Chapter 1

Introduction

1.1. CANCER

The term “disease” refers to an abnormal condition that affects the normal metabolic process of an organism. It is often characterized as a medical condition which includes specific symptoms and signs. Throughout the history, epidemics have caused the extinction of whole populations and still remains as a threat. Over the last century, man has discovered many causative factors that triggers diseases in humans and has learned how to protect from them either by prevention or treatment.

Among those, the term “cancer” may be defined as a complex disease involving numerous tempo-spatial changes in cell physiology, which ultimately lead to malignant tumors (Seyfried and Shelton, 2010). The disease of cancer dates back as far as prehistoric times. There is an evidence of cancer-affected animals long before humans were on earth. Studies of the remains of a cretaceous dinosaur and Pleistocene cave bear indicated the existence of tumors of the vertebrae (Brothwell, 1967). The numbers of cases in prehistoric and ancient tumors were very small but they support the assumption that cancer is a very old disease afflicting animals and man.

Cancer can spread to other parts of the body through the blood and lymph systems. It is not just one disease but collection of more than 100 diseases. There are more than 200 different types of cancer (Cancer Research UK, 2011). Most cancers are named for the organ or type of cell in which they start, for example, cancer that begins in the skin is termed as skin cancer and if it begins from lungs, then termed as lung cancer. Cancer may affect people of all ages, even fetuses, but in general, the risk for more common varieties tends to increase with age. All cancers begin in cells, the body's basic unit of life. The body is made up of many types of cells. These cells grow and divide in a controlled way to keep the body healthy. When the cells become old or damaged, they die and are replaced with new cells. However, sometimes this orderly process goes wrong and thereby the genetic material (DNA) of a cell can become damaged or changed, producing mutations that affect normal cell growth and division. When this happens, cells do not die when they should and new cells form
when the body does not need them. These extra cells may thus form a mass of tissue called as “tumors”.

![Diagram of Different Types of Cancer](source: www.cancertypes.in)

**Fig. 1.1:** Different types of cancer named accordingly from which they originate from the body (source: www.cancertypes.in).

Cancer causes about 13% of all deaths. According to International Agency for Research on Cancer (IARC), 7.6 million people died from cancer in the world during 2008 (American Cancer Society, 2011). Nearly all cancers are caused by abnormalities in the genetic material of the transformed cells. Hanahan and Weinberg (2000) proposed that there are six essential physiological characteristics that will get transformed in a cancer cell. These include self-sufficiency in growth signals, insensitivity to growth inhibitory signals, evasion of programmed cell death or apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis. These abnormalities may be caused by various factors like carcinogens, such as tobacco smoke, radiations, chemicals or infectious agents.

There are other cancer-promoting genetic abnormalities which may randomly acquire through errors in DNA replication or inherited and thus present in all cells from birth. Complex interactions between carcinogens and the host genome may able to explain the reason for developing cancers in some cases after exposure. New
aspects of the genetics of cancer pathogenesis, such as DNA methylation and microRNAs are increasingly being recognized as important.

Not all tumors are cancerous. Tumors can be benign or malignant.

- **Benign tumors** aren’t cancerous. They can often be removed and in most cases, the chance of getting it again was very rare. Cells in benign tumors do not spread to other parts of the body.

- **Malignant tumors** are cancerous. Cells in these tumors can invade nearby tissues and spread to other parts of the body. The spread of cancer from one part of the body to another is called metastasis. Metastasis is the major cause of cancer related deaths and accounts for greater than 90% of cancer mortality (Mehlen and Puisieux, 2006).

### 1.2. SKIN BIOLOGY

The skin is the largest organ in human body and it comprises about 15% of the total body weight. It primarily involved in body’s defence mechanisms against ultraviolet (UV) radiations. Development and maintenance of healthy skin with a functional skin barrier is having crucial role in normally developed and matured skin (Madison, 2003). The skin consists of an outer squamous epithelium, the epidermis and an inner connective tissue, the dermis.

![Fig. 1.2: A typical skin cross section of human skin.](image-url)
The skin barrier is composed mainly of epidermis, which is continuously renewed by the mitotic activities of the stem cells in the basal layer, which provides new keratinocytes. After the division, basal keratinocytes detach from the basement membrane and goes through a terminal differentiation program to become corneocytes in the outer layers of the epidermis. During transition from the granular to cornified layer, an elevation in intracellular calcium occurs which activates transglutaminases. They are a widespread group of enzymes involved in protein modification and take part in cell death process (Griffin et al., 2002). At the final stage of differentiation, the keratinocytes lose their organelles, including the nucleus and become the dead, flattened corneocytes. This cell death program called cornification is well orchestrated since the dead cells fulfill a specific function by acting as an essential barrier. Hence, this complex process in skin is described as “planned cell death” (Brian et al., 2002). Finally, corneocytes are shed from the skin by a process called desquamation. Melanocytes, also residing in the epidermis, are neuroectoderm-derived cells that produce melanin, which provides skin pigmentation. Imbalances in the delicate physiological turn-over of proliferating or differentiating keratinocytes can disrupt the barrier function of the skin and are accountable for many skin disorders/diseases (Lippens et al., 2009).

Fig. 1.3: Epidermal differentiation process. Once keratinocytes comes out of cell cycle, it undergoes differentiation process in various layers of epidermis and finally forms skin barrier.
1.3. SKIN CANCER

Skin cancer in humans represents about 30% of all new cancers reported annually (Yusuf et al., 2007). It is a disease caused due to the uncontrolled growth of abnormal skin cells. It occurs when unrepaired DNA damage to skin cells (most often caused by ultraviolet radiation) triggers mutations that lead the skin cells to multiply rapidly. It begins from the lower layer of the epidermis. Tumors of the skin are often referred to as lesions. It is the most common of all cancers, affecting more than two million Americans each year, and this number is rising rapidly. The risk is highly related to the amount of sun exposure and lack of pigmentation in the skin. Prolonged exposure to the sun with lighter skin, may increase the chances of getting skin cancer. It is also curable if diagnosed and treated early. If left untreated, skin cancer can result in disfigurement and may even cause death.

1.3.1. TYPES OF SKIN CANCER

1.3.1.1. NON-MELANOMA SKIN CANCER

The most common form of cancer in fair-skinned individuals is non-melanoma skin cancers (NMSCs), which has a predicted prevalence equal to that of all other cancers combined (Madan et al., 2006). The two main forms of NMSCs are basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) which occur at a frequency of 4:1 respectively.

- Basal cell carcinoma (BCC)

It is the most common NMSCs and found to be least lethal among other types of skin cancers. They typically exhibit non-aggressive behavior, with a metastatic rate of 0.0028% to 0.55% (Wong et al., 2003). In the United States, BCC accounts for 90 percent of all skin cancers. It is mostly seen in people over 45 years of age and almost twice in men than women. It effects far more prevalently among Caucasians. The most common type of BCC is nodular basal cell carcinoma, a flesh-colored (cream to pink), round or oval translucent nodule with overlying small blood vessels and a pearly-appearing rolled border. Metastasis is rare in BCC, but local invasion destroys underlying and adjacent tissue. In 90% of all cases, the lesion is seen between the hairline and the upper lip.
Fig. 1.4: Individual figures represent; (A) Basal cell carcinoma; (B) Squamous cell carcinoma; (C) Melanoma.

- Squamous cell carcinoma (SCC)

It is the second most common cancer of the skin. SCC represents about 20% of the non-melanoma skin cancers. It usually occurs on chronically sun exposed areas of the skin, with 70% of cases found on the head and neck (Nguyen and Yoon, 2005). The incidence of SCC varies with age, gender, race and genetics. The people around 50-70 years of age were found to be generally effected. Males are highly affected than females in the ratio of 2:1. SCC tends to grow and spread more than basal cell cancers in epidermis. They are more likely to invade fatty tissues just beneath the skin and are more likely to spread to lymph nodes and/or distant parts of the body.

1.3.1.2. MELANOMA

It is the most aggressive form of skin cancer which develops from a neoplastic transformation of melanocytes, the cells that produce melanin in our skin. Melanoma accounts for only 4% of skin cancers, but it is a far more serious skin cancer and causes about 73% of skin cancer deaths as it tends to spread throughout the body quickly (American Cancer Society, 2007).

There are four major types of melanoma:

- **Superficial spreading melanoma**: It is the most common type and usually appears as flat and irregular in shape and color with different shades of black and brown. It is mostly common in Caucasians.

- **Acral lentiginous melanoma**: It is the least common form. It usually occurs on the palms, soles or under the nails and is more common in African Americans.
- **Lentigo maligna melanoma**: It usually occurs in the elderly people. It is common in sun-damaged skin on the face, neck and arms.

- **Nodular melanoma**: It usually starts as a raised area that is dark blackish-blue or bluish-red. However, some types may not have any color.

1.3.2. **SYMPTOMS OF SKIN CANCER**

BCC may be seen as growths that are flat or small, elevated pink or red, translucent, shiny areas that may bleed following with minor injuries. Sometimes small blood vessels can be seen within the tumor. Crusting and bleeding in the center of the tumor occurs frequently.

SCC may appear as growing lumps often with a rough surface or as flat, reddish patches that grow slowly. Ulceration and bleeding may also occur. Another sign of basal and squamous cell skin cancers is a sore that doesn’t heal.

<table>
<thead>
<tr>
<th>Normal Mole</th>
<th>Melanoma</th>
<th>Sign</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Normal Mole Image" /></td>
<td><img src="image2" alt="Melanoma Image" /></td>
<td>Asymmetry</td>
<td>when half of the mole does not match the other half</td>
</tr>
<tr>
<td><img src="image1" alt="Normal Mole Image" /></td>
<td><img src="image2" alt="Melanoma Image" /></td>
<td>Border</td>
<td>when the border (edges) of the mole are ragged or irregular</td>
</tr>
<tr>
<td><img src="image1" alt="Normal Mole Image" /></td>
<td><img src="image2" alt="Melanoma Image" /></td>
<td>Color</td>
<td>when the color of the mole varies throughout</td>
</tr>
<tr>
<td><img src="image1" alt="Normal Mole Image" /></td>
<td><img src="image2" alt="Melanoma Image" /></td>
<td>Diameter</td>
<td>if the mole’s diameter is larger than a pencil’s eraser</td>
</tr>
</tbody>
</table>

**Fig. 1.5**: Various characteristic features of normal mole and melanoma (NCI).
Most melanomas are brown to black looking lesions. Unfortunately, a few melanomas are pink, red or fleshy in color; these are called amelanotic melanomas. These tend to be more aggressive and the changes that occur for a few days are usually not cancer but changes that progress over a month or more should be evaluated by a doctor.

1.3.3. CAUSES OF SKIN CANCER

Several epidemiological studies of NMSC indicated an association of tumour occurrence with fair skin, sun exposed skin and populations from decreasing latitude, which are all indicative of UV radiation as a causative agent (Hussein, 2005). Among UV radiations, it was more likely found that the UV-B rays which is having a wavelength of 280 to 320 nm, triggers skin cancers in more than 70% of the cases.

In certain cases, the Persistent Human Papilloma Virus (HPV) infection found to promote tumor progression by interfering with an individual's response to UV radiation-induced DNA damage and thereby plays a synergistic role in the development of BCC and SCC.

Other factors includes,
- Ionizing radiations - X rays
- Increased ozone depletion levels
- Chemical carcinogens- for e.g., DMBA (7,12-dimethylbenz[a]anthracene)
- Smoking
- Genetic syndromes
- Aging
- Diet

1.3.4. STATISTICS FOR SKIN CANCER

GLOBAL SCENARIO

Skin cancer is the most common form of cancer in the United States (US). More than 3.5 million skin cancers in over two million people are diagnosed annually (Rogers, 2010). Nearly 800,000 Americans are living with a history of melanoma and 13 million are living with a history of non-melanoma skin cancer, typically diagnosed as BCC or SCC (Altekruse, 2011). An estimated 114,900 new cases of melanoma
were diagnosed in the US in 2010, among those 46,770 are noninvasive and 68,130 are invasive with nearly 8,700 resulting in death.

Incidences of NMSCs in white population are associated with residence in areas with high levels of solar UV radiation. The highest incidences rates, ranging from 161 per 100,000 in Tasmania to 823 per 100,000 have been reported in Australia (Kaldor et al., 1993). Worldwide, the incidence for NMSCs varies widely with the highest rates in Australia (> 1000/100,000 person/year for BCC) and the lowest rates in parts of Africa (< 1/100,000 person/year for BCC). The average incidence rates in England were 76.21/100,000 person/year and 22.65 /100,000 person/year for BCC and SCC respectively, with highest rates in the South-West of England (121.29/100,000 person/year for BCC and 33.02 /100,000 person/year for SCC) and lowest rates by far in London (0.24 /100,000 person/years for BCC and 14.98 /100,000 person/years for SCC) (Lomas et al., 2012).

![Fig. 1.6: Ten leading cancer types for the estimated new skin cancer cases and death by sex, US, 2011 (Siegel et al., 2011).]

**Fig. 1.6:** Ten leading cancer types for the estimated new skin cancer cases and death by sex, US, 2011 (Siegel et al., 2011).

- **INDIAN SCENARIO**

In India, the exact incidence of skin cancer has not been recorded (Panda, 2010). Although national surveys and cross-country data are unavailable, several small reports indicate that Non-melanoma skin cancers (NMSCs) may be on rise in India. According to various cancer registries in India, the incidence of skin cancer
ranges between 0.4 – 2%, among which 0.3 % has been reported for death from less
developed areas (Ahmedin et al., 2011).

BCC, a type of NMSC is the most prevalent skin cancer reported worldwide,
but various studies from India reveals SCC as the most common skin malignancy.
Primarily SCCs is caused due to exposure to direct sun, but in India, SCC often
occurs in sites that have not been exposed to the sun and is often aggressive (Howe
and Lang, 1988). The following conditions are reported as the morphological
expressions of SCC: Bowen's disease caused due to arsenical and radiation keratoses
(Kanwar and Dhar, 1993), verrucous carcinoma (Kotwal et al., 2005),
keratoacanthoma (Krishnan et al., 1999) and proliferating trichoelomal cysts
(Heaphey and Ackerman, 2000). Among other unusual sites of SCCs include, leg
(Karthikeyan et al., 2003), Kangri cancer (Teli et al., 2009) and burns scar (Yesudian
et al., 1995). Development of SCC has also been associated with certain
dermatological conditions in which the pathogenesis is akin to the development of
SCC in scars such as discoid lupus erythematosus (DLE) (Sadhu and Sengupta, 1992;
Dawn et al., 1994; Ghosh et al., 1997) and lupus vulgaris (LV) (Salodkar et al., 1992;
Betti et al., 2002; Das et al., 2007). Other uncommon occurrences of SCC on pre-
existing dermatological conditions are, lichen planus (LP)[44] including LP
hypertrophicus, (Dogra et al., 1997) necrobiosis lipoidica diabeticorum (NLD)
(Pavithran, 1998) lichen simplex chronicus (LSC), (Masood and Manzoor, 2000)
psoriasis, (Kumar et al., 2004) chronic lymphedema (Abhyankar et al., 2010) and
disseminated porokeratosis (Sengupta et al., 2005).

1.3.5. THERAPIES AND TREATMENTS FOR SKIN CANCER

Typical treatments include: Surgery, radiation therapy, immunotherapy,
targeted therapy and chemotherapy.

- Surgery

Thin melanomas can be completely cured by a minor surgery called as simple
excision. The tumor can be excised out along with a small portion of normal non-
cancerous skin at the edges. The normal healthy skin around the edges of the cancer is
termed as “margin”. Simple excision differs from an excisional biopsy. The margins
are much wider and vary depending on the thickness and size of the tumor. Hence, surgery has got its own limitations.

- **Radiation therapy**

  Radiation therapy employed high energy rays or particles to destroy tumor cells. External beam radiation therapy targets from external of the body on to the skin tumors. Side effects of radiation therapy depend on where it is targeted in the body. This might include sunburn-like skin problems, hair loss, fatigue, nausea and vomiting.

- **Immunotherapy**

  Immunotherapy may be defined as treatment for a disease by inducing, enhancing, or suppressing an immune response in the cells. Melanoma is one of the few cancers that can spontaneously regress in the presence of infiltrating and circulating T cells. This emerging field initially promised great success rate in melanoma treatment. The current immunotherapy for melanoma consists of Interferon-α (IFN-α) and interleukin-2 (IL-2). While both regimens have perceived a positive response rate of 10% to 20%, the severe toxicities with high doses has limited the use of these immunotherapies.

- **Targeted therapy**

  Targeted therapy is a type of medication that blocks the growth of cancer cells by interfering with specific targeted molecules needed for carcinogenesis and tumor growth. Most of the melanomas have changes (mutations) in the BRAF gene. These mutations cause the alterations in the gene and signal the melanoma cells to grow and divide quickly. Drugs that target the BRAF protein are not likely to work in patients whose melanomas have a normal BRAF gene. For example, vemurafenib (Zelboraf) is a drug that targets melanoma cells with the altered BRAF gene. It causes tumors to shrink, in about half of the people whose metastatic melanoma has a BRAF gene change.

- **Chemotherapy**

  Chemotherapy involves the drugs that can kill cancer cells. These drugs are usually injected into a vein or taken by mouth in the form of pills. They go through the bloodstream to all parts of the body and destroy cancer cells. Since these drugs
reach all parts of the body, it is termed as “systemic therapy”. Although it is usually not as effective as in melanoma, it may still relieve symptoms or extends survival rates for some patients. Chemotherapy was given in cycles. Each period of the treatment was followed by a resting period to allow the body to recover. Several FDA (food and drug administration) approved chemo drugs that were currently in use to treat melanoma and some of them are listed below:

- Dacarbazine (also called DTIC)
- Temozolomide
- Paclitaxel
- Carmustine (also known as BCNU)
- Cisplatin
- Carboplatin
- Vinblastin

1.3.6. SIDE EFFECTS OF SYNTHETIC DRUGS

The drugs used in the treatment of cancer attack the rapidly proliferating cancer cells by various pathways. But normal cells in the body, such as those in the bone marrow, the lining of the mouth and intestines and the hair follicles, also divides quickly and these cells were also likely to get effected once the treatment gets started. The side effects of chemotherapy depend on the type, dose, period and mode of treatment. Commonly seen side effects were given below:

- Hair loss
- Mouth sores
- Loss of appetite
- Nausea and vomiting
- Diarrhea
- Increased risk of infection
- Easy bruising or bleeding
- Fatigue
Hence researchers now focus more on drugs of natural origin which can overcome these side effects especially by targeting molecules in cancer pathway and thereby develop a safe and efficient anticancer agent against skin cancer.

1.4. MEDICINAL PLANTS IN SKIN CANCER TREATMENT

Medicinal plants are considered to be the main sources of biologically active compounds that can be used for the treatment of various ailments including cancers. Out of 250,000-500,000 plant species on the earth, only 1-10% have been studied chemically and pharmacologically for their potential medicinal value (Borris, 1996). The era of chemotherapy began in 1940s with the first use of nitrogen mustards and antifolate drugs (Chabner and Roberts, 2005). Thereafter, cancer drug discovery and development have been the major research endeavour around the globe as evidenced by several peer-reviewed papers in the scientific literature (Suresh et al., 2006).

Chemotherapy for cancer control is based on the presumption that cancer develops through a multi-step process. Thus, the design and development of chemopreventive agents that act on specific and/or multiple molecular and cellular targets have gained support as a rational approach to control cancer. In the continuing search for agents that may treat or ameliorate the affliction of cancer, plants have provided an endless supply of active compounds that are increasingly being exploited (Deorukhkar et al., 2007). Various properties like chemical diversity, structural complexity, affordability, lack of substantial toxic effects and inherent biological activities of plant compounds made them ideal candidates for new therapeutics. This rapidly moving field now concentrates in particular, on the modulating effects; these agents can have on cellular signalling pathways involved in the development of UV-induced premalignant and malignant lesions, apoptotic, proliferative and angiogenic processes. “Chemoprevention” in context of skin cancer may also be defined as “the use of agents capable of ameliorating the adverse effects of UV-B on the skin” by natural compounds, represents a new concept in the attempt to control the carcinogenesis process (Zhao et al, 1999). Various plant compounds were already tested successfully under in vitro and in vivo conditions and some of them were listed below:
Table 1.1: Shows the mechanism of action of different plant compounds on various skin cancer cells. ↓ indicates down regulation, ↑ indicate up regulation and ~ indicate modulation.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Models</th>
<th>Target/Mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGCG</td>
<td>C3H/HeN</td>
<td>↓ LPO, ↓ Protein oxidation</td>
<td>Pinnell, 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ NF-κB, ↓ AP-1</td>
<td>Katiyar et al., 2007</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>NHEK</td>
<td>↓ NF-κB</td>
<td>Adhami et al., 2003</td>
</tr>
<tr>
<td>Curcumin</td>
<td>A431</td>
<td>↓ oxidative stress, ~ Apoptosis</td>
<td>Chang et al., 2003</td>
</tr>
<tr>
<td></td>
<td>HaCaT</td>
<td>↓ COX, ↓ AP-1, ↑ Caspases, ↑</td>
<td>Cho et al., 2005</td>
</tr>
<tr>
<td>Apigenin</td>
<td>HaCaT</td>
<td>↑ Apoptosis, ↑ Cytochrome c</td>
<td>Abu et al., 2008</td>
</tr>
<tr>
<td>Sanguinarine</td>
<td>HaCaT</td>
<td>Anti-proliferative,~ Apoptosis</td>
<td>Reagan et al., 2006</td>
</tr>
<tr>
<td></td>
<td>A431</td>
<td>↑ Apoptosis, ↑ p53,</td>
<td>Lu et al., 2000</td>
</tr>
<tr>
<td>Dihydrocaffeic acid</td>
<td>HaCaT</td>
<td>↓ Interlukin pro-inflammatory IL-6</td>
<td>Poquet et al., 2008</td>
</tr>
</tbody>
</table>

1.5. PLANT COMPOUNDS & THEIR RATIONALE FOR SELECTION FOR PRESENT STUDY

1.5.1. LUTEOLIN

Luteolin (LUT), a naturally occurring flavonoid, is abundant in our daily dietary intake. The chemopreventive effect and associated mechanisms of LUT in the JB6 P+ cell line and the SKH-1 hairless mouse model were described by Sanguine et al. (2010). It has been shown to delay or block the development of cancer cells both in vitro and in vivo, by protecting from carcinogenic stimuli, inhibiting tumor cell proliferation, inducing cell cycle arrest and apoptosis via intrinsic and extrinsic signalling pathways (Gunter et al., 2008). The potential for LUT against malignant keratinocytes was studied by Verschoote et al. (2010) and George et al. (2013). LUT inhibits the activation of protein kinase C, the enzyme involved in tumor promotion which made it as a candidate for preventing skin cancer (Horiuchi et al., 1986). Further, in vivo skin penetration studies with human volunteers have shown the
adsorption of LUT at the skin surface and penetrants of it into deeper skin layers, which is important for their topical use as antiphlogistic agents in dermatology (Seelinger et al., 2008).

1.5.2. OLEANOLIC ACID

Oleanic acid (OA), is a naturally occurring triterpenoid which is widely distributed in food and medicinal plants. Its anticancer effects has been reported in several cancer cell lines (Li et al., 1999; Chiang et al., 2005; Pui et al., 2009; Biswas et al., 2010). Further, OA have been found to be active in various stages of tumor development, including inhibition of tumor promotion, invasion and metastasis (Shishodia et al., 2003; Miao et al., 2007). An In vivo study by Lucio et al. (2011) reported the apoptotic potential of OA in non-small cell lung cancer cell lines and reduced metastasis of a B16F10 melanoma cells. More over treatment with OA increases the expression of the pro-apoptotic protein Bax, altering the Bcl-2/ Bax balance towards a pro-apoptotic profile with decreased expression of the angiogenic vascular endothelial growth factor (VEGF). Further, OA has been studied for its potential to induce apoptosis in HaCaT keratinocytes with non-cytotoxic doses (George et al., 2012) that enable it to consider as a potent template for skin cancer therapy.
1.5.3. BETULINIC ACID

Betulinic acid (BA), a pentacyclic triterpene of plant origin has been reported for the first time against melanoma cells and other malignant cells of neuroectodermal origin by Pisha et al. (1995). However, the molecular mechanism involved was studied later by many investigators. Tan et al. (2003) studied the possible MAPK activation pathway in which BA initiates cell death in melanoma cells. The ability of BA to induce apoptosis in HaCaT (immortalized human keratinocyte) cells was corroborated by Galgon et al. (2005), which makes it as an attractive candidate for skin cancer therapy.

[Fig. 1.9: Structure of BA]

1.5.4. PARTHENOLIDE

Parthenolide (PN), an active sesquiterpene lactone of feverfew, Tanacetum parthenium, has been reported to inhibit proliferation and induce apoptosis in a variety of cancer cells (Monica et al., 2005). PN has also shown to reduce the number of viable adherent cells in melanoma cultures. The cytotoxicity of PN was also tested in melanocytes, as well as melanoma cells directly derived from a surgical excision (Zhang et al., 2005; Bedoya et al., 2008). PN had been reported for its chemopreventive activity against UVB-induced skin cancer (Saadane et al., 2005) and its possible mechanism of action were demonstrated in mice model (Sheehan et al., 2002). Mice fed with PN (1 mg/day) showed a delayed onset of papilloma incidence, a significant reduction in papilloma multiplicity (papilloma/mouse) and sizes when compared to the UVB-only group (Yen-Kim et al., 2004).
1.5.5. VITAMIN D\textsubscript{3} AND RATIONALE FOR ITS USE AS A POSITIVE CONTROL

Vitamin D is a group of fat-soluble secosteroids. In humans vitamin D is unique because it functions as a prohormone and the body can synthesize it (as vitamin D\textsubscript{3}) when sun exposure is adequate. Topical treatment of psoriatic lesions (a type of skin disease) with a vitamin D\textsubscript{3} analogue resulted in a decrease of the psoriatic phenotype and an increase in caspase expressions particularly caspase-14 (key element in planned cell death mechanisms in skin) in the parakeratotic plugs (Lippens et al., 2004). \textit{In vitro}, caspase-14 is only expressed when keratinocyte are forced to differentiate by growing them post-confluently or in suspension or by adding vitamin D\textsubscript{3} (Pistritto et al., 2002). The active form of vitamin D\textsubscript{3}, 1,25-dihydroxyvitamin D\textsubscript{3}, has a concentration-dependent inhibitory effect on proliferation, but enhances differentiation in keratinocyte cell cultures (Bikle and Pillai, 1993). Vitamin D\textsubscript{3} was also reported for induction of apoptosis in various other epidermal cancer cell lines (Guzey et al., 2002).
1.6. OBJECTIVES OF THE STUDY

The continued increase in the incidence of skin neoplasia and the side effects caused by the synthetic drugs forced researchers to develop much safer drugs from natural origin by specifically targeting various molecules in skin cancer pathway. Induction of terminal differentiation is a novel target, specifically using plant compounds, which may lead to the eventual elimination of tumorigenic skin cells and retain normal cellular homeostasis. Hence, the present study intends to test the following hypothesis, “The Compound(s) might possess the ability to induce terminal differentiation by eliciting the expression of caspase-14 in the human skin cancer cells, thus leading the cells towards cell-annihilation” after analyzing the antioxidant, cytotoxicity and apoptotic induction potentials of the compound(s).