CHAPTER II

LITERATURE REVIEW

A. *Artocarpus Lakoocha.* Roxb. and Puag-Haad

Artocarpus Lakoocha. Roxb. is a tropical tree belonging to the family Moraceae and locally known as Ma-Haad (Figure 1). It is widely distributed in the northern, northeastern and central part of Thailand as well as in South and Southeast Asian countries.

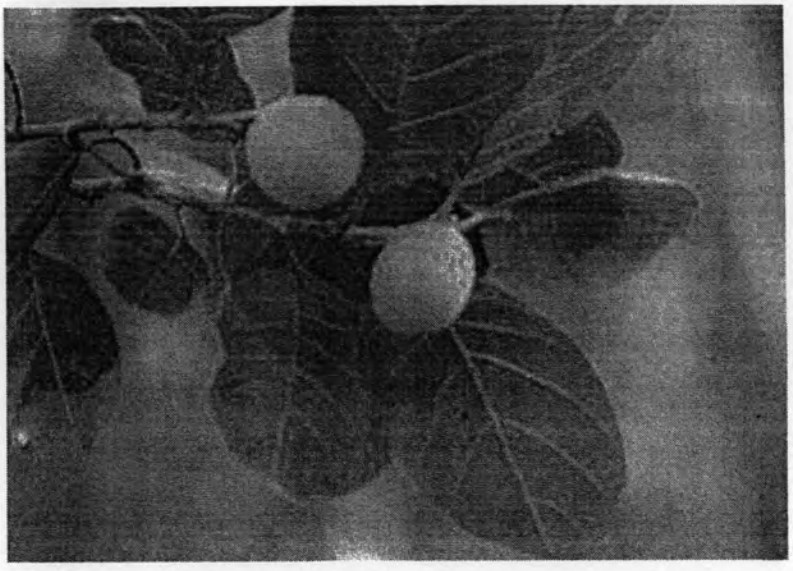
Ma-Haad is a large deciduous tree reaching 15-18 m in height with a spreading head; bark rough, grey; young shoots thin, densely clothed with a soft grey, tawny or rusty tomentum. Leaves cariaceous, 10-30 by 5-15 cm, oblong, elliptic or subovate, entire (the young ones sometimes serrate), obtuse, cuspidate, glabrous, and shining above, softly pubescent beneath, base broad or narrow, truncate or rounded; main nerves 6-12 pairs with reticulate venation between; petioles 1.3-2.5 cm long, lanceolate tawny pubescent. Flower in auxillary globose shortly pedunculate heads; bracteoles peltate. Male flower: Sepals 2-3, triangular, truncate, puberulous . Stamen 1; filament broad below, tapering upwards; anther exserted, short, broad, 2-called. Female flowers: Anthocarps completely units. Fruit 5-7.5 diameter, lobulate, smooth, velvety, yellow, edible. Seed oblong, few, broad, about 13 mm across (Kirtikar and Basu, 1980).

They are cultivated for medicinal use. The claimed efficacies in Thailand traditional textbooks are as follows (Farnsworth and Bunyapraphatsara, 1992):

Roots: as an antipyretic, anthelmintic; for allervation of toxic symptoms and treatment of urinary stones.

Wood: an antiflatulence, carminative and laxative; treatment of skin rash; chronic gartrointestinal ailments of children between the ages of 5 to 13 characterized by marked malnutrition and usually associated with intestinal parasitism; round and tape worm infestation; menstrual disorders; fainting; and any disorders or diseases which cause cachexia, disorder of flatulence and tendomyopathy.

Bark: as antipyretic.



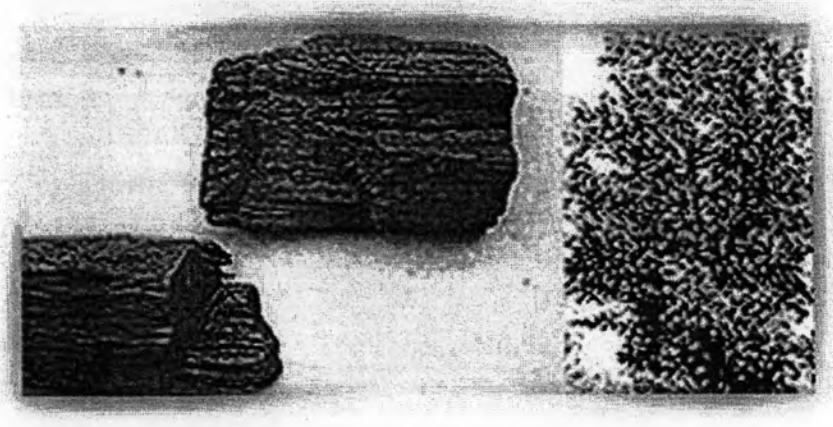


Figure 1. *Artocarpus lakoocha* Roxb. (Ma-Haad) (Joshee et al., 2002)

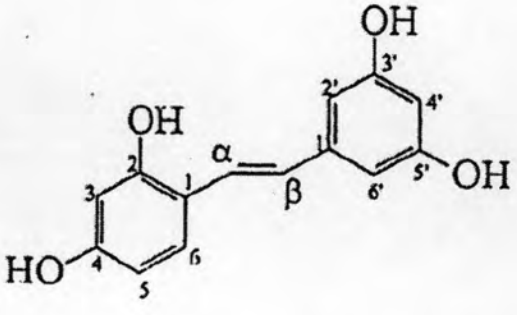


Figure 2. Chemical structure of oxyresveratrol or 2, 4, 3′, 5′-tetrahydroxystilbene (Sritularuk, 1998)

Pith: treatment of menstrual disorders; any disorders or diseases which cause cachexia; nephropathy; distension of abdomen due to peritonitis or paralytic ileus; insomnia; malnutrition syndrome in children due to intestinal parasitism; splenomegaly; eye irritation; dissipate hematoma; oropharyngeal symptom from gastroenteric disease; dyspepsia caused by wind element; cramps; clouded mind; incontinent urination; as antidiarrheal, anthelmintic, taenifuge, antituberculosis, analgesic and for increasing appetite.

In Thai traditional medicine, a dried aqueous extract of *A. lakoocha* heartwood, locally known as "Puag-Haad", has been used as an anthelmintic and antipruritic. The main component of the extract or Puag-Haad powder is 2,4,3',5'-tetrahydroxystilbene, which is also known as oxyresveratrol (Figure 2) (Mongkolsuk et al., 1957). Yodhabandu (1960) and Poopyruchpong et al. (1978) found oxyresveratrol in 51 to 70 percent yield of Puag-Haad. Tiptabiankarn (1967) reported that the main constituent of Puag-Haad (oxyresveratrol) is considered to be an effective antioxidant by delaying rancidity of lard as compared to Tenox II (Tenox II contains 20% BHA , 6% propyl gallate, 4% citric acid and 70% propylene glycol). Oxyresveratrol has been reported to exert an anthelmintic activity (Charoenlarp et al., 1981; Preuksaraj et al., 1983) and exhibit good safety profile in cytotoxicity test (Nilvises et al., 1985; Ngamwat et al., 1987). Morever, the pharmacokinetic properties in human studies have also been investigated (Tanunkat, 1990).

Recently, Sritularak et al.(1998) reported a potent inhibitory effect of the methanolic extract of *A. Lakoocha* on enzyme mushroom tyrosinase *in vitro* using L-DOPA as a substrate . Further comparison of its active constituent, 2, 4, 3', 5'-tetrahydroxystilbene (oxyresveratrol), showed that the compound had a concentration causing 50% enzyme inhibition (IC_{50}) of about 1.5 μM, which was 17.9 times higher than kojic acid (Sritularak, 1998; Sritularak et al., 1998). The IC_{50} value of oxyresveratrol was in agreement with Shin et al. (1998) and Kim et al. (2002), who reported the value of 1.0 and 1.2 μM, respectively. Following, the *in vitro* study, the *in vivo* skin whitening efficacy of the extract was evaluated in guinea pigs and human volunteers (Tengamnuay et al., 2003). The result of the study clearly demonstrated that the heartwood extract of *A.Lakoocha* could reduce melanin formation in both guinea pigs and humans. Comparing to other tyrosinase inhibitor commonly used in commercial whitening product such as kojic acid and licorice extract, the data were in agreement with the *in vitro* tyrosinase inhibitory effect, which showed that

oxyresveratrol demonstrated the highest anti-tyrosinase activity (Sritularak, 1998). Also, the anti-HIV and Herpes simplex virus activity has recently been reported (Sritularak, 1998; Likhitwitayawuid et al., 2003). Despite the above findings about *A.Lakoocha* heartwood extract, however, its many other beneficial properties, especially for cosmetics and dermatological applications are not widely known or studied.

Puag-Haad

Puag-Haad (Figure 3) is a dried aqueous extract of the heartwood of *A.Lakoocha* and its activities come from 2, 4, 3', 5'-tetrahydroxystilbene, the major constituent (Poopyrunchpong et al., 1978; Farnsworth and Bunyapraphatsara, 1992). Puag-Haad usually appears in the local herb market as a brown lump, which can be ground to give a light yellow powder. It is prepared by boiling chips of *A.Lakoocha* wood in water and the aqueous extract is concentrated by gentle heat. On cooling a yellow-brown powder of Puag-Haad is separated. The precipitate is filtered and dried near the fire (Mongkolsuk et al., 1957).

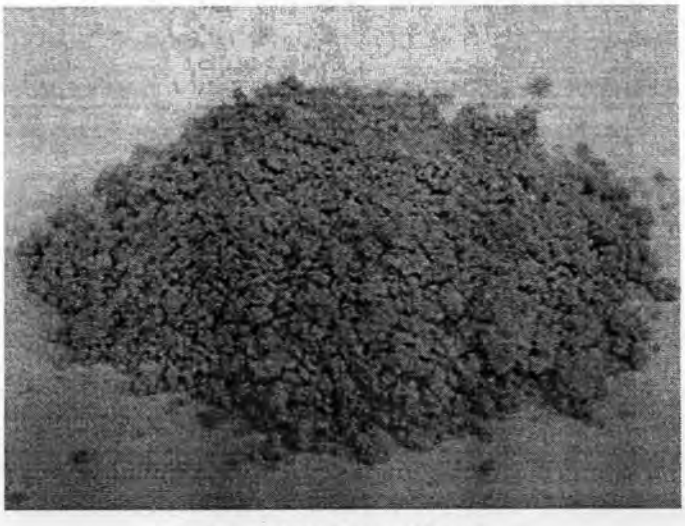


Figure 3. Puag-Haad

Due to its polyphenolic structure, one study has been carried out to determine the antioxidant properties of oxyresveratrol and its derivative from *A.Lakoocha* (Tiptabiankarn, 1967). The extract was evaluated in terms of its anti-rancidity in lard using the active oxygen method and Wheerer method. It was found that

oxyresveratrol could increase the stability of lard by delaying rancidity and is considered to be an effective antioxidant compared to Tenox II. Recently, the antioxidant and free radical scavenging effect of oxyresveratrol have been reported (Lorenz et al., 2003). They found that oxyresveratrol was a more potent scavenger of DPPH (2, 2-diphenyl-1-picryl-hydrazyl) and nitric oxide radicals than resveratrol, a related substance well known for its strong antioxidant activity. They thus suggested that it may have important therapeutic application such as in neuropathologies where oxidative/nitrosative stress is involved. Others have reported about the inhibitory effect effect of oxyresveratrol on cyclooxygenase (Shin et al., 1998b) and rat liver mitocondria ATPase (Nimmanpisut et al., 1976). However, little is still known of the many aspects of antioxidative/free radical scavenging activities of the extract or oyresveratrol, especially regarding the cosmetic applications.

Recently, the reactive oxygen species (ROS) scavenging properties of Puag-Haad have been investigated in more detail by comparing its inhibitory activities on DPPH, superoxide anion radical, hydroxyl radical and singlet oxygen (Wachiranuntasin, 2005). She found that Puag-Haad and oxyresveratrol were effective in scavenging these ROS and some of their effects were comparable or even superior to epigallocatechin gallate (EGCG) or pine bark extract, the well-known potent antioxidants in commercial use. It thus would be interesting to further investigate if the *in vitro* antioxidant activities of Puag-Haad could result in improvement or delaying the skin aging conditions upon application *in vivo*.

B. Skin and Aging

1. Skin

The skin is a complex, dynamic organ of many cell types and specialized structure serving multiple functions crucial to health and survival (Allen, 1967; Epstein, 1988). Skin covers entired surface of the body and protects it from various types of external stimuli and damages as well as from moisture loss. An adult has a surface area of about 1.6 m^2 (Mitsui, 1997). The skin provides a number of unique functions.

1. Protection

The skin protects deeper cells from potential damage that may occur from exposure to a number of hazardous environment (i.e, desiccation, chemical and

mechanical injury, microbial and fungal invasion, damaging effects of ultraviolet radiation) (Marieb, 1995; Wilkinson and Moore, 1982; Parker, 1991).

2. Thermoregulation

The skin regulates and helps maintain body temperature (Masoro, 1987). It adjusts the body temperature by changing the amount of blood flowing through the skin by dilation and constriction of the skin blood capillary and by the evaporation of perspiration (Mitsui, 1997).

3. Sensory perception

The skin senses change in the external environment and is responsible for the skin sensations (Mitsui, 1997)

4. Vitamin D production

The skin is the site of vitamin D3 production which results from the ultraviolet-mediated conversion of precursors. The body's entire vitamin D3 requirement is easily met by the skin and it is therefore conceivable that skin also protects toxicity from ultraviolet by prevention of hypervitaminosis (Thody and Friedmann, 1986).

5. Other functions

The skin also plays a role indicating emotional state, such as blushing, and fright (paleness and erect hair), and can be described as an organ signaling emotion (Mitsui, 1997).

The skin is divided into three layers called epidermis, dermis, and the subcutaneous layer (Figure 4).

The epidermis is composed of several cell layers about 0.1-0.3 mm thick. From the external surface inwards, these layers are called stratum corneum, stratum spinosum, stratum lucidum, stratum granulosum, and stratum basale (Figure 4). The principle cells of the epidermis are keratinocytes whose main purpose is to produce the fibrous protein keratin, which protects against frictional forces. The basal keratinocytes undergo cell division. One of the newly divided cell remains at basal layers and others move towards to outer epidermis, beginning the keratinization process. The horny cells are created continuously, the oldest cell are shed from the outer surface of the skin but they are replaced from below thereby maintaining the thickness of the horny layer. This type of continuous replacement of the cell layer is called "turnover". The turnover rate varies with the site and age, but it has been estimated to be approximately 26-28 days (Mitsui, 1997; Rongone, 1997). When the

skin is penetrated by a foreign object or the horny layer is damaged, the division of the cells in the basal layer increases in response causing the turnover rate to increase thereby expelling the foreign object and promoting recovery. In addition, repeated chemical or physical stimulation increases the thickness of the horny layers. These responses protect the epidermis from external stimuli.

In addition to these keratinocytes, the epidermis also contains melanocytes which produce the pigment melanin. The melanocytes are scattered between the basal cell and the basal layer. Melanocytes produce melanin for skin pigmentation, which is partially protective against UV radiation. Melanosomes (pigment containing granules produced within the melanocytes) are presented in melanocyte dendrited and are transferred to surrounding keratinocytes. Melanin synthesis begins with the oxidation of tyrosine by enzyme tyrosinase to form 3, 4-dihydroxylphenylalanine (dopa) within the melanosomes. A second oxidation, also under the control of tyrosinase, forms dopaquinone, which undergoes additional non-enzymatically mediated oxidation and polymerization leading to the formation of the final product, i,e., either eumelanin (brown or black) of phaeomelanin (red, yellow) pigment. Phaeomelanin is formed by the addition of cysteine to dopaquinone. The epidermis also contains Langerhans cells which have immune response functions as a protective mechanism against invasion of foreign material (Fenke and Lober, 1986; Thody, 1986; Marieb, 1995).

The dermis is composed predominantly of collagen and elastin fibers which are embedded in an interfibrillar gel of gylcosaminoglycan. Collagen is the major component and is secreted by fibrolasts which are the principle cells of the dermis. The dermis is divided into the superficial papillary dermis which interlock with a rate ridge of the epidermis, and the deeper zone called reticular dermis. The former is generally the thinner, being composed of finer collagen and elastin fibers which allow the dermis to mould to the contours of the overlying epidermis in such a way that its interface represents an exact mirror image of the undersurface of the epidermis. The dermal papillae which dovetail into the rate ridges of the epidermis have a richblood supply and contain many of the sensory nerve endings of the skin. The reticular dermis, on the other hand, is relatively avascular and acellular. Its collagen and elastin fiber are much thicker than those in the papillary dermis and form a denser lattice network, which depending upon its degree of packing, confers great strength and flexibility. This enables the skin to adapt to the various movements of the body and in addition, to resist mechanical damage. There are profound regional variations

in the dermal texture rendering it appropriate to the local requirement-thus it is thin and flexible over joints but very thick and tough on back (Fenke and Lober, 1986; Thody, 1986; Marieb, 1995).

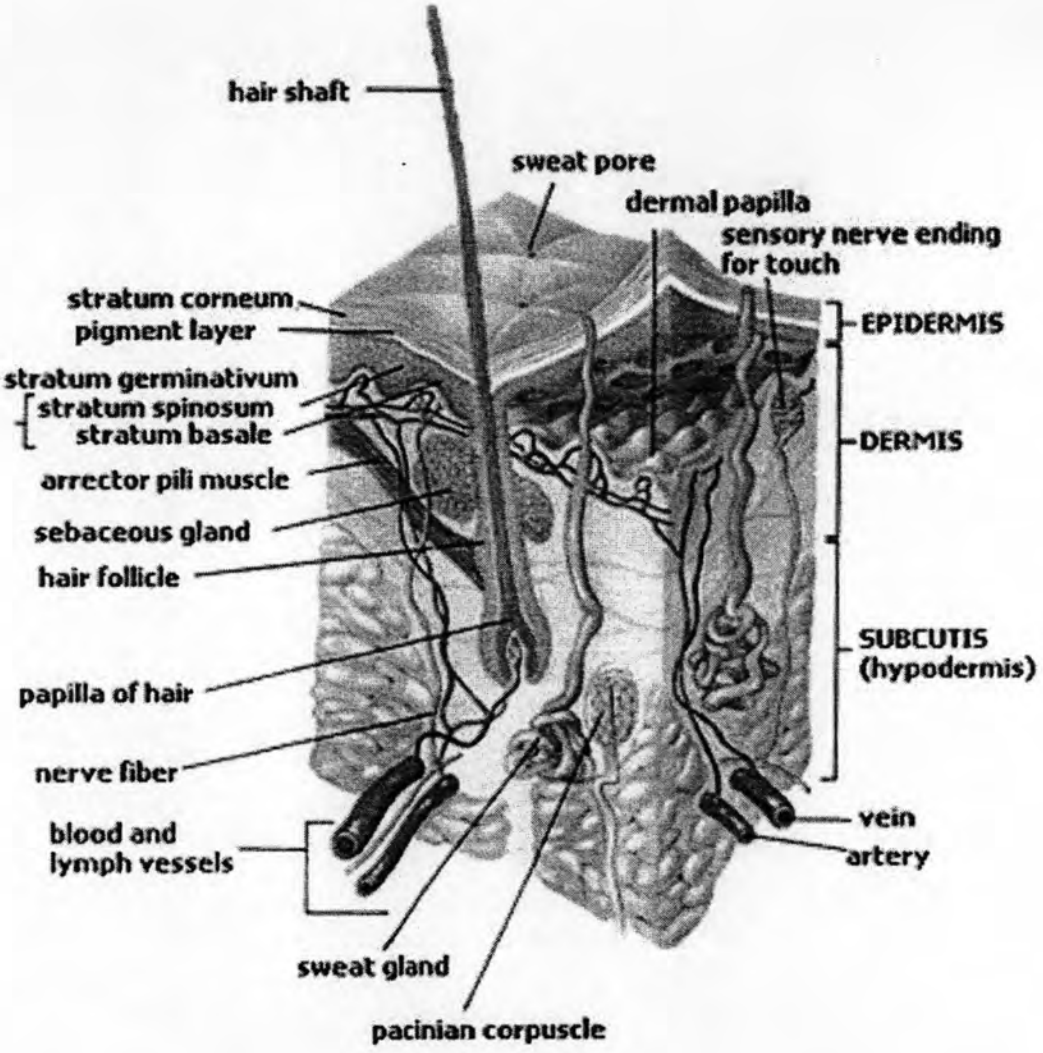
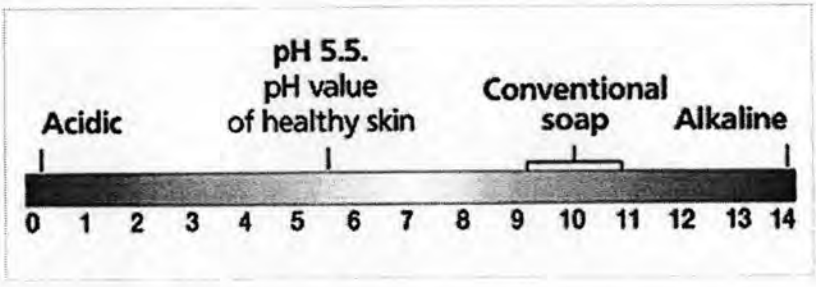


Figure 4. Basic structure of the skin

Beneath the dermis, there is subcutaneous layer or hypodermis which contains many adipose cells in and between the connective tissue. The subcutaneous tissue protects the skin from blunt and pressure-related trauma and serves as an insulator of heat loss. The loss of this protective padding result in an increase in problems of weight-bearing and pressure prone surfaces, and other injuries, as well as the risk of hypothermia (Montagna and Parakkal, 1974; Balin, 1992).

Skin Surface pH (Gil and Howard, 1996)

The skin has a special feature: its surface is slightly acidic. On average the pH value of the skin is 5.5. The slightly acidic pH value of the surface of the skin wards off pathogens that cause disease. The balance of the ecosystem of the surface of the skin promotes harmless micro-organisms well-adapted to humans which are found in thousands on every square centimetre of the skin. Harmful bacteria and fungi do not tolerate the acidic pH value and are displaced by normal micro-organisms. In addition, the acidic pH value also stabilizes the skin's function as a barrier. It ensures that lost fat in the horny layer is replenished quickly through reproduction. Another effect of the pH value of 5.5 is that the lipids in the horny layer retain their labyrinthine structure. This prevents water loss from the inside and penetration of pollutants or irritants from the outside. Thus the skin's acid mantle performs an important protective function.



Changes in the pH value of the skin weaken the protective function of the acid mantle. This promotes skin infection, dehydration, skin irritations and allergies.

2. Aging

2.1 Current theories on aging mechanism

The aging process is one of the most complex biological phenomena. Inevitably, all organism undergo a progressive deterioration in their functions, and concurrent with the passage of time, an increase in their inability to withstand challenges or stress. The nature of aging process and their underlying mechanism are difficult to define with our current knowledge (Yu, 1983). To explain these intricate changes, several theories have been advanced in the past, but no single theory is able to provide mechanism which satisfies all the age-related phenomena observed in different species. This review focuses on two major hypotheses which are probably

most satisfactory in delineating the cellular and molecular mechanisms underlying the aging and pathogenic processes (Pugliese, 1987).

2.1.1 The genetic or biological clock theory

The "genetic" theory has a variety of names, but the essential concept centers on the belief that maximum life span is controlled by the genetic material, DNA, and therefore is fixed in the time (Pugliese, 1987). A more unifying theory probably consists of concepts based on genetic instability as a cause of aging. It seems that the genetic contribution to the aging process is foremost in the determination of the life spans characteristic of various species. The genetic basis for aging is partly predicted on the observation that the range of maximum life spans of different species is much greater than the range of individual life spans within a species.

Genetic instability as a cause of age changes might include the progressive accumulation of faulty copying in dividing or otherwise functioning cells, or the accumulation of errors in information-containing molecules. The progressive accumulation of errors in the function of either fixed postmiotic cells or actively dividing cells could act as a clock. This process would intiate secondary types of mischief, which would ultimately be revealed as biological aging. Thus, aging may be the special case of morphogenesis; perhaps cells are programmed simply to run out of program (Hayflick, 1979)

2.1.1.1 Cellular aging as a programmed phenomenon (the program theory of aging)

This is one of the earliest of genetic theories, proposed by Hayflick in 1961. During embryonic development, tissues and organs undergo extensive and continuous remodeling. This is brought about by the orderly death of some cells and the activation of other cell lines controlled by genetic means. It is proposed that all cells, except the germ cells and transformed cells, bear specific "death" genes which are programmed to switch off some cellular processes in a sequential fashion to produce in the tissue the aggregate sign of aging. In this way, cellular aging and death are the ends of cellular differentiation. This theory states that the life span of animals is predetermined by a genetic program, or a so-called biologic clock (Hayflick and Moorhead, 1961; Kenny, 1982; Saxon, 2002).

2.1.1.2 The error theory

This theory is also based on the genetic information systems of the cell, DNA and RNA. It is proposed that the conversion of the information borne by these molecules into enzyme and protein synthesis becomes increasingly subject to errors, thus leading to the accumulation of inappropriate molecules that are unable to support the cell metabolism. This theory has been invoked as the mechanism underlying the fact that the life span of a species is inversely correlated with the rate of metabolism. The faster rate of metabolism affects to the faster rate of material turnover and thus the greater chance for biochemical errors (Kenny, 1982). Thus, aging and death are presumed to be the result of errors that occur and are transmitted at the cellular level. Research has not yet provided support for this theory, and it is generally no longer accepted. However, it has stimulated a great deal of research (Saxon, 2002).

2.1.1.3 Repair failure

Errors in the transcription of DNA, such as may be caused by experimental irradiation of the cell or by *in vivo* production of free radicals, can be corrected by repair processes. Two lines of evidence support the notion that aging is rooted in this mechanism: (1) the rate of DNA repair is related to the life span of the species, and (2) in cultured human cells, the rate of repair decreases as the cells age. The consequence, therefore, would again be the production of inappropriate molecules that are unable to support cell metabolism (Kenny, 1982).

2.1.1.4 Redundancy failure

The genetic message borne by the DNA molecule has a high degree of redundancy. Less than 1% of the information carried by the DNA is used by the cell, and gene sequences are repeated many times along the molecule. The theory supposes that as errors occur in gene synthesis, a supply of correct genes is available to take over from the ones damaged by error. As the cell ages, the supply of redundant (and correct) genes becomes exhausted and errors are then free to express themselves (Kenny, 1982).

2.1.1.5 The "killer hormone"

This theory invokes a hormone derived from the pituitary gland that depresses the responsiveness of peripheral cells to the thyroid hormone. Two systems for which

adequate thyroid activity appears to be necessary are the immune and the cardiovascular systems. Depression of the peripheral effects of the thyroid hormone by the pituitary factor may produce the decline and ultimate failure of these two major systems. This putative killer hormone appears to begin to be secreted at puberty, at which time it may buffer the tissues against the endocrine surge that occurs and it may restrain what otherwise would be an excessive metabolic response and burn out. Starvation, which when started before puberty delays it, extends the life span and also delays the appearance of this factor (Kenny, 1982).

2.1.2 The free radical theory

The free radical theory is the most viable and the most important concept in aging mechanism yet proposed (Pugliese, 1987; Pugliese, 1996). It is also one that we can both test and utilize in a practical manner with intermediate benefit. The free radical concept may be classified as an environment cause of aging as opposed to the genetic cause. The major difference between the two concept assume a fixed and relatively immutable life span, while the environmental concept sees adverse aging as a result of exogenously produced or initiated damage to the cell systems resulting in impairment of normal function (Pugliese, 1987).

- *Source of free radical*

It has been said that oxygen respiring organisms are both blessed and cursed by oxygen. They are blessed because oxygen supports life by providing fuel to generate energy. In contradiction, they are cursed because oxygen create free radicals that impinge on the normal biological function of organism. This dichotomous nature of life provided a biochemical basis for free radical theory of aging as proposed. This hypothesis predicts that the aging process is characterized by a decline in the physiological function with an increased incidence of disease due to free radical damage. To appreciate the biological mechanism and interactions of free radical and aging, a brief description of free radical chemistry is presented.

Oxidation of substrates by oxygen molecules produce several species of reactive intermediate such as superoxide radical, hydrogen peroxide and hydroxyl radical. These are products of reactions inherent to the atomic structure of a biradical oxygen having two unpaired electrons in its orbitals. These unpaired electrons are in parallel spin, forcing incoming paired electrons to also be in parallel spin. This

constrained electron configuration favors a univalent one-electron transfer pathway. The sequential one-electron transfer reaction generates reactive free radicals and reactive species as shown below:

oxygen+ one electron = superoxide radical ($^{\bullet}O_2^-$)
superoxide radical + one more electron + hydrogen = hydrogen peroxide (H_2O_2)
hydrogen peroxide + one more electron + hydrogen = hydroxyl radical ($^{\bullet}OH$)
hydroxyl radical + one more electron + hydrogen = water (H_2O)

Superoxide radical is a byproduct of various enzyme reactions (particularly in the mitochondria and chloroplast electron transport system), and can also be caused by environmental factors such as UV light, ultrasound, X- and gamma-rays, toxic chemicals and metal ions (Brocklehurst, Tallis, and Fillit, 1992). This free radical is known to attack enzymes and cell membrane. Within the cell membrane, the fatty acid decomposes and damages the integrity of the membrane which alters the control of materials in and out of the cells. Further decomposed products of the fatty acids form an amorphous yellow complex called lipofusin. It is lipofusin that give the skin an ugly yellow hue as people grow older or if they smoke tobacco. The attack of superoxide radical on the fatty acid is a sequential process that produces a lipid peroxide. The process, therefore, is called lipid peroxidation (Pugliese, 1996).

Hydrogen peroxide, a reactive oxygen species, is destroyed by enzymes called peroxidases which convert the hydrogen peroxide to oxygen and water. While hydrogen peroxide is not powerful oxidant, it is relatively dangerous because of two factors. The first is that it can diffuse rapidly, and cross both the cell membrane and the nuclear membrane. The second is that hydrogen peroxide can be converted to the hydroxyl radical easily in the presence of iron (Fe^{++}) (Pugliese, 1996).

The hydroxyl radical is one of the most potent oxidants. Hydroxyl radical can react with almost any compounds in the body such as enzymes, proteins, carbohydrates and lipids. It can attack lipids and produce lipid peroxides, and crosslink a number of proteins, particularly to the DNA in the nucleus (Pugliese, 1996).

In summary, these free radicals are consequently highly reactive and capable of reacting with a variety of biologically important macromolecules, including DNA, protein, and lipid (Halliwell and Gutterridge, 1999).

2.2 Skin aging

The major changes of cutaneous aging both instrinsic or chronological aging and actinic or photoaging are summarized as follow:

Table 1 shows skin changes associated with instrinsic aging. The most obvious sign of increasing age is epidermal atrophy. In the basal cell region there is increased cellular atypia. The epidermis becomes thinner, with fewer cell layers, basically owing to retraction of the rigdes. The decrease in the epidermal rete ridges leads to a reduced area of contact between the dermis and epidermis, resulting in an epidermis that separates from underlying dermis more easily than in the younger individual. Simple trauma such as application and removal of a sticking plaster, or a tightly fitting shoe may peel off the epidermis in older persons (Balin, 1992).

The number of horny cells does not diminish with age. It is important that the thickness of the stratum corneum does not change with age. On the other hand, the number of melanocytes and Langerhans cells decrease with age (Mark, 1981; Montana and Carlisle, 1979; Smith, 1989; Balin, 1989).

Aged skin retains its ability to reduce water loss, but damage to the stratum corneum barrier by physical or chemical agents may result in greater than normal water loss (Wilhelm, Cua, and Maibach, 1991; Potts, Burus, and Chrisman, 1984). In addition, the time necessary to repair the stratum corneum barrier increases considerably with age. It has been estimated that reepithelialization takes about twice as long for patients over 75 years old than for those around 25.

The renewal of the epidermis, like the stratum corneum, is slower in persons older than 60 years and epidermal wound healing takes longer. The longer renewal time means that irritant and sensitizing substances that contact the skin will remain longer and that substances, including medications, placed on the skin take longer to be shed (Balin, 1992; Balin and Pratt, 1989).

The epidermal response to photodamage differs from that seen in intrinsic aging. The initial response to UV light is a hyperproliferative response to injury and a thickening of the epidermis. Late effects of severe UV irradiation injury result in marked epidermal atrophy. Melanocytes are irregularly dispersed along the basement membrane, and epidermal Langerhans cells are markedly reduced in number compared with sun-protected sites of the same person (Fenske and Lober, 1986; Balin and Pratt, 1989). Table 2 shows structure differences between instrinsic aging and photoaging in epidermis.

In addition to all these changes in the epidermis, the aging process also acts on the dermis. The dermis becomes thinner with age. In addition, it becomes less cellular and less vascular. Measurements of total amount of dermal collagen reveal a decrease of about 1 percent per year. The remaining collagen fibers thicken, become less soluble, have less capacity to and become more resistant to digestion by collagenase. Collagen imparts tensile strength to skin, therefore, the loss of integrity of collagen fibers may explain the proclivity of skin in elderly persons to tear under even moderate stress (West, 1994).

The changes in elastic fibers differ in sun-protected and sun-exposed skin. While the amount of elactotic material is increased in sun-exposed skin, there is a spontaneous and progressive degeneration of the elastin fibers in protected aged skin. During aging, the skin thickness decreases and the skin becomes atrophic, while in photoaged skin, the thickness increases owing to the accumulation of the elastic material. While there is an accumulation of elastic material in sun-exposed skin, in protected skin the elastic fibers become fragmented and their functions become deficient because of loss of elastic fiber. The most obvious difference seen in seriously photodamaged skin is the presence of massive quantities of thickened, tangled accretions of degraded elastic fiber, which may finally degenerate into an amorphous mass. These elastic changes are not seen in normal, protected skin of even very old people. In addition, they can be quite advanced before the extensiveness of the damage becomes visible clinically. The degree of elastic-tissue damage in different regions is directly proportional to the amount of sunlight received. This is clearly demonstrates by the sharp border in the V-area of the neck between exposed and unexposed skin (Timiras, 1998; Takema et al., 1994; Fazio, Olsen, and Uitto, 1989). Table 3 shows structure differences between intrinsic aging and photoaging in dermis.

Because of the increased cutaneous laxity, the skin becomes more prone to wrinkling. The wrinkle formations are caused by various internal and external factors. UV light is known to be important cause, but there are also other causes such as environmental stresses on the skin including dryness, and physical and chemical stress (Gilchrest et al., 1983; Bhawan et al., 1993).

There are two types of wrinkles. The first is a permanent type that present as a deep wrinkle on sun-exposed skin, such as the face and the neck, which does not disappear on stretching. Microscopically, there is less elastotic change in the upper

dermis in the area of the wrinkle than in that of the surrounding skin. The other type is a fine, shallow wrinkle that developed in sun-protected skin, such as the abdomen and buttocks, which disappears on stretching. Microscopically, there is a decrease of the elastic fibers in the papillary dermis, but there are no differences in the upper dermis between the area of the wrinkles and the surrounding skin (Fazio, Olsen, and Uitto, 1989; Baran and Maibach, 1994; Gilchrest, 1989; Hazzard et al., 1999). Table 4 shows clinical differences between intrinsic aging and photoaging.

Table 1. Skin changes associated with intrinsic aging

Compartment	Component	Change	Biological Consequence
Epidermis	Keratinocytes	Decreased proliferative potential	↓ Would healing ,↓ barrier
		Decreased response to environment	↓ Cytokine, growth factor and vitamin D production
	Melanocytes	Decreased 10% - 20% per decade	↓ Photoprotection, white hairs
	Langerhans cells	Decreased as much as 40%	↓ Delayed hypersensitivity reactions
	Basement membranes	Decreased surface area	↓ Epidermal-dermal adhesion, ↑ blistering
Dermis	Fibroblast	Decreased collagen/elastin	↓ Tensile strength, ↓ elasticity
	Blood vessels	Decreased	↓ Thermoregulation, response to injury
	Mast cells	Decreased	↓ Immediate hypersensitivity reactions
	Neural elements	Decreased by one third	↓ Sensation, ↑ pain threshold
Subcutaneous	Fat	Decreased	↓ Mechanical protection and insulation
Appendages	Eccrine glands	Decreased number and output	↓ Thermoregulation
	Apocrine glands	Decreased number and output	Unknown
	Sebaceous glands	Increased size, decreased output	Unknown
	Hair	Decreased number and growth rate	Cosmetic

Table 2. Structural differences between intrinsic aging and photoaging in the epidermis

Item	Photoaged skin	Instrinsic-aged skin
Epidermal thickness	Thick epidermis	Thin epidermis
Epidermal cells (keratinocytes)	Non-uniform cells Cells distributed randomly (similar to precancerous condition) Loss of polarity Frequent enlargement Diversified melanosomes (melanosomes lacking cells)	Uniform cells Defined cell distribution Polarity maintained Usually atrophied Melanosomes uniformly distributed
Stratum corneum	Increased number of cell layers Diversified form, staining properties and size of corneocytes	Normal cell layers Uniform corneocyte size
Melanocytes	Increased cell number Diversified cells Increased melanosome production	Cell number reduction Uniform cells Poor melanosome production
Langerhans cells	Marked reduction in cell number Diversified cells	Slight reduction in cell number Normal cells

Table 3. Structural differences between intrinsic aging and photoaging in the dermis

Item	Photoaged skin	Instrinsic-aged skin
Glycosaminoglycans	Markedly increased	Slightly decreased
Elastic tissues	Tremendously increased Degenerated into amorphous mass	Increased but almost normal
Collagen	Markedly decrease of bundles and fibers	Bundles thick and disoriented
Reticular dermis	Thickened	Thinner
Fibroblasts	Increased and hyperactive	Decreased and inactive
Mast cells	Increased	Decreased
Inflammatory cells	Inflammatory cell penetration	No inflammary cells
Papillary dermis	Grenz zone of new collagen (repair zone)	Non grenz zone of new collagen
Capillary vessel	Small vessels great loss Abnormal vessel Telangiectatic	Moderate loss Normal Non-telangiectatic
Lymphatics	Practically absent	Moderate loss

Table 4. Clinical differences between intrinsic aging and photoaging

Sun-protected skin	Sun-exposed skin*
Fine wrinkling	Coarse wrinkling and furrowing
Atrophy of dermis	Thickening of the skin
Reduced subcutaneous adipose tissue	Elastosis
Reduced resilience	Reduced elasticity

* In sun-exposed skin, changes due to actinic and innate aging are superimposed.

C. Antioxidant Mechanisms

An antioxidant is any substance that when present at low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substrate (Halliwell and Gutteridge, 1995). The term "oxidizable substrate" include almost everything found in living cells, including proteins, lipids, carbohydrates, and DNA. On the other hand, antioxidants are molecules that interact with the "free radicals" thereby neutralizing them, which results in protecting normal tissue and DNA from potential damage. Because of the seriously damaging potential of reactive oxygen species, cells depend on elaborate defense mechanisms to effectively neutralize or metabolize these toxic intermediates and to prevent significant free radical-induced injury. Fortunately, the normal body mechanism has its own antioxidants to neutralize "free radicals" (Cho, 2002). Basically, the mechanisms of antioxidants involve in three different ways; (1) act as preventive antioxidant which reduces the rate of initiation of free radicals, (2) act as chain-breaking antioxidant which interacts rapidly with the radicals after chain-reaction is initiated, and converted to the stable free radicals and inhibit the propagation phase, (3) repair compounds to their original state or degrade them to non-functional compounds (apoptosis) where enzymes reaction are also involved (Bidlack et al., 1998). More recently, one has provided a convenient summary of the sequence of events involved in free radical damage and antioxidant mechanism as shown in Figure 5 (Ternay and Sorokin, 2000).

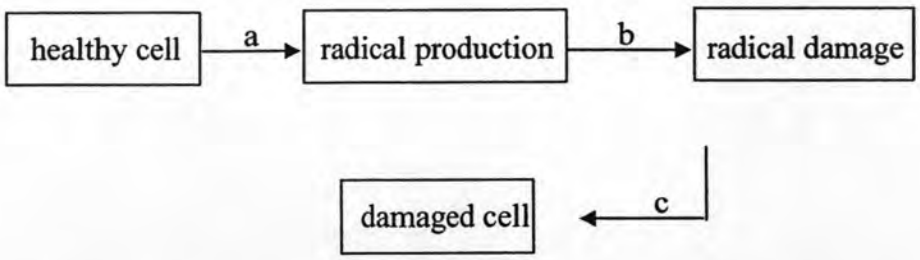
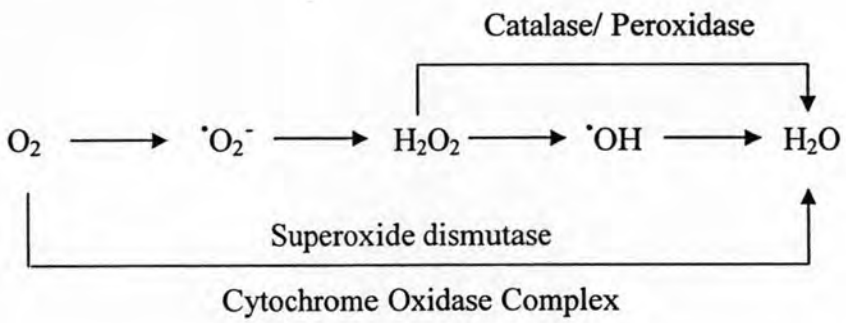


Figure 5. Diminishing radical-induced cell damage; a = radical formation prevention; b = radical scavenging; c = repair of radical-induced damage

Skin is particularly exposed to oxidative free radical aggressions, but cell possess an array of protective enzymes and compounds (Jay et al, 1998).

1. Protective enzymes



- Superoxide dismutase are group of metalloenzymes that catalyze the disproportionation of two superoxide molecules to hydrogen peroxide (H_2O_2) and water (H_2O).
- Catalase and peroxidase destroy hydrogen peroxide (H_2O_2) by converting them to water and oxygen.

2. Non enzymatic agents

Non enzymatic systems include lipophilic and hydrophilic antioxidants. Endogeneous small molecules also play an important role in the removal of toxic oxygen species. Substances which react quickly with the free radicals are commonly named radical scavengers – they act stoichiometrically with radicals, and a molecule of these protectors can only activate one or two free radicals. Generally, the body's natural antioxidant systems can effectively neutralize the radicals or oxidized products up to a certain limit. However, massive oxidative

stress and aging induced by an overproduction of reactive oxygen species (ROS) can lead to a disruption of cellular functions. Under these circumstances, there is an imbalance between oxidants and antioxidants necessitating the addition of exogenous antioxidants. Therefore, diets rich in antioxidants such as vitamin C, vitamin E, vitamin B_2, B_6, β-carotene and flavonoids have played an important role. Moreover, considerable attention has been emphasized on naturally occurring materials that can protect against ROS and their antioxidant activities have been identified (Cho, 2002).

Vitamin C (l-ascorbic acid)

Ascorbic acid or vitamin C is one of the most important water soluble antioxidants and present in high amounts in the skin. While most species are able to produce ascorbic acid, human lacks enzymes necessary for its synthesis. Deficiency in ascorbic acid causes scurvy, a disease already described in the ancient writing of the Greeks (Sauberlish, 1994). Aparts from the pure antioxidant function ascorbic acid is an essential co-factor for different enzymes. The antioxidant capacity of vitamin C is related to its unique structure (Figure 6). Due to its pKa of 4.25 it is present as a monoanion at physiological pH, which can undergo a one electron donation to form the ascorbyl radical with a delocalized electron and can be further oxidized to result in dehydroascorbic acid. Dehydroascorbic acid is relatively unstable and breaks down if it is not regenerated. Ascorbic acid can scavenge many types of radicals *in vitro* including the hydroxyl- (°OH), the superoxide- ($°O_2^-$) and water soluble peroxyl- (ROO°) radicals as well as other reactive oxygen species such as O_3, and quenches singlet oxygen. Due to their relative reduction potentials, ascorbate can reduce Fe(III) to Fe(II), which in turn can decompose hydrogen peroxide (H_2O_2) to the dangerous hydroxyl radical. Therefore, vitamin C can exert pro-oxidant effects in the presence of unbound iron (Fenton chemistry).

In the skin, vitamin C is found in all layers. In SC it forms a similar gradient as vitamin E with decreasing concentrations towards the outside. Vitamin C is depleted by O_3, UV radiation and benzoyl peroxide. One of the earliest discoveries of vitamin C benefits in the skin was the observationthat it stimulates collagen synthesis in dermal fibroblasts (Murad et al., 1981). Recently a pretranscriptional role of vitamin C had been described (Davidson et al., 1997). Also, vitamin C is essential in

the formation of competent barrier lipids in reconstructed human epidermis (Ponec et al., 1997).

Several studies have investigated protective effects of vitamin C against oxidative stress. UVB-induced immunotolerance, as a marker of damage to the immune system, could be abrogated by topical application of the vitamin C to murine skin (Nakamura et al., 1997). UVB-induced sunburn cell formation was mitigated by vitamin C in porcine skin (Darr et al., 1992). While one study reported a postadministrative protective effect of vitamin C-phosphate against UV-induced damage in mice (Kobayashi et al., 1998), another study found no such effect in humans (Dreher et al., 1999). Systemic application of vitamin C in combination of vitamin E protected against UV-induced erythema in humans (Eberlein-Konic, Placzek and Przybilla, 1998). In a keratinocyte cell culture system vitamin C reduced UVB-induced DNA damage (Stewart, Cameron and Pence, 1996). In mice, an anticarcinogenic effect of vitamin C was described (Pauling, 1991). However, no data regarding such benefits exists in humans.

Since vitamin C is not very stable, it is difficult to incorporate it into topical formulations. Esterification with phosphate is used to circumvent this limitation. Such as Mg-ascorbyl-2-phosphate and sodium ascorbyl phosphate.

Sodium Ascorbyl Phosphate (STAY-C®50) (Roland and Anna, 2002)

STAY-C®50 is a stable form of vitamin C (ascorbic acid). It is a sodium salt of the monophosphate ester of ascorbic acid (Sodium Ascorbyl Phosphate) (Figure 6) and is supplied as a white powder. The most important attributes of STAY-C®50 are as follows:

- Stable provitamin C which bioconverts to vitamin C in the skin
- *In vivo* antioxidant that is applicable to skin care, sun care and hair care products (not approved for oral care use in the US)
- Stimulates collagen production and is, therefore, an ideal active in anti-aging and skin firming products
- Reduces melanin formation that is applicable in skin brightening and anti age-spot treatments
- Has mild anti-bacterial activity and is, therefore, an ideal active in oral care, anti-acne and deodorant products

Glutathione

Glutathione (GSH) is an important water soluble antioxidant and reducing agent, presents intracellularly at millimolar concentration. Oral GSH is poorly absorbed and is not required to be provided by dietary intake (Witschi et al., 1992). In cells, glutathione is synthesized from glutamate, cysteine, and glycine (Meister and Anderson, 1983). It acts as a substrate for numerous reducing enzymes, among them glutathione peroxidase and the phospholipids hydroperoxide glutathione peroxidase. Therefore, the absence of glutathione may lead to an accumulation of lipid hydroperoxide (Briviba and Sies, 1994). Importantly, glutathione also protects cells by reacting directly with reactive oxygen species such as singlet oxygen, hydroxyl radical and superoxide radical.

In mice, ascorbate supplementation increases GSH levels in lung epithelial tissue (Jain et al., 1992), and glutathione deficiency increases hepatic ascorbic acid synthesis (Martensson and Meister, 1992), suggesting that the antioxidant actions of glutathione and ascorbate are closely linked. In humans, who are dependent on dietary vitamin C intake, this link remains to be clarified.

Vitamin E (α -tocopherol)

Alpha-tocopherol is the main component and the most active form of vitamin E. It is well accepted as the major endogenous lipid-soluble, chain breaking antioxidant in human plasma and LDL (Liu, et al., 2000). The structure is shown in Figure 6. Moreover, it serves as the preventing lipid peroxidation and modulating the metabolism of the arachidonic acid cascade initiated by lipoxygenase and/or cycloxygenase, and an increased intake of vitamin E is recommended for heart disease prevention and, on current hypothesis; it could be protective against cancers where N-nitroso compounds are implicated. Other isomers of vitamin E, such as β -, γ - and δ -tocopherols, are either present in very low concentrations or not detectable at all. Judging by their rate of reaction with peroxyl radicals, the antioxidant activity decreases in the order of $\alpha > \beta > \gamma > \delta$, in analogy with the biological potencies of these different forms of vitamin E. Bowrey, Ingold, and Stocker (1992) point out recently that tocopherol might be come a prooxidant via the so-called tocopherol mediated peroxidation, α - tocopheroxyl radical, in LDL particles in the absence of other endogenous antioxidants such as vitamin C and uniquinol-10 (Sies and Stahl, 1995; Giacosa and Filiberi, 1996; Jacob and Burri, 1996; Punchard and Kelly, 1996).

Other antioxidants

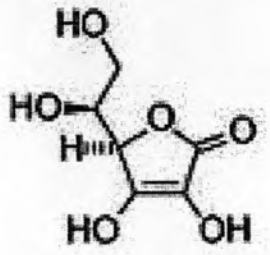
In addition to those natural antioxidants, a huge range of synthetic antioxidants are available such as those used in the rubber industry to prevent copper-catalyzed oxidative degradation of polypropylene, or in the polymer industry to prevent UV induced degradation of plastics, and for foodstuff to protect food lipid against oxidative damage (and consequent rancidity) during storage, in heat sterilization, or sterilization by ionizing radiation. Several synthetic antioxidants have long been used in biology and food technology such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate, etc. Many of these antioxidants also have properties other than a chain-breaking action. For example, most phenolic antioxidants have metal ion complexing ability, especially those antioxidants with adjacent –OH groups. However, the chain-breaking action is predominant in peroxidizing lipid systems, causing phenolic antioxidants to be powerful inhibitors of peroxidation process.

Several products of plant origin like some flavonoids and polyphenols have chain-breaking antioxidant activity. Examples are curcumin, catechin, quercetin, kaempferol and caffeic acid, etc. Several of these compounds, such as quercetin and catechin, also have metal-binding capacity.

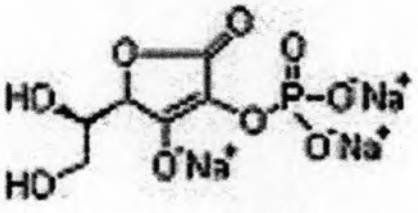
EGCG

EGCG or(-)-epigallocatechin-3-gallate is the main polyphenolic component of catechin in green tea, which has been consumed as a popular beverage in Asian countries for many centuries (Baumann, 2002). Its structure is shown in Figure 6. Topical EGCG reduced UVB-induced inflammatory responses and infiltration of leukocytes in human skin. Moreover, topical application of EGCG also inhibited carcinogenesis and selectively increased apoptosis in UVB-induced skin tumors in mice (Pinnell, 2003). EGCG enhances the activity of superoxide dismutase (SOD) and catalase in mouse striatum thus suggesting that flavan-3-ols can also exhibit their neuroprotective effect via regulation of gene expression. The high antioxidant activity of EGCG is explained by the presence of galloyl moiety attached to flavan-3-ol at the 3 position, adding three more hydroxyl groups (Soobrattee et al., 2005). EGCG has been represented as a powerful radical scavenger, as investigated by many *in vivo* and *in vitro* techniques (Geetha et al., 2004; Hsu, 2005; Soobrattee et al., 2005). However, a low concentration of EGCG increased amounts of double base

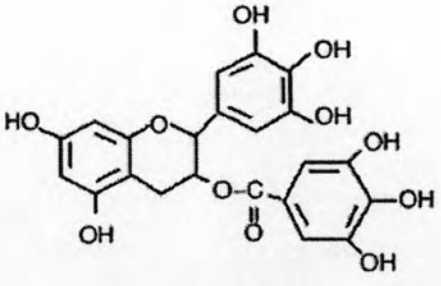
lesions of DNA, especially 8-oxodG in HL-60 cells, further supporting the involvement of H_2O_2 in cellular DNA damage. These results suggested that EGCG can act not only as an antioxidant, but also as a pro-oxidant in the presence of metal ions (Furukawa et al., 2003). Green tea has caused so much excitement in the media that many pharmaceutical and cosmetic companies are supplementing their skin care products with green tea extracts. It will be interesting to see the long term results of green tea therapy for photoprotection (Baumann, 2002).



Vitamin C



STAY-C®50



EGCG

Figure 6. Structure of Vitamin C, STAY-C®50 and EGCG

D. Stability of Cosmetic Preparations and Role of Antioxidants

The stability of a cosmetic product may refer to both the physical and chemical stabilities (Mithal, 1980). The most common goal is to preserve the products that are particularly susceptible to oxidation which causes the formation of objectionable degradation products with unpleasant odor or color. Many active components in cosmetic preparations are claimed to have an anti-aging effect through their antioxidant activities on the skin. Most of these compounds are phytochemicals and plant extracts. Their chemical structures are often flavonoids or polyphenols in nature. However, these substances are also prone to oxidation, especially from the atmospheric oxygen. Fortunately, this aspect can be taken care of by inclusion of appropriate synthetic antioxidants. These additives have appreciable benefits by providing protection for other oxygen sensitive compounds like those phytochemicals that are the active components of the products (Carter, 1975).

In general, the antioxidants used as a product stabilizer can be categorized into:

1. True antioxidants (by breaking the free radical chain). They are effective against oxidation by atmospheric oxygen (autooxidation). For example, 0.01-0.1% butylated hydroxyanisole (BHA), 0.005-0.15% propyl gallate and 0.01-0.1% tocopherol (Boylan, Chowhan and Cooper, 1986).

2. Reducing agents; they are not preferentially oxidized but act by blocking an oxidative chain reaction. For example, 0.01-0.15% sodium metabisulfite and 0.01-0.02% sodium sulfite (Carter, 1975).

3. Synergists; these antioxidants generally have little effect by themselves but enhance the action of the true antioxidants either by removing pro-oxidant metals or by regenerating the antioxidant by reduction. For example, ethylenediaminetetraacetic acid (EDTA) and calcium. EDTA is a chelating agent. It forms stable water-soluble complexes (chelates) with alkaline earth and heavy metal ions. The chelated form has few of the properties of the free ions, and for this reason chelating agents are often described as "removing" ions from solution (also called sequestering). The stability of the metal-EDTA complex depends on the metal ion involved and also on the pH. Antioxidant synergists have been used both alone and in combination with true antioxidants. Concentrations in the range of 0.005-0.1% have been employed (Boylan, Chowhan and Cooper, 1986).

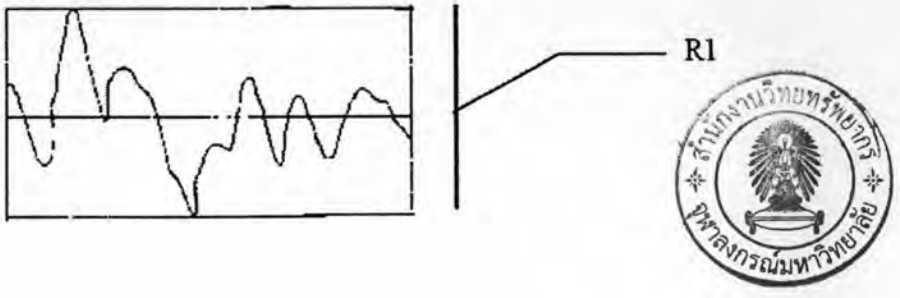
E. Instrumental Principle and Measurement for Evaluating Skin Conditions

1. Skin roughness (CK electronic GmbH, 2005)

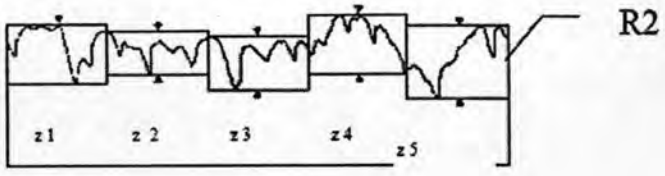
Skin roughness is measured by Visioscan® VC 98 (Figure 7). A measurement where the skin can be monitored optically using an image digitalization process without using replica is a great process in scientific research, done by Prof. Tronnier from the University of Witten in Germany. This new method is called SELS (Surface Evaluation of the Living Skin). It is based on a graphic depiction of the living skin under special illumination and the electronic processing and evaluation of this image according to four clinical parameters. The parameters correspond quantitatively and qualitatively to the physiological condition of the skin surface. Those parameters are, according to Prof. Tronnier, the skin smoothness (SEsm), which is the calculation from the average width and depth of the wrinkles. Evidently, the tenser the skin is, the better (e.g. in its youth). The second parameter is skin roughness (SEr), which seems to be the opposite parameter to the first. The scaliness (SEsc) which shows the level of dryness of the stratum corneum, and the wrinkles (SEw) calculated from the proportion of horizontal and vertical wrinkles, are the third and fourth parameters, respectively. The picture taken by Visioscan® VC 98 was also shown in Figure 7.

Roughness (R1-R5) were another important parameters to evaluate the skin surface. These parameters derive originally from the metal industry and are leaned on to the directive DIN 4762-4768 as Ra-Rz. Choose if the lines should be arranged horizontally, vertically or circularly. The advantage of circularly arranged lines is that an influence from the direction of the wrinkles is compensated.

Skin Roughness R1: The roughness R1 is the distance between the highest mountain and the lowest value, referred to as a reference length l. In the DIN norm this parameter is known as Rt.

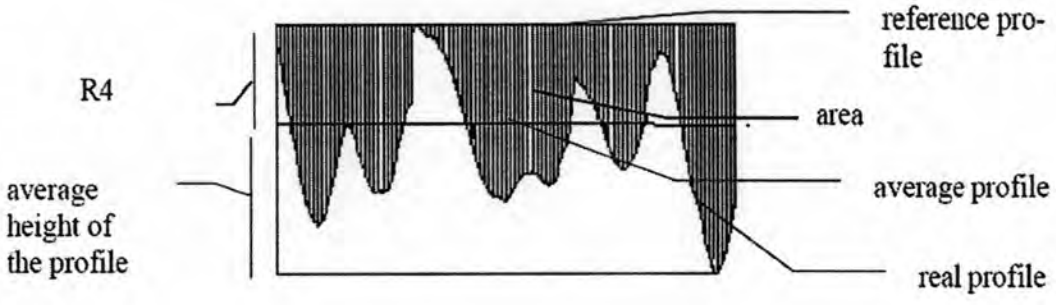


Skin Roughness R2 : R2 is the biggest roughness of the different segment roughnesses. In DIN norm this parameter is known as Rm or Rmax.

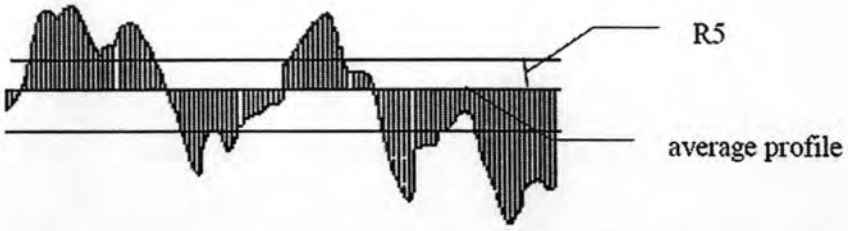


Average Roughness R3 : The average roughness is the arithmetic average of the different segment roughnesses calculated from 5 succeeding measurement segments of the same length. In contrast to R1, R3 is not that much influenced by artifacts due to calculating the average. In the DIN norm this parameter is known as Rz.

Smoothness Depth R4: This parameter describes the area above the real profile and a line drawn above the highest mountain (reference profile). This area is divided by the average height of the profile, therefore R4 is distance. In the DIN norm this parameter is known as Rp.



Arithmetic Average Roughness R5 : The area surrounded by the profile and the average profile is calculated. The average profile is the profile on the average height between the real profile and the reference profile drawn on top of the highest mountain (see above). The area is devided by the average height, thus R5 is a distance: the distance describing the standard deviation of the reference profile to R4. In the DIN norm this parameter is known as Ra.



In this study, the average roughness R3 was chosen as a key parameter. Circularly arranged lines (circular roughness) and horizontal + vertical lines (mean roughness) were used as roughness parameters.

The measurement starts by putting the probe of Visioscan® VC 98 onto the skin area to be measured. Put the probe vertically on the measurement area. Then, a specialized software calculates standard parameters relating to specific surface characteristic.

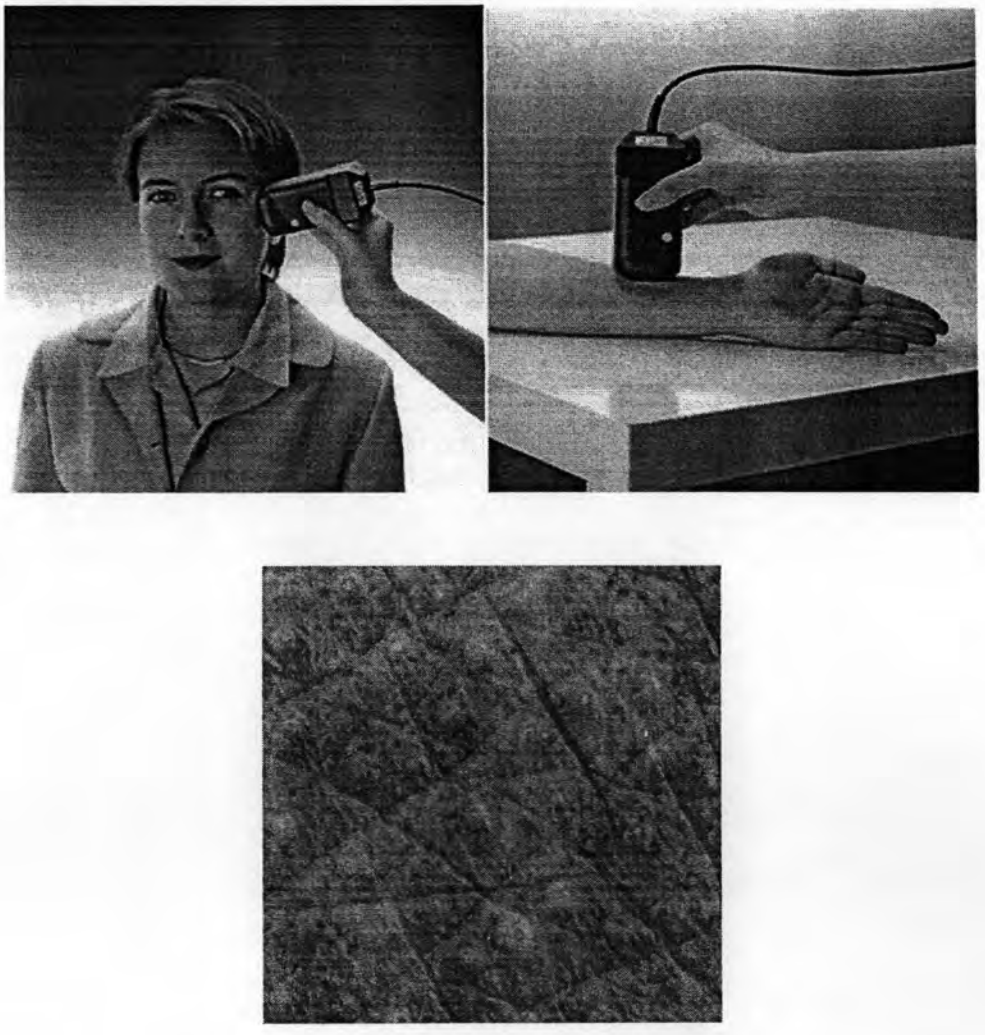


Figure 7. Visioscan® VC 98 and picture taken by Visioscan® VC 98

2. Skin moisture

Corneometer® CM 825 (Figure 8) measures skin moisture by capacitance method. The measurement is based on the completely different dielectric constant of water (Lund, 1994) and other substances (mostly<7). The measuring capacitor shows changes of capacitance according to the moisture content of the samples. A glass lamina separates the metallic tracks (gold) in the probe head from the skin in order to prevent current conduction in the sample. An electric scatter field penetrates the skin during the measurement and dielectricity is determined. One track builds up a surplus of electrons (minus charge), the other a lack of electrons (plus charge). An electric field between the tracks with alternating attraction develops. During the measurement the scatter field penetrates the very first layer of the skin and determines the dielectricity. In contrary to the impedance measurement, no galvanic relation between the device and the measuring object and no polarization effect exists (Mitsui, 1997; Wilhelm, Cua, and Maibach, 1991)

The measurement starts by putting the probe onto the skin area to be measured. Put the probe vertically on the measurement area according to the pressure of the spring inside the probe head. The measurement values are displayed immediately as spots with numeric value in a system of co-ordinates. The value detected by Corneometer® CM 825 is "H" value.

Figure 8. Corneometer® CM 825

3. Melanin value and erythema value

Melanin value and erythema value are measured by Mexameter® MX 18 (Figure 9). The measurement is based on the absorption principle. The special probe of the Mexameter® MX 18 emits light of three defined wavelengths. A receiver measures the light reflected by the skin. The position of emitter and receiver guarantees that only diffuse and scattered light is measured. As the quantity of emitted light is defined, the quantity of light absorbed by the skin can be calculated.

The melanin is measured by two wavelengths. These wavelengths have been chosenin order to achieve different absorption rates by the melanin pigments.

For the erythema measurement, two different wavelengths are used to measure the absorption capacity of the skin. One of these wavelengths corresponds to the spectral absorption peak of hemoglobin. The other wavelength has been chosen to avoid other color influences (e.g. bilirubin). The higher the values the more melanin or erythema is detected (Wilhelm, Cua, and Maibach, 1991; Harnisch et al, 1999; Weichers et al., 1998).

The measurement starts by simply press the probe on the skin surface. Press it tightly according to the pressure of the spring in the probe. It should be placed straight and quickly on the skin. Record data as "n" and "e" for melanin value and erythema value, respectively.

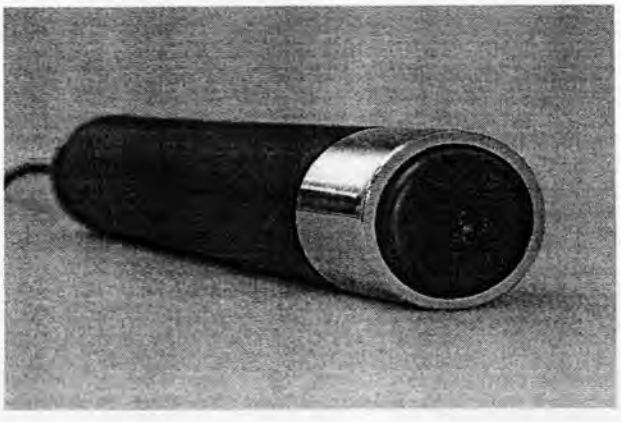


Figure 9. Mexameter® MX 18

4. Skin elasticity

The mechanical properties of the skin have always been a very important subject. The biomechanical parameters of the skin like the elasticity are characteristic for the different body sites. They vary extremely during the aging process or certain diseases of the skin. Physiologic skin aging starts at the age of thirty. Indications for that are flabbiness, wrinkles, dehydration, blotchy pigmentation and loss of elasticity.

Our skin is viscoelastic, i.e. elastic as well as plastic properties are present. The following examples explain these characteristics:

1. An inflated balloon is fully elastic. It is deformed by pressure applied to the surface, but it regains its original shape, when the pressure is stopped. Depending on how much the balloon is inflated the deforming pressure results in different amplitudes. If the surface is tight due to high inflation, the amplitude is small but the deformation is elastic in any case.

2. Modelling clay, e.g. plasticine is completely plastic. Pressure deforms plasticine easily. The deformation stays when the pressure is stopped. It can be continued by applying the pressure again.

A young skin with good blood circulation is very elastic, but does not resemble a balloon. The skin does not regain its original state immediately after application of force, but stays slightly deformed. This phenomenon is called hystereses. In old skin with bad blood circulation the plastic deformation dominates. Different parts of the body do not only have different degrees of elasticity and plasticity but in elastic skin the amplitudes also differ.

High losses of the elastic properties of the skin are generally due to anomalies in the collagen biosynthesis. In vivo measurements of the elasticity are of great interest for the quantification of the stiffness of the skin and finding a correlation with the course of illness. Elasticity measurement is indispensible for testing the efficiency of cosmetic and pharmaceutical products.

Skin elasticity is measured by Cutometer MPA 580 (Figure 10). The measuring principle is based on suction and elongation. The device generates negative pressure which can be varied between 20-500 mbar. The skin area to be measured is drawn into the aperture of the probe due to the negative pressure. The penetration depth of the skin into the aperture is determined without contact by an optical measuring system. This system consists of a light emitter and light accepter. Two opposing glass prisms transmit the light from emitter to acceptor. The light ratio

changes propotionally to the penetration depth of the skin (Wilhelm, Cua, and Maibach, 1991; Takema et al., 1994; Harnisch et al., 1999).

The measurements resulting in a typical deformation curve (Figure 10). Distinction can be made between a purely elastic part (Ue), a viscoelasticity part (Ua-Ur) and finally. A pure viscous part (i.e., delayed distension (Uv)). Finally distention and immediately retraction are indicated by Uf and Ur, respectively. Ua is the ability of redeformation of the skin. Ratios are used to exclude the influence of skin thickness. Ur/Ue (R5) is a measure for elastic function; Uv/Ue (R6) for viscoelasticity index. Ua/Uf (R2) and Ur/Uf (R7) are measured for the biological elasticity and the elastic recovery, respectively. In this study, biological elasticity(R2) was used to measure the skin elasticity, the closer the value is to 1 (100%) the more elastic the curve (Van de Vijver et al., 2003)

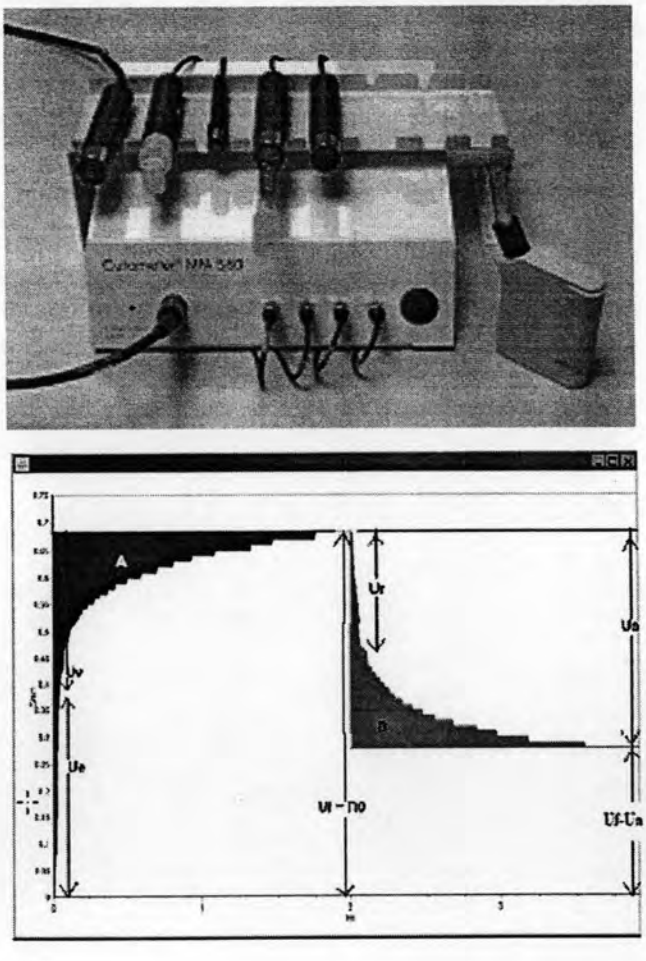


Figure 10. Cutometer® MPA 580 and its deformation curve