and small moth larvae (25-35 mm in length). Field tests with naturally occurring sawfly larvae were conducted using betulonic acid dissolved in ethanol, which was sprayed on small trees (2 m high) with plenty of feeding larvae on the branches.

Main result from the screening assays was that most of the tested extracts from different tree species showed some bioactivity by deterring the feeding of insects. Almost all tested solutions significantly deterred the feeding of P. brassicae larvae, and also the beetle species, while S. littoralis was the most tolerant to the provided extracts. Still, several extracts also deterred significantly the feeding by S. littoralis larvae at least during the first 24 hours. Usually the efficiency of the extracts seemed to decrease with time. With the extracts from UNIS it was notable that the dichloromethane and ethyl acetate extracts had generally much higher Feeding Deterrence Indexes than the methanol extracts. Moreover, the extracts based on ethanol as solvent showed better antifeedant properties than the water based extracts.

Figure x. Bioassay of feeding deterrence of Colorado potato beetle (Leptinotarsa decemlineata Say) after 22 hours of starting the experiment. A and B are control leaves treated only with 95 % ethanol (solvent) and C and D are treated with abietic acid (100 µg/cm2) on both sides of the leaves.
In the Dendrolimus experiments betulonic acid at three different concentrations were tested (5, 10 and 25 mg in 10 ml of ethanol), and bornyl, epimannool, piceatannol, rhaponticin, cathecin, and three variants of dehydroabietic acid. Only betulonic acid showed significant feeding reduction by this insect, at all tested concentrations. Further studies with this system might be useful.

In the field test, betulonic acid treatment did not have any effect on the number of Acantholyda larvae remaining on the twigs one week after the treatment, while neem and quassia extracts practically cleaned the twigs from the larvae. This was expected, as betulonic acid is not insecticidal (unlike neem and quassia), but only a feeding deterrent. Inspecting the amount of pine needles eaten at the time of the assay reveals that betulonic acid was as effective in protecting the needles as neem and quassia. This raises the interesting question of whether it might be ecologically beneficial that the insects are not killed, but stop feeding and remain on the branches e.g. as food for natural enemies. The use of betulonic acid in this context might be very interesting to study further.

The experiments showed that there is good potential to utilize the byproducts of forestry for pest management purposes, via extraction of antifeedants for plant protection. Still, there remain questions such as how to produce extracts in an economically feasible way, and how to lengthen their period of activity in the field. Also the possible environmental and other effects remain unclear, while it is known that some of these compounds can cause irritation. Clearly, bark represents an exciting source of new bioactive molecules and extracts that can be used in plant protection.

3.3.2. Suppression of fungal diseases on plants
The aim of the work was to identify new compounds as provided by FORESTSPECS partners as fungicides or resistance elicitors and to quantify their efficacy. Downy mildew of grapevine (Plasmopara viticola) and scab (Venturia inaequalis) on apples were used as standardised test systems under controlled conditions.

The work consisted of primary, secondary, and field screening of the extracts. For the primary screening, established bio-assays with seedlings of apple (cv. Jonagold) and grapevine (cv. Chasselas) were used to quantify the activity of test substances against Venturia inaequalis and Plasmopara viticola. The standard disease assessment included disease incidence (proportion of leaves showing symptoms) and disease severity (proportion of infected leaf surface) and/or lesion diameter. These tests also allowed the identification of dose-response curves, rain fastness or resistance induction, as foreseen in further screening steps. In the secondary screening the range of activity of selected substances and the narrowing down of their putative mode of action (i.e. inhibition of spore germination, mycelial growth, elicitor activity) was accomplished in a micro-screening with Phytophthora infestans, Pseudomonas syringae pv. tomato and Venturia inaequalis. Additionally, one new compound was tested in the experimental vineyard under field conditions at FiBL, Frick, Switzerland. The experimental setup fully complies with the EPPO requirements for fungicide screening. The plot consists of 576 plants of the susceptible grapevine varieties ‘Riesling-Sylvaner’ (‘Müller-Thurgau’) and ‘Chasselas’ (‘Gutedel’). Twelve different treatments were arranged in a „Randomized Block Design“, with four replicates, each consisting of six plants per variety. Test products and references were deployed weekly with an air assisted knapsack sprayer until near run-off. During the growing season, visual disease scoring was carried out several times by recording disease incidence (proportion of leaves with symptoms) and disease severity (proportion of diseased leaf area) of P. viticola on leaves and by recording disease severity (%) on grapes.
Within the frame of Forestspecs, a total of 105 substances/extracts/derivatives were supplied by partners to FiBL. A limited number of substances with a significant fungicidal activity were identified, based on the experimental screening, and taken forward. Substances with limited potential were excluded from further evaluation due to (i) lack of activity, (ii) phytotoxic effects and (iii) extreme difficulty to obtain a ready-to-use formulation for spray application as a plant protection product. From the bark extracts provided by WP2 (partners UNIS and UHEL) it was possible to identify four new substances with potential use as fungicides in plant protection. From the natural wood extracts and the betulin- and abietin-derivatives provided by WP2 (partner VTT) it was possible to identify four substances or derivatives that show a very interesting pattern of activity and an interesting level of efficacy. Additionally the formulated natural wood extract (RAM) yielded very promising results in the 2012 field trial. Improved formulation for better handling and higher efficacy, quantification of effects on non-target organisms and studies on environmental fate and ecotoxicity are needed to further develop this substance into a plant protection product.

Figure Efficacy of some of the plant extracts in controlling the Downy mildew of grapevine in the primary screening. Kocide = copper reference.

Within these extracts and substances it was possible to identify some with interesting activity against plant pathogens. Due to the feedback from our activity screening to the partners providing the material it was possible for them to produce advanced extracts. Such a partnership is necessary to combine strengths of different sciences for developing products from natural sources such as wood bark. As a consequence of the prolongation of the project, it was possible to test one formulated substance under field conditions on grapevine. The availability of raw material, costs for synthesis and extraction and time limitations proved to be limiting factors for field screening. However, based on screening results under controlled conditions, additional four candidate substances might be evaluated under field conditions in the future. Plant extracts that show effectual fungicidal activity worth to be further investigated and potentially developed into plant protection products were identified. As a next step a roadmap for further testing and developing the identified substances may be created, containing efficacy and toxicity testing as well as economic and feasibility analyses.

3.3.3. Tests for herbicidal activity
Total of 95 extracts/compounds which were produced in ForestSpeCs were tested for their ability to inhibit the germination of test seeds. The test substances were dissolved in acetone to a concentration of 5 μg/μl (pure substances) or 10 μg/μl (extracts); 100 μg of the substances per well/filter paper were used. Juglone with a concentration of 5 μg/μl was used as a positive control. Filter papers for the untreated control were only treated with acetone. As test species seeds from Lactuca sativa as a representative for dicotyledonous weeds and Lolium perenne as a representative of monocotyledonous weeds were used. Some of the test substances did not inhibit the germination at all but delayed it. In order to check whether those substances might have a long term effect, after 7 d on treated filter paper the germinated seeds were transferred into soil, and cultivated in a climatic chamber at 21 ºC, 70% RH, 16:8 Light:Dark. Four weeks later the development of the plants was assessed. 

During the screening of fungicidal activity one test substance, TOFA 2, provoked severe symptoms of phytotoxicity, when applied on potted grape plants. This and compounds which showed in the germination test good effects were tested for their ability to have effects on young plants. Seeds were sown in soil in
plastic pots measuring 37cm x 57cm. In one half of the form salad seeds, and in the other half grass seeds were sown. After the development of the first two leaves (salad) the plants were sprayed with a determined amount of the test substances in different concentrations and assessed one week later. As control a commercial non-selective contact herbicide “Finalsan” with pelargonic acid as active substance was used. Most of the 94 tested extracts/compounds had no effect on the germination ability of the seeds of salad, and therefore cannot be used as a biological herbicide. Only with the extract TOFA and the compound dehydroabietyl amine a sufficient herbicidal activity could be observed. Evaluations regarding acute and ecotoxicity still have to be done for these. Dehydroabietyl amine is commercially available (Sigma Aldrich) and therefore a material safety data sheet (MSDS) exists. Unfortunately no data for acute toxicity or ecotoxicity are given in this MSDS. It is only known that dehydroabietyl amine causes skin irritation, serious eye irritation and may cause respiratory irritation. It is classified under Regulation 1272/2008 with skin irritation category 2, eye irritation category 2 and Specific target organ toxicity – single exposure category 3. For TOFA we do not know anything about the toxicity either regarding acute, or ecotoxicology. Therefore studies concerning the impact on humans and the environment should be done before thinking about a further development of TOFA and dehydroabietyl amine as biological herbicides, since these data are essential for the registration of Plant Protection Products.

3.3.4. Protection of wood and fibre products

In the present work, selected ForestSpeCs bioactive compounds and extracts were tested for their antifungal efficiency to prevent the growth of the several mould fungi and a blue-stain fungus causing damage in wooden material. First, the ability of bioactive products to prevent the mycelial growth of selected mould fungi and one blue stain fungus (Aureobasidium pullulans) was determined by screening tests carried out on Petri dishes. The mould test was performed by applying small amount of bioactive material (dipping, brushing) on filter paper samples. After the treatments the samples were placed on malt agar plate and were treated with a mixed spore/hyphal suspension of different mould fungi. The mould growth was examined visually using the mould index (0–5). The total incubation time was 5 weeks. The resistance against blue stain fungus Aureobasidium pullulans was studied using the method mentioned above.

Antifungal testing of selected compounds and extracts was performed using the principles of the standard EN 15457 with a fungal mixture containing six different fungi, including the mould fungi Aspergillus niger, Penicillium funiculosum, Chaetomium globosum, Paecilomyces variotii, Trichoderma viride, and the blue-stain fungus Aureobasidium pullulans.

Unfortunately the antifungal activity of bark extracts and betulin derivatives was poor in the filter paper tests as compared with the IPBC fungicide (3-iodoprop-2-ynyl-N-butylcarbamate). Only very few of the test compounds showed any activity. Because of these results in the initial screening, there is no reason to continue testing as originally planned in the DoW with planting pots made from recycled paper. Without doubt the results would not be significantly different. As a conclusion it seems to be difficult to find natural or semisynthetic, environmentally benign and feasible products to replace the fungicides, which are marketed today and accepted against mould growth.

3.3.5. Formulation and application technology for plant protection

Trifolio-M investigated formulation technology in ForestSpeCs using Resin Acid Mixture (RAM) FOR90S as the active ingredient. In total about 25 different formulations were developed. The amount of FOR90S, which is composed mainly of tricyclic diterpene carboxylic acids, varied between 12.5% and 25% in the
formulations. Previous tests at FIBL showed that the concentrations of RAM have to be between 0.1% and 0.5% in the spraying solution to control e.g. the infestation of downy mildew in grapes. Therefore the application rate of the formulations was around 20 g per liter of spraying solution.

The developed formulations should meet the following criteria:

- Formation of a stable suspension in water
- Manageability of the formulation should be given
- Actives should be protected against UV-radiation
- Rainfastness should be given
- Storage stability should be preferably about two years at room temperature

To achieve these goals different solvents, emulsifiers and stabilizing agents were used to formulate FOR90S. Trifolio-M has already developed different test designs in former projects to investigate the UV stability and the rainfastness both in vitro and in vivo. Trifolio-M also has developed a test design for the evaluation of fungicidal activity of test substances against different phytopathogenic fungi (P. infestans, B. cinerea, etc.) on potted tomato plants.

Figure x. Efficacy of formulations of FOR90S against P. infestans on potted tomato plants

Thirteen different formulations containing the RAM (FOR90S) were tested for the efficacy against P. infestans on young tomato plants. The best efficacies were achieved with the formulations EC7b and EC10 (Fig. x). These formulations showed a moderate to good efficacy of about 80% against P. infestans on potted tomato plants.

A problem which occurred during the tests was phytotoxicity. Some of the formulations led to strong damage of the tomato leaves even after one application. Therefore the concentration of FOR90S cannot be raised, since the phytotoxicity was not due to the formulation additives. Blank formulations without the active ingredient did not show any phytotoxicity. Therefore improvements in the formulation concerning efficacy will have to be made through the use of further adjuvants.

Some of the formulations were also tested in field trials against P. viticola on grapes. Therefore 2-3 kg of the formulation was prepared and sent to the project partner FIBL, who performed the tests. The results are promising as indicated in the section 3.3.2 (above). It is worth noting that it was no problem to produce larger amounts of the formulations.

3.4. Applications for environmental remediation

3.4.1. Bioremediation and biotechnology

Four different experiments by three ForestSpeCs partners (UNIS, UHEL, FEFRI) were conducted to study the properties of bark based composts as detoxifying materials for heavy metal contaminated soils. Three of them were carried out with potted plants or seedlings in the greenhouse, with specific heavy metals added to the growth substrate; the fourth study utilized toxic waste from tin-ore mining. Wheat, ryegrass, white clover, meadow fescue grass, and seedlings of the trees Larix amurensis, Ulmus parvifolia, Salix caprea and Salix rorida were used in these assessments.

A study was conducted to evaluate the ability of the four composts produced in ForestSpeCs and their humic substances to bind to Pb, Zn, Cu, Mn, Ni and Cd. The hypothesis was that the composts and/or humics can bind to heavy metals, therefore can be used in detoxifying environments polluted by heavy metals. Different composts have been demonstrated earlier to immobilize heavy metals, however, composts prepared from birch and spruce bark have not been evaluated. The four composts B, BT, P and
PT and their humics successfully immobilized the elements that were studied. The composts immobilized more Pb than the other elements, whereas the humics immobilized more Zn followed by Ni. It can be concluded from this experiment that the four composts can be used as detoxifiers of environment polluted by heavy metals.

The heavy metal levels in our second experiment was not high enough to be phytotoxic to the tested plant species, but we nevertheless could clearly demonstrate that the composts based on birch and spruce bark improved plant growth in contaminated soils, and that the composts and the humic substances which they contain immobilize harmful heavy metals. The addition of Trichoderma into the composts enhanced these effects, and will be a useful technique in bioremediation of soils contaminated with heavy metals.

The experiment conducted with actual toxic waste from the mining industry demonstrated convincingly that using mixed bark compost prepared from timber processing waste, and amended with Trichoderma, appears to provide a successful solution of the problem of negative effects of tin-ore processing toxic wastes on the surrounding environment, and to increase the efficiency of re-cultivation and landscaping. The beneficial effect of bark compost on the soil structure and on plant growth and development on toxic soil was shown, and should be further developed and applied for re-vegetation of such soils.

As overall conclusion from these experiments we can state that composts based on birch and spruce bark will improve plant growth in contaminated soils, and that the composts and the humic substances which they contain will immobilize harmful heavy metals. The addition of Trichoderma into the composts enhanced these effects, and will be a useful technique in bioremediation of soils contaminated with heavy metals. The potential of using bark compost, amended with Trichoderma, to help in restoration of damaged soils for example due to mining activity, was demonstrated in this project. The beneficial effect of bark compost on the soil structure, and on plant growth and development on toxic soil was shown, and should further be developed and applied in re-vegetation of such soils.

3.4.2. Soil conditioners and specialty fertilizers

Soil conditioning value of bark materials

Poor soils deficient of organic matter and nutrients affect the lives of hundreds of millions of people all over the world, for example in northern climates as well as in arid areas threatened by desertification. With the advance of climate change this becomes an increasingly serious issue in both cases: northern soils would become good agricultural land but often lack organic matter and nutrients, and desertification advances threatening even southern Europe within the next 50 years, further accelerating climate change. Stopping desertification and returning poor, arid lands to support vegetation to bind CO2 is of utmost importance to all of humanity. This process will need huge amounts of organic matter from renewable sources – waste materials such as bark from forest industry can play a key role in providing such inputs.

In arid conditions the capacity of the soils to retain water is a critical feature, often determining whether plants can survive on the soil or not. Soil water retention capacities on application of composts to soil in arid and semiarid areas are not well studied. In this laboratory study we have compared the soil water retention and soil water holding capacities for sandy soil mixed with four types of composts prepared from birch and spruce barks. The composts were prepared in two ways: One set of composts were prepared in the standard composting way and are denoted as birch (B) and spruce (P) whereas another set was composted with the addition of Trichoderma to give birch bark (BT) treated with Trichoderma and spruce bark (PT) treated with Trichoderma. The treatments contained 100% compost; 75% compost + 25% sand; 50% compost + 50% sand; 25% compost + 75% sand and 100% sandy soil. For the experiment the required sandy soil mixed with the relevant amount of compost was placed in plastic pots (the container
could hold 150 g of sandy soil or 50 g of compost) that had two small holes on the base covered by a filter paper. The plastic containers were soaked in water until saturated and left to stand for 30 minutes. The masses of wet containers containing 100% compost, 100% sandy soil and sandy soil mixed with the four types of compost were measured. The containers were left on the bench for 24 hours and masses measured. The masses of the containers were recorded each day for the next 14 days, and followed less frequently until day 38.

Adding bark composts to the sandy soil significantly increased the soil moisture content and its water holding capacity (Fig. x). The capacity to hold more water was retained for a long period of time (38 days in the study; Fig. x). Sandy soil mixed with P compost at 75% level had the highest moisture closely followed in the order by sandy soils mixed with B, PT and P compost at the same level over 38 days.

Figure x. Soil water content (%; left side graph) and water holding capacity (% water held; right side) of sandy soils mixed with four types of composts (B, BT, P and PT). S (0) refers to 100% sandy soil, 25 is 25% compost: 75% sandy soil, 50 is 50% compost: 50% sandy soil, 75 is 75% compost: 25% sandy soil and 100 is 100% compost.

Figure x. Soil water retention study over 38 days. Sand = sandy soil by itself; B = sandy soil mixed with birch bark compost at 25%, 50% and 75% of volume; P = sandy soil mixed with spruce bark compost; BT = sandy soil mixed with birch bark compost treated with Trichoderma; BP = sandy soil mixed with spruce bark compost treated with Trichoderma.

Nutrient value of bark materials as non-traditional organic fertilizers

Studies carried out at the University of Helsinki on the nutrient value of bark composts used two very different kinds of plants: the ryegrass, and white clover. We specifically wanted to investigate the utility of amending the compost at the start with a well-known beneficial fungus Trichoderma harzianum. This fungus is a plant growth and health promoting fungus, controlling also several soil-borne diseases and furthermore, used to decontaminate soils suffering from heavy metal or organic pollutants. Because the fungus grows particularly well on cellulose (wood), its use in composting wood waste such as bark is of particular interest. Four types of composted bark were produced. Shredded spruce bark (230 l) was mixed with 10 l of chicken manure, and either amended with 180 g of Trichoderma (strain T22, Trianum, Koppert) or not. After 4 weeks, additional 10 l of chicken manure was provided to the compost. The same was done with shredded birch bark. The composts were turned and mixed at the beginning once per week, later once per month, and water was added as needed. At the time of the experiment, the composting had lasted for 20 months.

Figure x. Overview of the experimental setup of the tests for nutrient value of composts.

Ten treatments, control (without any fertilizer), synthetic fertilizer, birch bark compost (BBC), BBC + Trichoderma, BBC + heavy metals, BBC + Trichoderma + heavy metals, spruce bark compost (SBC), SBC + Trichoderma, SBC + heavy metals, and SBC + Trichoderma + heavy metals, were added before sowing on the top five cm of 7.5 L pots filled with 5 kg of fine sandy soil on basis of dry matter. Half kg DM of birch and spruce treatments was added to each pot. Germination, macro and micro elements in the substrate, nutrient uptake and plant biomass, and the total N and C contents were analysed for each pot.
Figure x. Accumulated plant biomass (per pot) of ryegrass (left side graph) and white clover (right side) grown with different treatments of birch and spruce composts.

From the performed analyses it can be concluded that birch and spruce composts have highly interesting characteristics and clearly are suitable for use as a nutrient source for crops. They increase plant productivity (see Fig. x), and amendment of the composts with Trichoderma appears to give exciting opportunities to tailor composts for specific uses. In terms of key nutrient uptake/availability for the plants it may be of great significance that at least under some conditions, phosphorus (often the key limiting nutrient) uptake was enhanced in the presence of Trichoderma.

3.4.3. Large scale production of bark based composts as an alternative to peat-based substrates in horticulture

Forest soils are diverse and their cultivation for any purpose needs to be carried out only after detailed analysis of soil conditions, which define the choice of species and appropriate technology to maximally utilise their potential fertility. In farming and forestry the arable layer soil is considered favourable if soil volume weight, or density, does not exceed 1.2 g/cm³, and the total porosity totals 50-70%. Peat applied to soil does not provide the necessary level of mineral nutrition, as pure peat is poor with mobile nutrients. Wood waste (bark, sawdust, shavings, and chips) is regarded not only as sorbent-filling compound, but also as a carrier of certain nutritive value, biologically active soil ameliorant, and humus-former. An important advantage of wood bark is practical sterility from pathogenic microorganisms and weed seeds. A disadvantage of wood bark is its low nitrogen content (not more than 0.3-0.4% by a.d.m.) and very wide C:N ratio (120-200). An appropriate composting process of wood bark reduces these disadvantages.

Application of bark composts to soil increases its carrying capacity and counteracts mechanical deformations. Wood bark contains all main nutrients (calcium, magnesium, significant amounts of phosphorous, potassium, manganese), which become available to plants during the process of bark decomposition. Compost has also an effect on pH-value of soil; this effect is positive for plants growing in pH-neutral soils, but can be a drawback to use compost for acidic plants like blueberries. A very important advantage of bark compost is its disease suppression efficacy, which is already known for a long time.

The most widespread and reasonable way of wood waste processing for fertilizers is composting it under natural field conditions, or in special commercial plants. Based on centuries-old agricultural experience and comparatively low capital costs, this method possesses entire advantages over application of fresh materials to soil, as it doesn’t cause absorption (immobilization) of nitrogen by microorganisms, or toxic effects on plants and soil microflora. The effect of organic fertilizers on soil fertility and crop capacity of fruit-berry plants using bark-manure composts (rate of 100-150 t/ha) was shown to last for 6 years.

Wood bark and chicken manure represent mutually complementary raw material pair for preparation of bark-manure fertilizers (BMF). Wood bark is poor with nitrogen but contains a lot of easily decomposable organic substances and possesses good hydrophysical and sorption properties. Chicken manure is characterized with high content of nitrogen (up to 4-6%, mainly up to 60-65% in water soluble form),
phosphorous (up to 2%), potassium (up to 1%), calcium (2-2.5%), and other elements in easily digestible forms, however, its application is complicated with unfavourable physical-mechanical properties, high moisture content, danger of infection and weed seed distribution, and putrid smell. Negative qualities of chicken manure are significantly decreased during the process of composting with bark. It was shown in ForestSpeCs trials that use of chopped materials, thorough mixing with manure and optimal (calculated) ratio of components provide rapid biothermal composting at above zero air temperatures, and the use of thoroughly stirred mixtures allows reducing composting time to 1 month. During the period at high temperatures (up to 55–65°C), all necessary processes for obtaining the final fertilizer proceed: fixing of nutrients, decomposition of the bulk of soft compounds of the mixture, oppression of pathogenic microorganisms from chicken manure, and suppression of weed seed germinating ability. Reduction of composting time significantly decreases nitrogen losses and decrease of composting mass.

Commercial production of BMF should proceed from unbiased agrochemical characteristics of raw materials: bark–moisture content, ammonia absorption capacity, manure–moisture content, content of total and mobile nitrogen. These initial data are used for calculation of reasonable proportions of the components. An important condition of the technology is thorough and even mixing of bark and manure. Bark-manure fertilizers are recommended to be used on newly cultivated and old-arable soils of various mechanical composition and fertility levels. Under their impact hydro physical soil properties are improved, its biological activity rises, and content of humus and nutrients increases. These fertilizers beneficially affect soil and fruit-berry cultures yield during 5-7 years. Based on the results of vegetation experiments, BMF are able to facilitate the growing of these cultures without the use of mineral fertilizers.

Small fruit production in Russia and Europe has expanded substantially in the past years and demand for fresh locally grown fruit has increased. The cultivation of bush fruits could significantly contribute to the diversification and sustainability of the rural economy. Application of bark-manure compost is an important measure for increasing soil fertility, especially in forest nurseries and plantations for cultivation of fruit-berry plants such as rowan, viburnum, hawthorn, serviceberry, black currant, and especially sea buckthorn and rose hips. In the ForestSpeCs project, recommendations and instructions for creating plantations for a range of fruit-berry plants (16 species) are provided, based on the application of bark composts.

3.5. Innovation and feasibility
3.5.1. Feasibility of developed conversion technologies

Economic evaluation of primary process solutions for separation of bioactive compounds
The production of betulin from birch bark is taken as a case study for economic feasibility. In the production of betulin the main part of the operating costs come from losses of extraction solvent acetone, which is lost with the solid bark residue. Some additional losses of acetone occur in the evaporation procedure. Also some additional operating costs come from crystallisation of crude betulin extract (purity 62%) with 2-propanol-water azeotrope to yield high quality betulin (purity 96%). The total operating costs, calculated based on laboratory experiments, for production of 1000 kg of betulin with 96% purity are 1300 € (13 €/kg). Labour and investments costs are not included in this figure.

The extraction and purification costs for specific bark compounds from other bark species are likely to be roughly similar to that of betulin extraction per ton of bark. The decisive factor for the economics is the amount of the target substance in the bark: while pure betulin yield is around 12% of the bark dry weight, the yield for most other compounds of interest is around 1% (0.5-2.0%) of bark dry weight. This means
that the operation costs per kg of these compounds will be roughly around 100-150€.

Economic evaluation of primary modification techniques, especially betulin oxidation, suberin hydrolysis, and separation of humic substances

Betulin oxidation to produce betulonic and betulinic acids has proven to be demanding, and no practical and safe production method has been found. At the laboratory scale the oxidation of betulin to betulonic acid can be achieved with K2Cr2O7, but its toxicity and carcinogenic effects prevent its large-scale use. Very rough operating cost estimation for manufacturing of betulonic acid in this way is 890 €/kg. Because of the highly toxic oxidation reagent, this method is not suitable for large scale manufacturing of oxidized betulin derivatives.

In the case of hydrolysis of birch bark to produce suberin fatty acid Na-salts, and suberin fatty acids, most of the operating costs come from loses of the organic solvents 2-propanol and diethyl ether. Operating cost estimation for production of suberin fatty acid as Na-salts are 14 €/kg, and for suberin fatty acids 25 €/kg.

In the case of production of humic acids from peat, organic solvents are not required. The only needed reagents are NaOH and HCl, and estimated operating costs are as low as 0,5 €/kg making these substances highly attractive as industrial raw materials.

3.5.2. Feasibility of developed materials and/or products

The most promising new uses for extractives and compounds obtained and identified in ForestSpeCs are:

Health:
• Possible hepatoprotective effect in rats with suberin
• Anti-inflammatory efficacy of peat distillates and nasal spray administration for humidification of tissues
• Pine knotwood extract decreased erythema and oedema
• Several interesting extracts with antibacterial/antifungal activities
• Interesting abietic acid and dehydroabietic acid derivatives eg. dehydroabietyl amine

Cosmetics:
• Some betulin cinnamates appear excellent for sunscreens/UV protection

Specialty chemicals:
• Suberin sulphate esters for surfactant applications
• Suberin maleates for binder applications
• Suberin methoxy methyl esters for lubricant and plasticizer applications
• Humic acids as chelating agents for metals such as Ca2+, Cu2+ and Cd2+
• Humic acids as adhesives in phenol-formaldehyde type resins

Plant protection products:
• Larixyl acetate, lariciresinol acetate, lariciresinol, 15-hydroxydehydroabietic acid showed good activity as a fungicide against grapevine downy mildew
• Benzoyl dehydroabietylamide showed good fungicidal activity
• Picea abies D, Populus tremula E30, Pinus sylvestris EA, 7β,15-dihydroxydehydro-abietic acid, 15-hydroxy-7-oxodehydroabietic acid, abietic acid, Larix gmelinii essential oil 10%, and betulonic acid as antifeedants against several insect species
• dehydroabietyl amine appears as a promising compound for herbicidal applications

Two of the most potential options for commercial exploitation for the moment appear to be
Humaric acids in phenol-formaldehyde resins

One of the main uses for phenol is phenol-formaldehyde resins (PF), which include synthetic thermosetting resins such as obtained by the reaction of phenols with formaldehyde. Phenolic resins are mainly used in the production of circuit boards, moulded products, and as coatings and adhesives. Base-catalysed phenol-formaldehyde resins are called resols. Resoles are major polymeric resin materials widely used for gluing and bonding building materials. Exterior plywood, oriented strand boards, engineered laminated composite lumber are some of their typical applications.

Even though phenol is used in huge quantities, it has several drawbacks such as its toxic and harmful properties to human health. This complicates the usage of phenol and in addition phenol (and formaldehyde) as such can be present in residual quantities in phenol-formaldehyde resin products. Thus there is demand to replace this chemical with more environmentally benign alternatives. Global production of phenol is almost exclusively based on the cumene-based acetone co-product process (98% in 2005) and produced ~8.25 M tonnes in 2004 of which 30.6% went for use as phenolic resins. Phenol price in 2011 was approx. 1100-1150 €/ton. Operating costs (labour or investments costs are not included) for production of humic acids were calculated to be 464 €/ton. Adhesive tests on veneer and paper indicate that humic acid content can be as high as ca. ~44 % of all solidifiable/ polymerisable matter (HA, phenol, formaldehyde, and NaOH) to still possess adhesive strength. Thus partial replacement of phenol with humic acids in phenol-formaldehyde resins could be economically feasible and the market potential for this is huge.

Bark-based substances as fungicides in agriculture

In the screening work within ForestSpeCs we found at least five candidate substances or extracts that have the potential to be further developed into effective fungicidal products for use against plant diseases in agriculture. Particularly in organic agriculture these products have the potential to replace at least part of the use of copper based fungicides. The main uses of copper in organic farming systems in Europe currently include grapevine, pome and stone fruit, olive, potato, and some vegetable crops. In the EU/EFTA countries, where copper use is still allowed (i.e. all countries except for Netherlands, Denmark, Norway, and Sweden) the total areas in organic farming systems grown in 2009 with (i) pome and stone fruit were: 60'403 ha, (ii) grapevine 155'413 ha, and (iii) olive 845'874 ha. The annual copper use depends on crop/disease complex, pedo-climatic conditions, and the legal framework. Overall it is estimated that fungicidal products to replace copper in organic and low input farming systems may be applied on approximately 400'000 ha annually. Cost of product per application and hectare may vary between 20 Euro and 200 Euro. In addition, effective copper replacement will also be used to some degree in conventional agriculture, with huge market potential.

3.5.3. Feasibility via integrated economies

The ForestSpeCs project as part of the FP7-KBBE programme was designed to incorporate at least two Russian partners from different regions of Russia (instrument: CP-SICA); we in fact had three partners from three regions: SPSMA, NRIF and FEFRI. One aim from such arrangement has been to integrate EU and Russian research groups around common interests, but also, in the end, to facilitate a deeper economic integration via enhancing joint ventures and other economic activity within the specific areas of
research. This section examines some of the possibilities and constraints to such deeper integration within the topic areas of ForestSpeCs.

The Russian forest industry could serve as a source of bark for the applications identified in ForestSpeCs – either for processing and upgrading within Russia, or for exporting to other countries, e.g. within joint venture companies. Considering that about 15% of the tree volume is on the average bark, Russia’s 150-200 million m³ annual harvest of roundwood represents a huge volume of potentially valuable bark resource for extraction of bioactive or specialty chemical components, and/or for production of growth substrate, as explored in ForestSpeCs. Of particular interest is the species composition of Russia’s forest resources, with some 35% of larch, followed by pine (16%), birch (13%) and spruce (11%). Thus the potential for commercial exploitation by Russian companies and/or European companies of these resources clearly exists (see ForestSpeCs Deliverables 6.4 6.5 and 6.6) but we are not aware of any planned joint ventures or other forms of economic collaboration in these areas. Intense developments, on the other hand, have taken place in exploiting the traditional forest resources in the form of timber, pulp and paper production.

Overall, several forms of economic integration opportunities would appear to rise from the ForestSpeCs project between Russian and European entrepreneurs, including FDI and joint venture activities e.g. in the area of raw materials sourcing, processing, cosmetics, speciality chemicals production, health care processes, equipment and products, plant protection products, and in production and marketing of growth substrates and remediation materials.

In order to develop these innovations further, and to exploit their economic potential, the corresponding IPR-owners, investors, and interested business parties have to find each other and to seriously tackle the next steps in the creation of such new business and value chains based on upgrading forestry byproducts. The ForestSpeCs consortium is eager to pursue some of the leads, and hopes that most if not all of the project findings will be able to materialize the potential which we have shown to be inherent in them.

Potential Impact:

4. The potential impact

In section 4.1. of the report we wish to refer directly to the approved DoW of ForestSpeCs, at the beginning of the project. In this section, the normal text is directly copied from the ForestSpeCs DoW, while our comments to achieving the stated, expected impacts, are highlighted in yellow.

4.1 Expected impact

The work programme lists as expected impacts from the studies funded by this call:
“Contribution to enlarging the economic potential of the forest-related industry and better understanding of the potential of humic substances”

In ForestSpeCs we have identified and demonstrated numerous ways of upgrading the bark waste fraction into highly valuable products, which have the potential to substantially enlarge the economic potential of forest-related industry. Similarly, the economic potential of humic substances in several important, identified areas of application as specialty chemicals, is huge.

4.1.1 Contribution of FORESTSPECS towards the expected impacts

The FORESTSPECS project addresses the issues defined in the 7th Framework Programme, and those included in the European Forest-Based Sector Technology Platform’s “2030 vision” by providing research
based biological and technical understanding and solutions needed for upgrading wood related residues and humic substances to value-added chemicals and materials. Only through such knowledge-based approach it is possible to develop innovative applications to the potentially huge variety of bioactive substances available in the wood-related wastes and humic substances, which currently are largely converted into energy by burning. These substances, however, represent rich sources of aromatic and other complex structures whose upgrading to value-added speciality chemicals and materials will provide an opportunity to the sector to create new types of forest-based value chains. Innovative application opportunities for such products include for example medical, pharmaceutical, agricultural, and environmental remediation uses.

The project is entirely consistent with the overall objectives and expected outcomes of the EC’s 7th Framework Programme on “Knowledge-Based Bio-Economy, KBBE” to exploit new and emerging research opportunities for addressing environmental and economical challenges, and the growing demand for sustainable use of renewable resources. The project will fully contribute to the objectives of area 2.3.3. Environmental biotechnologies, Use of waste and by-products, which emphasizes the potential of biotechnology to prevent, treat and remove pollution, and calls for maximising the economic value of waste and by-products through new, innovative processes. FORESTSpeCS project makes use of biotechnology and other suitable techniques to convert side products (waste) of the forest-based industries into value added products and materials that can be used among other applications to treat and remove pollution (bioremediation of contaminated soils).

The European Forest-Based Sector Technology Platform in its “2030 vision” clearly identifies its role in the society and the need for adjustments. The forest-based sector comprises a competitive, knowledge-based industry that fosters the extended use of renewable forest resources and plays a key role in a sustainable society. It strives to ensure its societal contribution in the context of a bio-based, consumer-driven and globally competitive European economy, while meeting the multifunctional demands on forest resources and their sustainable management. The industry, represented by the technology platform, realizes that the forest industry has a challenging period of adjustment ahead of it, and that the industry has no other alternative than to keep optimising its activity and to search for solutions from new products in order to improve profitability.

The forest-based sector needs to be innovative, which requires strong links between science and the sector. Some of the ideas put forth by the platform as partial solutions to the problems by 2030 include:

- Deepening the sector's scientific basis, including taking advantage of emerging sciences
- Development of innovative products
- Focus on specialities and new business

ForestSpeCs has achieved this in several innovative applications

- Innovative and sustainable use of forest resources

ForestSpeCs results may significantly contribute to achieving this

- Enhancing availability and use of forest biomass for products and energy ForestSpeCs results may significantly contribute to achieving this, and furthermore, we maintain that it is not wise to use bark biomass for energy because of the low added value, and because even after extraction of the most valuable compounds, the remaining biomass is best used for the production of growth substrates and materials for soil conditioning (e.g. bioremediation)
• Improved cooperation between EU countries and Russia

ForestSpeCs has demonstrated the clear potential for such scientific and economic co-operation; however, practical realities of operating joint ventures and constraints to and difficulties in FDI investments hamper the materialization efforts.

• Advance technology for the Western European and Russian forest industry

ForestSpeCs has contributed to the technology of dealing with the side-streams of forest industries (bark, possibly other side-products)

• Participate in supply chains from cost competitive regions

Russia likely would present a cost-competitive source of bark-based products such as those identified in ForestSpeCs, either as supplier of raw materials, or of upgraded products such as extracts or final products.

When we compare the expected impacts as envisaged by the work programme of the call, as well as the defined needs and visions for solutions as expressed by the Forest-Based Sector Technology Platform, with the work plan and expected results of the FORESTSPECS project, it becomes clear that this project expects to make a significant scientific and practical contribution towards reaching the stated goals (and occasionally, beyond them). The expected results from the project include:

• innovative new ways to utilize wood residues and peat to produce speciality chemicals and polymers, complementing or replacing some current products and materials based on oil

Demonstrated in ForestSpeCs

• value-added new options for total usage of wood bark and peat

Demonstrated in ForestSpeCs

• novel transformations for production of

  o derivatives of wood residue-based compounds (e.g. triterpenoids) to be used as pharmaceuticals, additives in cosmetics, and biological plant protection agents

  o poly- and oligoesters from suberin to be used as binders for coatings, adhesives and composites and as plasticizers, surfactants and lubricants

  o humic acid derivatives to be used as chelating agents in water purification and in mining and as wood adhesives

All demonstrated in ForestSpeCs

• new bioactive fractions, materials and compounds for high value-added applications e.g. in medicine, cosmetics, and plant protection

All demonstrated in ForestSpeCs

• improved profitability of the forest-based sector, including companies, forest owners, and local economies

Remains to be seen, but potential demonstrated in ForestSpeCs

• improved export possibilities for European based SMEs and other companies utilizing the improved processes, materials and speciality chemicals

Remains to be seen, but potential demonstrated in ForestSpeCs

• decreased risks of environmental contamination via applications based on natural substances and improved technical/biotechnical processes (e.g. in plant protection, remediation)

Significant potential for widespread use, applications, and impacts demonstrated in ForestSpeCs

• improved sustainability of agriculture, horticulture and forestry via replacement of products and processes using non-renewable resources by rapidly renewable materials (e.g. plastic or peat based potting materials used in horticulture)
Significant potential for widespread use, applications, and impacts demonstrated in ForestSpeCs
- creating the knowledge, techniques and materials for the reversal of processes contributing to
desertification and climate change (improved biomass production in currently unproductive areas,
‘greening of the desert’, etc.)

Significant potential for widespread use, applications, and impacts demonstrated in ForestSpeCs
We can identify that the expected impacts from the FORESTSPeCS project affect different levels, and
many different segments of the society, either directly or indirectly. These impacts can be seen at the
micro-level, meso-level, and at the macro-level.

Micro-level
Impacts at the micro-level affect directly at least the following:
- the forest-based industries (as those who can add value to their existing materials/waste)

This was demonstrated as an example – out of the many possibilities identified in ForestSpeCs – for the
production of Larix-bark extract based fungicide (‘Larixyne’): over 1000 Central-European sawmills
handling Larix could benefit from this development, and of course also the forest owners with larch forests
[Further details provided in ForestSpeCs Deliverable 6.5]
- companies benefiting as users of the new value-added raw materials (medical, pharma, etc.)
For example, in the case of Larixyne: the SME company producing Larixyne will gain significant
advantage, and farmers will be more competitive and will be able to produce higher quality (priced)
products (e.g. organic wines), and will need to deal less with synthetic chemical pesticides (health and
environmental benefits to growers). Similar benefits can be foreseen to be associated with the other
ForestSpeCs identified value-added materials.
- commerce

Meso-level
Impacts at the higher level include tangible benefits to the:
- local communities via improved economic activity
- consumers buying and using safer, improved, higher-quality products (e.g. medicines, cosmetics, food)
- improved agricultural and forest productivity in areas with poor soils
These all follow from improved local economies, from the replacement of hazardous pesticides or other
products by those identified and tested in ForestSpeCs, and from the adoption of composts and
remediation materials as pioneered in ForestSpeCs

Macro-level
Macro-level benefits from the project can be expected in the long run:
- EU general competitiveness improved; export opportunities (e.g. to regions already suffering from
desertification)
- increased ecological/environmental sustainability: replacement of non-renewable raw materials by
rapidly renewable resources
- long-term benefits to humanity via development of technology (based on rapidly renewable raw
materials), which is essential for limiting desertification and combating climate change

Increased sustainability and long-term benefits to humanity are realistic and logical consequences of
replacing fossil-based or slowly renewable raw materials (e.g. oil, peat) by rapidly renewable organic
matter, and from substituting toxic or carcinogenic substances in industrial as well as in agricultural processes by benign substances based on bark extracts or humic substances. Furthermore, increasing productivity on poor soils and limiting desertification are urgently needed to bind CO2 and to ameliorate the impacts of changing climate. The basic technologies to achieve these have been explored and demonstrated in ForestSpeCs.

4.1.2. Steps needed to bring about these impacts
The prerequisite to obtaining these impacts of course is that the FORESTSPECS project gets fully funded, and is thus able to produce the initial outcomes envisaged. It is also clear that during the lifetime of the project only the potential of the approach can be established (and part of the knowledge-base), and some of the most promising leads can be followed to a stage, where commercialisation of the results is possible.

One example: the core ForestSpeCs partners have submitted a proposal (‘ProLarix’) to the EU, call KBBE.2013.1.4-07: Boosting the translation of FP projects’ results into innovative applications in the field of agriculture, forestry, fisheries and aquaculture, in order to take one of the most promising leads fully into commercialization. Without this transitional step, likely not even that result will lead into practical use of the ForestSpeCs outcomes. It will then require further R&D by the companies interested to develop such products to realise in practise the economic benefits which can be obtained from it. In order to fully exploit the opportunities offered by the approaches taken here, a long term R&D commitment and support is required from not only the industry itself, but also from the public bodies and organizations concerned about the meso- and macro-level processes and impacts (listed in the above section). With the involvement of only the commercial companies in the development process, the impacts are likely to be smaller, and restricted mainly to the micro-level benefits.

The visions expressed at the time of application for ForestSpeCs are still perfectly valid, and likely even more acute than we could envisage.

4.2. Selected case studies for potential impact
4.2.1. Larix extracts for plant protection
In the ForestSpecs project, several Larix bark compounds were found to have good activity against Plasmopara viticola (downy mildew) on grapevine under controlled conditions, outperforming and/or equalling the activity to the copper reference compound used. The structures of these compounds have been determined. These compounds are present in moderate amounts from the dichloromethane extract (1% and 0.5% respectively, but these yields have not yet been optimised.

A conservative estimate of the market potential of Larixyne indicates that (i) Larixyne will have a market size in European agriculture of about 68 M Euro per annum (total sales), and (ii) a substantial amount of the available larch bark from the forest industry can be used to produce a high-value product. An estimate of the market potential of Larixyne indicates that 428 tonnes of a.i. will be used in agriculture within a few years after product registration. This corresponds to approximately 13% of the copper usage in 2003 and is thus considered a realistic estimate. The quantities of raw material needed to produce this amount of Larixyne will depend on the concentration in the raw material and the extraction efficiency. We have used a conservative estimate and assume that 1% a.i. is available in the bark, and will be extracted. If bark turns out to be the best raw material, then 52000 tonnes of bark will be needed for extraction. This corresponds to the total amount of bark that is currently produced in the DE, AT and CH larch industry. However, other sources (e.g. branches, saw dust, resin-pockets in larch wood) may also prove to be additional excellent sources for extraction. Furthermore, sources from Scandinavia and/or Russia may also be tapped in the future stages to increase the supply of raw material. Worldwide, the amount of Larix bark is virtually
unlimited.

Larixyne will make an ideal showcase for the novel registration process of botanicals. We believe that Larixyne is an ideal candidate as the active compounds are present in the European environment already in vast amounts, and since there is a long history of use of Larix products in households and industry. The registration process of Larixyne will serve as a case study, and it will open the avenue for further renewable, plant derived plant protection products in the EU, thus facilitating business opportunities for the plant protection industry and the supplier sector.

The detailed feasibility study of developing Larix-bark based bioactive compounds into botanical plant protection products for the control of fungal diseases is presented in Deliverable 6.5. and shows excellent potential of successful economic exploitation. The outlook is highly promising from many points of view, and the development is expected to provide an urgently needed alternative to traditional plant protection products. It also will significantly increase value to the forest-based production chain, and improve the economics of forestry, timber processing, and the associated SMEs, which was one of the main aims of the ForestSpeCs project.

4.2.2. Betulin compounds in cosmetics
Betulin and betulinic acid have already found some cosmetic use as hair care additives in shampoos for regulating hair loss, as well as in skin lotions and sunscreens against aging and wrinkling. Furthermore, betulinic acid can be used in sun creams for prevention of detrimental effects of the UV light. Betulin possesses significant anti-inflammatory activity, and betulinic acid induces programmed cell death (apoptosis) in human melanoma cells. We have found that other simple betulin derivatives e.g. betulonic acid may have even higher bioactivities. Aromatic esters of betulinic acid, such as benzyl betulinate, are also useful as photoaging inhibitors. Increased melanoma is a growing problem, but betulinic/betulonic acid applied e.g. in sunscreens can cause apoptosis and control of melanoma cells. A cosmetic preparation for sunscreens/ after sun would thus be highly beneficial for older people with skin cell damage. Betulin derivatives combined with antibacterial and UV-protection properties are targeted, and the potential impact of these new products in sunscreen application area is remarkable.

The main problem for any industry wishing to utilize betulin or its derivatives is that no industrial-scale production of these compounds exists, and that only small amounts of them are available. A specific project would be needed to solve that problem, and to initiate pilot production, which then could serve as a supply of high-quality betulin products. ForestSpeCs SME partner Granula Ltd. has suitable products where betulin compounds can immediately be used, and they have extensive experience with betulin via earlier research projects. The specific steps needed to facilitate the bringing to the market of new products, mainly for cosmetics applications, based on betulin and its derivatives will need to:

- Up-scale processing and extraction techniques from laboratory to pilot industrial scale
- Optimise extraction and oxidation techniques
- Produce new pilot products for cosmetics based on betulin and its derivatives
- Prepare regulatory dossiers and to perform studies required for them
- Test market the new products and to prepare an updated business plan for them
- Protect the accumulated IPR

Several of Granula’s existing products can be developed to contain betulin or betulin derivatives to offer multifunctionality. This would be a natural progression. Moreover, new dedicated products can be developed if sufficient amount of material can be produced. Also, products classified as medical device can be developed.
4.2.3. Pine and spruce knot-wood extracts as food additives
Lignans from knot wood extraction are very interesting due to their multi-functionality and the huge untapped sources for raw material. Besides antioxidant effects, anti-inflammatory, antimicrobial, wound healing, and anti-cancer effects have been found.
ForestSpeCs SME-partner Granula Ltd has patented many different wood extracts for use in cosmetics, food, feed and technical applications, and several university groups are working on the health benefits of lignans and their role in preventing different cancers (breast, prostate, etc). Despite of that, very few products are on the market. The main problem also here is that no actual production of these materials exists - only small amounts are available for commercial applications. That problem needs to be solved first in order to facilitate the uptake of these health-benefitting products by the market – based on forest extracts.

4.2.4. Rhaponticin for medical applications
Rhaponticin is a stilbene glucoside compound that is found naturally mainly in rhubarb rhizomes, and currently obtained from cultivation of medical rhubarb species such as Rheum undulatum, R. officinale, R. palmatum, and R. rhaponticum. However, in the ForestSpeCs project we have shown that rhaponticin occurs in significant amounts in the bark of the Norway spruce Picea abies, and we have developed a simple and efficient method of extraction for it This may be one of the most exciting outcomes of the ForestSpeCs-project.
Rhubarb rhizomes and roots have been known and used in traditional Chinese medicine for some 5000 years. About 2000 years ago it spread into the Middle East and Europe, where its use as a laxative has continued until the present time. Recently, many highly interesting medical applications for rhaponticin have been discovered, assuring an increasing interest in this natural molecule. A dry extract from the roots of rhapontic rhubarb (Rheum rhaponticum) [ERr] has been commercially available in Germany for over two decades to treat menopausal symptoms, and similar products are sold also in the USA. The extract consists mainly of rhaponticin (90%) and aglycones (5%) of rhaponticin and desoxyrhaponticin. Importantly, no adverse events have been observed in human applications of the ERr, although the synthetic estrogen diethylstilbestrol with a structural similarity of hydroxystilbenes exerts deleterious transplacental effects in humans.

Many other applications of rhaponticin in medicine and cosmetics are foreseen. Several recent studies show that rhaponticin has good antidiabetic effects and could be potentially used as a new agent to treat type 2 diabetes mellitus and its complications. Recent studies on the biological activities of rhaponticin, related hydroxystilbenes, and the metabolite of rhaponticin (rhapontigenin), have furthermore shown that these substances possess significant antiallergenic and antithrombotic activity. Additionally, a most exciting new potential application for rhaponticin is in the treatment of Alzheimer’s Disease. Other potential uses for rhaponticin, as reported in the literature, include applications for sun care (sun protection, after-sun & self-tanning), and skin care (facial care, facial cleansing, body care, baby care).
Commercially rhaponticin is available only as an extract of medicinal rhubarb, exclusively obtained from China. The extract contains significant amounts of other hydroxystilbenes; analytical grade of >99% rhaponticin is offered by several specialist suppliers at an extremely high price (e.g. Extrasynthese-company in France: 73 € for 10 mg of rhaponticin).
Several attempts at cultivating medicinal rhubarb have been made in Europe and in the USA, in order to
avoid problems associated with the supply from (and dependence on) China. These have not led to commercial activity as yet. Growing rhubarb for obtaining root extracts as a source of rhaponticin seems to be difficult and input intensive, and hence, limiting the potential applications of rhaponticin in medical and cosmetic sectors. Rhubarb root yields can only be harvested from the second year after establishment, and the best root quality is obtained only after 6 years. In addition, the crop requires management with pesticides (especially herbicides, but also insecticides) for proper yields, which interferes with product quality (residues) and with the image of „natural medicine“. Clearly, separation and purification of rhaponticin from rhubarb root extracts is a difficult and expensive process.

In the ForestSpeCs project we have shown that rhaponticin occurs in significant amounts in the bark of Picea abies, and we have developed a simple and efficient method of extraction for it. We believe that this very abundant natural source can be exploited for commercial production of high-quality rhaponticin, making it available for further development by pharmaceutical industry for the applications presented above – and possible further uses.

4.2.5. Peat distillate for medical applications
Peat and various peat preparations have been successfully used in peat therapy since historic times. Recently new types of peat therapy have been developed, and the use of peat in connection with the Finnish sauna has become especially popular. In the FORESTSPeCS project the anti-inflammatory effect of peat distillates was experimentally demonstrated in the model of adjuvant-induced arthritis, and in the carrageenan air pouch model in rats.

Taking in account long story of peat sauna and positive effect of peat distillates described in literature it is reasonable to develop medical device of peat distillate in spay for nose. The main purpose for nasal spray is to moisturize the nasal membrane, not to clean the nose. It provides water particles instead of water stream to moisturize the nasal membrane.

In summary nasal spray:
• The main purpose is to moisturize the nasal membrane, not to clean the nose.
• It should be convenient to carry on, to be used as often as needed.
• Recommend to use 3~6 times per day, even once per hour as instructed by medical professionals.
• The amount of water utilized is small.
• More acceptable than nasal wash without the scare of water choking.
• Is helpful for various rhinitis and sinusitis as instructed by medical professionals.
• Can be used for young children, even infants as instructed by medical professionals.
• Entering the nose in the form of water particles - by sprayer, moisturizer or nebulizer, etc.

Nasal spray with peat distillate could be used as Medical Device:
• to moisten dry nasal membranes
• as additional therapy in case of cold, rhinitis
• in case of difficulty in breathing through the nose
• clear debris from the nasal passageway, and improve mucous membrane function
• also frequently used after sinus or nasal surgery.

In order to commercialize results obtained in ForestSpeCs concerning the benefits of humic substances obtained by distillation of peat, specific objectives need to be addressed:
• Optimizing raw material supply and quality
• Chemical characterization and quality assurance of peat distillates for the medical devise
• Development of suitable formulation for the active substances
• Safety and efficacy needs to be assured
• Market acceptability needs to be assessed

ForestSpeCs Russian partner SPSMA is planning to develop this technology further, and to patent the relevant findings.

List of Websites:
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