CHAPTER I

INTRODUCTION

People's lifestyles have changed in recent decades to reflect the fashion to obtain a `healthy' tan, by frequent sunshine holidays, increased leisure time and wearing minimal clothing as soon as the sun shines. Thus at the present time, it is likely that people are being exposed to more solar UV than previously and that this situation will continue well into the new century.

Due to depletion of the ozone layer, there is also the possibility of increased UV-B in sunlight reaching the surface of the Earth in amounts sufficient to have important biological consequences to the skin and eyes. UV radiation stimulates the production of reactive oxygen species in the cells and skin. The skin possesses an elaborate antioxidant defense system to deal with UV-induced oxidative stress. However, excessive exposure to UV can overwhelm the cutaneous antioxidant capacity, leading to oxidative damage and ultimately to skin cancer, immunosuppression, and premature skin aging. Although the pigment melanin in human skin is a major defense mechanism against ultraviolet light of the sun, the production of abnormal pigmentation such as melasma, freckles,senile lentigenes and other forms of melanin hyperpigmentation could be a serious aesthetic problem.

Green tea which is a natural product in the market claims to give wide protection against UV-irradiation. In green tea, polyphenols are present as catechin flavonoids such as epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). The most active antioxidant is EGCG (Salah *et al,*. 1995). A growing amount of research has demonstrated that green tea polyphenols have powerful antioxidants with anticarcinogenic properties (Brown, 1999). Evidence has been reported on the stronger scavenging effects of green tea components on the superoxide anion radical (Hatano et al., 1989) and the DPPH radical (Nanjo *et al*., 1996; Yoshida *et al*., 1988 ; Hatano *et al*., 1989) when compared

with α-tocopherol. The outcome of the several experimental studies suggested that green tea possesses anti-inflammatory and anti-carcinogenic potential, which can be exploited against a variety of skin disorders (Katiyar, Ahmad, and Mukhtar, 2000). Inhibition of mushroom tyrosinase by green tea has been studied. The results showed that all catechins with gallic acid group (EGC, GCG, EGCG and EGC) possess tyrosinase inhibitory activity (No *et al.*, 1999).These experimental evidences concerning green tea strongly suggested the possibility of its future use as a significant pharmacological agent for the prevention and treatment of a variety of human skin disorders caused by ultraviolet radiation.

Green tea polyphenols (GTP_S) in alkali solutions are extremely unstable and degrade almost completely in a few minutes, whereas in acidic solution they are very stable (Zhu *et al.*, 1997). Therefore extraction solvents with different pH may affect the polyphenol contents in green tea extract. To compare the content of GTP_S in different green tea leaves or get high yield extracts, the optimum extraction procedure is necessary to assure the materials of interest do not degrade during the process.

Many laboratory studies of green tea have been conducted on animal models. For ethical reasons, over the past few years there has been a worldwide movement to introduce *in vitro* testing to identify the activities of new compounds. Much of this work uses monolayer cultures of a single cell type as a first step, then progresses to reconstituted 3-dimentional human skin (3D skin) or followed by excised human skin which is close to *in vivo* testing but less complicated. *In vivo* there is an interaction between cell types and the hemapoietic system. While we cannot introduce a hemapoietic system we are able in the case of skin testing to produce a 3D skin equivalent model that consists of dermal fibroblasts in a collagen raft seeded with epidermal cells on the apical surface which under the correct culture conditions will grow and differentiate into granular and squamous cells very similar to normal human epidermis. Aside from the ethical considerations, this skin model is in some ways a better experimental vehicle because from one sample of human skin, usually foreskin, many identical test vehicles can be cultured and used for assays eliminating intra-experimental variations due to different donors.

Melanocyte-keratinocyte co-culture protocol is a reliable model for testing melanogenic regulators (Lei *et al.*, 2002). There is no published evidence of *in vitro* studies with cultured cells to see the potential effects on pigmentation of green tea.

The goal of this research is the improvement of the green tea extraction method, the preparation of primary cell cultures and the living skin equivalent model. The healthful cutaneous effects of green tea extract were studied by imitating the *in vivo* skin environment with an *in vitro* system. Individual cell components were tested first, then the project progressed to assay the effects when the cells were differentiated in the skin model and finally, the results were compared to excised human skin.

This study has focused on UV protection and inhibition of melanin synthesis activity of green tea extract. Green tea leaves were extracted using different pH solvents (citrate buffer pH 3, 3.5, 4, 4.5 and distilled water). The extracts were analysed for catechin contents by HPLC assay method. L929 cells (mouse lung adenocarcinoma cell line) are epithelial like cells which have morphology similar to keratinocytes. They are widely used in standard method for cytotoxicity tests (follow BSEN 30993-5 and ISO 10933-5: Biological evaluation of medical devices part 5; Test for cytotoxicity, *in vitro* methods). In this study, L929 cells were used to determine toxicity of GTE and EGCG before working with human epidermal cells (NHFM, NHFK and NHFF). Keratinocyte, melanocyte and fibroblast cultured cells were prepared from human skin biopsies and used to determine safe, non toxic concentrations of green tea extract compared to the pure catechin (EGCG). UV protection activity of green tea extract was conducted on keratinocytes. Melanocyte / keratinocyte cocultures were prepared and used to study inhibition of melanin synthesis activity of green tea extract. 3D skin containing melanocytes and keratinocytes seeded on collagen rafts were prepared to study UV protection activity of green tea extracts. The UV protection effect of the extract was studied on excised human skins and the results compared with 3D skin.

The improvement of the extraction procedure can be expected to produce high yield of catechins for further studies. This study will provide information of safety

concentration for green tea extract. *In vitro* studies with 3D skin model and excised human skin will enhance the testing facility in the field of skin care industry and reduce the need of animal using.