Phytochemical analysis of avocado seeds (Persea americana Mill., c.v. Hass)
1. INTRODUCTION

1.1. Objectives of phytochemical analysis

During thousands of years of development of human cultures the use of medicinal plants with curing properties influenced people around the world. For European people, active plant species were precious and an important reason for settlement and trade (Rätsch, 1997).

The ethnomedicine covers healthcare systems that include beliefs and practices related to diseases and health, which are products of indigenous development and are not explicitly derived from a conceptual framework of modern medicine (Iwu and Wootton, 2002).

The indigenous groups have used curing plants as their personal phytomedical remedies and also for spiritual reasons. The herbal medicines are a readily available resource for primary health care (Ankli et al., 2002). Nowadays, Mexico is still a country where ethnomedicine is very popular and socially of high importance. People of the tribes Tepehuan (cf. Fig. 1-1), Mayan, Huichol and other Mexican folk cultures believe in the direct relation between herbal activity and the healers in contact with God.
During the acquisition of phytomedicinal knowledge of healing, many people died by ingestion of overdosed preparations made of extracts using bioactive plants containing toxic ingredients.

Many cultures tried to preserve their acquired phytomedical knowledge in illustrated or written form, e.g. on stone tablets or later in pharmacopoeias.

Through the close relationship between people and plants some scientific disciplines were born. The pharmacologists evaluate the benefits and risks of using potentially active medicinal plants, botanists classify plants according to morphological and chemotaxonomical data, and chemists elucidate the structures of molecules responsible for activity (Balick and Cox, 1997).

The phytochemistry was born first as part of the pharmacognosy with the aim of isolation of plant substances and their systematic structural elucidation. The discovery of substances having biological activities started with the industrial development in the second half of the 19th century. The morphine alkaloid was first isolated by F.W. Sertürner. The principles of the antibiotic action of penicillin (not of plant origin but from fungus) was discovered in 1928 by Sir A. Fleming. Both
discoveries may serve as good examples for the beginning of the phytochemistry area (Nuhn, 1997).

The growing possibilities using chromatography and spectroscopy in phytochemical analysis made it possible to find new bioactive natural products.

Ingredients used in the ethnomedical remedies provide attractive templates for the development of new pharmaceutical products. Nowadays, there is an enormous natural product pool containing substances of well known bioactivities and scientists have the opportunity to investigate structure-activity relationships and mechanisms of action.

Synthetic variation of the natural lead-structures have led in various cases to the discovery of more active compounds being possible new drugs of the future and therapeutic models in the treatment of so far incurable diseases.

Another way the ethnomedical preparations possess self vital healing energy and can be developed into dietary supplements and phytomedicines with defined characteristics but with different pharmacodynamic properties as the pharmaceuticals containing isolated single chemical compounds.

In the tropical rain forests there are still many plant species waiting for a thorough phytochemical investigation. This natural pharmacy is endangered by fastly moving destruction of the vital biosystems by emerging uncontrolled timber logging for wood production and generation of new farm land. Time seems to run out for many species and making needs to awake and stop the elimination of natural resources.

Avocado fruits (Persea americana Mill.) are a very popular and recognized healthy fruit (Knight, 2002) cultivated in many subtropical and tropical areas of the world. The origin of the plant is Southern Mexico (Hegi, 1958; Rodríguez-Suppo, 1982; Ochse et al., 1986; De Luna and Flores, 1994). Although avocado grows in wild forms, the variety Hass is preferably cultivated in Mexico due to a higher content of fruit flesh and superior sensory attributes (Ramos et al., 2004a).

After industrial fruit processing, avocado seed material is generally disposed, although it could be a potential source for food supplements and for medicinal products. It is noteworthy that ethnopharmacology of the Aztec and Maya cultures (cf. Fig. 1-2) used decocts of avocado seeds as a potent agent to treat mycotic
and parasitic infections. They are used also against diabetes (Lozoya and Lozoya, 1982; Kunow, 2003). Additionally, local anesthetic effects of avocado seed preparations are known to decrease muscle pain. The seed is also used as anti-inflammatory drug (Argueta-Villamar et al., 1994; Cabrera, 1996).

![Figure 1-2. Aztecan ethnomedicine. A. Some used plants (“peyote”) (Rätsch, 1998). B. Ancient Aztec healers and some of the medicinal plants they used (Balick and Cox, 1996). C and D. Aztecan medicine and fertility goddess (Codex Borgia, 68; http://www.urologiaaldia.com/imagenes/aztecas/23.jpg; http://fis.ucalgary.ca/aval/321/Mexico.html).]

1.2. Current knowledge about avocados

Previous phytochemical studies of avocado seeds identified various classes of natural products, such as phytosterols, triterpenes (Werman et al., 1990; Lozano et al., 1993), fatty acids with olefinic, and acetylenic bonds (Kashman et al., 1969),
furanoic acids (Farines et al., 1995), dimers of flavanols (Geissman and Dittmar, 1965), and oligomeric proanthocyanidins (Valeri and Gimeno, 1953; Thompson et al., 1972).

Different in vivo assays with animals (Werman et al., 1989, Werman et al., 1991; Oelrichs et al., 1995; Alvizouri et al., 2003), and humans (Carranza et al. 1995) as well as in vitro assays with virus (de Almeida et al., 1998), bacteria (Sugiyama et al., 1982), enzymes (Hashimura et al., 2001), fungus (Domergue et al., 2000), insects (Rodríguez-Saona et al., 2000), and cells (Henrotin et al., 1998; Kim et al., 2000a, Kim et al., 2000b) have been used to test the biological activities from avocado tissues (fruit, leaves, seeds, and bark). Variable results have been obtained depending on the system used.

1.2.1. Known biological activities from avocado (Persea americana Mill.).

The avocado leaves’ aqueous extract can be very toxic for horses but not for cows, while the lipophilic extracts are toxic (Oelrichs et al., 1995). In case of humans the aqueous extracts are reported to have positive influences on human health (Adeyemi et al., 2002). Some lipids isolated from the avocado fruit have shown a selective activity against human prostate adenocarcinoma (Oberlies et al., 1998).

Details about the known biological activities from Persea americana will be described below and the structures of the involved substances responsible for these activities will be explained on pages 28 to 31.

1.2.2. Pharmacological and toxicological profiles

From avocado fruit four principal groups of lipophilic substances are known: acetylenic, vinylic, trihydroxylated and furanoic compounds. The last group are called avocatins (Farines et al., 1995) or avocadofurans (Rodríguez-Saona et al., 2000), and the rest are called acetogenins. In the polar fraction proanthocyanidins have been identified (Valeri and Gimeno, 1953; Geissman and Diettmar, 1965; Thompson et al., 1972).

Some of the known biological activities from avocado tissue extracts are known as rooting promoters, exhibiting insecticidal-, antifungal- and antimicrobial-activities.
Also cholesterol lowering effects, enzyme inhibition, anti-inflammatory, analgesic, antitumor, vasorelaxant and hypotensive activities were described (Adeboye et al., 1999).

An excellent avocado rooting promoter is 16-heptadecylene-1, 2, 4-triol (56) (cf. Fig. 2-13, p. 29) in its (2R, 4R) natural form (Becker et al., 1990).

A strong suppressive effect has been shown for the liver toxin D-galactosamine, in rats liver system by the acetogenins persenone A [(5E,12Z,15Z)-2-hydroxy-4-oxoheneicosa-5,2,15-trienyl-acetate] and persin [(2R,12Z,15Z)-2-hydroxy-4-oxoheneicosa-12,15-dienyl-acetate] (Kawagishi et al., 2001). The last one also showed a high insecticidal (Rodríguez-Saona et al., 1998 and 1999) and antifungal activity (Bull and Carman, 1994; Ardi et al., 1998; Domergue et al., 2000).

Antifungal and antibacterial activities have also been shown by 1-acetoxy-2, 4-dihydroxy-n-heptadec-16-ene (60) that is a growth inhibitor (Bittner et al., 1971). Its trihydroxy homologue showed antibacterial activity (Sugiyama et al., 1982). Similar compounds with antifungal activity possessing (2S, 4S) configuration (cf. Fig 2-14, p. 31) have been identified by Domergue et al. (2000).

Adikaram et al., (1992) suggested that the acetylenic partial structures in some of these compounds seem to be the active side and reason for the fungicidal and insecticidal activity. In this study, both persin (66) and persenone A (64) were tested. The only difference (cf. general chapter Fig. 2-14 p. 31) between both compounds is an additional double bond conjugated with a keto group in case of persenone A (the less active) and therefore it remains unclear which chemical group is responsible for the observed activities.

With regard to its nutritional value, avocado fruit is one of the richest in the content of unsaturated fatty acids and people consuming a special avocado based diet showed lower cholesterol levels. Also beneficial skin healing effects may be due of the positive influence of this kind of fatty acids (Carranza et al. 1995).

The enzyme acetyl-CoA carboxylase (ACC), which is a key enzyme in fatty acid biosynthesis, is strongly inhibited by persenone A, persin and as well by (2R,4R)-1-acetoxy-2,4-dihydroxy-heptadec-16-ene (61) and (2R,4R)-1-acetoxy-2,4-dihydroxy-heptadec-16-ene (58a) (cf. Fig. 2-14 p. 31) found in the fruits and also in the seeds. It seems to be likely that the ACC inhibition is not related to the
unsaturated terminal bond (in the case of vinylic and acetylenic groups) but to the chain-length. It has been also proposed that the non-toxic ACC-inhibiting substances could be a beneficial tool to suppress fat accumulation and hence to avoid obesity (Hashimura et al., 2001).

In our present study on avocado seed, the brine shrimp assay (*Artemia salina* L.) revealed for the lipid fractions high toxicity values (cf. p. 206). For the further use of these components, e.g. therapy of obesity, it has to be assured that there is no toxicity related to these inhibitors of ACC enzymes.

Against the human prostatic carcinoma (PC-3), the compounds 1, 2, 4-trihydroxy-nonadecane (54), 1, 2, 4-trihydroxy-heptadec-16-ene (55) and 1, 2, 4-trihydroxy-heptadec-16-yne (56) (cf. Fig. 2-13, p. 29) had shown high activity and good selectivity. According to their structure-activity-relationship, the compounds with terminal alkyne groups are more potent than the terminal alkene. A terminal alkane is less active than all other substances under investigation (Oberlies et al., 1998).

Persenone A and persenone B could be possible agents to prevent inflammation-associated diseases including cancer by suppression of the expression of the inducible form of the enzymes (i.e. nitric oxide synthase and cyclooxygenase in macrophages) implicated in this biochemical processes (Kim et al., 2000a and 2000b).

The osteoarthritis is an inflammatory process and some of the used therapies are the administration of drugs for symptomatic relief; drugs that stimulate production of cartilage matrix components; and drugs that inhibit cartilage destruction. Henrotin et al. (1998) reported about the effects of avocado/soybean oils mixture acting as potent inhibitors of substances responsible for inflammation processes in human articular chondrocytes (i.e. neutral protease activity, proinflammatory cytokines and prostaglandine-E2). Furanoic compounds from the unsaponifiable avocado fruit-oil are considered to be responsible for such anti-inflammatory activities. Their structures are described later (cf. Fig. 2-12 p. 28).

For chronic diseases such as hepatic fibrosis and lung fibrosis, the activity of the enzyme lysyl oxidase is increased accompanied with collagen accumulation. The unsaponifiable avocado fruit oil components are able to inhibit this enzyme.
Therefore it could be considered to be used in the treatment of disorders of connective tissue (Werman et al., 1990).

Anti-inflammatory and analgesic activities have also been shown by the aqueous extracts from the avocado leaves (Adeyemi et al., 2002) as well as a strong inhibition on herpes simplex virus type 1 (HSV-1), Aujeszky’s disease virus (ADV) and adenovirus type 3 (AD3) (de Almeida et al., 1998).

The leaves of *Persea americana* have been studied as source for vasorelaxant activity (Adeboye et al., 1999). Hence the aqueous extracts from the leaves could be considered also for treatment of high blood pressure (Owolabi et al., 2005).

With regard to the toxicological profiles, avocado fruits and their fruit oils are considered to be safe (Werman et al., 1990), whereas the unsaponifiable oils from avocado seeds are potentially hepatotoxic (Valeri and Gimeno, 1953; Werman et al., 1989; Werman et al., 1991; Werman et al., 1996). The natures of the substances responsible for this activity are unknown.

Persin (in its *R*-configuration) was responsible for the mammary cell necrosis in lactating mice (Oelrichs et al., 1995; Carman et al., 1995). This activity was also tested with derivatives in which the acetyloxy group at C1 was substituted with a butoxy or phenoxy group by changing the ester linkage for an ether linkage, or replacing the ketone group at C4 with a hydroxyl group. It was found that only persin was active (Oelrichs et al., 1995; Oelrichs et al., in Garland et al., 1998).

It would be interesting to know which of these chemical groups are responsible for this activity because structurally related substances (i.e. with the same functional groups such as persin but not having a longer extension of the carbon chain) were not active. A combination of the chain-length and substitution pattern could therefore be important factors for this activity (Hashimura et al., 2001).

More details about the chemical structure of these substances will be discussed in the general chapter (cf. p. 27).

### 1.3. General objectives

The avocado is a tropical fruit. Mexico continues to be the most important producing nation, accounting for nearly 40 percent of total world production (http://www.fao.org/docrep/006/y5143e/y5143e1a.htm). The production amounts