Phytochemical and Biosynthetic Studies of Lignans, with a Focus on Indonesian Medicinal Plants

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Chapter 1

Aims and scope of the thesis

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The use of plants, plant extracts and plant-derived chemicals in the treatment of diseases, in supplementing foods and in making cosmetics is firmly rooted in the past and still developing. Many drugs used in contemporary medicine have been derived from plants and were originally discovered through the traditional use by indigenous people. Podophyllotoxin, vincristine, vinblastin, camptothecin, taxol, artemisinin, aspirin, atropine, ephedrine, quinine, reserpin and digoxin are well known examples of such drugs.

Progress in science and technology boosts the further development of medicinal plants as valuable sources of drugs and drug leads. Modern analytical methods, biotechnology approaches, genomics, proteomics and metabolomics are nowadays applied in medicinal plant research and contribute to the advancement of the field. Quite frequently a relatively low yield of active components and difficulties in standardization are bottlenecks in medicinal plant exploitation. Efforts have been made worldwide to enhance the production of bioactive component using a biotechnology approach. In this thesis we aim to study phytochemical and biosynthetic aspects of lignans in selected medicinal plants used in Indonesia and in European *Linum* species.

In chapter 2, we review *jamu*, the traditional Indonesian medicine based on the popular use of medicinal plants. Indonesia is one of the biggest biological diversities in the word. Indonesian people have been using *jamu* to treat various diseases since long. An introduction to *jamu*, its present status in Indonesia, legislative and regulatory aspects, economical perspective and rational therapy with *jamu* are highlighted as well as ethical considerations that should be taken into account in the development of medicinal plants. This review aims to give comprehensive information about the biological activity and therapeutic value of the most commonly used medicinal plants (and plant constituents) in *jamu*. This knowledge can be used to further develop *jamu* in Indonesia in a rational way.

In the chapter 3 we studied lignans from cell suspension cultures of *Phyllanthus niruri*, an important medicinal plant in Indonesia. Feeding experiments using the biosynthetic precursors of lignans, caffeic acid and ferulic acid, were carried out in order to enhance the lignan production and to study their biosynthetic pathway. A phytochemical study of another important medicinal plant in Indonesia, *Piper cubeba*, was conducted as well. A systematic profiling of lignans was made using TLC, HPLC, GC and GC-MS of different parts of this plant (chapter 4). So far, only fruits from *P. cubeba* are used medicinally and as a food supplement. To complete the phytochemical study we also analyzed the essential oil composition of *P. cubeba* using GC and GC-MS (chapter 5). Lignans and the essential oil are the main classes of secondary metabolites of *P. cubeba*.

The discovery of podophyllotoxin, the starting compound for the semisynthetic anticancer drugs etoposide®, teniposide® and etophopos® stimulates the research of lignans including derivatives of podophyllotoxin and its biosynthetic intermediates. In chapter 6 we present the enhancement of the production of 6-methoxypodophyllotoxin in cell suspension cultures of *Linum flavum* using ethylenediamine tetra acetate (EDTA) and other glucosyltransferase inhibitors. The enzyme glucosyltransferase converts coniferyl alcohol to coniferin that accumulates in the cells in high amounts. Coniferyl alcohol is a biosynthetic precursor of 6-methoxypodophyllotoxin and other lignans. We carried out feeding experiment in order to inhibit the coniferin formation and to enhance the 6-methoxypodophyllotoxin production. The lignan biosynthesis in cell suspension cultures of *L. flavum* was also investigated at a genetic level. We tried to clone the glucosyltransferase in *Escherichia coli* and to study the enzyme further in order to better understand the biosynthesis pathway of lignans (chapter 7). The ultimate goal is to develop a tool to enhance the production of cytotoxic lignans.

The production of a cytotoxic lignan, justicidin B, using hairy roots cultures of genetically modified *Linum leonii* (transformation by *Agrobacterium rhizogenes*) is described in chapter 8. Justicidin B is an arylnaphthalene lignan which exerts cytotoxic, antiviral, fungicidal, antiprotozoal and

antiplatelet properties. In addition to the production we also study the cytotoxic activity of justicidin B against three chronic myeloid leukemia-derived LAMA-84, K-562 and SKW human cell lines.

This thesis covers a broad range of lignan studies including phytochemistry, biotechnology and genetic engineering in order to better understand and to enhance the production of lignans. Subjects of our study were two selected Indonesian plants used in *jamu*, and two European *Linum* species that contain cytotoxic lignans.