"Non-food Crops-to-Industry schemes in EU27"

WP3. Bio-based products

D3.4 Other specialty products that can be produced in EU27

Lead beneficiary: NATIONAL INSTITUTE FOR CHEMICAL PHARMACEUTICAL R&D BUCHAREST, ROMANIA

Authors: Alice Grigire, Misu Moscovici

February 2012

The project is a Coordinated Action supported by

Grant agreement no. 227299

Table of Contents

Introduction

The general target of WP3

The main target of this WP3 is to explore the potential and feasibility of the European industry to manufacture high-value biobased products from renewable agriculture and forestry feedstocks and biotechnological routes. The work is divided in four tasks: Task 3.1 Oils, Task 3.2 Fibres, Task 3.3 Resins, Task 3.4 Pharmaceutical and other specialty products.

In this WP, the bio-industry demands in oils, carbohydrates, resins, pharmaceutical and other specialty products is reported and restricting factors that inhibit broader industrial use of the feedstocks are to be identified. Research gaps, prospects and recommendations to procure bio-based products are tackled.

Task 3.4 Other specialty products

Tasks in WP3 Bio-based products:

1. ***Review on the product yielding capacity from various industrial crops streams***

2. ***Identify desirable quality characteristics that feedstock has to meet for mature industrial processes***

3. ***The report on raw materials from non-food crops as alternative to fossil, petroleum-based and chemical resources***

4. ***Set prospects to widen the range of potential feedstocks for the understudy industrial uses, based on the technology improvements***

5. ***Identify restricting factors that inhibit broader industrial use of the biomass feedstocks (supply, costs, physical traits, consistency in quality, technical performance, research gaps, etc)***

6. ***Set forth research gaps, prospects and recommendations to procure bio-based products will be tackled***

1 Plantago lanceolata L. (Plantaginaceae)

1.1 Review on the product yielding capacity from various industrial crops streams

According to Plant for Future Platform (1), the main uses for Plantago lanceolata are:

Table 1-1. Most common raw materials and products obtained from flax

Agriculture Raw Materials	**Directions of use**	**Product example**
Leaves Root Seeds	Phytotherapy	-remedy for bleeding, it quickly staunches blood flow and encourages the repair of damaged tissue -remedy for bite of rattlesnakes -treatment of parasitic worms, laxative
Leaves, seeds	Cosmetics	-beauty products (shampoo, soap, crème, lotions, etc)
Leaves Seed coats	Textile industry	Fibre Fabric stiffner
Leaves	Veterinary	Occasionally grown as fodder crop
Seeds	Other uses	Source of a low-cost gelling agent for tissue culture

1.2 Identify desirable quality characteristics that feedstock has to meet for mature industrial processes

Guidelines for pharmaceutical products:

Plantaginis lanceolatae folium has monograph in European and British Pharmacopoeia, ESCOP, German Commission E and French Avis aux fabr. monographs and draft report published on EMEA (Herbal Medicinal Products committee assessment)

Plant material of interest for pharmaceutical purposes: whole or fragmented, dried leaf and scape of *Plantago lanceolata* L

Herbal substance (equivalent to the term "herbal drug" as defined in the European Pharmacopoeia)

The product is usually used in dried form but sometimes fresh.

- Definition: whole or fragmented, dried leaf and scape of *Plantago lanceolata* L. It is also important to know the geographical source(s) and the conditions under which the herbal substance is obtained.

- Characters: a qualitative statement about the organoleptic character(s) where characteristic and the macroscopic and microscopic botanical characters of the herbal substance.

Microscopic characteristics

The leaf is up to 30 cm long and 4 cm wide, yellowish-green to brownish-green, with a prominent, whitish-green, almost parallel venation on the abaxial surface. It consists of a lanceolate lamina narrowing at the base into a channeled petiole. The margin is indistinctly dentate and often undulate. It has 3, 5 or 7 primary veins, nearly equal in length and running almost parallel. Hairs may be almost absent, sparsely scattered or sometimes abundant, especially on the lower surface and over the veins. The scape is brownish-green, longer than the leaves, 3-4 mm in diameter and is deeply grooved longitudinally, with 5-7 conspicuous ribs. The surface is usually covered with fine hairs. (2)

Powdered plant material

The powder is yellowish-green and shows the following diagnostic characters: fragments of epidermis, composed of cells with irregularly sinuous anticlinal walls, the fragments from the scape with thickened outer walls and a coarsely ridged cuticle; stomata mostly of the diacytic type and sometimes anomocytic; the multicellular, uniseriate, conical covering trichomes are highly characteristic, with a basal cell larger than the other epidermal cells followed by a short cell supporting 2 or more elongated cells with the lumen narrow and variable, occluded at intervals corresponding to slight swellings in the trichome and giving a jointed appearance; the terminal cell has an acute apex and a filiform lumen; the glandular trichomes have a unicellular cylindrical stalk and a multicellular, elongated, conical head consisting of several rows of small cells and a single terminal cell; dense groups of lignified fibro-vascular tissue with narrow, spirally and annularly thickened vessels and slender, moderately thickened fibres. (*2*).

Other general identity tests: identification testing optimally should be able to discriminate between related species and/or potential adulterants/substitutes, which are likely to be present (for ex. presence of *Digitalis lanata* leaves) (2).

Purity tests:

Foreign organic matter - Maximum 5 per cent of leaves of different colour and maximum 2 per cent of other foreign matter (2)

Total ash - Maximum 14.0 per cent (2)

Loss on drying - Maximum 10.0 per cent, determined on 1.000 g of the powdered drug (355) (2.9.12) by drying in an oven at 105 ° C for 2 h (2).

Chemical assays

Minimum 1.5 per cent of total ortho-dihydroxycinnamic acid derivatives expressed as acteoside (dried drug) (2).

The main constituents:

Iridoidglycosides: The herbal substance contains about 2-3 % iridoidglycosides with aucubin and catalpol as the main compounds, as well as asperuloside, globularin and desacetylasperuloside-acid methylester. The iridoid content depends on the maturity of the leaves. Young leaves contain up to 9%, while in the older ones iridoids are present only in traces. In young leaves, catalpol is the dominant constituent, and in older leaves, aucubin is the major compound (3).

Mucilage: Other drug constituents include 2-6.5% mucilage. An arabinogalactan, a glucomannan and a rhamnogalacturonan with an arabinogalactan side-chain as well as a rhamnoarabinogalactan and a linear (1-6)-α-D-glucan have been isolated (3).

Flavonoids: Flavonoids include apigenin and luteolin as well as their derivatives with the main compounds apigenin-6,8-di-C-glucoside and luteolin-7-O-glucuronide, luteolin-7-O-glucoside and 7-O-glucuronide-3'-glucoside, in addition to the 7-O-glucuronyl-glycosides of apigenin and luteolin as well as apigenin-7-O-glucoside and 7-O-glucuronide (3).

Other constituents: The herbal substance also contains 6.5% tannins, phenolic carboxylic acids including p-hydroxybenzoic-, protocatechuic, gentisinic-, chlorogenic- and neochlorogenic acid, among others. The coumarin aesculetin, the xanthophyll decomposition product loliolide and small amounts of a hemolytic and antimicrobial saponin are also present, as well as volatile oil. Inorganic constituents include 1% silicilic acid and mineral salts with a high proportion of zinc and potassium (3).

Herbal preparation (equivalent to the term "herbal drug preparation" as defined in the European Pharmacopoeia) of ***Plantago lanceolata*** are obtained by subjecting vegetal material to treatments such as extraction, distillation, expression, fractionation, purification, concentration or fermentation. These preparations include comminuted or powdered herbal substances, tinctures, extracts.

Preparations are given in various dosage forms including tablets, liquids (in ethanol-water mixtures or other), capsules, and dried extracts (in tablets or capsules). Plantain extracts are used in combinations with many other herbal substances / herbal preparations.

According to EMEA (draft monograph), herbal preparation for traditional use are:

- Herbal substance, comminuted
- Herbal substance, powdered
- Dry extract (3-6:1); extraction solvent: water
- Liquid extract (1:0.8-1.2); extraction solvent: ethanol 20%-40% (V/V)
- Soft extract (1.5-1.7:1); extraction solvent ethanol 20% (m/m)
- Expressed juice (1:0.5-0.9) from the fresh herb
- Liquid extract (1:11); extraction solvent water

Herbal medicinal products (the term includes "traditional medicinal product")

The following tests and acceptance criteria are considered generally applicable to all herbal medicinal products:

-Description: A qualitative description of the dosage form should be provided (e.g., size, shape, colour). If colour changes occur during storage, a quantitative procedure may be appropriate.

-Identification: Identification tests should establish the specific identity of the herbal substance(s) and/or herbal preparation(s), in the herbal medicinal product and optimally should be discriminatory with regard to substitutes/adulterants (ex. *Digitalis lanata* leaves) that are likely to occur Identification solely by chromatographic retention time, for example, is not regarded as being specific; however, a combination of chromatographic tests (e.g. HPLC and TLC-densitometry) or a combination of tests into a single procedure, such as HPLC/UV-diode array, HPLC/MS, or GC/MS may be acceptable. In the case of herbal medicinal products containing powdered or comminuted herbal substances, microscopical and macroscopical characterisation could be used for identification in combination with other methods, if justified.

-Assay: In the case of products containing herbal substances and/or herbal preparations with constituents of known therapeutic activity, validated assays of the content of these constituents are required along with details of the analytical procedure(s). Where appropriate, a specific, stability-indicating procedure should be included to determine the content of the herbal substance(s) and/or herbal preparation(s) in the herbal medicinal product. In cases where use of a non-specific assay is justified, other supporting analytical procedures should be used to achieve overall specificity. In the case of herbal medicinal products containing herbal substance(s) and/or herbal preparation(s) where the constituents with known therapeutic activity are not known, validated assays of active or analytical markers or other justified determinations are required. The choice of such markers should be

justified. In cases where a specific assay of each active substance of a herbal medicinal product is not possible other justified determinations are required (for example, in multi-component traditional herbal medicinal products for human use the same markers may be present in more than one herbal substance/preparation).

-Impurities: Refer to the ICH/VICH Guidelines on impurities in new drug products/Guidelines on impurities in new veterinary products (CPMP/ICH/2738/99 and CVMP/VICH/838/99 as revised) and the European Pharmacopoeia General text on Residual Solvents for detailed information.

Impurities arising from the herbal substance(s) and/or herbal preparations e.g. Contaminants such as pesticide/fumigant residues, heavy metals, if controlled during the testing of the herbal substance/preparation, it is not necessary to test for these in the herbal medicinal product.

Similarly, residual solvent arising from the manufacture of the herbal preparation (e.g. an extract) need not be controlled in the herbal medicinal product provided it is appropriately controlled in the extract specification. However, solvents used for example in tablet coating will need to be controlled in the dosage form.

In cases where degradation products of the herbal substance/preparation are evident, they should be monitored in the herbal medicinal product. Acceptance limits should be stated for such degradation products.

In addition to the universal tests listed above, the following tests may be considered applicable to *herbal medicinal products* on a case by case basis. Individual tests/criteria should be included in the specification when the tests have an impact on the quality of the herbal medicinal product for batch control.

Additional tests and acceptance criteria generally should be included for particular herbal medicinal products. The specific dosage forms addressed include **solid oral** herbal medicinal products, and **liquid oral** herbal medicinal products.

-Tablets (coated and uncoated) and hard capsules (One or more of these tests may also be applicable to soft capsules and granules) – dissolution/ disintegration, hardness/friability, uniformity of dosage units (this term includes both uniformity of content and uniformity of mass; a pharmacopoeial procedure should be used), water content, microbial limits (Microbial limit testing is seen as an attribute of Good Manufacturing Practice, as well as of quality assurance. Reference should be made to the European Pharmacopoeia general text on the Microbiological Quality of Pharmaceutical Preparations for guidance on acceptable limits. Periodic testing may be appropriate.)

-Oral liquids – uniformity of dosage units (This term includes both uniformity of content and uniformity of mass. Generally, acceptance criteria should be set for weight variation, fill volume, and/or uniformity of fill. Pharmacopoeial procedures should be used), pH, microbial limits, antimicrobial preservative content (The lowest specified concentration of antimicrobial preservative should be demonstrated to be effective in controlling micro-organisms by using the European Pharmacopoeia antimicrobial preservative effectiveness test. Antimicrobial preservative effectiveness should be demonstrated during development, during scale-up, and throughout the shelf-life), antioxidant preservative content, extractables (Generally, where development and stability data show no significant evidence of extractables from the container/closure system, elimination of this test may be proposed), alcohol content, dissolution, particle size distribution, redispersibility (for oral suspensions, which produce sediment), rheological properties, specific gravity, reconstitution time (Acceptance criteria for reconstitution time should be provided for dry powder products, which require reconstitution. The choice of diluent should be justified), water content (for oral products requiring reconstitution).

According to EMEA (draft monograph), pharmaceutical forms for *Plantago lanceolata* leaves are:

Traditional use: Comminuted herbal substance as herbal tea, powdered herbal substance in a solid dosage form and other herbal preparations in liquid or solid dosage forms for oral and/or oromucosal use.

According to EMEA (draft monograph), traditional uses for *Plantago lanceolata* leaves are: "Indications for the internal administration are catarrhs of the respiratory tract and inflammation of oral

and pharyngeal mucosa. Externally applied it is used for inflammation of the skin. "

Herbal Medicinal Products containing exclusively herbal substances **(e.g. herbal teas)**

One or more of these tests may be applicable to herbal medicinal products containing exclusively herbal substances:

-loss on drying (To be specified depending on the plant parts present in the herbal medicinal product, if not performed on the herbal substance),

-identification (Identification tests must establish the specific identity of the herbal substance(s) in the herbal medicinal product and optimally should be discriminatory between the different herbal substances and with regards to substitutes/adulterants that are likely to occur.

-Microscopical and macroscopical characterisation can be used to support identification, if justified), purity (Relevant adulterants and substitutes should be determined), uniformity of mass/average mass of the sachet (The dosage unit is considered to be the typical dose taken by the patient. Pharmacopoeial procedures should be used),

-assay (In the case of such herbal medicinal products containing herbal substances with constituents of known therapeutic activity, validated assays for these constituents are required along with details of the analytical procedure(s),

-particle size,

-microbial quality.

A key issue in manufacturing herbal products and medicines is **standardization.** Standardization is the process of producing herbal extracts or phytochemicals in which product potency is guaranteed through consistency in active compound content level. This process requires high knowledge in phytochemical analysis and process technology to ensure the quality assurance required.

Product value increases in the following order: fresh material < dried powder < non-standardized extract < freeze/spray dried extract < standardized extract < phytomedicine.

In the case of cosmetics, there are some standards for high quality products:

-BDIH "Certified Natural Cosmetics" - herbal extracts and essential oils and aromatic materials from controlled biological cultivation or controlled biological wild collection

-COSMOS standard for organic cosmetics

-ECOCERT standard for natural and organic cosmetics (ECOCERT certifies organic farming as well)

1.3 Raw materials from non-food crops as alternative to fossil, petroleum-based and chemical resources

There are 45 registered international patents concerning *Plantago lanceolata* leaves and just a part are applied in industry.

1.3.1 *Herbal medicinal preparations and products*

Usually herbal products are adjuvant in classical medicine or have a preventive action. In pharmacological testing, the activity is compared to synthetic drugs.

A broad spectrum of different herbal preparations has been marketed so far in EU. According to the overviews of the market in the Member States of the European Union there were herbal preparations with a well-established use status and there were also herbal preparations under traditional use. With respect to the overall evaluation of the existing data on efficacy the monograph addresses only the traditional use.

Herbal preparations which have been reported to be marketed so far under well-established use (although EMEA doesn't approved a well-establish use for Plantaginis folium due to the lack of consistent data concerning efficacy):

i. Herbal substance, cut

ii. Dry extract (3-6:1); extraction solvent: water

iii. Liquid extract (1:0.9-1.1); extraction solvent: ethanol 35% (V/V)

iv. Liquid extract (1:1); extraction solvent: ethanol 25% (V/V)

v. Liquid extract (1:1); extraction solvent: ethanol 20% (V/V)

vi. Liquid extract (1:1); extraction solvent: ethanol 24.6% (V/V)

vii. Liquid extract (1:1); extraction solvent ethanol 40% (V/V)

viii. Liquid extract (1:0.9-1.1); extraction solvent: ethanol 40% (V/V)

ix. Soft extract (1.5-1.7:1); extraction solvent: ethanol 20% (m/m)

x. Expressed juice from the fresh herb (1:0.5-0.7)

xi. Expressed juice from the fresh herb (1:0.6-0.9)

Herbal preparations which have been reported to be traditionally used:

i. Herbal substance, cut

ii. Powdered herbal substance

iii. Liquid extract (1:0.8-1.2); extraction solvent: ethanol 40% (V/V)

iv. Liquid extract (1:1); extraction solvent: ethanol 35% (V/V)

v. Liquid extract (1:11); extraction solvent water

-Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

-European manufacturers use Plantaginis folium (alone or in combination with other plants) for various medicinal purposes: digestion (Frantsila, Fares), expectorant (KRKA, Aboca, Fares, Plantavorel, Hofigal), antimicrobial, astringent, soothing irritations (Phytopharm, Asta Medica) or in various forms: herbal tea (Plantavorel, Fares), tablets (Plantavorel), syrup (Fares), etc.

-Homeopathy - drugs with medical prescription - *Plantago lanceolata* drops, granules (VSM Geneesmiddelen B.V., Netherlands)

1.3.2 *Cosmetics*

Plantago lanceolata leaves are used in cosmetic industry by many European manufacturers: Alban Muller Group -France, Martina Gebhardt Naturkosmetik-Germany, Ilcsi -Hungary, Phytopharma-Poland, Farmec-Romania, Calendula -Slovakia, KRKA-Slovenia in a large variety of products: creams, lotions, solution for spa use, etc.

Ex. SEBOCLEAR, a complete concept containing *Plantago lanceolata* water extract, *Mahonia aquifolium* and salicylic acid with antibacterial, keratolytic and antiinflammatory action. SEBOCLEAR is effective as a treatment comedos and pustules in a soft fashion. Application methods: cleansing facewash, hair lotion, cleansing masks, cover-up pens, facial care for especially oily skin, cleansing facial gel, products for deep cleaning, acne swabs or spot pads. (Rahn Cosmetics AG)

Ex. PLANTAGO AO *Plantago Lanceolata* Leaf Extract Enhances collagen production, wound healing, antioxidant.(Pentapharm Switzerland)

1.3.3 Veterinary products

Leaves are edible and sometimes eaten as vegetable. *Plantago lanceolata* is occasionally grown as a fodder crop and considered to be of better quality than *Plantago major*.

1.3.4 Other intermediary products

European manufacturers produce a large variety of intermediary products:

Hydroalcoholic extract -Plantavorel, Hofigal-Romania, Phytosan-France

Liquid extract -Phytex-Bulgaria, Phytopharm-Poland

Dry extract – Phytex-Bulgaria, Alban Muller, Phytopharm-Poland, Calendula-Slovakia

Mother tincture-Phytosan-France

Glycerine extract - Phytosan-France

Ex. SEDOX® is a fluid extract of *Plantago lanceolata* L., standardized in verbascoside, manufactured by ethanolic extraction (general method pursuant to European Pharmacopoeia 6.1 dated 4/2008); the final ethanol content is: 26.0 e 30.0 % (v/v). (EPO Istituto Farmochimico Fitoterapico, Italy). It can be applied in many different final products, from cosmetics to nutritional supplements.

1.4 Set prospects to widen the range of potential feedstock for the understudy industrial uses, based on the technology improvements

Technologies improving the quality of preliminary raw material (e.g. breeding varieties with better gene expression together with improvement of technology of: cultivation, harvesting and processing) as factor determining further application.

New and improved varieties

In vitro cultures

-explants for plant regeneration and micropropagation

-callus culture - Leaf and root mucilage content in the intact plant of *P. lanceolata* is 10% g/g dw and in seeds is 5% g/g dw while in the callus is about 14.75%g/g dw. Callus could have up to 3 times more mucilage than seeds, leaf and root parts.(4)

-hairy root cultures (transformed by *Agrobacterium rhizogenes*) generated from root and leaf explants. Transformed root clones can be maintained on medium without growth regulators, they are genetically more stable than cell cultures and show wide production field and high level of metabolite production over a long time course. Hairy root cultures are able to biotransform an outer precursor such as cinnamic acid into a phenolic derivative. Further investigation should be carried out in order to optimize the culture conditions and increase the bioproduction of biologically active metabolites.

-study of the biotransformation processes correlated with the polyphenolic metabolism

-to select mutants with high stress or salt resistance, freezing tolerance or with high bioactive compound contents

New products based on improvement of extraction technology, formulation technology and identification of new medicinal applications

Materials

-New type of nanomaterials has been synthesized using iridoidic extract derived from *Plantago* sp. The iridoidic compounds were separated from *Plantago lanceolata* by successive extraction in aqueous media. The composition of the stable nano-emulsion used for nanomaterials synthesis has been chosen from the pseudo ternary phase diagram and the dimensions of the emulsion were confirmed by Dynamic Light Scattering measurements. The

obtained nanodrops were then encapsulated in silica resulting porous core - shell particles which were characterized by Dynamic Light Scattering and electronic microscopy confirming the nanostructure of the new biomaterials. Nanomaterials obtained from iridoidic extract were used for preclinical tests performed on mice in order to establish the influence of biomaterials on the cicatrisation and diarrhoea. The results obtained revealed a positive action on the cicatrisation process, and the crude extract processed as nanopowder showed a protective action against diarrhoea disorder.

-These days modified polysaccharides have been the major area of scientific research. These polysaccharides being cost effective, biodegradable and quite efficient towards various technological processes provides a better option for the artificial synthetic materials. Lot of work has been carried-out on various polysaccharides such as starch, cellulose, chitosan, dextrin, guar-gum, psyllium and many more. The end product obtained were found to be the quality products as they were efficiently used in different technological processes viz. drug- delivery, agriculture (insecticide and pesticide delivery), water treatment (removal of toxic metal ions from waste water and flocculation) and membrane technology.

New pharmaceutical applications which were already proved on animal models:

-immunostimulant effect

-antitoxic effect

-procoagulant effect

-antiheminthic effect

-antiulcerous activity For ex. An international patent (A61K36/00; A61P1/04; A61K36/00; A61P1/00) belonging to Romanian researchers from NCPRI describes a bioactive product with antiulcerous activity comprising a mixture of the species *Centaurea cyanus* L-herba and *Plantago lanceolata* L-folium. This product is not yet commercialized, is registered as dietary supplement and has a registration dossier for approval as gerbal medicine at Romanian Medicine Agency.

1.5 Identify restricting factors that inhibit broader industrial use of the biomass feedstock (supply, costs, physical traits, consistency in quality, technical performance, research gaps, etc.)

5.1 Agricultural raw material

Physical traits

-*Plantago lanceolata* is a common weed; some of the manufacturers use wildcrafted raw material

-The contents of aucubin and acteoside are extremely lower in plants grown in the shade.

-Nitrogen application enhances the growth of the cultivars, especially the top fresh weight; on the other hand, it significantly diminished the top dry-matter content. Generally, the contents of aucubin and acteoside are apparently lower in the plants treated with nitrogen than in those that did not receive it.

-Depending on the time of harvesting the content of aucubin and catalpol varies. Before the flowering period the content of aucubin is very low in every organ and reaches its maximum in autumn with aucubin at levels of 1-3% and catalpol up to 1% .

-After harvesting the herb has to be dried directly to avoid fermentative processes. After hydrolysis aucubin is converted to dark brown polymers, which are responsible for the dark coloration of improperly dried drug material. The herbal substance is commonly dried at temperatures of 40-50°C. During this process the content of aucubin decreases. Drying at room temperature results in aucubin contents twice as high.

Costs

- for most applications raw material must be free of impurities or other plant parts (especially when harvest is done mechanically) and requires additional operations incurring additional costs

-primary processing which ensures the product quality

1.5.2 Industrial raw material

Research gaps

-There are no human data available regarding pharmacodynamic and pharmacokinetic properties of *Plantago lanceolata* leaves. Also, dose response studies have not been performed in clinical trials.

-In literature there is also evidence of a traditional use of *Plantago lanceolata* for the external treatment of irritations of the skin, but so far only one medicinal product has been registered in Poland. This preparation, however, does not fulfil the requirement of a traditional use for at least 30 years.

-There is sufficient evidence in literature for the traditional oral and oromucosal use in the above mentioned indication. Although various pharmacological effects have been

described for the total extract of Plantago lanceolata and constituents thereof, these effects have never been verified in controlled clinical studies. Also, for children and adolescents no data are available. Thus, the oromucosal administration should be limited to adults. A well-established use of the herbal substance thus cannot be postulated.

-Plantain (*Plantago lanceolata*) pollen is generally considered as one of the most important dicotyledons that cause allergic diseases in Europe. Further studies on polyamine and allergy relation with other genus having different species, which have different allergy degrees to be able to strength, the results of this research are in progress.

Physical traits

-Mucilage content is lower than in *Plantago ovata* which gives about 25% of good quality mucilage. This mucilage has various industrial applications (thickener, hydrocolloidal agent) as well as medicinal properties (source of dietary fibres, hypocholesterolemic and antidiabetic activities). Because of the poor yield, *P. lanceolata* mucilage is not suitable for industrial production.

-Ribwort plantain is palatable to cattle and is recommended in pastures including grasses and legumes as it may improve Cu content. However, it is less consumed than a large variety of grasses or legumes such as prairie grass, kikuyu, lucerne or white clover.

- It proved to be suitable in combination with grass in swards to sustain finishing lambs growth. It may also be recommended as an alternative to hay. However, it gave poorer results than chicory (*Cichorium intybus*) on live weight gain, hot carcass weight. It had lower effect than chicory in reducing lambs parasites.

-Ribwort plantain could be fed to weaned piglets as it is a source of fibre. It could be included up to 8% DM dietary level without deleterious effect on pig performances. There have been attempts to use ribwort plantain in mixtures of herbs acting as growth promotors in pigs in order to replace antibiotics or probiotics, but the results were not very consistent.

Costs

-high costs for clinical trials

-high costs for organic cultivation and for classic cultivation too, comparing to wildcrafting.

-equipment and facilities for specific processing – extractors, spray-dryers, freeze-dryers

-equipment for conditioning – special machines for tablets and capsules, for oral solutions, for products with topical application

-equipment for packaging (vacuum technology)

1.6 Set forth research gaps, prospects and recommendations to procure bio-based products will be tackled

1.6.1 Agricultural raw material

Basic research

-select mutants with high stress or salt resistance, freezing tolerance or with high bioactive compound contents

-optimizing post-harvest processes to maintain a good yield of active principles

-large scale organic cultivation

Applied research

-technology improvement for processing

1.6.2 Industrial raw material

Basic research

-pharmacological testing - dose response studies; controlled clinical studies

-Further studies on polyamine and allergy relation with other genus having different species, which have different allergy degrees.

-The compounds found in Plantago lanceolata have potential for use in selectively targeting plant-parasitic nematodes in pest management systems. Further research is needed to isolate and identify *Plantago*-specific compounds, to determine their toxicity to additional plant-parasitic nematodes, and to understand the fate of these compounds in soil.

-new applications in veterinary medicine

Applied research

-modern procession technologies – microwave-assisted extraction, ultrasound extraction, accelerated solvent extraction, superfluid extraction

-technology improvement for chemical analysis and standardization

-technology improvement for conditioning and formulation drugs and cosmetics (for ex. immediate or delayed release capsules, effervescent tablets, tea bags, dispensers for local application, etc).

Prospects and recommendations

-financial help for cultivation; it is important to understand the importance of controlled conditions on final product quality

-Need for legislative harmonization in EU27 countries. The same drug is sold as medicine in a country and as dietary supplement in other.

-know the nature of the desired product. In the case of medicinal plants which are used directly as pharmaceuticals, the quality and thus the concentration of active compounds is much more relevant than the total yield.

-for pharmaceutical products - ensuring product quality by standardization

-valorification of all plant parts; good management of wastes

-modern processing technologies in order to reduce heat degradation, reduce processing costs, faster extraction, much lower energy usage, less solvent usage

-new technologies for conditioning pharmaceutical and cosmetic products to achieve a targeted and better absorption

-strong marketing strategy for natural, innovative and high quality products

References

- Plant for Future Database www.pfaf.org
- British Pharmacopoeia, 2009
- European Medicine Agency - Assessment report on *Plantago lanceolata* L., folium, Draft, 2010
- M. Mirmasumi, H. brahimzadeh, S. Tabatabaei - Mucilage Production In Tissue Culture of *Plantago lanceolata* , , J. Agric. Sci. Technol. (2001) Vol. 3: 155-160

2 Lavandula angustifolia Mill. (Lamiaceae)

2.1 Review on the product yielding capacity from various industrial crops streams

According to Plant for Future Platform (1), the main uses for *Lavandula angustifolia* are:

Agriculture Raw Materials	**Directions of use**	**Product example**
Flowers	Phytotherapy	-local antiseptic to help heal wounds, restorative and tonic -antihalitosis, powerfully antiseptic, antispasmodic, aromatic, carminative, cholagogue, diuretic, nervine, sedative, stimulant, stomachic and tonic
	Veterinary products	-shampoos and other products as an insect repellent, especially for fleas
	Cosmetics	-Lavender water, lavender vinegar, lavender bath, pot-pourris, scented candles, beauty products, herbal pillows, lavender bags, therapeutic bath salts
	Ornamental	
Volatile oil	Phytotherapy	-local antiseptic to help heal wounds, restorative and tonic -antihalitosis, powerfully antiseptic, antispasmodic, aromatic, carminative, cholagogue, diuretic, nervine, sedative, stimulant, stomachic and tonic
	Food industry	-flavouring agent
	Veterinary products	-shampoos and other products as an insect repellent, especially for fleas
	Cosmetics	-Lavender water, lavender vinegar lavender bath, scented candles, beauty products, perfumery, therapeutic bath salts
	Repellent	
Leaves	Repellent	

2.2 Identify desirable quality characteristics that feedstock has to meet for mature industrial processes

Guidelines for pharmaceutical products:

Lavandulae flos has a monograph in European and British Pharmacopoeias, ESCOP, WHO and French Avis aux fabr. monographs and draft report under discussion on EMEA (Herbal Medicinal Products committee assessment)

Lavandulae aetheroleum has a monograph in European and British Pharmacopoeias, ESCOP and WHO monographs and draft report under discussion on EMEA (Herbal Medicinal Products committee assessment)

Plant material of interest for pharmaceutical purposes: dried flower of *Lavandula angustifolia* Mill

Herbal substance (equivalent to the term "herbal drug" as defined in the European Pharmacopoeia) - which includes Lavandulae flores

The product is usually used in dried form but sometimes fresh. A comprehensive specification must be developed even if the starting material for the manufacture of the herbal substance is required unless justified. The specification should be established on the basis of recent scientific data and should be set out in the same way as the European Pharmacopoeia monographs. The general monograph "Herbal drugs" (herbal substances) of the European Pharmacopoeia should be consulted for interpretation of the following requirements.

-Definition: dried flower of *Lavandula angustifolia* Mill. It is also important to know the geographical source(s) and the conditions under which the herbal substance is obtained.

-Characters: a qualitative statement about the organoleptic character(s) where characteristic and the macroscopic and microscopic botanical characters of the herbal substance.

Microscopic characteristics

Calyx and corolla bear glandular hairs with a very short unicellular stalk and a head of four to eight cells, of a labiaceous type, and characteristic branching unicellular and multicellular non-glandular hairs with pointed ends and a somewhat streaked or warty cuticle. Corolla bears also, on the inner surface at the throat, characteristic glandular hairs with a unicellular, glandular head and a bicellular stalk, its basal cell being long and knotted and the other cell short and cylindrical. Anthers covered with whipshaped, unicellular, non-glandular trichomes; pollen grains, almost rounded, with six germ pores.

Powdered plant material

Grey-blue with fragments of calyx, elongated epidermal cells with wavy anticlinal walls, and multicellular non-glandular covering trichomes. Encapsulated labiate oil glands. Corolla fragments, almost oval and slightly wavy-walled epidermal cells, labiate oil glands and branched covering hairs; unicellular glandular hairs. Pollen grains spherical to ellipsoidal, 24–30 µm in diameter, with six furrows, six germ pores and lines of pits radiating from the poles. Leaf fragments, almost straight-walled epidermal cells, covering branched trichomes and labiate oil glands, glandular hairs with a unicellular stalk and a bicellular head.

Odour: fragrant, aromatic; **taste**: aromatic, bitter, somewhat camphoraceous .

Other general identity tests: identification testing optimally should be able to discriminate between related species and/or potential adulterants/substitutes, which are likely to be present. Microchemical tests, and thin-layer chromatography for the presence of linalyl acetate and linalool.

Purity tests:

Foreign organic matter - Not more than 2.0%.(3)

Total ash - Not more than 9.0% (3)

Acid-insoluble ash - Not more than 1.0% (4).

Water-soluble extractive - Not less than 18.0% (4).

Alcohol-soluble extractive - Not less than 12.0% (4.).

Moisture - Not more than 10.0% (3.).

Pesticide residues: The recommended maximum limit of aldrin and dieldrin is not more than 0.05 mg/kg (3).

Chemical assays

-Contains not less than 1.3% (v/w) essential oil determined by steam distillation (3.).

The main constituents: 1.0–3.0% essential oil, of which the major constituents are linalyl acetate (30–55%) and linalool (20–50%). Two hydroxycinnamic acid esters, rosmarinic acid and chlorogenic acid, are regularly present in the leaves of *Lavandula* species.

Herbal preparation (equivalent to the term "herbal drug preparation" as defined in the European Pharmacopoeia) of *Lavandula angustifolia* are obtained by subjecting vegetal material to treatments such as extraction, distillation, expression,

fractionation, purification, concentration or fermentation. These preparations include comminuted or powdered herbal substances, tinctures, extracts, essential oils.

The following tests and acceptance criteria are applicable for all the above mentioned preparations.

Example for volatile oil:

-Definition: Essential oil obtained by steam distillation from the flowering tops of *Lavandula angustifolia*. The ratio of the herbal substance to the genuine herbal preparation must be stated.

-Characters: A clear colourless or pale yellow liquid, miscible with 90% alcohol, ether and fatty oils.

-Odour: characteristic, fragrant, aromatic; **taste:** aromatic, slightly bitter.(2)

-General identity tests: Macroscopic examinations; refractive index, specific gravity and optical rotation measurements; thin-layer chromatography for the presence of linalyl acetate and linalool, and gas chromatography.

-**Purity tests:**

Relative density 0.878–0.892.

Refractive index 1.455–1.466

Optical rotation -12.5–7o

Acid value not more than 1.0 (3).

Pesticide residues The recommended maximum limit of aldrin and dieldrin is not more than 0.05 mg/kg. (3)

Chemical assays: Official analysis by gas chromatography shows the following composition: linalyl acetate (25–46%), linalool (20–45%), terpinen-4-ol (1.2–6.0%), lavendulyl acetate (> 1.0%), 1,8-cineole (1,8-cineol, cineol, cineole, eucalyptol) (< 2.5%), 3-octanone (< 2.5%), camphor (< 1.2%), limonene (< 1.0%), and α-terpineol (< 2.0%).(2)

Herbal medicinal products (the term includes "traditional medicinal product")

Lavandula herbal medicinal products contain as active substances one or more herbal substances or one or more herbal preparations, or one or more such herbal substances in combination with one or more such herbal preparations.

The following tests and acceptance criteria are considered generally applicable to all herbal medicinal products:

-Description: A qualitative description of the dosage form should be provided (e.g., size, shape, colour). If colour changes occur during storage, a quantitative procedure may be appropriate.

-Identification: Identification tests should establish the specific identity of the herbal substance(s) and/or herbal preparation(s), in the herbal medicinal product and optimally should be discriminatory with regard to substitutes/adulterants that are likely to occur Identification solely by chromatographic retention time, for example, is not regarded as being specific; however, a combination of chromatographic tests (e.g. HPLC and TLC-densitometry) or a combination of tests into a single procedure, such as HPLC/UV-diode array, HPLC/MS, or GC/MS may be acceptable. In the case of herbal medicinal products containing powdered or comminuted herbal substances, microscopical and macroscopical characterisation could be used for identification in combination with other methods, if justified.

-Assay: In the case of products containing herbal substances and/or herbal preparations with constituents of known therapeutic activity, validated assays of the content of these constituents are required along with details of the analytical procedure(s). Where appropriate , a specific, stability-indicating procedure should be included to determine the content of the herbal substance(s) and/or herbal preparation(s) in the herbal medicinal product. In cases where use of a non-specific assay is justified, other supporting analytical procedures should be used to achieve overall specificity. In the case of herbal medicinal products containing herbal substance(s) and/or herbal preparation(s) where the constituents with known therapeutic activity are not known, validated assays of active or analytical markers or other justified determinations are required. The choice of such markers should be justified. In cases where a specific assay of each active substance of a herbal medicinal product is not possible other justified determinations are required (for example, in multi-component traditional herbal medicinal products for human use the same markers may be present in more than one herbal substance/preparation).

-Impurities: Refer to the ICH/VICH Guidelines on impurities in new drug products/Guidelines on impurities in new veterinary products (CPMP/ICH/2738/99 and CVMP/VICH/838/99 as revised) and the European Pharmacopoeia General text on Residual Solvents for detailed information.

Impurities arising from the herbal substance(s) and/or herbal preparations e.g. Contaminants such as pesticide/fumigant residues, heavy metals, if controlled during the testing of the herbal substance/preparation, it is not necessary to test for these in the herbal medicinal product.

Similarly, residual solvent arising from the manufacture of the herbal preparation (e.g. an extract) need not be controlled in the herbal medicinal product provided it is appropriately controlled in the extract specification. However, solvents used for example in tablet coating will need to be controlled in the dosage form.

In cases where degradation products of the herbal substance/preparation are evident, they should be monitored in the herbal medicinal product. Acceptance limits should be stated for such degradation products.

-Microbial limits: There is a need to specify the total count of aerobic micro-organisms, the total count of yeasts and moulds, and the absence of specific objectionable bacteria. These limits should comply with the European Pharmacopoeia. The frequency of testing should be justified.

In addition to the universal tests listed above, the following tests may be considered applicable to *herbal medicinal products* on a case by case basis. Individual tests/criteria should be included in the specification when the tests have an impact on the quality of the herbal medicinal product for batch control.

Additional tests and acceptance criteria generally should be included for particular herbal medicinal products. The specific dosage forms addressed include **solid oral** herbal medicinal products, and **liquid oral** herbal medicinal products.

-Tablets (coated and uncoated) and hard capsules (One or more of these tests may also be applicable to soft capsules and granules) – dissolution/ disintegration, hardness/friability, uniformity of dosage units (this term includes both uniformity of content and uniformity of mass; a pharmacopoeial procedure should be used), water content, microbial limits (Microbial limit testing is seen as an attribute of Good Manufacturing Practice, as well as of quality assurance. Reference should be made to the European Pharmacopoeia general text on the Microbiological Quality of Pharmaceutical Preparations for guidance on acceptable limits. Periodic testing may be appropriate.)

-Oral liquids – uniformity of dosage units (This term includes both uniformity of content and uniformity of mass. Generally, acceptance criteria should be set for weight variation, fill volume, and/or uniformity of fill. Pharmacopoeial procedures should be used), pH, microbial limits, antimicrobial preservative content (The lowest specified concentration of antimicrobial preservative should be demonstrated to be effective in controlling micro-organisms by using the European Pharmacopoeia antimicrobial preservative effectiveness test. Antimicrobial preservative effectiveness should be demonstrated during development, during scale-up, and throughout the shelf-life), antioxidant preservative content,

extractables (Generally, where development and stability data show no significant evidence of extractables from the container/closure system, elimination of this test may be proposed), alcohol content, dissolution, particle size distribution, redispersibility (for oral suspensions, which produce sediment), rheological properties, specific gravity, reconstitution time (Acceptance criteria for reconstitution time should be provided for dry powder products, which require reconstitution. The choice of diluent should be justified), water content (for oral products requiring reconstitution).

Herbal Medicinal Products containing exclusively herbal substances **(e.g. herbal teas)**

One or more of these tests may be applicable to herbal medicinal products containing exclusively herbal substances:

-loss on drying (To be specified depending on the plant parts present in the herbal medicinal product, if not performed on the herbal substance),

-identification (Identification tests must establish the specific identity of the herbal substance(s) in the herbal medicinal product and optimally should be discriminatory between the different herbal substances and with regards to substitutes/adulterants that are likely to occur.

-Microscopical and macroscopical characterisation can be used to support identification, if justified), -purity (Relevant adulterants and substitutes should be determined), uniformity of mass/average mass of the sachet (The dosage unit is considered to be the typical dose taken by the patient. Pharmacopoeial procedures should be used),

-assay (In the case of such herbal medicinal products containing herbal substances with constituents of known therapeutic activity, validated assays for these constituents are required along with details of the analytical procedure(s)),

-particle size,

-microbial quality.

A key issue in manufacturing herbal products and medicines is **standardization.** Standardization is the process of producing herbal extracts or phytochemicals in which product potency is guaranteed through consistency in active compound content level. This process requires high knowledge in phytochemical analysis and process technology to ensure the quality assurance required.

Product value increases in the following order: fresh material < dried powder < non-standardized extract < freeze/spray dried extract < standardized extract < phytomedicine.

Cosmetic

In the case of cosmetics, there are some standards for high quality products:

-BDIH "Certified Natural Cosmetics" - herbal extracts and essential oils and aromatic materials from controlled biological cultivation or controlled biological wild collection

-COSMOS standard for organic cosmetics

-ECOCERT standard for natural and organic cosmetics

Food additive

The Joint FAO/WHO Expert Committee on Food Additives considered linalool and linalyl acetate, constituents of *Lavandulae aetheroleum* as part of a group a substances and an estimated ADI of 500μg/ kg bw i.e. 3000μg/ person was established for citral, geranyl acetate, citronellol, linalool and linalyl acetate expressed as citral.

Veterinary medicine

Lavandulae aetheroleum for use in veterinary medicine is approved by Committee of Veterinary Medicinal Products of European Medicine Agency. The application relates to the volatile oil which is intended for use in all food-producing animals for topical use only.(4)

2.3 The report on raw materials from non-food crops as alternative to fossil, petroleum-based and chemical resources

2.3.1 Herbal medicinal preparations and products

Usually herbal products are adjuvant in classical medicine or have a preventive action. In pharmacological testing, the activity is compared to synthetic drugs.

There are 44 registered international patents concerning *Lavandula angustifolia* flowers or volatile oil and just a part are applied in industry.

Ex. The efficacy of tincture of *L. angustifolia* was compared with imipramine in the treatment of mild to moderate depression and the possible adjuvant effect of this tincture in a 4 week double-blind, randomized trial was evaluated. A combination of imipramine and lavandula tincture was more effective than imipramine alone and one of the advantages of this combination is a better and earlier improvement. A large-scale trial is justified.(5)

Ex. Silexan (active substance of Lasea drug commercialized in Germany) contains a quality-selected, well-defined volatile oil preparation from *Lavandula angustifolia* in an immediate release capsule. The results from a multi-centre, double-blind, randomised phase III study demonstrate that silexan is not less effective than lorazepam in the treatment of patients with generalized anxiety disorder.

Ex. Ephydrol, solution, OTC, crème for the treatment of excessive sudorific secretion, (CS Dermatologie, France)

Ex. International patent concerning a composition for the topical treatment of labial herpes lesions containing essential oils of *Mellissa officinalis* and *Lavandula angustifolia*, glycyrrhizic acid, beta-glucan, D-pantenol and gelifiers. (IST Farmaterapico IT SPA, Italy)

Ex. Human data: Perillyl alcohol (found in cherries and mint as well as lavender) is in Phase I clinical trials for use as a chemoprotective and chemotheraputic agent against advanced breast, ovarian and prostrate cancers.

For traditional use, *Lavandula angustifolia* is sold in various forms: herbal tea (Natura Biertan, Fares-Romania), tincture (Fares-Romania), volatile oil (Hofigal, Fares-Romania), tablets (Fares, -Romania), syrup (Fares-Romania), combinations (Natura Biertan, Hofigal, Fares-Romania)

Cosmetics

Lavandula angustifolia flowers are used in cosmetic industry by many European manufacturers: Ryor, Alpa s.r.o-Czech Republic-Czech Republic, Frantsila Farm –Finland, Florame , L'Occitane, Yves Rocher –France, Kneipp-Germany, Apivita, Electra soap –Greece, Silvestris&Szilas, Ilcsi , Herbaria –Hungary, Agronatura , Arco Cosmetici-Italy, Original Cosmetics-Netherlands, Bielenda, Phytopharma , ASA Ltd-Poland, Hofiga, Elmiplant-Romania, Kozmetika Afrodita-Slovenia, Naetura-Spain, Curiosa Neways-Sweden in a large variety of products: creams, lotions, solution for spa use, etc.

-At the beginning of the century, lavender was apparently first incorporated into men's fragrances in Italy. Nowadays, lavender is still used as a top note in men's eau de toilettes, to add freshness to the blend.

-Lavender has a fresh clean, slightly herbal note, and has also enjoyed considerable revival of fashion with the popularity of aromatherapy, where the relaxing effect of lavender is used in consumer products. Lavender is the most common essential oil used by

aromatherapists due to its pleasant odour, relaxant effect on most people and above all its low price.

-Therapeutic bath salts. A mixture of lavender oil, ylang ylang oil, rosewood oil and patchouli oil with epsom salts, LiCl and Copper gluconate have been patented by McLean (1999) as a muscle relaxant. The calming and relaxing action of essential lavender oil is used in Dr.Hauschka Lavender Bath and in the protecting and fortifying Moor Lavender Body Oil (Wala Heilmittel GmbH)

2.*3.2 Synthetic repellents and insecticides*

-The efficacy of lavender volatile oil or leaves extract falls short when compared to synthetic pesticides although there are specific pest contexts where control equivalent to that with conventional products has been observed. Essential oils also require somewhat greater application rates (as high as 1% active ingredient) and may require frequent reapplication when used out-of-doors. Even so, from the ecological point of view is preferable to choose a natural product.

-Fly and mosquito repellent. Lavender absolute, benzoin, dimethylbenzylcarbinyl acetate, with jasmine absolute, racemic borneol, d-limonene and/or hydrolinalool is patented by Warren (1997).

2.3.3. Natural food flavours

-Lavender oil, absolute and even concrete are used as natural food flavours. Reported uses in the food industry include: baked goods, frozen dairy, soft candy, gelatin, pudding, non-alcoholic and alcoholic beverages.

2.4 Set prospects to widen the range of potential feedstock for the understudy industrial uses, based on the technology improvements

Technologies improving the quality of preliminary raw material (e.g. breeding varieties with better gene expression together with improvement of technology of: cultivation, harvesting and processing) as factor determining further application.

New and improved varieties

-breeding – there is a need to develop new varieties with 50% increased levels of linalyl-acetate and linalool. It has to be clarified if - and maybe to what extent - the putative gain in quality by increasing the secondary plant product concentration by applying deliberately drought stress would be compensated by decreasing yields in biomass.

-polyploidy - to increase lavender yields, increasing flower size, inflorescence size and the number of leaves. This has also produced varieties more resistant to disease and extreme conditions.

Improving the yield and quality of volatile oil and its constituents

-tissue-culture-based screening for selection of high biomass and phenolic producing clonal lines of lavender using Pseudomonas species and azetidine-2-carboxylate

-cell suspension fir rosmarinic acid biosynthesis

New products based on improvement of extraction technology, formulation technology and identification of new medicinal applications

Extraction technology

-Maximum efficiency of harvesting and distillation in order to preserve raw material qualities. In modern systems, mechanical harvesters load the flowers into special trailers which themselves become the distilling vessels. Mechanical harvesters usually break some of the superficial glands and the oil will evaporate and be lost from these if the herb is subsequently exposed to the open air. So the flowers must be fully ripe before mechanical harvesting is started, because they cannot be laid out in the sun to wilt. Some of the modern trailer systems have retained the cylinder and grid principle but in an advanced form. Others are large rectangular boxes where the steam is introduced through an array of sparge pipes on the trailer floor.(Fig.1)(6)

Fig. 1 Filling still cylinder in the field (6)

` -Microwave-assisted extraction (MAE) is highly efficient for obtaining extracts under mild conditions. MAE is particularly important since the active components which are thermally labile can be recovered without any damage. Two types of separation processes – adsorptive and membrane-based pervaporation – are useful in recovering practically all the oil that is lost with the condensate water. The recovered oil can be sold as such or blended with the main oil fraction to yield a much more natural aroma and hence a high value.

-Phytonics process – suitable for the extraction of high-quality essential oils

New pharmaceutical applications which were already proved on animal models:

-antispasmodic/ digestive aid

-hypoglycemic effect

-antineoplastic

New pharmaceutical forms with targeted delivery

-nanotechnology (silver nanoparticles)

-liposome technology

-development of modern extraction technologies (microwave, supercritical CO2, fluid bed granulation)

2.5 Identify restricting factors that inhibit broader industrial use of the biomass feedstock (supply, costs, physical traits, consistency in quality, technical performance, research gaps, etc.)

2.5.1 Agricultural raw material

Physical traits

-the yield of oil from lavender varies greatly with the season, the cultural conditions (lavender has high demands regarding the soil and light and the geographical location)

-where lavender plantations are established from seed, significant variation in oil yield and quality between plants is to be expected due to natural cross-pollination and hybridization. (7)

-primary processing plays an essential role in final product quality (for. ex. for glycosides obtainment, a maximum drying temperature of 100°C is recommended and for essential-oil 35 to 45°C)

Costs

-for most applications raw material must be free of impurities or other plant parts (especially when harvest is done mechanically) and requires additional operations incurring additional costs

2.5.2 Industrial raw material

Lavandulae aetheroleum

Physical traits

-*distillation procedure* Free water in contact with the oil during the preheating stage of distillation reduces oil quality and extraction efficiency. If the pressure or the temperature is too high it may change the molecular structure of the fragrance molecule, altering the chemical constituents. The yield of the oil may vary considerably from one season to the next, as the age of the bushes and the weather will affect both the quantity and quality of the product.

-Lavender, by its very nature is not as prone to decrease in oil yield and quality after harvest and thus there is more opportunity for stockpiling the crop prior to extraction (2 days max), or for transporting it to distilleries nearby.

-Although present in teas and as a flavoring in some foods, lavender oil is toxic if ingested in large quantities

Consistency in quality

-A smaller quantity of lavender concretes is produced by solvent extractions. By succesive processing from concretes a wax-free residue called an absolute is obtained. There is a frequency of 50 % loss from concrete to absolute. Absolutes are more widely used in fine perfumery.(6)

-adulteration - addition of cheaper lavandin (*Lavandula* x *intermedia*) oil varieties; the addition of spike lavender oil (*Lavandula latifolia*); the addition of ho oil rectified (*Cinnamomum* spp) and or acetylated lavandin oils etc.

Research gaps

-There are no human data concerning the efficacy of Lavender oil or flowers on gastrointestinal/ hepatic disorders, hyperglycemia, inflammation. Although lavender has

traditionally been used for its sedative, anxiolytic and analgesic properties, several small case studies have had mixed results.

-regarding insecticidal effect, in a pilot study of 20 British children with persistent head lice resistant to pharmaceutical treatment, a mixture of six essential oils including lavender was reportedly "fully effective"131. No controlled trials of lavender as an insecticidal agent have been reported.

-Little is known about specific interactions between lavender and other herbs and pharmaceuticals.

2.6 Set forth research gaps, prospects and recommendations to procure bio-based products will be tackled

2.6.1 Lavandulae herba

Basic research

-breeding new varieties that suit the climate and yield well, have characteristics that are increasingly desirable to end users and a good disease resistance.

-large scale organic cultivation

-new applications in veterinary medicine and new products with insecticidal properties

Applied research

-increasing efficiency of processing infrastructure (modular construction so as to permit increase in capacity and function by duplicating or adding modules; simultaneous processing for more than one product)

2.6.2 Lavandula volatile oil

Basic research

-breeding new varieties that provide a volatile oil of good quality and yield

-development of partnerships between research centres and producers for increasing yield and quality of oil

-new methods for detecting adulterated oils

-evaluation of insecticidal properties and mechanisms of action

-new technologies for conditioning pharmaceutical and cosmetic products to achieve a targeted and better absorption

Applied research

-development of high standards facilities and equipment for distillation

-modern procession technologies – microwave-assisted extraction, ultrasound extraction, accelerated solvent extraction, superfluid extraction

-The determination of adulteration of essential oils was perfected by the use of special enantiomeric or chiral columns, mainly composed of an -cyclodextrin phase and by biological evaluation using several different parameters. Detection of marker compounds (α-Santalene, (-) -lavandulol, (-) -lavandulyl acetate etc) or impurities in synthetic linalol made via the older acetylene or β-pinene routes (e.g. dehydrolinalol, dihydrolinalol, tetrahydrolinalol, plinol & others), which don't occur naturally in linalol-containing essential oils such as lavender is made by modern methods like enantiomeric analysis or enantioselective multidimensional gas chromatography-mass spectrometry (enantio-MDGC-MS). By these analysis, lavender oil should contain -(4S)-(+)-linalol usually <5% but up to 15% during very prolonged hydrodistillation; over 15% of 4S-(+)- linalol indicates adulteration with racemic linalol and -4R)-(-)-linalyl acetate >99%; (4S)-(+)-linalyl acetate <1.0%

Prospects and recommendations

-development of cooperatives for reducing distillation related costs. There are around 400 producers of lavender in France, 80% of which belong to producer organizations. These collectives play an important role in the organizing and management of production, and provide distillation and storage services, marketing and technical support.

-financing research for large-scale use of lavender oil as insecticidal product. Funds from the French government (ONIPPAM) supported research, development and demonstration trials, subsidies for new plantings and promotion of commercial and tourism potential. The focus has been on the development of new varieties more of interest to the perfumery industry in terms of composition, with good oil yield and improved disease resistance.

-modern and eco-friendly use of wastes - It is recommended that the production cycle should be wasteless - after processing the utilized plant materials are reused as material for compost and fertilization or they are dried and used as ecological fuel for steam production instead diesel.

-ensuring product quality by standardization

-strong marketing strategy for natural, innovative and high quality products

-tourism development - Value-adding large-scale production by building a tourism enterprise around them.

References

- 1.Plant for Future Database www.pfaf.org
- WHO monographs on selected medicinal plants, 2007, vol. 3, p. 229-235 (Lavandulae flos), p. 219-228 (Aetheroleum lavandulae)
- European pharmacopoeia, 3rd ed. Suppl. 2001. Strasbourg, Council of Europe, 2000
- Central Council for Research in Unani Medicine. Standardization of single drugs of Unani medicine – part III. New Delhi, Ministry of Health and Family Welfare, 1992.
- Prog Neuropsychopharmacol Biol Psychiatry. 2003 Feb;27(1):123-7.Comparison of Lavandula

3 Calendula officinalis L. (Asteraceae)

3.1 Review on the product yielding capacity from various industrial crops streams

Agriculture Raw Materials	**Directions of use**	**Product example**
The whole plant, but especially the flowers and leaves	Phytotherapy	-antiphlogistic, antiseptic, antispasmodic, astringent, cholagogue, diaphoretic, emmenagogue, skin stimulant and vulnerary herbal product
Leaves, blossoms, buds	Homeopathy	-antiinflammatory, cicatrizing
Flowers, volatile oil,	Cosmetics	-beauty products (shampoo, soap, creme, lotions, etc)
Flowers		- food colouring due to carotenoid content
Flowers	Tinctorial	-yellow dye
Seed oil	Painting	Paint additive
Flowers	Agriculture	-alternative ingredient of "Quick Return" compost activator. This is a dried and powdered mixture of several herbs that can be added to a compost heap in order to speed up bacterial activity and thus shorten the time needed to make the compost
Whole plant	Agriculture	-allelopathic properties in relation to dicotyledons and weaker activity to monocotyledons
Herb	Repellent	-reduces the soil eelworm population -exert various effects on growth and physiology of *Spodoptera litura* (Fab.), a serious polyphagous pest
Flowers	Other uses	-weather forecasting (the flowers close when wet weather is likely to occur)
Flowers	Ornamental	

3.2 Identify desirable quality characteristics that feedstock has to meet for mature industrial processes

Guidelines for pharmaceutical products:

Calendulae herba has a monograph in German Commission E monographs (BfArM)

- published successively until 1994 (corrections until 2002)

Calendulae flos has a monograph in European and British Pharmacopoeias, ESCOP, WHO, German Commission E, French Avis aux fabr. monographs and final report on EMEA (Herbal Medicinal Products committee assessment)

Plant material of interest for pharmaceutical purposes: Whole or cut, dried, and fully opened flowers that have been detached from the receptacle of the cultivated, double-flowered varieties of *Calendula officinalis* L.

As regards herbal medicines, European Pharmacopoeia and accepted monographs have specific terms for each vegetal product obtained.

Herbal substance (equivalent to the term "herbal drug" as defined in the European Pharmacopoeia) - which includes Calendulae flos

The product is usually used in dried form but sometimes fresh. The specification should be established on the basis of recent scientific data and should be set out in the same way as the European Pharmacopoeia monographs.

-Definition: Whole or cut, dried, fully opened flowers, which have been detached from the receptacle, of the cultivated, double-flowered varieties. Is also important to know the geographical source(s) and the conditions under which the herbal substance is obtained.

-Characters: a qualitative statement about the organoleptic character(s) where characteristic and the macroscopic and microscopic botanical characters of the herbal substance.

Microscopic characteristics

Inner epidermal cells of ray floret elongated, rectangular and almost straightwalled, cuticle faintly striated; stomata absent; outer epidermal cells similar, but with 3 or 4 anomocytic stomata; trichomes very numerous on the tube, biseriate; stigma epidermal cells straight-walled, polygonal. In disc floret, outer epidermal cells elongated, straight or slightly sinuous-walled, stomata absent; abundant trichomes on area below point of insertion of the stamens, mainly glandular, uniseriate or biseriate. Within the upper part of the anthers, a layer of isodiametric to elongated, moderately thick-walled, lignified and pitted cells; pollen grains spherical, up to 45µm in diameter, with 3 germinal pores, exine finely granular with numerous short spines; apex of stigma covered by short, bulbous papillae (2).

Powdered plant material

Yellow-green; fragments of corollas containing light yellow oil droplets; some corollas with fairly large anomocytic stomata, others containing prismatic and very small clusters of calcium oxalate crystals. Covering trichomes biseriate, multicellular and conical; glandular trichomes with a uniseriate or biseriate, multicellular stalk and a large, ovoid, biseriate, multicellular head. Spherical pollen grains up to 45µm in diameter, exine finely granular with numerous short spines and with 3 germinal pores; occasional fragments of stigmas with short, bulbous papillae (*2*).

Odour: faint, pleasantly aromatic; **taste**: bitter (*2*).

Other general identity tests: microchemical tests, and thin-layer chromatography for flavonoid content. (2)

Purity tests:

Foreign organic matter - Not more than 5% bracts and not more than 2% other foreign matter(3)

Total ash - Not more than 10% (3)

Acid-insoluble ash - Not more than 2% (3).

Water-soluble extractive - Not less than 20% (2).

Loss on drying - Not more than 10.0% (3).

Pesticide residues: The recommended maximum limit of aldrin and dieldrin is not more than 0.05 mg/kg (*2*).

Chemical assays

Contains not less than 0.4% flavonoids, calculated as hyperoside, by spectrophotometry (3).

The main constituents:

Triterpene saponins: 2-10% derivatives of the oleanolic acid with glucuronic acid on C3

Triterpene alcohols: free and esterified (with fatty acids) mono-, di- and triols of the y–taraxene-, taraxene-, lupine- and ursine-type. Approximately 0.8% Monols (a- and b-amyrin, lupeol, taraxasterol, y-taraxasterol), approx. 4% Diols, mostly in form of the mono esters (faradiols and arnidiol esters).

Carotenoids: up to 4.7%; predominately lutein and zeaxanthine (together up to 92% of total carotenoids).

The sesquiterpene lactone calendine is not a genuine constituent, the structure is identical with the xanthophyll degredation product loliolide.

Flavonoids: 0.3-0.8%; glycosides of isorhamnetin, quercetin

Coumarins: scopoletin, , umbeliferone, aesculetin

Volatile oil: 0.2-0.3%, mostly sesquiterpenes (e.g., a cadinol)

Water soluble polysaccharides: up to 15% (4)

Herbal preparation (equivalent to the term "herbal drug preparation" as defined in the European Pharmacopoeia) of *Calendula officinalis* are obtained by subjecting vegetal material to treatments such as extraction, distillation, expression, fractionation, purification, concentration or fermentation. These preparations include comminuted or powdered herbal substances, tinctures, extracts.

According to EMEA, accepted *herbal preparations for traditional use* are:

- Liquid extract 1:1, solvent ethanol (40-50% v/v).

B) Liquid extract: 1:1.8-2.2, extraction solvent ethanol 40-50% (v/v): ointments containing this liquid extract in a concentration of 10% have been on the Austrian market for more than 30 years, and in a concentration of 4% on the German market.

C) Tincture 1:5, solvent ethanol (70-90% v/v). Tincture contains not less than 0.1% flavonoids calculated as hyperoside.

D) Liquid extract: 1:10, solvent fatty vegetable oil, e.g. olive oil . Peanut oil, which is also mentioned in literature, is not recommended because of the higher probability of adverse reactions.

E) Calendula ointment: 1:5 – 1:25, extraction by digestion on the water bath using traditionally lard or hardened vegetable fat or petroleum jelly. The herbal substance may be moistened with ethanol prior to digestion. The ointment base is melted, subsequently the herbal substance is added. The time for extraction is up to 16 hours. After digestion the still liquid mixture is filtrated, the filtrate congeals with falling temperature.

F) Comminuted herbal substance for infusion.

Extracts prepared with supercritical CO2 and liquid solvents different to water or ethanol (e.g., isopropylmyristate, propyleneglycol, glycerol, diethylenglycol, polyethylenglycol) do not fulfil the requirements for traditional use. The same is true for the so called LACE-extract (laser activated Calendula extract, Jimenez-Medina E *et al* (2006)).

Calendula ointments prepared with liquid extracts or tinctures are not discussed as particular herbal preparations.

According to EMEA, approved *therapeutic indications* for Calendulae flos are:

Traditional use

-Traditional herbal medicinal product for the symptomatic treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds.

-Traditional herbal medicinal product for the symptomatic treatment of minor inflammations in the mouth or the throat.

Herbal medicinal products (the term includes "traditional medicinal product")

Calendula herbal medicinal products contain as active substances one or more herbal substances or one or more herbal preparations, or one or more such herbal substances in combination with one or more such herbal preparations.

Calendula flowers and extracts are used in combinations with many other herbal substances / herbal preparations.

The following tests and acceptance criteria are considered generally applicable to all herbal medicinal products:

-Description: A qualitative description of the dosage form should be provided (e.g., size, shape, colour). If colour changes occur during storage, a quantitative procedure may be appropriate.

-Identification: Identification tests should establish the specific identity of the herbal substance(s) and/or herbal preparation(s), in the herbal medicinal product and optimally should be discriminatory with regard to substitutes/adulterants that are likely to occur Identification solely by chromatographic retention time, for example, is not regarded as being specific; however, a combination of chromatographic tests (e.g. HPLC and TLC-densitometry) or a combination of tests into a single procedure, such as HPLC/UV-diode array, HPLC/MS, or GC/MS may be acceptable. In the case of herbal medicinal products containing powdered or comminuted herbal substances, microscopical and macroscopical characterisation could be used for identification in combination with other methods, if justified.

-Assay: In the case of products containing herbal substances and/or herbal preparations with constituents of known therapeutic activity, validated assays of the content of these constituents are required along with details of the analytical procedure(s). Where appropriate , a specific, stability-indicating procedure should be included to determine the content of the herbal substance(s) and/or herbal preparation(s) in the herbal medicinal product. In cases where use of a non-specific assay is justified, other supporting analytical procedures should be used to achieve overall specificity. In the case of herbal medicinal

products containing herbal substance(s) and/or herbal preparation(s) where the constituents with known therapeutic activity are not known, validated assays of active or analytical markers or other justified determinations are required. The choice of such markers should be justified. In cases where a specific assay of each active substance of a herbal medicinal product is not possible other justified determinations are required (for example, in multi-component traditional herbal medicinal products for human use the same markers may be present in more than one herbal substance/preparation).

-Impurities: Refer to the ICH/VICH Guidelines on impurities in new drug products/Guidelines on impurities in new veterinary products (CPMP/ICH/2738/99 and CVMP/VICH/838/99 as revised) and the European Pharmacopoeia General text on Residual Solvents for detailed information.

Impurities arising from the herbal substance(s) and/or herbal preparations e.g. Contaminants such as pesticide/fumigant residues, heavy metals, if controlled during the testing of the herbal substance/preparation, it is not necessary to test for these in the herbal medicinal product.

Similarly, residual solvent arising from the manufacture of the herbal preparation (e.g. an extract) need not be controlled in the herbal medicinal product provided it is appropriately controlled in the extract specification. However, solvents used for example in tablet coating will need to be controlled in the dosage form.

In cases where degradation products of the herbal substance/preparation are evident, they should be monitored in the herbal medicinal product. Acceptance limits should be stated for such degradation products.

-Microbial limits: There is a need to specify the total count of aerobic micro-organisms, the total count of yeasts and moulds, and the absence of specific objectionable bacteria. These limits should comply with the European Pharmacopoeia. The frequency of testing should be justified.

In addition to the universal tests listed above, the following tests may be considered applicable to *herbal medicinal products* on a case by case basis. Individual tests/criteria should be included in the specification when the tests have an impact on the quality of the herbal medicinal product for batch control.

Additional tests and acceptance criteria generally should be included for particular herbal medicinal products. The specific dosage forms addressed include **solid oral** herbal medicinal products, and **liquid oral** herbal medicinal products.

-Tablets (coated and uncoated) and hard capsules (One or more of these tests may also be applicable to soft capsules and granules) – dissolution/ disintegration, hardness/friability, uniformity of dosage units (this term includes both uniformity of content and uniformity of mass; a pharmacopoeial procedure should be used), water content, microbial limits (Microbial limit testing is seen as an attribute of Good Manufacturing Practice, as well as of quality assurance. Reference should be made to the European Pharmacopoeia general text on the Microbiological Quality of Pharmaceutical Preparations for guidance on acceptable limits. Periodic testing may be appropriate.)

-Oral liquids – uniformity of dosage units (This term includes both uniformity of content and uniformity of mass. Generally, acceptance criteria should be set for weight variation, fill volume, and/or uniformity of fill. Pharmacopoeial procedures should be used), pH, microbial limits, antimicrobial preservative content (The lowest specified concentration of antimicrobial preservative should be demonstrated to be effective in controlling micro-organisms by using the European Pharmacopoeia antimicrobial preservative effectiveness test. Antimicrobial preservative effectiveness should be demonstrated during development, during scale-up, and throughout the shelf-life), antioxidant preservative content, extractables (Generally, where development and stability data show no significant evidence of extractables from the container/closure system, elimination of this test may be proposed), alcohol content, dissolution, particle size distribution, redispersibility (for oral suspensions, which produce sediment), rheological properties, specific gravity, reconstitution time (Acceptance criteria for reconstitution time should be provided for dry powder products, which require reconstitution. The choice of diluent should be justified), water content (for oral products requiring reconstitution).

According to EMEA, there are no data available from controlled clinical studies using herbal preparations, containing the herbal substance Calendulae flos, as defined in the European Pharmacopoeia. In conclusion, Calendulae flos preparations can be regarded as traditional herbal medicinal products.

Herbal Medicinal Products containing exclusively herbal substances **(e.g. herbal teas)**

One or more of these tests may be applicable to herbal medicinal products containing exclusively herbal substances:

-loss on drying (To be specified depending on the plant parts present in the herbal medicinal product, if not performed on the herbal substance),
-identification (Identification tests must establish the specific identity of the herbal substance(s) in the herbal medicinal product and optimally should be discriminatory between the different herbal substances and with regards to substitutes/adulterants that are likely to occur.
-Microscopical and macroscopical characterisation can be used to support identification, if justified), -purity (Relevant adulterants and substitutes should be determined), uniformity of mass/average mass of the sachet (The dosage unit is considered to be the typical dose taken by the patient. Pharmacopoeial procedures should be used),
-assay (In the case of such herbal medicinal products containing herbal substances with constituents of known therapeutic activity, validated assays for these constituents are required along with details of the analytical procedure(s)),
-particle size,
-microbial quality.

A key issue in manufacturing herbal products and medicines is **standardization.** Standardization is the process of producing herbal extracts or phytochemicals in which product potency is guaranteed through consistency in active compound content level. This process requires high knowledge in phytochemical analysis and process technology to ensure the quality assurance required.

Product value increases in the following order: fresh material < dried powder < non-standardized extract < freeze/spray dried extract < standardized extract < phytomedicine.

Veterinary medicine

The homeopathic mother tincture for use in veterinary medicine is prepared according to homeopathic pharmacopoeias by ethanolic extraction of the fresh flowering aerial parts. The application relates to the homeopathic mother tincture which is intended for use in all food-producing animals for topical use only.(5)

Cosmetics

In the case of cosmetics, there are some standards for high quality products:

-BDIH "Certified Natural Cosmetics" - herbal extracts and essential oils and aromatic materials from controlled biological cultivation or controlled biological wild collection

-COSMOS standard for organic cosmetics

-ECOCERT standard for natural and organic cosmetics

Based on the available data, the CIR Expert Panel concluded that *Calendula officinalis* extract, *Calendula officinalis* flower, *Calendula officinalis* flower extract, *Calendula officinalis* flower oil and *Calendula officinalis* seed oil are safe as used in cosmetics and personal care products.

3.3 The report on raw materials from non - food crops as alternative to fossil, petroleum - based and chemical resources

There are 195 registered international patents concerning *Calendula officinalis* and just a part are applied in industry.

3.3.1 Herbal medicinal preparations and products

Usually herbal products are adjuvant in classical medicine or have a preventive action. In pharmacological testing, the activity is compared to synthetic drugs.

Ex. Because the effectiveness of nonsteroid topical agents for the prevention of acute dermatitis during adjuvant radiotherapy for breast carcinoma has not been demonstrated, the effectiveness of calendula (Pommade au Calendula par Digestion; Boiron Ltd, Levallois-Perret, France) with that of trolamine (Biafine; Genmedix Ltd, France), which is considered in many institutions to be the reference topical agent was compared. Calendula is highly effective for the prevention of acute dermatitis of grade 2 or higher and should be proposed for patients undergoing postoperative irradiation for breast cancer.(*4)*

Ex. Controlled study of three ointments for the local management of 2nd and 3rd degree burns Randomized, controlled, open study with parallel groups (only adults > 18 years of age; 53 patients treated with Pommade au Calendula par digestion, 53 patients treated with Elase (proteolytic ointment), 50 patients treated with vaseline (control treatment)). A marginally significant difference in favour of Calendula over vaseline was observed. Calendula was significantly better tolerated than the other treatments.(4)

Ex. Antiinflammatory activity-The hydro-alcoholic extract had a mild dose dependent effect, whereas the CO2 extract gave a 70% better efficacy when added on a drug-

equivalent basis and a 350% better efficacy based on the absolute extract dosage. Pure indometacin, one of the most potent non-steroidal anti-inflammatory drugs, was found to be only 10 times more active than the Calendula CO2 extract.(4)

- For traditional use, *Calendula officinalis* is sold in various forms: herbal tea (Natura Biertan, Fares, Plantavorel, Hofigal -Romania), tincture (Hofigal, Fares-Romania), tablets (Plantavorel-Romania), combinations (Natura Biertan, Hofigal, Fares -Romania)

-Homeopathy-*Calendula officinalis*, oral drops, solution, suppositories, ointment, tablets, granules, oral powder, Boiron (legal status-prescription only medicinal products)

3.3.2 Paint additive

The use of modified unsaturated vegetable oils as reactive diluents in solid in high-solid paints used to increase resistance or to decrease drying time. *Calendula oficinalis oil* contains fatty acids with conjugated triene functionality (calendic acid or A8t, lot, 12c-octadecatrienoic acid) very similar to the elaeostearic acid in tung oil from *Aleuritis fordii.* This oil presents an alternative source of these fatty acids, and can be utilized in the same applications as tung oil. The film-forming properties and film characteristics are expected to be very comparable to those of tung oil. The process in which vegetable oil from C. officinalis are transesterified to methyl esters for use in paint formulations is patented by DSM resins BV (European Patent EP 600 546, 1994). By following this route, the last 15-30% of organic solvents present in high solids can be replaced by methyl esters with conjugated double bonds, which become part of the polymer network upon exposure to air.

3.3.3 Cosmetics

-Cosmetic studies revealed interesting that *Calendula* stimulates the regeneration of skin cells and exerts a calming and relaxing effect, especially in irritated and inflamed skin. Therefore many cosmetic preparations, like face lotions, sun protection products and skin care preparations for babies contain *Calendula*.

-It is suggested that toothpaste containing *Calendula* flower alcoholic extract for gingivitis could be a useful aid to obtain a significant reduction compared to the placebo paste. It can be recommended as an adjuvant treatment to daily oral hygiene procedures. (6)

-Calendula also has the rare quality of keeping its color during soapmaking. Unlike most herbal additives, calendula will not turn brown or black in a bar of herbal soap.(Hofigal)

Calendula officinalis is used in cosmetic industry by many European manufacturers: Ryor, Alpa s.r.o-Czech Republic, Frantsila Farm , Glaeniline Lab-Finland, Alban Muller Group ,Sicobel Lab-France, Krautervital Kosmetik, Logona , Martina Gebhardt Naturkosmetik-Germany, Apivita, Benostan , Korres –Greece, Kelen Cosm, Silvestris&Szilas-Hungary, Arco Cosmetici, Aboca –Italy, Madara –Latvia, Original Cosmetics-Netherlands, Farmona, Bielenda, Phytopharma , ASA Ltd-Poland, Genmar, Plantavorel –Romania in a large variety of products: creams, lotions, solution for spa use, etc.

3.3.4 Agriculture

Utilizing allelopathic plants to suppress the weed infestation is the most cost-effective and environment-friendly method of weed control. Activity of allelochemical compounds varies with several external factors (temperature, photoperiod, water and soils) as well as with their initial concentration, compound structure and operation processes.

Allelopathic crops offer strong potential for the development of cultivars that are more highly weed suppressive in managed settings. 3-O-monoglucoside of oleanolic acid secreted to the soil by *Calendula* species possesses very strong allelophatic properties in relation to the dicotyledons and weaker activity to the monocotyledons.

3.3.5 Veterinary

VAGIZAN® (foam with Calendula extract) is a high quality natural product for the skin care and is appropriate for different animal species. It is particularly recommended for the skin care between udder and inner thigh of dairy cows. In this area the skin is often overstrained through friction.(Casa Verde Naturprodukte)

3.3.6 Other intermediary products

Hydroalcoholic extract: Phytosan-France

Liquid extract -Phytex-Bulgaria, Epo Srl-Italy, Phytopharm-Poland

Dry extract– Phytex-Bulgaria, Epo Srl-Italy, Phytopharm-Poland

Mother tincture- Epo Srl-Italy

Glycerine extract- Phytosan-France, Epo Srl-Italy, Calendula-Slovakia

CO2 Extract - Standardized

3.4 Set prospects to widen the range of potential feedstock for the understudy industrial uses, based on the technology improvements

Technologies improving the quality of preliminary raw material (e.g. breeding varieties with better gene expression together with improvement of technology of: cultivation, harvesting and processing) as factor determining further application.

New and improved *Calendula* varieties

- new *Calendula* cultivars resistant to aphids attack

Improving the yield and quality of active principles

Plant cell cultures

- Ex. *Trichoderma viride* homogenate for induction of oleanolic acid synthesis in *Calendula* cell suspension culture. Rhizobacteria that exert beneficial effects on plant growth and development are referred to as Plant Growth Promoting Rhizobacteria (PGPR). PGPR can affect plant growth either indirectly or directly; indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effects of one or more phytopathogenic organisms; while direct promotion of plant growth by PGPR involves either providing plants with a compound synthesized by the bacterium or facilitating the uptake of certain nutrients from the environment..*(7*)

-*In vitro* shoot regeneration and micropropagation (8)

-Innovative and controlled culture methods – hydroponics and aeroponics

-Elicitation and molecular studies for the development of improved sources for commercial supply of bioactive saponins. The enhancement of saponin yields by methyl jasmonate in plants and cell cultures in several species indicates the involvement of these metabolites in plant defense mechanisms. Yields of bioactive saponins in various plant species and experimental systems have been successfully increased by treating cells and tissues with jasmonate or by exposing these to oxidative stress.

New products based on improvement of extraction technology, formulation technology and identification of new medicinal applications

Extraction technology

-High-pressure solvent extraction (HPSE) is becoming increasingly popular in the chemical, food and pharmaceutical areas. Extraction by means of CO2, extensively studied in the last decade, is a good technique for the production of flavors and fragrances from plant material. Conventional processes, such as steam distillation, solvent extraction, *etc.,* often require additional steps, such as separating the extract, and their selectivity is usually inferior to that of CO2. Due to the lower temperature and low water content in HPSE, thermal degradation and hydrolysis are avoided. The extract obtained in this manner contains all active compounds unaltered from the plant and exhibits a scent more similar to the starting material. The amounts of essential oil with respect to the plant material obtained from the CO2 extracts under the investigated conditions is considerably higher (1.52–2.70 times) than the one determined by the official procedure. This type of extraction is suitable for *Calendula officinalis* L., in which an important part of essential oil is dissolved in fatty oil, waxes and resins. Using steam distillation, the official procedure for the determination of the content of essential oil according to all world Pharmacopoeias, only the "free" essential oil from the plant, accessible to steam, is extracted. Application of high pressure CO2 extraction enables, beside the "free" essential oil extraction, fatty oil, waxes and resins (carrying essential oil dissolved in them) to be extracted. (9)

-Extraction of marigold with supercritical CO2 is known to yield extracts with a high content in triterpenoid esters. The concomitant extraction of pigments such as carotenoids, however, is disadvantageous for a subsequent isolation of triterpenoid esters. An extract with a high content in triterpene fatty acid esters could be obtained by a combination of supercritical fluid extraction (SFE) with CO2 and online adsorption of carotenoids and other pigments. Extraction provides a satisfactory compromise between yield of triterpenoids (approx. 75% of content in the herb extracted) and removal of pigments (approx. 95% of colored matter). Starting from an optimized SFE extract, followed by filtration over silica gel, the LPLC (low-pressure liquid chromatography) separation afforded highly enriched triterpene ester fractions in multigram quantities.(10)

-new method for preparing high-content lutein crystal with a pot marigold extract, which can be applied to the fields of food colorants, food additives, pharmaceutical health products.

New pharmaceutical applications which were already proved on animal models:

Some potential pharmacological effects of various parts of *Calendula* need to be further investigated on clinical trials:

-virucidal effect

-immunostimulation

-antitumour

-spasmogenic and spasmolytic

-hepatoprotective

New pharmaceutical forms with targeted delivery

-nanotechnology

-liposome technology

-development of modern extraction technologies (microwave, supercritical CO2, fluid bed granulation)

Other uses

Recent studies revealed considerable efficiency of chemical enhancement and correspondingly increased potential of *Calendula officinalis* L. for applications of phytoremediation of Cd contaminated sites.

Also it was showed that the fluid extract of marigold possess a certain aphicidic activity (the mean corrected mortality reached 51 %), but the efficacy is much less than that of the synthetic insecticides usually used to control apple aphid.

3.5 Identify restricting factors that inhibit broader industrial use of the biomass feedstock (supply, costs, physical traits, consistency in quality, technical performance, research gaps, etc.)

3.5.1 Agricultural raw material

Physical traits

-supply – depends on weather conditions

-*Calendula* harvest is a difficult operation that requires attention and manual labor. The operation is done in several phases, from June to October, as the first flowers have opened their ligulate inflorescences, starting at about 65-70 days after rising.

-Saline irrigation water decreases the fresh and dry weights of flower heads, and pigment contents (total flavonoids and total carotenoids) but increased essential oil yield and its main components (α-cadinol, γ- and Δ-cadinene) of *Calendula officinalis*. Fresh and dry weights of flower heads and essential oil increased towards the full bloom stage of flowering while pigment content, such as total flavonoids and total carotenoids, increased.(11)

Consistency in quality

-Morphological variability is seen in cultivars of *Calendula officinalis* L with the appearance of plants with flowers of varied size and coloration, compromising the standardization of the material of interest. This marked variation in plant anatomy allied to the diverse forms of cultivation employed in the field means that the quality and quantity of bioactive products in this plant remain unknown.

- colour is important and must be retained by careful drying.

Costs

-the main costs for production are the specialised harvest which requires manual labor. Hand-picking results in a superior product as the comb method will capture buds, over mature flowers, and seed heads. The combed product either has to be picked over to remove the unwanted material or must be sold at a lower price.

-if the desired raw material is represented by seeds, transportation over any type of distance will be a big issue. This product has such a low bulk density that it will be uneconomical to produce and transport far from the processor.

- for most applications raw material must be free of impurities or other plant parts (especially when harvest is done mechanically) and requires additional operations incurring additional costs

3.5.2 Industrial raw material

Research gaps

-There are no clinical studies on the efficacy of Calendulae flos, but just in combination with other plants, given no reason for the inclusion on the well-established use. (that is why EMEA doesn't included a well-established use for Calendulae flos in the monograph)

-researches concerning understudied compounds like polysaccharides.

Costs

-High costs of clinical trials, research for new applications and innovative products development

3.6 Set forth research gaps, prospects and recommendations to procure bio - based products will be tackled

3.6.1 Agriculture raw material

Basic research

-Organic Certification – In general, small growers will end up marketing their product directly to consumers who want organic production.

-The decreased cost of synthetic fertilizer and pesticide inputs, along with the higher prices that consumers pay for organic produce, contribute to increased profits.

-The maximum content of carotenoid pigments, a main component of the drug in marigolds, is maximal in the inflorescences of orange varieties (like Bellezza del Pacifico), which pleads for their initiation of marigold cultures.

-Breeding work should focus on varieties with a greater number of ray florets in order to improve the quality of herbal medicinal products derived from marigold or on varieties with high carotenoid content

Applied research

-Improvement of the harvest technology

3.6.2 Industrial raw material

Basic research

-cost-effective and environment-friendly method of weed control using allelopathic plants like *Calendula* to suppress the weed infestation

-clinical studies for herba, volatile oil effectiveness

Applied research

-modern processing technologies – microwave-assisted extraction, ultrasound extraction, accelerated solvent extraction, superfluid extraction

-technology improvement for chemical analysis and standardization

-technology improvement for conditioning and formulation drugs and cosmetics (for ex. immediate or delayed release capsules, effervescent tablets, tea bags, dispensers for local application, etc).

Prospects and recommendations

-financial help for cultivation; it is important to understand the importance of controlled conditions on final product quality

-Need for legislative harmonization in EU27 countries. The same drug is sold as medicine in a country and as dietary supplement in other.

-know the nature of the desired product. In the case of medicinal plants which are used directly as pharmaceuticals, the quality and thus the concentration of active compounds is much more relevant than the total yield.

-ensuring product quality by standardization

-modern processing technologies in order to reduce heat degradation, reduce processing costs, faster extraction, much lower energy usage, less solvent usage

-good management of wastes

-strong marketing strategy for natural, innovative and high quality products

References:

- Plant for Future Database www.pfaf.org
- WHO monographs on selected medicinal plants, 1999, vol. 2, p. 35-44 (Calendulae flos) European pharmacopoeia, 3rd ed. Suppl. 2001. Strasbourg, Council of Europe, 2000
- British Pharmacopoeia, 2009
- European Medicine Agency – Community herbal monograph on *Calendula officinalis* L., flos, 2008
- European Medicine Agency – Calendula officinalis, use in veterinary homeopathy, summary report, 1999
- B. Amoian, A. Moghadamnia, M. Mazandarani, M. Amoian, S. Mehrmanesh -The effect of Calendula extract toothpaste on the plaque index and bleeding in gingivitis., Research Journal of Medicinal Plant 4(3):132-140, 2010
- *S. Sekar, D. Kandavel* -Interaction of plant growth promoting rhizobacteria (PGPR)

and endophytes with medicinal plants – new avenues for phytochemicals**.,** *Journal of Phytology 2010, 2(7): 91-100*

- A. Cocu, S. Uranbey, A. Ipek, K. Khawar, E. Sarihan, M. Kaya, I. Parmaksiz, S. Ozcan- Adventitious Shoot Regeneration and Micropropagation in Calendula officinalis L. , Biologia Plantarum, vol. 48, no. 3, 449-451, 2004
- L.Petrovic, I. Lepojevi, V. Sovilj, D. Adamovic, V. Tesevic -An investigation of CO2 extraction of marigold *Calendula officinalis* L., *J. Serb. Chem. Soc. 72* (4) 407–413, 2007
- M. Hamburger, S. Adler, D. Baumann, A. Forg, B. Weinreich -Preparative purification of the major anti-inflammatory triterpenoid esters from marigold *Calendula officinalis*,, Fitoterapia 74 328–338, 2003
- K. Khalid, J. Teixeira da Silva -Yield, essential oil and pigment content of *Calendula officinalis* L. flower heads cultivated under salt stress conditions,, Scientia Horticulturae, vol. 126, issue 2, p. 297-305, 2010

4 Echinacea angustifolia DC (Asteraceae)

4.1 Review on the product yielding capacity from various industrial crops streams

According to Plant for Future Platform (1), the main uses for *Echinacea angustifolia* are:

Agriculture Raw Materials	**Directions of use**	**Product example**
The whole plant, but especially the root	Phytotherapy	-immunostimulant -treatment of sores, wounds, burns -adaptogen, alterative, antiseptic, depurative, digestive
Plant	Cosmetics	-beauty products (shampoo, soap, crème, lotions, etc)
Plant, especially the root	Veterinary	Herbal feed supplement with immunostimulant, coccidiostatic and antibiotic activity
Flowers	Ornamental	

4.2 Identify desirable quality characteristics that feedstock has to meet for mature industrial processes

Guidelines for pharmaceutical products:

Calendulae herba has a monograph in German Commission E monographs (BfArM)

- published successively until 1994 (corrections until 2002)

Calendulae flos has a monograph in European and British Pharmacopoeias, ESCOP, WHO, German Commission E, French Avis aux fabr. monographs and final report on EMEA (Herbal Medicinal Products committee assessment)

Plant material of interest for pharmaceutical purposes: Whole or cut, dried, and fully opened flowers that have been detached from the receptacle of the cultivated, double-flowered varieties of *Calendula officinalis* L.

As regards herbal medicines, European Pharmacopoeia and accepted monographs have specific terms for each vegetal product obtained.

Herbal substance (equivalent to the term "herbal drug" as defined in the European Pharmacopoeia) - which includes Calendulae flos

The product is usually used in dried form but sometimes fresh. The specification should be established on the basis of recent scientific data and should be set out in the same way as the European Pharmacopoeia monographs.

-Definition: Whole or cut, dried, fully opened flowers, which have been detached from the receptacle, of the cultivated, double-flowered varieties. Is also important to know the geographical source(s) and the conditions under which the herbal substance is obtained.

-Characters: a qualitative statement about the organoleptic character(s) where characteristic and the macroscopic and microscopic botanical characters of the herbal substance.

Microscopic characteristics

Inner epidermal cells of ray floret elongated, rectangular and almost straightwalled, cuticle faintly striated; stomata absent; outer epidermal cells similar, but with 3 or 4 anomocytic stomata; trichomes very numerous on the tube, biseriate; stigma epidermal cells straight-walled, polygonal. In disc floret, outer epidermal cells elongated, straight or slightly sinuous-walled, stomata absent; abundant trichomes on area below point of insertion of the stamens, mainly glandular, uniseriate or biseriate. Within the upper part of the anthers, a layer of isodiametric to elongated, moderately thick-walled, lignified and pitted cells; pollen grains spherical, up to 45µm in diameter, with 3 germinal pores, exine finely granular with numerous short spines; apex of stigma covered by short, bulbous papillae (2).

Powdered plant material

Yellow-green; fragments of corollas containing light yellow oil droplets; some corollas with fairly large anomocytic stomata, others containing prismatic and very small clusters of calcium oxalate crystals. Covering trichomes biseriate, multicellular and conical; glandular trichomes with a uniseriate or biseriate, multicellular stalk and a large, ovoid, biseriate, multicellular head. Spherical pollen grains up to 45µm in diameter, exine finely granular with numerous short spines and with 3 germinal pores; occasional fragments of stigmas with short, bulbous papillae (*2*).

Odour: faint, pleasantly aromatic; **taste**: bitter (*2*).

Other general identity tests: microchemical tests, and thin-layer chromatography for flavonoid content. (2)

Purity tests:

Foreign organic matter - Not more than 5% bracts and not more than 2% other foreign matter(3)

Total ash - Not more than 10% (3)

Acid-insoluble ash - Not more than 2% (3).

Water-soluble extractive - Not less than 20% (2).

Loss on drying - Not more than 10.0% (3).

Pesticide residues: The recommended maximum limit of aldrin and dieldrin is not more than 0.05 mg/kg (*2*).

Chemical assays

Contains not less than 0.4% flavonoids, calculated as hyperoside, by spectrophotometry (3).

The main constituents:

Triterpene saponins: 2-10% derivatives of the oleanolic acid with glucuronic acid on C3

Triterpene alcohols: free and esterified (with fatty acids) mono-, di- and triols of the y–taraxene-, taraxene-, lupine- and ursine-type. Approximately 0.8% Monols (a- and b-amyrin, lupeol, taraxasterol, y-taraxasterol), approx. 4% Diols, mostly in form of the mono esters (faradiols and arnidiol esters).

Carotenoids: up to 4.7%; predominately lutein and zeaxanthine (together up to 92% of total carotenoids).

The sesquiterpene lactone calendine is not a genuine constituent, the structure is identical with the xanthophyll degredation product loliolide.

Flavonoids: 0.3-0.8%; glycosides of isorhamnetin, quercetin

Coumarins: scopoletin, , umbeliferone, aesculetin

Volatile oil: 0.2-0.3%, mostly sesquiterpenes (e.g., a cadinol)

Water soluble polysaccharides: up to 15% (4)

Herbal preparation (equivalent to the term "herbal drug preparation" as defined in the European Pharmacopoeia) of *Calendula officinalis* are obtained by subjecting vegetal material to treatments such as extraction, distillation, expression, fractionation, purification, concentration or fermentation. These preparations include comminuted or powdered herbal substances, tinctures, extracts.

According to EMEA, accepted *herbal preparations for traditional use* are:

- Liquid extract 1:1, solvent ethanol (40-50% v/v).

B) Liquid extract: 1:1.8-2.2, extraction solvent ethanol 40-50% (v/v): ointments containing this liquid extract in a concentration of 10% have been on the Austrian market for more than 30 years, and in a concentration of 4% on the German market.

C) Tincture 1:5, solvent ethanol (70-90% v/v). Tincture contains not less than 0.1% flavonoids calculated as hyperoside.

D) Liquid extract: 1:10, solvent fatty vegetable oil, e.g. olive oil . Peanut oil, which is also mentioned in literature, is not recommended because of the higher probability of adverse reactions.

E) Calendula ointment: 1:5 – 1:25, extraction by digestion on the water bath using traditionally lard or hardened vegetable fat or petroleum jelly. The herbal substance may be moistened with ethanol prior to digestion. The ointment base is melted, subsequently the herbal substance is added. The time for extraction is up to 16 hours. After digestion the still liquid mixture is filtrated, the filtrate congeals with falling temperature.

F) Comminuted herbal substance for infusion.

Extracts prepared with supercritical CO2 and liquid solvents different to water or ethanol (e.g., isopropylmyristate, propyleneglycol, glycerol, diethylenglycol, polyethylenglycol) do not fulfil the requirements for traditional use. The same is true for the so called LACE-extract (laser activated Calendula extract, Jimenez-Medina E *et al* (2006)).

Calendula ointments prepared with liquid extracts or tinctures are not discussed as particular herbal preparations.

According to EMEA, approved *therapeutic indications* for Calendulae flos are:

Traditional use

-Traditional herbal medicinal product for the symptomatic treatment of minor inflammations of the skin (such as sunburn) and as an aid in healing of minor wounds.

-Traditional herbal medicinal product for the symptomatic treatment of minor inflammations in the mouth or the throat.

Herbal medicinal products (the term includes "traditional medicinal product")

Calendula herbal medicinal products contain as active substances one or more herbal substances or one or more herbal preparations, or one or more such herbal substances in combination with one or more such herbal preparations.

Calendula flowers and extracts are used in combinations with many other herbal substances / herbal preparations.

The following tests and acceptance criteria are considered generally applicable to all herbal medicinal products:

-Description: A qualitative description of the dosage form should be provided (e.g., size, shape, colour). If colour changes occur during storage, a quantitative procedure may be appropriate.

-Identification: Identification tests should establish the specific identity of the herbal substance(s) and/or herbal preparation(s), in the herbal medicinal product and optimally should be discriminatory with regard to substitutes/adulterants that are likely to occur Identification solely by chromatographic retention time, for example, is not regarded as being specific; however, a combination of chromatographic tests (e.g. HPLC and TLC-densitometry) or a combination of tests into a single procedure, such as HPLC/UV-diode array, HPLC/MS, or GC/MS may be acceptable. In the case of herbal medicinal products containing powdered or comminuted herbal substances, microscopical and macroscopical characterisation could be used for identification in combination with other methods, if justified.

-Assay: In the case of products containing herbal substances and/or herbal preparations with constituents of known therapeutic activity, validated assays of the content of these constituents are required along with details of the analytical procedure(s). Where appropriate , a specific, stability-indicating procedure should be included to determine the content of the herbal substance(s) and/or herbal preparation(s) in the herbal medicinal product. In cases where use of a non-specific assay is justified, other supporting analytical procedures should be used to achieve overall specificity. In the case of herbal medicinal products containing herbal substance(s) and/or herbal preparation(s) where the constituents with known therapeutic activity are not known, validated assays of active or analytical markers or other justified determinations are required. The choice of such markers should be justified. In cases where a specific assay of each active substance of a herbal medicinal product is not possible other justified determinations are required (for example, in multi-component traditional herbal medicinal products for human use the same markers may be present in more than one herbal substance/preparation).

-Impurities: Refer to the ICH/VICH Guidelines on impurities in new drug products/Guidelines on impurities in new veterinary products (CPMP/ICH/2738/99 and CVMP/VICH/838/99 as revised) and the European Pharmacopoeia General text on Residual Solvents for detailed information.

Impurities arising from the herbal substance(s) and/or herbal preparations e.g. Contaminants such as pesticide/fumigant residues, heavy metals, if controlled during the testing of the herbal substance/preparation, it is not necessary to test for these in the herbal medicinal product.

Similarly, residual solvent arising from the manufacture of the herbal preparation (e.g. an extract) need not be controlled in the herbal medicinal product provided it is appropriately controlled in the extract specification. However, solvents used for example in tablet coating will need to be controlled in the dosage form.

In cases where degradation products of the herbal substance/preparation are evident, they should be monitored in the herbal medicinal product. Acceptance limits should be stated for such degradation products.

-Microbial limits: There is a need to specify the total count of aerobic micro-organisms, the total count of yeasts and moulds, and the absence of specific objectionable bacteria. These limits should comply with the European Pharmacopoeia. The frequency of testing should be justified.

In addition to the universal tests listed above, the following tests may be considered applicable to *herbal medicinal products* on a case by case basis. Individual tests/criteria should be included in the specification when the tests have an impact on the quality of the herbal medicinal product for batch control.

Additional tests and acceptance criteria generally should be included for particular herbal medicinal products. The specific dosage forms addressed include **solid oral** herbal medicinal products, and **liquid oral** herbal medicinal products.

-Tablets (coated and uncoated) and hard capsules (One or more of these tests may also be applicable to soft capsules and granules) – dissolution/ disintegration, hardness/friability, uniformity of dosage units (this term includes both uniformity of content and uniformity of mass; a pharmacopoeial procedure should be used), water content, microbial limits (Microbial limit testing is seen as an attribute of Good Manufacturing Practice, as well as of quality assurance. Reference should be made to the European Pharmacopoeia general text on the Microbiological Quality of Pharmaceutical Preparations for guidance on acceptable limits. Periodic testing may be appropriate.)

-Oral liquids – uniformity of dosage units (This term includes both uniformity of content and uniformity of mass. Generally, acceptance criteria should be set for weight variation, fill volume, and/or uniformity of fill. Pharmacopoeial procedures should be used), pH, microbial limits, antimicrobial preservative content (The lowest specified concentration of

antimicrobial preservative should be demonstrated to be effective in controlling micro-organisms by using the European Pharmacopoeia antimicrobial preservative effectiveness test. Antimicrobial preservative effectiveness should be demonstrated during development, during scale-up, and throughout the shelf-life), antioxidant preservative content, extractables (Generally, where development and stability data show no significant evidence of extractables from the container/closure system, elimination of this test may be proposed), alcohol content, dissolution, particle size distribution, redispersibility (for oral suspensions, which produce sediment), rheological properties, specific gravity, reconstitution time (Acceptance criteria for reconstitution time should be provided for dry powder products, which require reconstitution. The choice of diluent should be justified), water content (for oral products requiring reconstitution).

According to EMEA, there are no data available from controlled clinical studies using herbal preparations, containing the herbal substance Calendulae flos, as defined in the European Pharmacopoeia. In conclusion, Calendulae flos preparations can be regarded as traditional herbal medicinal products.

Herbal Medicinal Products containing exclusively herbal substances **(e.g. herbal teas)**

One or more of these tests may be applicable to herbal medicinal products containing exclusively herbal substances:

-loss on drying (To be specified depending on the plant parts present in the herbal medicinal product, if not performed on the herbal substance),

-identification (Identification tests must establish the specific identity of the herbal substance(s) in the herbal medicinal product and optimally should be discriminatory between the different herbal substances and with regards to substitutes/adulterants that are likely to occur.

-Microscopical and macroscopical characterisation can be used to support identification, if justified), -purity (Relevant adulterants and substitutes should be determined), uniformity of mass/average mass of the sachet (The dosage unit is considered to be the typical dose taken by the patient. Pharmacopoeial procedures should be used),

-assay (In the case of such herbal medicinal products containing herbal substances with constituents of known therapeutic activity, validated assays for these constituents are required along with details of the analytical procedure(s)),

-particle size,
-microbial quality.

A key issue in manufacturing herbal products and medicines is **standardization.** Standardization is the process of producing herbal extracts or phytochemicals in which product potency is guaranteed through consistency in active compound content level. This process requires high knowledge in phytochemical analysis and process technology to ensure the quality assurance required.

Product value increases in the following order: fresh material < dried powder < non-standardized extract < freeze/spray dried extract < standardized extract < phytomedicine.

Veterinary medicine

The homeopathic mother tincture for use in veterinary medicine is prepared according to homeopathic pharmacopoeias by ethanolic extraction of the fresh flowering aerial parts. The application relates to the homeopathic mother tincture which is intended for use in all food-producing animals for topical use only.(5)

Cosmetics

In the case of cosmetics, there are some standards for high quality products:

-BDIH "Certified Natural Cosmetics" - herbal extracts and essential oils and aromatic materials from controlled biological cultivation or controlled biological wild collection

-COSMOS standard for organic cosmetics

-ECOCERT standard for natural and organic cosmetics

Based on the available data, the CIR Expert Panel concluded that *Calendula officinalis* extract, *Calendula officinalis* flower, *Calendula officinalis* flower extract, *Calendula officinalis* flower oil and *Calendula officinalis* seed oil are safe as used in cosmetics and personal care products.

4.3 The report on raw materials from non - food crops as alternative to fossil, petroleum - based and chemical resources

There are 88 registered international patents concerning *Echinacea angustifolia* and just a part are applied in industry.

4.3.1 Herbal medicinal preparations and products

Usually herbal products are adjuvant in classical medicine or have a preventive action. In pharmacological testing, the activity is compared to synthetic drugs.

Ex. ECHIGENA PluS is the only biotech extract (cell cultures) with a defined and standardized composition profile that is uniquely able to guarantee a batch-to-batch reproducible efficacy. A clinical test performed on athletes has demonstrated that the daily consumption of Echigena PluS significantly controls the oxidative stress damage by reducing the plasma liperoxide levels more than 30% after a 30-day treatment.(IRB, Istituto di Ricerche Biotecnologiche, Italy)

Ex. PolinaceaTM is a new immunomodulating E. angustifolia standardized extract. Considering the multiplicity of activities referred to Echinacea the biological effects may not be attributed to a single component: the standardized extract from E. angustifolia developed and patented* by Indena has a triple standardization that makes it different from all other Echinacea derivatives. The extract is obtained from its roots. It contains echinacoside (≥2%), and a very unique high molecular weight polysaccharide characterized by a backbone of a partially carboxymethylated and partially acetylated polygalacturonic acid with a hairy region of rhamnogalacturonan (≥5%), named IDN 5405. IDN 5405 is a polysaccharide that has been highlighted for the first time in the root of E. angustifolia. (Indena, Italy)

Ex. ECHINACEA LIPOSPRAY 4% STANDARDIZED, 60 MG PER SPRAY This product has an excellent Echinacea angustifolia taste in a natural fruit flavor base. Great tasting for kids.(Clinical Resolution Laboratory, USA)

Ex. ANXIOFIT-1, tablets, dietary supplement, based on *Echinacea angustifolia* roots. The anxiety relieving effects are produced at low doses by active compounds that work only in a narrow therapeutic window of activity. (EuroPharma USA)

For traditional use, *Echinacea angustifolia* (herb and roots) is sold in various forms: herbal tea (Fares, Plantavorel-Romania), tablets (Plantavorel-Romania), syrup (Fares-

Romania), combinations (Fares-Romania)

-Homeopathy - *Echinacea angustifolia* granule for oral use - Prescription-only medicinal products (Boiron)

4.3.2 Cosmetics

Echinacea angustifolia is used in cosmetic industry by many European manufacturers: Glaeniline Lab-Estonia, Hankintatukku-Finland, Krautervital Kosmetik, Logona, Salus – Germany, Korres –Greece, Herbaria –Hungary, Phytopharma-Poland, Hofigal-Romania, Calendula -Slovakia in a large variety of products: creams, lotions, solution for spa use, etc.

Ex. Preparation of 20% of *Echinacea angustifolia* stem cells in plant glycerine, without preservatives. Echinacea angustifolia stems G increases hydration by 28% and elasticity by 11%. Cosmetic uses: improving dull and tired skin, replenishing and restructuring treatments, prevention of collagen loss. Recommended concentration: 0,5 – 3% (IRB, Istituto di Ricerche Biotecnologiche, Italy)

4.3.3 Veterinary

Ex. Immuzime (*Echinacea angustifolia* root and *Echinacea pallida* root extract)-oral prescription animal drug

Echinacea effectively stimulates equine immunocompetence, and the plant extract behaves, in equine subjects, as a haematinic agent, i.e. one which improves the quality of blood by increasing haemoglobin levels and the number of erythrocytes and which, by virtue of their effects on oxygen transport cells, are considered to improve parameters of exercise physiology and performance.(5)

Results from experiments comparing *Echinacea* application with that of subtherapeutic antibiotics suggest that *Echinacea* may be a good substitute for antibiotics in feed, and results in equal or better performance parameters. It would be of great value to repeat these studies with an *Echinacea* product that is both standardized and characterized, in order to enhance repeatability by other investigators.

A German-language publication describes an *in vivo* study comparing the effect of a drug complex containing 30% *Echinacea angustifolia* extract, and pure *E. angustifolia* extract in the humoral immune response of intact and immunodeficient chickens. In normal chickens, the administration of the complex drug resulted in a rise in serum immunoglobulin concentration, as well as an increase in the three classes of antibodies. In immunodeficient chickens, the complex drug caused a slight production of IgG.

4.3.4 Other intermediary products

Hydroalcoholic extract –Epo Srl-Italy, Alban Muller, Phytosan-France

Liquid extract- Epo Srl-Italy, Calendula-Slovakia

Dry extract – Alban Muller-France, Epo Srl-Italy, Calendula-Slovakia

Mother tincture-Phytosan-France, Epo Srl-Italy

Glycerine extract- Phytosan-France, Plantavorel, Calendula-Slovakia

4.4 Set prospects to widen the range of potential feedstock for the understudy industrial uses, based on the technology improvements

Technologies improving the quality of preliminary raw material (e.g. breeding varieties with better gene expression together with improvement of technology of: cultivation, harvesting and processing) as factor determining further application.

New and improved varieties

-*Echinacea angustifolia* hybrids with large flowers with wide petals (for ornamental value), great resistance to wet soils and shade and resistant to diseases.

Improve yield and quality of constituents

Even *Echinacea* in cultivation for many years contains considerable phytochemical variation between individual plants. Quality assurance can be partially addressed through germplasm enhancement to develop consistently characterized plant materials and to minimize phytochemical variation in crop situations. For instance, identification of specific genetic markers leading to phytochemical variation may enable optimal selection of genetic resources for cultivation. Also, the environmental variation imposed externally in the plant production process needs to be factored into improved cultivation methods.(6)

In vitro studies

According to the provision of Article 5 of Regulation (EC) 258/97 on novel foods and novel food ingredients, the Commission for Nutrition and Dietetics has recognised the substantial equivalence of the extracts from plant cell cultures compared with the traditional extracts from plants. Products are obtained by the exclusive HTN (High Tech Nature) technology, an innovative industrial process able to shorten the distance to the ideal profile of the active plant ingredient:

• Free from any type of pollutant
• Guaranteed high titre and reproducible composition profile
• Unlimited and planned availability
• Fully eco-sustainable production

- Liquid cell cultures (derived from callus or directly from explant material) provide a source of material for various metabolic studies or for transformation studies
- Micropropagation and callus production may for extraction of medicinally important compounds. T
- Hairy roots culture for production of alkamides
- Microarray analysis and metabolomics may answer these questions through identification of active genes and secondary metabolites associated with plant developmental stages and as influenced by environmental treatments and controls.
- Controlled cultivation methods – for ex. hydroponic culture

New products based on improvement of extraction technology, formulation technology and identification of new medicinal applications

New pharmaceutical applications which were already proved on animal models:

Some potential pharmacological effects of *Echinacea angustifolia* roots and aerial parts need to be further investigated

-antimicrobial activity

-antineoplastic activity

-alkamides as cannabinomimetics

Applications in veterinary medicine need to be further investigated to determine posology, mechanism of action and route of administration.

All studies concerning the efficacy of *Echinacea* should test the same preparation obtained by the same extraction procedure and having the same content of marker compounds.

New pharmaceutical forms with targeted delivery

-nanotechnology

-liposome technology

-The use of phytosomes is a new advanced modern dosage formulation technology to deliver herbal products and drugs by improved better absorption and, as a result, produce better results than those obtained by conventional herbal extracts. This phytosome technology is a breakthrough model for marked enhancement of bioavailability, significantly greater clinical benefit, assured delivery to the tissues, and without compromising nutrient safety. They permeate the non-lipophillic botanical extract to allow better absorption from the intestinal lumen, which is otherwise not possible. The formulation of phytosomes is safe and the components have all been approved for pharmaceutical and cosmetic use. This technology offers cost-effective delivery of phytoconstituents and synergistic benefits when used as functional cosmetics to protect the skin against exogenous or endogenous hazards in normal as well as stressful environmental conditions. They can be also used for enhanced permeation of drug through skin for transdermal and dermal delivery (application in cosmetics).

4.5 Identify restricting factors that inhibit broader industrial use of the biomass feedstock (supply, costs, physical traits, consistency in quality, technical performance, research gaps, etc.)

4.5.1 Agricultural raw material

Physical traits

-cultivation factors, both growing site and harvest stage, can have major effects on alkamide and phenolic quality indicator levels,

-echinacoside content is affected by potassium supply

-roots could be harvested after 3 years. Some farmers prefer to harvest herba after 1-2 years for a yearly income (in this case the quality of roots decreases).

- mechanization is the main mechanism involved in large-scale cultivation of *Echinacea.* Without proper machinery, the cultivation areas and the quantity of the harvested raw materials remain small and costly, and labor intensive.

-metabolite degradation may occur in roots which do not undergo dehydration, and the best posthandling process is oven-drying at 50°C or higher temperature.

Consistency in quality

- most of the loss of active constituents arises from suboptimal handling and drying practices.

Costs

-specialised harvest

- for most applications raw material must be free of impurities or other plant parts (especially when harvest is done mechanically) and requires additional operations incurring additional costs

-if the desired raw material is represented by roots, it needs time and additional costs for the plant to mature

4.5.2 Industrial raw material

Physical traits

- active compounds purification procedures may not be always feasible at industrial level

- processing methodology will affect the level of the different components extracted. The level of polysaccharides will be much lower if alcohol extraction is used during preparation.

Consistency in quality

-Quality control is a serious problem in *Echinacea* phytomedicines with multiple activities, species, and formulations.

-The use of caffeic acid derivatives (CADs) for product standardization has been shown to be problematic due to the polyphenol oxidases (POs) present in the plant material. POs can lead to the enzymatic degradation and oxidation of CADs in hydroalcoholic solutions

during the extraction process. Recent studies show that Echinacea markers are unstable in juice, during harvesting and drying, and in storage.

-No routine methods are available for the determination of polysaccharides or glycoproteins .

-There are no quantitative standards for *Echinacea angustifolia* herba

-Commercial *Echinacea* samples and marketed *Echinacea* products may contain one or more of the three species, and analysis of samples of raw material and products has shown that some do not meet recognized standards for pharmaceutical quality.

Research gaps

-Of the four *Echinacea* monographs published by Commission E, two are positive (i.e., approved) (*E. pallida* root and *E. purpurea* herb) and two are negative (i.e., unapproved) (*E. purpurea* root and *E. angustifolia* root). The latter were given negative assessments due to lack of clinical trials for the specific plant parts. Therefore, lack of current pharmacological and clinical studies on *E. angustifolia* root and *E. angustifolia*/*E. pallida* aerial parts resulted in the issuance of a negative monograph until further supporting scientific information becomes available

-As studies concerning the efficacy of Echinacea as a growth promoter and immune enhancer in productive livestock are limited, no final conclusions are possible yet.

-Regarding safety administration: certainly continuous use is not advised by several sources. Also it is often written that Echinacea is contraindicated in autoimmune disease. The origin of this highly cautious approach to what is a relatively benign agent needs to be critically examined.

Costs

-High costs of clinical trials, research for new applications and innovative products development

4.6 Set forth research gaps, prospects and recommendations to procure bio - based products will be tackled

6.1 Agricultural raw material

Basic research

-optimizing choices of chemotype, growing area (for ex. choosing a friable soil with relatively low clay content can make a substantial difference to the ease of root washing

which will reduce additional costs) and harvest stage; improved handling of the crop before drying and use of more efficient drying technology;

-large scale organic cultivation

-new applications in veterinary medicine

-breeding new varieties with great resistance to wet soils and shade and resistant to diseases

Applied research

- increasing efficiency of processing infrastructure

6.2 Industrial raw material

Basic research

- glycoproteins as marker compounds in *Echinacea* products (MALDI-TOF analysis)

-there is a need for solid scientific data concerning efficiency and safety based on clinical trials; evaluation of dosage amount and of dosage timing using standardized extracts; bioavailability and pharmacokinetics

-researches concerning understudied compounds like polysaccharides.

-studies concerning the efficacy of Echinacea in productive livestock

Applied research

-modern procession technologies – microwave-assisted extraction, ultrasound extraction, accelerated solvent extraction, superfluid extraction

-ensuring the basis for standardized and pharmacologically characterized extracts

-implementation of modern technologies for target drug delivery

Prospects and recommendations

-financial help for cultivation; it is important to understand the importance of controlled conditions on final product quality

-Need for legislative harmonization in EU27 countries. The same drug is sold as medicine in a country and as dietary supplement in other.

-know the nature of the desired product. In the case of medicinal plants which are used directly as pharmaceuticals, the quality and thus the concentration of active compounds is much more relevant than the total yield.

-modern processing technologies in order to reduce heat degradation, reduce processing costs, faster extraction, much lower energy usage, less solvent usage

-good management of wastes

-for pharmaceutical products - ensuring product quality by standardization

-development of new technologies for conditioning pharmaceutical and cosmetic products to achieve a targeted and better absorption

-strong marketing strategy for natural, innovative and high quality products

References:

- Plant for Future Database www.pfaf.org
- WHO monographs on selected medicinal plants, 1999, vol. 1, p. 125-135 (Radix Echinaceae)
- British Pharmacopoeia, 2009
- European Medicine Agency – Echinacea, use in veterinary homeopathy, summary report, 1999
- W. O'Neill, S. McKee, A. Clarke - Immunological and haematinic consequences of feeding a standardised Echinacea (Echinacea angustifolia) extract to healthy horses, , Equine Vet J, 34(3):222-7, 2002
- Medicinal and aromatic plants-Industrial profiles, The Genus Echinacea, 2004, S. Miller Ed., CRC Press
- M. Hamburger, S. Adler, D. Baumann, A. Forg, B. Weinreich -Preparative purification of the major anti-inflammatory triterpenoid esters from marigold *Calendula officinalis*,, Fitoterapia 74 328–338, 2003
- K. Khalid, J. Teixeira da Silva -Yield, essential oil and pigment content of *Calendula officinalis* L. flower heads cultivated under salt stress conditions,, Scientia Horticulturae, vol. 126, issue 2, p. 297-305, 2010

5 Mentha x piperita L. (Lamiaceae)

5.1 Review on the product yielding capacity from various industrial crops streams

According to Plant for Future Platform (1), the main uses for *Mentha piperita* are:

Agriculture Raw Materials	**Directions of use**	**Product example**
Leaves, herb	Phytotherapy	-remedy for fevers, headaches, digestive disorders (especially flatulence) and various minor ailments -abortifacient, anodyne, antiseptic, antispasmodic, carminative, cholagogue, diaphoretic, refrigerant, stomachic, tonic and vasodilator
Leaves Oil	Cosmetics Perfumery	-Externally a lotion is applied to the skin to relieve pain and reduce sensitivity -antiseptic and strongly antibacterial, though it is toxic in large doses mouthwash, toothpaste, cream, lotions)
Plant, oil	Alimentary industry	Food additive
Plant	Repellent	Rat, mice, insects
Leaves	Ornamental	Pot-pourri

5.2 Identify desirable quality characteristics that feedstock has to meet for mature industrial processes

Guidelines for pharmaceutical products:

Menthae piperitae folium has monograph in Ph Eur (Menthae piperitae folium 406/5 (6.6) and

Menthae piperitae folii extractum siccum 2382/5(6.4), British Pharmacopoeia, ESCOP, WHO, German Commission E, French Avis aux fabr. and Chinese Pharmacopeia monographs and final report on EMEA (Herbal Medicinal Products committee assessment)

Menthae piperitae aetheroleum has monograph in Ph Eur, ESCOP, WHO, German Commission E and Chinese Pharmacopeia monographs and final report on EMEA (Herbal Medicinal Products committee assessment)

Plant material of interest for pharmaceutical purposes: dried leaves

As regards herbal medicines, European Pharmacopoeia and accepted monographs have specific terms for each vegetal product obtained.

Herbal substance (equivalent to the term "herbal drug" as defined in the European Pharmacopoeia) - which includes Menthae folium

The product is usually used in dried form but sometimes fresh. The specification should be established on the basis of recent scientific data and should be set out in the same way as the European Pharmacopoeia monographs.

-Definition: dried leaves. It is also important to know the geographical source(s) and the conditions under which the herbal substance is obtained.

-Characters: a qualitative statement about the organoleptic character(s) where characteristic and the macroscopic and microscopic botanical characters of the herbal substance.

Microscopic characteristics

Upper epidermis composed of large, clear epidermal cells with sinuous, vertical walls and possessing few or no stomata, few glandular trichomes present; palisade parenchyma, comprising a layer of columnar cells rich in chloroplasts; spongy parenchyma, of 4-6 layers of irregularly shaped chloroplastid containing cells and intercellular air-spaces. Lower epidermis of small epidermal cells with sinuous, vertical walls and numerous diacytic stomata; in the region of veins and midrib, exhibits non-glandular and glandular trichomes as outgrowths; non-glandular trichomes uniseriate, papillose, 1-8-celled; glandular trichomes have 1-2-celled stalk and 1-8-celled glandular head containing the essential oil. Calcium oxalate crystals absent; pollen grains spheroidal and smooth. (2)

Powdered plant material

Brownish-green. Fragments of leaf tissue with cells of epidermis having sinuous walls, cuticle striated over the veins, diacytic stomata present predominantly on the lower epidermis; epidermis fragments from near leaf margin with isodiametric cells showing distinct beading and pitting in anticlinal walls; covering trichomes short, conical, unicellular, bicellular or elongated, uniseriate multicellular (3-8 cells) with striated cuticle. Glandular trichomes of 2 types: either with unicellular base with small, rounded, unicellular head 15-25 µm in diameter; or with unicellular base with enlarged, oval multicellular head 55-70 µm in diameter composed of 8 radiating cells; dorsoventral mesophyll fragments with a single palisade layer and 4-6 layers of spongy parenchyma; yellowish crystals of menthol under the cuticle of secretory cells. Calcium oxalate crystals absent. (*2*).

Odour: characteristic, penetrating; taste: characteristic, aromatic (*2*).

Other general identity tests: thin-layer chromatography. Gas chromatography of the steam-distilled essential oil (*2*)

Purity tests:

Foreign organic matter - Not more than 5% stems, the diameter of which must be not more than 1.5 mm; not more than 8% leaves showing brown stains due to *Puccinia menthae* ; not more than 2% other foreign matter (*2*)

Total ash - Not more than 15% according to the *European pharmacopoeia*; not more than 12% according to the *African pharmacopoeia*

Acid-insoluble ash - Not more than 1.5% (2).

Pesticide residues- The recommended maximum limit of aldrin and dieldrin is not more than 0.05 mg/kg (2)

Other purity tests- Sulfated ash, water-soluble extractive, alcohol-soluble extractive, and loss on drying tests to be established in accordance with national requirements.

Chemical assays

Whole and cut leaves contain not less than 1.2% and 0.9% (v/w) essential oil, respectively, determined as described in the *European pharmacopoeia*.

The main constituents:

The major constituent of the leaves is the essential oil (0.5-4%), which contains menthol (30-55%) and menthone (14-32%). Various flavonoids are present including luteolin and its 7-glycoside, rutin, hesperidin, eriocitrin and highly oxygenated flavones. Other constituents include phenolic acids and small amounts of triterpenes (2)

Eriocitrin, with a concentration range of 6.6-15.0%, is the dominant flavonoid glycoside, accompanied by luteolin 7-0-rutinoside, hesperidin and rosmarinic acid, on a study of 40 clones of Mentha piperita (2).

About 75% of the polyphenolic compounds present in the leaves are extracted in an infusion (2).

According to EMEA (3), *therapeutic indication* for *Mentha piperita* leaves is: "traditional herbal medicinal product for the symptomatic relief of digestive disorders such as dyspepsia and flatulence".

According to EMEA (3), approved *pharmaceutical forms* for *Mentha piperita* leaves are:

-Traditional use: herbal substance for infusion or other herbal preparation in liquid or solid dosage forms for oral use

Herbal preparation (equivalent to the term "herbal drug preparation" as defined in the European Pharmacopoeia) of *Mentha piperita* are obtained by subjecting vegetal material to treatments such as extraction, distillation, expression, fractionation, purification, concentration or fermentation. These preparations include comminuted or powdered herbal substances, tinctures, extracts.

According to EMEA (4), accepted herbal preparation for traditional use is:

"Essential oil obtained by steam distillation from the fresh aerial parts of the flowering plant"

To determine if a natural oil or its isolate is a pure product, internationally accepted specifications have been developed. The main organizations that have broadly accepted specifications are the International Organization for Standardization (ISO), the Association Francais de Normalisation (AFNOR [1996a–c]) providing French essential oil standards, United States Pharmacopoeia (USP), European Pharmacopoeia (EUP), Essential Oils Association of the USA (EOA), and the USA National Academy of Sciences Food Chemical Codex (FCC). Additionally, some individual countries have their own sets of specifications, for example, India (Indian Standardized Specifications), the United Kingdom (British Standard Specifications), etc. Significantly, these individual specifications are closely related to the aforementioned international standards.

-Definition: Aetheroleum Menthae Piperitae is the essential oil obtained by steam distillation of the fresh overground parts of *Mentha* × *piperita* L. (Lamiaceae) (2) It is also important to know the geographical source(s) and the conditions under which the herbal substance is obtained.

-Characters: A colourless, pale yellow or pale greenish-yellow liquid (2).

-**Microscopic characteristics**: Not applicable

-**Powdered plant material**: Not applicable

-**Odour:** characteristic, penetrating; taste: characteristic, pungent, followed by a sensation of cold (*2*).

-**General identity tests**: Thin-layer and gas chromatography for characteristic monoterpene profiles

-**Purity tests:**

Acid value: not more than 1.4 (2).

Relative density: 0.900-0.916 (2).

Refractive index: 1.457-1.467 (2).

Optical rotation: -10° to -30° (2).

Solvent solubility: miscible with ethanol (96%), ether and methylene chloride (2)

Pesticide residues: The recommended maximum limit of aldrin and dieldrin is not more than 0.05 mg/kg (2)

Chemical assays: The monoterpene content determined by gas chromatography should be 1,8- cineole (6-14%), limonene (1-5%), menthone (14-32%), menthofuran (1-9%), isomenthone (2-10%), menthyl acetate (3-5%), menthol (30-55%), pulegone (not more than 4.0%) and carvone (not more than 1.0%). The ratio of 1,8- cineole to limonene should be greater than 2.0 (2)

The main constituents: The major constituents are menthol (30-55%) and menthone (14-32%).

Menthol occurs mostly in the free alcohol form, with small quantities as the acetate (3-5%) and valerate esters. Other monoterpenes present include isomenthone (2-10%), 1,8-cineole (6-14%), α-pinene (1.0-1.5%), β-pinene (1-2%), limonene (1-5%), neomenthol (2.5-3.5%) and menthofuran (1-9%)(2)

According to EMEA (4), approved *therapeutic indications* for *Mentha piperita* volatile oil are:

-for well-established use:

- Oral use (herbal medicinal product for the symptomatic relief of minor spasms of the gastrointestinal tract, flatulence and abdominal pain, especially in patients with irritable bowel syndrome.
- Cutaneous use (herbal medicinal product for the symptomatic relief of mild tension type headache)

-for traditional use:

-Cutaneous and transdermal use (for the relief of symptoms in coughs and colds; for the symptomatic relief of localized, muscle pain; for the symptomatic relief of localised pruritic conditions in intact skin)

-Inhalation (for the relief of symptoms in coughs and colds)

-Oromucosal use (for the relief of symptoms in coughs and colds)

According to EMEA (4), approved pharmaceutical forms for *Mentha piperita* volatile oil are:

-Well-established use : in gastro–resistant capsules (for oral use) and in liquid or semi-solid preparations (for cutaneous use).

-Traditional use : in liquid or semi-solid preparations (for cutaneous and transdermal use ; for inhalation; for oromucosal use).

Herbal medicinal products (the term includes "traditional medicinal product")

Preparations are given in various dosage forms including tablets, liquids (in ethanol-water mixtures or other), capsules, and dried extracts (in tablets or capsules). Mentha extracts are used in combinations with many other herbal substances / herbal preparations.

The following tests and acceptance criteria are considered generally applicable to all herbal medicinal products:

-Description: A qualitative description of the dosage form should be provided (e.g., size, shape, colour). If colour changes occur during storage, a quantitative procedure may be appropriate.

-Identification: Identification tests should establish the specific identity of the herbal substance(s) and/or herbal preparation(s), in the herbal medicinal product and optimally should be discriminatory with regard to substitutes/adulterants that are likely to occur Identification solely by chromatographic retention time, for example, is not regarded as being specific; however, a combination of chromatographic tests (e.g. HPLC and TLC-densitometry) or a combination of tests into a single procedure, such as HPLC/UV-diode array, HPLC/MS, or GC/MS may be acceptable. In the case of herbal medicinal products containing powdered or comminuted herbal substances, microscopical and macroscopical characterisation could be used for identification in combination with other methods, if justified.

-Assay: In the case of products containing herbal substances and/or herbal preparations with constituents of known therapeutic activity, validated assays of the content of these constituents are required along with details of the analytical procedure(s). Where appropriate , a specific, stability-indicating procedure should be included to determine the content of the herbal substance(s) and/or herbal preparation(s) in the herbal medicinal product. In cases where use of a non-specific assay is justified, other supporting analytical procedures should be used to achieve overall specificity. In the case of herbal medicinal

products containing herbal substance(s) and/or herbal preparation(s) where the constituents with known therapeutic activity are not known, validated assays of active or analytical markers or other justified determinations are required. The choice of such markers should be justified. In cases where a specific assay of each active substance of a herbal medicinal product is not possible other justified determinations are required (for example, in multi-component traditional herbal medicinal products for human use the same markers may be present in more than one herbal substance/preparation).

-Impurities: Refer to the ICH/VICH Guidelines on impurities in new drug products/Guidelines on impurities in new veterinary products (CPMP/ICH/2738/99 and CVMP/VICH/838/99 as revised) and the European Pharmacopoeia General text on Residual Solvents for detailed information.

Impurities arising from the herbal substance(s) and/or herbal preparations e.g. Contaminants such as pesticide/fumigant residues, heavy metals, if controlled during the testing of the herbal substance/preparation, it is not necessary to test for these in the herbal medicinal product.

Similarly, residual solvent arising from the manufacture of the herbal preparation (e.g. an extract) need not be controlled in the herbal medicinal product provided it is appropriately controlled in the extract specification. However, solvents used for example in tablet coating will need to be controlled in the dosage form.

In cases where degradation products of the herbal substance/preparation are evident, they should be monitored in the herbal medicinal product. Acceptance limits should be stated for such degradation products.

In addition to the universal tests listed above, the following tests may be considered applicable to *herbal medicinal products* on a case by case basis. Individual tests/criteria should be included in the specification when the tests have an impact on the quality of the herbal medicinal product for batch control.

Additional tests and acceptance criteria generally should be included for particular herbal medicinal products. The specific dosage forms addressed include **solid oral** herbal medicinal products, and **liquid oral** herbal medicinal products.

-Tablets (coated and uncoated) and hard capsules (One or more of these tests may also be applicable to soft capsules and granules) – dissolution/ disintegration, hardness/friability, uniformity of dosage units (this term includes both uniformity of content and uniformity of mass; a pharmacopoeial procedure should be used), water content, microbial limits (Microbial limit testing is seen as an attribute of Good Manufacturing Practice,

as well as of quality assurance. Reference should be made to the European Pharmacopoeia general text on the Microbiological Quality of Pharmaceutical Preparations for guidance on acceptable limits. Periodic testing may be appropriate.)

-Oral liquids – uniformity of dosage units (This term includes both uniformity of content and uniformity of mass. Generally, acceptance criteria should be set for weight variation, fill volume, and/or uniformity of fill. Pharmacopoeial procedures should be used), pH, microbial limits, antimicrobial preservative content (The lowest specified concentration of antimicrobial preservative should be demonstrated to be effective in controlling micro-organisms by using the European Pharmacopoeia antimicrobial preservative effectiveness test. Antimicrobial preservative effectiveness should be demonstrated during development, during scale-up, and throughout the shelf-life), antioxidant preservative content, extractables (Generally, where development and stability data show no significant evidence of extractables from the container/closure system, elimination of this test may be proposed), alcohol content, dissolution, particle size distribution, redispersibility (for oral suspensions, which produce sediment), rheological properties, specific gravity, reconstitution time (Acceptance criteria for reconstitution time should be provided for dry powder products, which require reconstitution. The choice of diluent should be justified), water content (for oral products requiring reconstitution).

Herbal Medicinal Products containing exclusively herbal substances **(e.g. herbal teas)**

One or more of these tests may be applicable to herbal medicinal products containing exclusively herbal substances:

-loss on drying (To be specified depending on the plant parts present in the herbal medicinal product, if not performed on the herbal substance),

-identification (Identification tests must establish the specific identity of the herbal substance(s) in the herbal medicinal product and optimally should be discriminatory between the different herbal substances and with regards to substitutes/adulterants that are likely to occur.

-Microscopical and macroscopical characterisation can be used to support identification, if justified), -purity (Relevant adulterants and substitutes should be determined), uniformity of mass/average mass of the sachet (The dosage unit is considered to be the typical dose taken by the patient. Pharmacopoeial procedures should be used),

-assay (In the case of such herbal medicinal products containing herbal substances with constituents of known therapeutic activity, validated assays for these constituents are required along with details of the analytical procedure(s),

-particle size,

-microbial quality.

A key issue in manufacturing herbal products and medicines is **standardization.** Standardization is the process of producing herbal extracts or phytochemicals in which product potency is guaranteed through consistency in active compound content level. This process requires high knowledge in phytochemical analysis and process technology to ensure the quality assurance required.

Product value increases in the following order: fresh material < dried powder < non-standardized extract < freeze/spray dried extract < standardized extract < phytomedicine.

Food additive

In 1976, FAO/WHO Joint Expert Committee on Foods Additives established an ADI of 0, 2 mg/kg body weight/day for menthol. In 2000, an ADI of 0-4mg/kg of body weight/day was allocated.

Maximum levels for pulegone in foodstuff and beverages to which flavourings or other food ingredients with flavouring properties have been added: 25 mg/kg in foodstuff, 100 mg/kg in beverages, with the exception of 250 mg/kg in peppermint or mint flavoured beverages and 350 mg/kg

in mint confectionery (Annex II of Directive 88/388/EEC). Pulegone may not be added as such to foodstuff.(3)

Cosmetic

In the case of cosmetics, there are some standards for high quality products:

-BDIH "Certified Natural Cosmetics" - herbal extracts and essential oils and aromatic materials from controlled biological cultivation or controlled biological wild collection

-COSMOS standard for organic cosmetics

-ECOCERT standard for natural and organic cosmetics

Because of the toxicity of pulegone, the CIR expert Panel limited it to ≤ 1% in cosmetic grade. Recent data reported that peppermint leaves are used in <0, 2% on formulations. (3)

5.3 The report on raw materials from non - food crops as alternative to fossil, petroleum - based and chemical resources

There are 223 registered international patents concerning *Mentha piperita* leaves or volatile oil and just a part are applied in industry.

5.3.1 Herbal medicinal preparations and products

Usually herbal products are adjuvant in classical medicine or have a preventive action. In pharmacological testing, the activity is usually compared to synthetic drugs.

Ex. In two double blind, crossover studies of irritable bowel syndrome with 16 and 29 patients respectively, capsules containing peppermint oil (0.2 mL/capsule) were considered better than placebo in relieving abdominal symptoms.

Ex. In a double blind, crossover study, 40 irritable bowel syndrome patients were treated orally for 2 weeks with peppermint oil in enteric-coated capsules (0.2 mL/capsule), hyoscyamine (0.2 mg) or placebo. Treatment with peppermint oil tended to have a more pronounced effect on symptoms than placebo or hyoscyamine, but this was not statistically significant.

Ex. A placebo controlled double-blind study has been studied in 69 woman in the treatment of abdominal distension and dyspepsia following routine gynaecological surgery, using Peppermint oil (Colpermin – Tillots Laboratories, St. Albans, Hertfordshire), in enteric coated capsules, 2 capsules, 3 times/day, during 5 days. No differences were found in abdominal distension, flatulence or abdominal pain between the two groups. Peppermint oil was not effective, but safe.

Ex. In a double blind, randomized, placebo controlled, multicentre, 4-week trial, 39 patients with dyspepsia (non ulcerative), with moderate to severe pain were given a combination (Enteroplant ®) of peppermint (90mg) and caraway oil (50mg). Decrease in pain intensity was significantly greater in the treatment group (15 days-84, 2% - p=0,002; 29 days – 89, 9% - p=0,015) than in the placebo group (15 days - 50%; 29 days – 45%)

Ex. Rowachol ® (Pinene 17mg, Camphene 5mg, Cineol 2mg, Menthone 6mg, Menthol 32mg, Borneol 5mg, Olive Oil 33mg – for each capsule of 100mg) enhances the cholesterol solubility of gall bladder bile ($p<0,001$) and human T-tube ($p<0, 05$) bile after the ingestion of 2 capsules three times daily for 48 hours (4).

Ex. A study was performed with 18 patients in a three condition experimental design, to investigate the efficacy of peppermint oil on the relief of postoperative nausea in

gynaecological surgical patients - (control group – no treatment; placebo – peppermint essence; experimental – peppermint oil), isolated from each others due to the volatile nature of the compound. The experimental group had an increased number of intra-abdominal procedures, received more opioid analgesia postoperatively and required less traditional antiemetics (4)

Ex. -The effect of a locally applied peppermint oil preparation on tension-type headache was examined in the design of a randomized, placebo-controlled double-blind crossover study. The preparation was tested against both the reference substance acetaminophen and to the corresponding placebo. The liquid test preparation contained 10 g of peppermint oil and ethanol (90%) ad 100 (test preparation LI 170, Lichtwer Pharma, Berlin); the placebo was a 90% ethanol solution to which traces of peppermint oil were added for blinding purposes. The reference preparation contained 500 mg acetaminophen; the placebo tablet was identical to the verum in size and appearance. Compared to the application of placebo, 10% peppermint oil in ethanol solution significantly reduced the clinical headache intensity already after 15 minutes . Acetaminophen, too, proved to be efficient compared to placebo ($p < 0, 01$). There was no significant difference between the efficacy of 1,000 mg of acetaminophen and 10% peppermint oil in ethanol solution (4).

Some of the pharmaceutical products containing Mentha leaves or volatile oil commercialized in European countries are:

-*Pinosol* Tocopherol + Eucalyptus oil + Pine silvesteris oil + Thymol + Peppermint oil + Guaiazulene (OTC, Zentiva)

-*Septolete* Benzalkonium chloride + Eucalyptus oil + Levomenthol + Peppermint oil + Thymol, (OTC, KRKA)

-*Iberogast*, oral drops, solution (*Iberis amara, Angelica archangelica, Matricaria recutita, Carum carvi, Silybum marianum, Melissa officinalis, Mentha piperita, Chelidonium majus, Glycyrrhiza glabra*) 2010-11-10. Approved indication: Herbal medicinal product for relief of symptoms of functional dyspepsia and IBS, such as bloating, epigastric pain and nausea. (Sweden, approved as Authorised Herbal Medicinal Products)

-*Mentha piperita tincture* (OTC, Hofigal Export-Import SA) for functional intestinal disorders.

For traditional use, *Mentha piperita* is sold in various forms: herbal tea (Natura Biertan, Fares, Plantavorel, Hofigal-Romania), tincture (Hofigal, Fares-Romania), volatile oil

(Hofigal, Fares-Romania), tablets (Hofigal, Fares, Plantavorel-Romania), syrup (Fares-Romania), combinations (Natura Biertan, Hofigal, Fares-Romania)

-Homeopathy - *Mentha piperita* tablets- Heel Belgium; *Mentha piperita* granules VSM Geneesmiddelen B.V. Netherlands, *Mentha piperita* granules, globules, drops for oral use– Boiron, France

5.3.2 Cosmetics

Mint oils and mint flavors are used to deliver a cooling sensation and a minty flavor to a number of oral care products such as toothpastes, mouthwashes, breath fresheners, etc. In fine fragrances, mint oils and mint aromas are used in perfumes, colognes, lipsticks, lip balms, shaving cream, hair lotions, face creams, soaps, shampoos, solution for spa use, etc. In household care products, mint oils and their fragrances are used in room deodorizers (air fresheners), cleaning products (including detergents and fabric softeners), soaps, etc.

5.3.4 Other uses

Synthetic (-)-menthol production accounts for approximately 30% of the world market of about 12,000 metric tonnes. Natural menthol comes from mint that is sourced mostly from India and accounts for about half the demand for menthol overall. Synthetic menthol is sold on the world market in relatively large quantities, generated from thymol, citronellal, menthone and β-pinene.

Table 1 - Use Levels (ppm) of peppermint oil and menthol (5)

Product	**Pepprmint oil (ppm)**	**Menthol (ppm)**
Hard candy	300-2500	500-1000
Soft candy	300-1200	500-1000
Chewing gum	6500-10000	100-5000
Extra strong mints	5000-20000	1000-5000
After dinner mints	1000-2000	200-500
Nonalcoholic beverages	40-100	
Alcoholic beverages	150-250	
Frozen diary products	90-110	
Baked goods	140-300	
Puddings	50-200	
Icing, toppings, cake frostings	5-650	

Jams and jellies	50-200	
Toothpaste	1000-4500	500-7000
Mouthwash	200-1200	100-2000
Breath fresheners	1000-6000	500-10000
Perfumes and colognes	5000	10000
Lipstick and lip balms	100-1000	100-1000
Shaving cream and hair lotions	100-1000	20000
Face cream and soaps	5000	10000-30000
Shampoos	1000-2000	2000-5000
Room deodorizer	100-1000	100-5000
Cleaning products	5000-10000	1000-5000
Soaps	5000-10000	1000-5000
Inhalants	1000-5000	≥30000
Liniments and creams	100-1500	≥20000
Preshave and aftershave lotions	100-1000	50-2000

-Mint oils and mint flavors and, more importantly, menthol are used in cigarettes, snuff, and chewing tobacco (including Pan Masala in India) at levels of 50–20,000 ppm.

-In 1893, William Wrigley introduced peppermint chewing gum while at the same time Colgate, Pepsodent, and Kolynos introduced toothpastes.

-Mint oil and their corresponding isolates are used in both nonalcoholic and alcoholic beverages (in particular the liqueur or cordial Creme de Menthe). They can also be found as flavorants in frozen dairy products such as ice cream and ice lollies, baked goods, icings, toppings, cake frostings, puddings, sauces, chutneys, etc (Table 1).

5.3.5 Other intermediary products

European manufacturers produce a large variety of intermediary products:

Hydroalcoholic extract–Epo Srl-Italy, Hofigal-Romania, Phytosan-France

Liquid extract -Phytex-Bulgaria, Epo Srl-Italy

Dry extract – Phytex-Bulgaria, Epo Srl-Italy

Mother tincture-Phytosan-France, Epo Srl-Italy

Glycerine extract - Phytosan-France

5.4 Set prospects to widen the range of potential feedstock for the understudy industrial uses, based on the technology improvements

Technologies improving the quality of preliminary raw material (e.g. breeding varieties with better gene expression together with improvement of technology of: cultivation, harvesting and processing) as factor determining further application.

New and improved varieties

-Extensive hybridization studies have been performed in the genus *Mentha*. Somatic hybridization is aimed either at modifying the composition of the oil, or at combining essential oil quality with disease resistance, production of varieties with abnormally large or increased numbers of oil glands or vastly increased leaf areas, production of varieties with upregulated enzymes responsible for biosynthesis of specific volatiles such as menthol or downregulated enzymes that catabolise such compounds, breeding programs using primitive species and varieties to insert 'new' disease resistance genes into highly bred stock, biological control mechanisms for Verticillium wilt, rust and other diseases.

-*Induced mutation* The polyploid peppermint (*Mentha*×*piperita*) variety "Multimentha" resulted from a genome mutation by mutation breeding. Almost 5000 progenies were screened for high yield, adequate chemical composition and resistance against *Puccinia menthae*, and this selection resulted in the high-performance cultivar "Multimentha", which is infertile due to a chromosome set of 2n = 96. Genetic stability is ensured because this variety can be propagated only vegetatively.

-*Clonal breeding* Clonal breeding has a particularly high importance for MAPs because this method shortens the breeding procedure considerably. Clone varieties are vegetatively propagated due to sterility of the species Mentha.

-Cell suspension culture for the enhancement of menthol yield (establishment of *Agrobacterium tumefaciens* (Ach5) gall-mediated calli, and consequently.(6)

-Chitosan elicitation may activate the conversion of pulegone to menthol in *Mentha piperita* cultured cells. (7)

Improving the yield and quality of volatile oil and its constituents

-Metabolic Engineering of the Terpenoid Pathway

Peppermint (*Mentha×piperita*) essential oil consists mainly of menthol and menthone, while high levels of menthofuran are considered detrimental to its quality. The gene coding for the enzyme menthofuran synthase has been silenced in peppermint, generating plants harboring an essential oil with a much lower menthofuran levels, and therefore of a much higher quality than the essential oil from control nontransgenic plants. Exploitation of this highly enriched library has further led to the cloning and functional expression of isopiperitenone reductase (IPR) and pulegone reductase (PR), isopiperitenol dehydrogenase (IPD), and the two menthol reductases (–)-menthone:(–)-(3*R*)-menthol reductase (MMR) and (–)-menthone:(+)-(3*S*)-neomenthol reductase (MNR). The entire complement of cDNAs encoding the redox enzymes of (–)-menthol biosynthesis in peppermint were discovered thus facilitating the production of new varieties.

-At first sight, it appears very promising to use optically active terpenoids as starting materials for the production of (-)-menthol, as it is not necessary to introduce optical activity by a resolution step or by asymmetric synthesis. However, the economy of such processes depends highly on certain crucial factors: availability and price of the natural raw material, its optical purity, synthetic steps of high regio- and stereoselectivities to retain the asymmetry of the starting material and to achieve a satisfactory overall yield.

New products based on improvement of extraction technology, formulation technology and identification of new medicinal applications

Extraction technology

-Peppermint oil can be used directly as the recovered oil from the steam distilled mint herbage or it can be further distilled or rectified (fractional distillation) in order to refine the character and adjust the overall profile. In practice, rectification schemes normally involve the removal of varying amounts of the volatile and high boiling fractions and then collecting a heart-cut or center fraction as the rectified oil. Through this process, significant volumes of by-products containing some very interesting ingredients are made available. The by-product streams are further processed and become a source of natural flavor chemicals for use by flavorists in new flavor formulations.

-At the present time, there are a number of non-conventional extraction methods in use that are all, in principle, solid-liquid extractions (SLE) but which introduce some form of additional energy to the process in order to facilitate the transfer of analytes from sample to solvent. The Microwave Assisted Extraction Process is a high-speed method used to selectively extract target compounds from various raw

materials. Microwave assisted extraction uses energy of microwave radiation to heat solvents quickly and efficiently. By using a closed system, extraction can be performed at higher temperatures and extraction times can be reduced drastically. It is an innovative solvent-extraction technology, offers a superior alternative to several thermal applications owing to its efficient volumetric heat production and has many advantages over conventional solid liquid extraction methods. The main advantages of MAE are shorter extraction times (typically 15 minutes), shorter cooling times (2 minutes) and less use of solvent (10 mL for MAE versus 250 mL for Soxhlet).(8)

-Phytonics Process - A new solvent based on hydrofluorocarbon-134a and a new technology to optimize its remarkable properties in the extraction of plant materials offer significant environmental advantages and health and safety benefits over traditional processes for the production of high quality natural fragrant oils, flavors and biological extracts. Advanced Phytonics Limited (Manchester, UK) has developed this patented technology termed "phytonics process". The products mostly extracted by this process are fragrant components of essential oils and biological or phytopharmacological extracts which can be used directly without further physical or chemical treatment. Unlike other processes that employ high temperatures, the phytonics process is cool and gentle and its products are never damaged by exposure to temperatures in excess of ambient; the process is carried out entirely at neutral pH and, in the absence of oxygen, the products never suffer acid hydrolysis damage or oxidation. The technique is highly selective, offering a choice of operating conditions and hence a choice of end products; it is less threatening to the environment, it requires a minimum amount of electrical energy, it releases no harmful emissions into the atmosphere and the resultant waste products (spent biomass) are innocuous and pose no effluent disposal problems. The solvents used in the technique are not flammable, toxic or ozone depleting. The solvents are completely recycled within the system.

-Introduction of a low cost tray dryer offers a promising alternative to reduce the excessive postharvest losses and also improve farm profitability by encouraging the cultural production of medicinal plants. The technical performance of the heated-air tray dryer in terms of drying efficiency, specific heat energy consumption is very good in the case of Mentha piperita. The capacity of the system is designed for on-farm use to eliminate the long transport distances to central large-scale dryers, incurring extra costs and loss of quality. Due to the modular design, each tray can be filled with a different species and the capacity of the system can be adapted to meet the varying needs of mechanical harvesters.

Further investigation are necessary to optimize the heated-air tray dryer in terms of loading density, fuel and electrical energy input and manpower requirements.

Food additives with antioxidant potential

-A food material having a high concentration of flavones (especially eriocitrin) from peppermint leaves as an antioxidant ingredient can industrially be produced at a low cost.

Insect and animal repellents

-larvicidal and mosquito repellent action

New pharmaceutical applications which were already proved on animal models:

Some potential pharmacological effects of *Mentha piperita* leaves and volatile oil need to be further investigated

-virucidal effect on HPV

-psychoactive action

New pharmaceutical forms with targeted delivery

-nanotechnology (silver nanoparticles)

-liposome technology

-development of modern extraction technologies (microwave, supercritical CO2, fluid bed granulation)

5.5 Identify restricting factors that inhibit broader industrial use of the biomass feedstock (supply, costs, physical traits, consistency in quality, technical performance, research gaps, etc.)

5.5.1 Agricultural raw material

Physical traits

-The equipment required for mint production, especially irrigation and specialized digging, planting and distillation equipment (even if the latter represents tubs only), are the main items that take the overall profits into losses per ha over at least the first 4 years of production.

-The yield of oil from mint varies greatly with the season, the cultural conditions and the geographical location.

-Peppermint is demanding from soil moisture, water is a limiting factor. For the entire period of vegetation plant consumes 6,000 m3 of water.

-There are significant disease problems in mint, especially *Verticillium* wilt (*Verticillium alboatrum*), a fungal disease which causes apical twisting and stunting, chlorosis, wilting and premature death, and rust (*Puccinia menthae* Pers).

-Determining the optimum harvest time is difficult since a visual observation (for example at a certain stage in flowering) may not truly reflect the internal oil composition (45% menthol content being critical). Due to the nature of the harvesting process, and the material itself, mint is particularly susceptible to loss of oil yield due to unfavourable weather.

Consistency in quality

-Besides the timing of harvest, the numbers of harvests per year greatly influence yield, and composition of oil. The essential oil from the first harvest is richer in menthol than that of the second harvest; first harvest is richer in menthol than that of the second harvest. In the second harvest all the leaves have a higher menthone and lower menthol content The amount of menthol increases as the plant matured. Harvesting of peppermint only once at the stage of full bloom (end of August) give the maximum oil yield of good quality. Furthermore, there is not enough time for the leaves to mature for the second cut.

Costs

-The main costs for production are the specialised planting and irrigation

-for most applications raw material must be free of impurities or other plant parts (especially when harvest is done mechanically) and requires additional operations incurring additional costs

5.5.2 Industrial raw material

***Mentha* leaves**

Research gaps

There are no clinical studies on the efficacy of Menthae piperitae folium alone, but just in combination with other plants, given no reason for the inclusion on the well-established use. (that is why EMEA doesn't included a well-established use for Mentha folium in the monography)

Costs

-equipment and facilities for specific processing – extractors, spray-dryers, freeze-dryers

-equipment for conditioning – special machines for tablets and capsules, for oral solutions, for products with topical application

-equipment for packaging (vacuum technology)

***Mentha* volatile oil**

Physical traits

-As mint oil is recovered from the mint herbage by steam distillation, there can be a tendency to have a small amount of water dissolved in the oil, which can later separate or cause a cloudiness to develop in the product.

Consistency in quality

-Adulteration can take many forms, such as:

1. The addition of synthetic (foreign) compounds unrelated to the oil composition
2. The addition of synthetic compounds related to the oil composition
3. The addition of oils or fractions of oils of similar composition to all or part of the oil
4. The addition of natural compounds produced enzymatically or from other oil sources
5. The addition of more than one of the above

For every kilogram of pure essential oil that is produced, it is estimated there are between 10 and 100 kilograms of synthetic oil created.

-End users need a careful monitoring of their source of supply. In recent years, export volumes of *M. piperita* oil far exceed the reported production volumes.

Costs

-Synthetic menthol could represent a real threat to some parts of the industry since it is a more consistent supply compared with menthol from natural sources, however in recent times the price has been greater than that of natural menthol. German synthetic menthol is less affected by price swings due to long-term contracts and demand for new products

-The important feature of mint production is distillation, which requires specialised equipment. This requires a large investment by the producer. Energy savings can be generated by re-using the water from the condensor which will be hot, to recharge the boiler. Mint oil when used as a flavour is generally tri-rectified (re-distilled); the rectified or fractionated oil is almost colourless and much less harsh than the raw oil.

5.6 Set forth research gaps, prospects and recommendations to procure bio - based products will be tackled

5.6.1 Agricultural raw material

Basic research

-large scale organic cultivation

-finding new applications in veterinary medicine

-breeding new varieties resistant to diseases and drought tolerant

Applied research

The equipment required for mint production, especially irrigation and specialized digging, planting and distillation equipment (even if the latter represents tubs only), are the main items that take the overall profits into losses per ha over at least the first 4 years of production.

5.6.2 Industrial raw material

***Mentha* leaves**

Basic research

-pharmacological testing - dose response studies; controlled clinical studies

Applied research

-increasing efficiency of processing infrastructure (modular construction so as to permit increase in capacity and function by duplicating or adding modules; simultaneous processing for more than one product)

-modern procession technologies – microwave-assisted extraction, ultrasound extraction, accelerated solvent extraction, superfluid extraction

-technology improvement for conditioning and formulation drugs and cosmetics (for ex. immediate or delayed release capsules, effervescent tablets, tea bags, dispensers for local application, etc).

Mentha volatile oil

Basic research

-breeding new varieties that provide a volatile oil of good quality and yield

-new methods for detecting adulterated oils (analytical measurements – chiral GC/MS, GC-MS, GC-FTIR, GC-13C NMR , HPLC-MS. Iisomenthone and isopulegol have become useful markers to distinguish the presence of cornmint oil and its by-products in mint flavor formulations. With reference to the data, elevated levels of these components become an especially good indicator of peppermint oil adulteration.

-reduce the menthofuran content in volatile oil

-evaluation of insecticidal and repellent properties and mechanisms of action

Applied research

-development of partnerships between research centres and producers for increasing yield and quality of oil

-development of high standards facilities and equipment for distillation -before storage, the oils should be examined for moisture and any residual water removed from the bottom of the drum. The drums should be filled to within 95% of their capacity to reduce the headspace above the oil while allowing space for the likelihood of temperature-induced expansion. Prior to sealing, it is preferable to purge the headspace of the drum with nitrogen or another inert gas to reduce the presence of oxygen. It is recommended that the drums be stored in a cool and protected location.

-Energy savings can be generated by re-using the water from the condensor which will be hot, to recharge the boiler. Mint oil when used as a flavour is generally tri-rectified (re-distilled); the rectified or fractionated oil is almost colourless and much less harsh than the raw oil.

-new technologies for conditioning pharmaceutical and cosmetic products to achieve a targeted and better absorption

Prospects and recommendations

-financial help for cultivation; it is important to understand the importance of controlled conditions on final product quality

-Need for legislative harmonization in EU27 countries. The same drug is sold as medicine in a country and as dietary supplement in other.

-know the nature of the desired product. In the case of medicinal plants which are used directly as pharmaceuticals, the quality and thus the concentration of active compounds is much more relevant than the total yield.

-development of cooperatives for reducing distillation related costs; thus, the specialized equipment required would be reduced.

-financing research for large-scale use of peppermint oil as insecticidal and repellent product

-modern processing technologies in order to reduce heat degradation, reduce processing costs, faster extraction, much lower energy usage, less solvent usage

-good management of wastes

-for pharmaceutical products - ensuring product quality by standardization

-development of new technologies for conditioning pharmaceutical and cosmetic products to achieve a targeted and better absorption

-strong marketing strategy for natural, innovative and high quality products

-tourism development - Value-adding large-scale production by building a tourism enterprise around them.

References:

- Plant for Future Database www.pfaf.org
- WHO monographs on selected medicinal plants, 1999, vol. 2, p. 199-205 (Menthae folium), p. 188-198 (Aetheroleum Menthae)
- European Medicine Agency – Community herbal monograph on *Mentha x piperita* L. folium, 2008
- European Medicine Agency – Community herbal monograph on *Mentha x piperita* L. aetheroleum, 2008
- Medicinal and aromatic plants-Industrial profiles, The Genus Mentha, 2007, B. Lawrence Ed., CRC Press
- A. Chakraborty, S. Chattopadhyay - Stimulation of menthol production in *Mentha*

piperita cell culture, , In Vitro Cellular and Developmental Biology - Plant 44(6):518-524. 2008

- J. Chang, J. Han Shin, I. Chung, H. Joo Lee - Improved menthol production from chitosan-elicited suspension culture of Mentha piperita, , Biotechnology Letters, Vol. 20, no. 12, 1097-1099, 1998
- Pare JRJ, Sigouin M, Lapointe J. - Microwave assisted natural products extraction U S patent no. 5 002 784, March 26, 1991

6 Summary and conclusions

Pharmaceutical and other specialty crops are the starting point for a wide range of products: essential oils, human and veterinary drugs, herbal health products, inks, colorants and dyes, perfumes, beauty products, novel plant protection products and also a range of intermediate products from which the above are manufactured.

Pharma industry

The importance of plants as a source of new drug molecules is illustrated by the fact that, in the past 20 years, 28% of new drug entities were either natural products or derived from them as semi-synthetic derivatives. Research on 'rich world' diseases, as they are sometimes called, which are quickly spreading. It has been projected that, by 2020, 75% of worldwide deaths due to stroke and 70% of deaths due to diabetes will occur in developing countries. It is advisable to look for new therapeutic strategies based on natural compounds for cardiovascular diseases, infectious diseases, diabetes, obesity, cancer and allergy. There are a wide range of medicinal preparations: tea (infusions or decoction), tinctures, glycerolates, medicinal oils, essential oils, compresses or plasters, eye washes, balsams, cataplasms, as well as a great number of pharmaceutical forms: tablets, capsules, syrups, ointments, hydrophilic gels, eyedrops (colliriums), nasal sprays and drops.

In Romania, research and development from cultivation to final product is conducted especially by small and medium enterprises. Over 75 years of its foundation, Fares is a private company with Romanian capital, leading on herbal tea (medicinal and aromatic) market in Romania. Medicinal plants are grown on surfaces over 180 hectares on both conventional and organic crops. Processing is done on modern production lines. The entire process, from cultivation and up to the finished product is held in compliance with the requirements of the Quality Management System ISO 9001:2000 and production flows meet the HACCP food safety requirements. Active substances content in fresh plants is monitored in a well equipped quality control laboratory and then controlled in the whole production flow so that the therapeutic effect of finished products is guaranteed. All Fares preparations are characterized by an optimal combination of components and precise dosing, as guaranteed by R & D team composed of physicians, pharmacists and chemists.

Cosmetics

In the last years a new concept has developed – cosmeceutics -cosmetic products that include ingredients designed not only to enhance the appearance but to also have a positive physiological effect at the cellular level. The use of new products is increasing: men's grooming products, anti-aging products, spa-at-home, detoxification products.

A recent study shows that cosmetic industry based on natural products could be very profitable(Market Demand and New Industry Formation: Eco-Products and Entrepreneurship in the "Natural Cosmetics Sector" in Greece, I. Katsikis, Workshop on "Environmental Innovation, Industrial Dynamics and Entrepreneurship", Utrecht, Netherlands 2009). A success Greek company, Korres SA Natural Products, set up in 196, aim to use its extensive scientific resources for the creation of beneficial and safe products, offers today a complete skin and haircare range, make-ul, sun care products and herbal preparations.

The keys to success were:

- Its capacity for innovation and new product development (500 new items from 1996 to 2009);
- Flexibility for corporate decision making, development and expansion into new markets;
- Global Image: the products can compete in international markets as an international brand name;
- Product and Firm Marketing: the company has received numerous awards for its packaging design and firm marketing activities;
- R&D activities: multi-level R&D activities, starting with analysing and studying the properties of plants, isolating the active ingredients, standardising the quality of the extract, developing lab-scale and industry-scale production methods;
  Innovative Products
- Quality and Control: The integrated production process provides high quality final products.

Another Greek company, Apivita Cosmetic Medicine Dietary SA, uses ingredients which fulfill the EU requirements (quality and technical) and are all developed and prepared in accordance with the European Union's Good Manufacturing Practices (GMP) for cosmetics, with strictly select natural ingredients whose proven effectiveness and safety have been supported by laboratory and clinical studies. Today, Apivita develops plant extracts from more than 60 different plants and is one of the few European companies have been certified

with ISO 900 for this purpose. The herbal extracts developed, produced and controlled by the firm itself.

The plants are collected, dried and stored in a specific area. These are then cut and suitable solvent for the extraction of active substances.

Then they are pressured and filtrated, the plant is removed and the solvent is extracted, which contains the active ingredient of the plant. After quality control, the extract is used as feedstock for the manufacture of herbal cosmetics.

According to the General Manager of the company, the great market response to Apivita's products in chains of luxury stores and the high export performance can be attributed to three benefits:

- Use of local resources
- Living tradition
- Quality control: The integrated production process adopted by firm, from the collection of the plant to produce the product provide high quality final products
- Aesthetics: The aesthetics of the final product and packaging, contribute significantly to the creation of a distinctive image. They aesthetic is evidenced by the many international prizes received for their packaging.

Dyes, colourants

There is an increasing development of new natural compounds able to substitute chemical additives for food and beverage industry. These compounds are used as antioxidants or colorants. For example, Naturex France has developed an extensive range of extracts which naturally protect food products against oxidation and therefore extend their shelf life as well as a wide range of special coloring formulations made by natural pigments like carotenoids, curcuminoids, chlorophylls and anthocyanins.

Natural dyes are rarely used in modern dyeing, except by specialist companies and craft dyers. It has become a common misconception that natural dyes only produce beiges and browns and washed-out shades. In reality, vibrant, fast, natural colours can be produced which are comparable with, and often surpass the colours of synthetics.

Because of the low fastness of the dye some of them, can be used as dyestuffs only for food, but not for textiles. Only a limited number of plant species exhibit the potential for largescale production. Method for obtain and purify compouds and stability studies must be developed.

Insecticides

Alternative insecticides normally mean the insecticides are less toxic to humans and breakdown more rapidly in the environment than conventional insecticides. They are often called "environmentally friendly". The most known natural insecticides are pyrethrins which come from certain species of chrysanthemums and limonene and linalool which are volatile molecules obtained from some vegetal species.

Selected crops and bio-based industry

Information on the specialty crops and crop products sector is difficult to analyze, because of its extreme diversity and variability and is limited by the reluctance of certain parts of the industry to document for commercial reasons. Literature data show that the 5 selected species have a wide range of applications :

Lavandula angustifolia	**Uses**	**Plant part used**
Phytotherapy	-local antiseptic to help heal wounds, restorative and tonic -antihalitosis, powerfully antiseptic, antispasmodic, aromatic, carminative, cholagogue, diuretic, nervine, sedative, stimulant, stomachic and tonic	flowering spikes, oil oil
Food industry	flavouring agent	lavender oil, absolute and even concrete
Veterinary products	-shampoos and other products as an insect repel-lent, especially for fleas	oil
Cosmetic	Lavender water, lavender vinegar lavender bath, pot-pourris, scented candles, perfumes, beauty products, perfumery, herbal pillows, lavender bags, therapeutic bath salts	Oil, flowers
Repellent	Repel mice and insects Acaricid, pediculicide	Leaves Essential oil
Calendula officinalis	**Uses**	**Plant part used**
Phytotherapy	-antiphlogistic, antiseptic, antispasmodic, aperient, astringent, cholagogue, diaphoretic, emmenagogue, skin, stimulant and vulnerary -homeopathic remedy	-The whole plant, but especially the flowers and the leaves -leaves, blossoms and buds
Dye	Yellow dye	flowers
Agriculture	-alternative ingredient of 'Quick Return' compost activator. This is a dried and powdered mixture of several herbs that can be added to a compost heap in order to speed up bacterial activity and thus shorten the time needed to make the compost -allelophatic properties in relation to the dicotyledons and weaker activity to the monocotyledons.	Flowers whole
Cosmetic	Beauty products	Flowers, oil
Repellent	-Reduces the soil eelworm population -exert various effects on growth and physiology of *Spodoptera litura* (Fab.), a serious polyphagous pest	herba
Other uses	Weather forecasting (The flowers close when wet weather is likely to occur)	Flowers

	Paint additive	oil
Mentha x piperita	**Uses**	**Plant part used**
Phytotherapy	-fevers, headaches, digestive disorders (especially flatulence) and various minor ailments -abortifacient, anodyne, antiseptic, antispasmodic, carminative, cholagogue, diaphoretic, refrigerant, stomachic, tonic and vasodilator -Externally a lotion is applied to the skin to relieve pain and reduce sensitivity -antiseptic and strongly antibacterial, though it is toxic in large doses	Leaves herb leaves oil
Cosmetic	Beauty products, perfumery Pot-pourri	Oil leaves
Repellent	Insects, rats, mice	Whole plant
Plantago lanceolata	**Uses**	**Plant part used**
Phytotherapy	-bleeding, it quickly staunches blood flow and encourages the repair of damaged tissue -remedy for the bite of rattlesnakes -treatment of parasitic worms, laxative	Leaves root seeds
Cosmetic	Beauty products	Leaves, seeds
Dye	Gold and brown dyes	Whole plant
Textiles industry	Fibre fabric stiffener	Leaves seed coats
Veterinary	Occasionally grown as a fodder crop	leaves
Other uses	Source of a low-cost gelling agent for tissue culture	seeds
Echinacea angustifolia	**Uses**	**Plant part used**
Phytotherapy	-general stimulatory effect on the immune system, treatment of sores, wounds, burns -adaptogen, alterative, antiseptic, depurative, digestive, sialagogue	plant root
Cosmetic Veterinary medicine	 Herbal feed supplement with immunostimulant, coccidiostatic and antibiotic activity	plant root

An internet survey regarding species selected – Calendula officinalis, Lavandula angustifolia, Mentha piperita, Echinacea angustifolia and Plantago lanceolata – and their various uses in different European countries showed that these herbs are used for various purposes and are presented in various forms.

Most of the raw material used by these manufacturers comes from own plantations: Florame (organic farms)-France, Sicobel Lab (organic farms)-France, Logona (herbs grown organically or wild-harvested)-Germany, Benostan (Greece organically-grown herbs)-Greece, Electra soap (most of the herbals come from Lesvos' mountains)-Greece, Korres (natural raw material of higher ecological quality or wild)-Greece, Herbaria (local wild and cultivated)-

Hungary, Ilcsi (organic plantation)-Hungary, Phytopharma (raw materials from all over the world)-Poland, Plantavorel (wildcrafted and cultivated by other cultivars)-Romania, Frantsila Farm (organic cultivation-own and other farms or wild organic certified) -Finland, Aboca (60 species of medicinal herbs are organic cultivated on own lands, while another 20 species are wildcrafted)-Italy, Madara (biological farms)-Latvia, Calendula (bio quality herbs)-Slovakia, Alban Muller Group (40% of its production comes from French cultures)-France, Martina Gebhardt Naturkosmetik (95% of our raw materials from certified organic cultivation or Demeter contract cultivation)-Germany, L'Occitane (organic farming)-France, Genmar (raw materials are entirely imported)-Romania, Salus (organic farming)-Germany, Agronatura (own organic cultivation), Yves Rocher (own organic cultivation and wildcrafting), Ecomaat (wild or organic farming), Interherba (wild, cultivated), Fares (own cultivation or wildcrafted), Herbacin (own cultivation) – Germany, Fytosan (own cultivation or from other farmers), Silvestris&Szilas (own cultivation) – Hungary, Bionorica (own cultivation)-Germany.