CHAPTER 1

INTRODUCTION

1.1 General introduction

Medicinal plants has been used to be the main natural source of drugs. The use of medicinal plants was based on the experience of many generations of physicians and traditional systems of medicine from different ethnic societies. The use of medicinal plants in modern medicine suffered from the fact that though hundreds of plants were used in the world to prevent or to cure diseases, scientific evidenced in terms of modern medicine was lacking in most cases. However, it is necessary to provide scientific proof as to whether or not it is justified to use a plant or its active principles (Ammon and Wahl, 1991).

The World Health Organization (WHO) has estimated that for some 3.4 billion people in the developing world, plants were represented to be the primary source of medicine. This represents about 88% of the world's inhabitants who rely mainly on traditional medicine for their primary health care. At least 119 compounds derived from 90 plant species can be considered as important drugs currently in use in one or more countries, with 77% of these being derived from plants used in traditional medicine (Farnsworth, 1988).

The WHO has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. Traditional medicine is the synthesis from therapeutic experience of many generations of practising physicians in indigenous systems for treatment. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. (Kamboj, 2000).

Natural products, polyphenols, alkaloids, flavonoids and other secondary metabolites, in medicinal plants will provide a scientific validation for the popular use of them

and serve as a guide which may help in the selection of the plants with anticarcinogenic activity (Kaur *et al*., 2005). The majority of these naturally occurring phenolics retain antioxidative and antiinflammatory properties, which appear to contribute to their chemopreventive activity (Pal *et al*., 2001).

Triphala is a Thai traditional medicine preparation used as adaptogen for body in summer season of Thailand. It is consisting of the dried fruits of three plants, *Phyllanthus emblica* Linn, *Terminalia chebula* Retz. and *Terminalia bellerica* Gaertn. in equal proportions. Triphala is also a laxative that rejuvenates the membrane lining the digestive tract and contributes to effective cleansing of the colon, a key condition to maintaining optimum health. It is a completely balanced energetic formula (Tierra, 1996).

Recent studies have been reported that the aqueous extract of Triphala possessed ability to induce cytotoxicity against human breast cancer cell line (MCF-7) but less toxic against normal breast epithelial cell (MCF-10) (Sandhya *et al*., 2006). The acetone extract showed a significant cytotoxic effect against two types of breast cancer cell Shionogi 115 (S115) mouse breast cancer cell line and human breast cancer cell line (MCF-7), two types of prostate cancer cell (PC-3 and DU-145) and this extract showed nonspecific with all these cell line (Kaur *et al*., 2005).

Other previous studies of each plant in Triphala formula are describe below:

Recently reports about *Phyllanthus emblica* Linn. Showed showed the butanol extract of the water fraction of *P. emblica* fruits at the dose of 100 mg/kg body-weight, orally administered to rats for 10 consecutive days, was found to enhance secretion of gastric mucus and hexosamine in the indomethacin induced ulceration of rats. This extract was also shown to have a protective effect of the stomach wall. An antioxidant property appears to be predominantly responsible for this cytoprotective action of the herb (Bandyopadhyay *et al*., 2000). The methanolic extract was also active and inhibited lipid peroxidaton with IC_{50} value of 13 µg/ml (KC and Müller, 1999). Numerous studies have suggested that *P. emblica* fruit possesses anticancer activity. A simple aqueous extract of *P. emblica* was shown to protect mice against the chromosome damaging effects of the well known carcinogen 3,4-benzo(a)pyrene. This carcinogen causes genotoxicity at least in part because it is an electrophilic reactant, which causes the formation of oxygen-derived free radicals with DNA-damaging potential (Nandi *et al*., 1997). The protective effect against this

type of damage therefore most likely involves the antioxidant activity of *P. emblica* fruit extract. An anticancer effect may be mediated by the immune system. An antitumour effect of *P. emblica* aqueous fruit extract was demonstrated in tumour-bearing mice, resulting in a 35% increase in life span. The antitumour activity was shown to be meditated primarily through enhanced natural killer cell activity and antibody-dependent cellular cytotoxicity (Suresh and Vasudevan, 1994). Another study showed an aqueous extract of *P. emblica* to significant reduce induced solid tumours in mice in a manner suggesting interaction with cell cycle regulation (Jeena *et al*., 2000). Extract of *P. emblica* fruit inhibited the proliferation of four human tumour cell lines *in vitro*. Pyrogallol was identified as an active component of the extracts. (Khan *et al*., 2002). An aqueous extract of the dried fruits of *P. emblica* protected mice against the effects of nickel chloride. Nickel is a major environmental pollutant with carcinogenic potential (Dhir *et al*., 1991). Alcoholic extract was also found to lack cellular toxicity in an assay using fresh sheep erythrocytes (Ahmad *et al*., 1998).

Recently reports about *Terminalia chebula* Retz. showed that the aqueous extract acts as a potent antioxidant by examining its ability to inhibit γ-radiation-induced lipid peroxidation in rat liver microsomes and damage to superoxide dismutase (SOD) enzyme in rat liver mitochondria (Naik *et al*., 2004). The cytotoxic activity of *T. chebula* extract that is used in the treatment of cancer in Asia and Africa. A 70 %methanol extract of *T. chebula* decreased the number of cell in immortalized and cancer cell lines by inhibiting the rate of cell proliferation and induced cell death. At low concentrations this extract is able to initiate cellular pathways that lead to apoptosis whereas at high concentrations the extract has toxic effects, which lead to rapid necrotic cell death. Among the the phenolics of the extract, chebulinic acid and ellagic acid showed moderate inhibition and they may be responsible for the inhibiting cell proliferation (Saleem *et al*., 2002).

Recently reports about *Terminalia bellerica* Gaertn showed that the aqueous extract inhibit the growth and some physiological functions of *Streptococcus mutans*. The extract investigated as a potential anticaries and antiplaque agent. The growth of *S. mutans*, which is directly involved in the development of dental caries through the production of enamel degrading acids, was strongly inhibited by the extract (Jagtap and Karkera, 1999).

1.1.1 Antioxidants

In recent years the role of free radicals and reactive oxygen species (ROS) in human degenerative diseases of aging such as cancer, cardiovascular diseases, cataracts, brain dysfunction and immune system decline has become apparent. Free radicals or oxidants are energetically unstable and highly dangerous molecules which are constantly generated during body functions such as respiration, oxidative energy metabolism and immune activity. Free radicals are also produced from other sources (UV radiation, smoke, pollution, heavy metals, rancid fatty acids, etc.). Molecule oxygen is an oxidising agent, that is, it takes electron from another species (Halliwell and Gutteridge, 1999). The destructive effects of free radicals are far-reaching, including cell membrane destruction via the interaction of fatty acid with oxygen to form dangerous peroxides (lipid peroxidation); genetic damage via DNA mutation; decline in immune function; increased inflammatory conditons; growth and spread of cancers; oxidation of LDL cholesterol leading to atherosclerosis hormone disruption, contributing to diabetes and other systemic disorders (Halliwell and Gutteridge, 1990; Benzie, 2000).

Oxygen is a double-edged sword: although we require oxygen to survive, certain oxygen species (such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen) are toxic to the body. In healthy aerobic organisms, production of reactive oxygen species is approximately balanced by the antioxidant defence system in the body. These endogenous antioxidants can protect from damage caused by these harmful molecules, as well as from free radicals mention above. The body has evolved its own natural free radical scavengers, which include the antioxidant vitamins (Vitamin A and beta-carotene, several of the B-complex vitamins, Vitamin C and Vitamin E), the mineral selenium and the antioxidant enzyme systems such as SOD (superoxide dismutase), glutathione peroxidase and catalase, which are the backbone of the cellular antioxidant defence system. Damage from free radicals can be prevented and even reversed if there are sufficient concentrations of antioxidants, which work individually and together in the body. However, the endogenous antioxidant system in body is not able to respond to a rapid increase in oxidative stress so the small exogenous antioxidant molecule such as α-tocopherol, ß-carotene , ascorbic acid or antioxidants from plant foods can prevent effects damage from oxygen free radicals (Dreher and Junod, 1996 , Thurnham, 1993).

Important causes of cancer are ingested substances such as tobacco, chemicals used in the processing of foods, (e.g nitrosamines), toxic waste products and chemicals, and also the products of food preparation, such as charcoal grilling and pickling. Scientists estimate that perhaps 40-80% of all human cancers, excluding UV-induced cancers, may be caused by the burning of aromatic hydrocarbons, including tobacco, gasoline, coal, fat, oil, etc since these product can produced free radicals in the body. Some 90% of lung cancers and 50% of urinary cancers occur in smokers, while some 90% of mouth, larynx, esophagus and liver cancers occur in smokers who also drink. The mechanism of oxygen free radicals in cancer is based on the fact that oxidative stress can stimulate expansion of mutated cells and promote proliferation in mammalial cells after the initiation of cancer development by radiation and chemical mutation. Researchers have succeeded in identifying key mechanisms behind the development of these types of cancers, including the generation of toxic oxygen species or free radicals; and the damage to cell membranes caused by the interaction of certain fats with oxygen to form toxic peroxides (lipid peroxidation) (Harman , 1982, Jones, 1992, Mates and Sanchez-Jemenez, 2000).

The discovery that many of the body's natural mechanisms to prevent and neutralize damage caused by free radicals are actually nutrients themselves – the antioxidant vitamins A, C, E, the mineral selenium, and certain essential fatty acids, has provided an impetus to the use of nutritional antioxidants in the clinical treatment of many cancers (Odeleye, 1992; Das, 1990). The mechanism of antioxidant in cancer can modify the delvelopment stage of cancer by preventing the escalation of radical production by blocking lipid peroxidation and neutralizing free radical damage in the initiation and promotion phases of cancer. Additionally, antioxidant will improve the resistance of tissues to oxidative damage and promote healing and so reduce hyperplasia. The dietary antioxidant have been proven to have immune stimulating properties which is critical in treatment of any infectious disease and diseases such as cancer (Thurnham, 1993; Gordon , 1996).

Therefore, the discovery of antioxidant compounds from plants which is prevention disease and make healthy body is necessary especially the plants which was used as diary food.

Antioxidants come in various forms. They are classified broadly into two groups: antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase

etc. and molecular antioxidants such as vitamin (C and E), mineral (selenium, zinc and manganese), carotenoids and flavonoids which are found in plants.

1.1.2 Cytotoxicity

The National Cancer Institute (NCI) in the USA has established the development of a method for initial screening and has defined the term “cytotoxic”. A cytotoxic agent is toxic to tumor cells *in vitro* which is the method for studying bioactive compounds from plants and allows large numbers of plant extracts to be screened for activity especially cytotoxic compounds against many types of cancer cell lines. This assay is particularly useful for bioassay-guided fractionation of plant extracts. It is not always possible to test against cancer in an animal model and is more sensitive to most antitumor agents than *in vivo* assay and also costs less and requires less test material and time.

1.2 Rational of this study

In Thailand, many people have used traditional medicine as an alternative treatment for cancer (Subchareon, 1998). Folk doctors in Thailand have used Triphala in cancer drug formulae (Itharat *et al.*, 1998). This traditional medicines are prepared by boiling the plant material in water or soaking in alcohol and are commonly used by Thai people to prepare the drug for oral intake. An investigation of Triphala which is adaptogen in a traditional drug formula for treatment of cancer patients. This preparation is used by folk doctors to treat cancer in Southern Thailand and it can improve the condition and make patients have good quality of life. Surprisingly, there is no research comparison biological activities of each plant with combine plants extract in form Triphala especially cytotoxic activity against breast, cervical and prostate cancer. Therefore, the objective of the study should be to test cytotoxic activity against breast, cervical and prostate cancer which cause high death rates in man and also test antioxidant activity. Bioassay guide fractionation was used for isolated cytotoxic compounds against these cancer cells

and also isolated antioxidant compounds. These results would provide additional useful data on the biological activities of these plants and support the use of folk doctors to treat cancer patients.

1.3 Review of Literatures

1.3.1 *Phyllanthus emblica* Linn.

Phyllanthus emblica Linn. (Syn. *Emblica officinalis* Gaertn.) is known in Thailand by the local names, Ma-Kham Pom. In English, the plant is known as Indian Gooseberry; alternative English names included, Emblic Myrobalan, Malacca tree (Benny *et al*., 2008). It is belonging to family Euphorbiaceae occurs throughout Thailand and also in India, Pakistan, Bangladesh, Ceyton, Malaya, Singapore, Malaysia, China, Sri Lanka and Peninsula (Benny *et al*. 2008, and Scartezzini *et al*., 2006).

The description of *Phyllanthus emblica* Linn., shown in Figure 1-1, is a small or medium sized tree, up to 20 m high, deciduous, with crooked trunk and spreading branches; bark greenish gray, peeling off in conchoidal flakes; branchlets glabrous or finely pubescent 10 to 20 cm long. Leaves imbricate when young, subsessile, 0.5 to 2.5 cm by 1.5 to 5.5 mm, closely set along the branchlets, distichous, light green, glabrous, narrowly-linear, obtuse, having appearance of pinnate leaves; stipules minute, ovate, finely acute. Flowers small, monoecious, apetalous, greenish yellow, in axillary fascicles on the leaf-bearing branchlets, often on the naked portion below the leaves, with fimbricate bracts at the base. Male flowers numerous, on short slender pedicle; calyx-lobes, oblong, obtuse, 1.2 mm long; anthers; filaments united in a short central column; disk-glands 6 alternating with the calyx-segments. Female flowers few, subsessile or sessile; calyx as in the male; ovary 3-celled, half immersed in the lacerate, cup-shaped disk; style connate at the base; stigma, bilobed, lobed dilated, recurved. Fruits sessile, 1.3 to 2.7 cm in diameter, fleshy, globose or depress globose, with 6 longitudinal faint lines, glabrous, lucid, pale yellow; endocarp of triangular cocci, bony, dehiscent, with 3 short bundles of vascular tissue at the base. Seeds, trigonous (THP, 2000).

Figure 1-1 *Phyllanthus emblica* Linn. (original picture)

1.3.2 *Terminalia chebula* Retz.

Terminalia chebula Retz. or Sa-Maw-Thai in Thai is one member of family Combretaceae. The fruit commonly known as black Myrobalans in English and Harad in Hindi, indigenous in Pakistan and India among many Asia and Africa countries (Saleem *et al*., 2002).

The description of *Terminalia chebula* Retz., shown in Figure 1-2, is a medium-sized or large tree, up to 30 m high, up to 1.3 m in girth; bark rough, scaly; shoots and young leaves usually rusty villous. Leaves simplex, opposite, coriaceous, broadly ovate to ovate-elliptic, 8 to 15 cm by 6 to 10 cm, glabescent; nerves obscure above, slightly raised and usually brownish pubescent beneath; apex acute or abruptly acuminate; base cuneate, slightly cordate or rounded; petiole 1 to 3 cm long, glabrous or sparsely pubescent with a pair of nodular glands near leaf-base. Inflorescences axillary or terminal panicles, usually with 3 to 6 spikes; spikes 3 to 6 cm long; rachis pubescent; flowers 2 mm long, 3 to 4 mm in diameter; bracts nearly glabrous, 1.5 to 2 mm long; calyx outside glabrous, inside densely villous, calyx-segments triangular; stamens 3 to 4 mm long; ovary glabrous, ovoid, 1 mm long; style glabrous, 2.5 to 3 mm long; disc lobed, densely villous. Fruit drupe, glabrous, subglobose to ellipsoid, 2.5 to 5 cm by 1.5 to 2.5 cm,

usually smooth or frequently 5-angulate ridged, wrinkled, turning blackish when dry. Seed, rough, ellipsoid, 1.5 to 2.0 cm by 0.5 to 0.7 cm, without ridges (THP, 2000).

Figure 1-2 *Terminalia chebula* Retz. (from THP, 2000)

1.3.3 *Terminalia bellerica* Gaertn.

Terminalia bellerica Gaertn. or Sa-Maw-Phi-Phek in Thai and Bahera in Hindi is one member of Combretaceae. It occurs throughout Thailand and also in India, Nepal, Sri Lanka and Malaysia.

The description of *Terminalia bellerica* Gaertn., shown in Figure 1-3, a large tree, up to 50 m high, decidous, up to 2 m in girth, usually with large buttresses; bark blackish, brittle, longitudinally fissured and cracked, thick, cut yellow. Leaves, coriaceous, obovate, 4 to 20 cm by 2 to 15 cm, glabrous; nerves widely spaced, 6 to 8 pairs; petiole glabrous, 3 to 9 cm long, usually with a pair of dotted glands at about the middle or near leaf-base, occasionally inconspicuous or hardly observed when dry. Inflorescences spike or raceme, 3 to 15 cm long, often crowed at ends of branchlets without leaves so as to form terminal panicles; flowers andromonoecious, male on the upper part, tomentose; calyx 1 to 2 mm long, 4 to 5 mm in diameter; calyx-segments recurved, deltoid, 1.5 mm long; stamens 3 to 3.5 mm long; ovary ellipsoid, 2 to 3.5 mm by 1.5 to 3 mm; style 4 mm long; disc densely, rusty villous. Fruits drupe,

subglobose to broadly ellipsoid, 2 to 3.5 cm by 1.5 to 3 cm, slightly 5-ridged, densely velvety pubescent, very hard when dry. Seed, ellipsoid, rough, 1.2 cm by 0.5 cm. (THP, 2000)

Figure 1-3 *Terminalia bellerica* Gaertn. (from THP, 2000)

1.4 Chemical constituents of the investigated species

The reports of chemical constituents of these three plants are shown in Table 1-1 to 1-3. Their chemical structures are showed in Figure 1-4 to 1-6.

Table 1-1 Chemical constituents found in *Phyllanthus emblica* Linn.

Botanical name	Part of plant used	Chemical constituents	References
Phyllanthus emblica Linn.	Fruit	ascorbic acid	THP, 2000; Scartezzini *et al*., 2006; Khopde *et al*., 2001
		chebulagic acid	Zhang *et al*., 2004; Zhang *et al*., 2003
		chebulanin	Zhang *et al*., 2001(a)
		chebulinic acid	Zhang *et al*., 2003
		elaeocarpusin	Zhang *et al*., 2004
		corilagin	Zhang *et al*., 2004; Zhang *et al*., 2003; Kumaran and Karunakaran, 2006
		ellagic acid	Zhang *et al*., 2003

Table 1-1 (Continued)

Botanical name	Part of plant used	Chemical constituents	References
Phyllanthus emblica Linn.	Fruit	emblicanin A	Ghosal *et al*., 1996; Bhattacharya *et al*., 2002
		emblicanin B	Ghosal *et al*., 1996; Bhattacharya *et al*., 2002
		emblicol	Scartezzini and Speroni, 2000; Khopde *et al*., 2001; Ghosal *et al*., 1996
		furosin	Kumaran and Karunakaran, 2006
		gallic acid	THP, 2000; Kumar *et al*., 2006; Bhattacharya *et al*., 2002; Zhang *et al*., 2003; Kumaran and Karunakaran, 2006; Chase, 2002
		ethyl gallate	Zhang *et al*., 2003; Khopde *et al*., 2001; Ghosal *et al*., 1996
		methyl gallate	Kumaran and Karunakaran, 2006
		gallo-ellagitannoid	Ghosal *et al*., 1996
		1-*O*-galloyl-ß-D-glucose	Zhang *et al*., 2004; Zhang *et al*., 2003; El-Mekkawy *et al*., 1995
		gallo-ellagitannoid	Ghosal *et al*., 1996

Table 1-1 (Continued)

Botanical name	Part of plant used	Chemical constituents	References
Phyllanthus emblica Linn.	Fruit	1-*O*-galloyl-ß-D-glucose	Zhang *et al*., 2004; Zhang *et al*., 2003; El-Mekkawy *et al*., 1995
		1-*O*-galloyl-ß-D-glucoside	Zhang *et al*., 2001
		1-di-*O*- galloyl-ß-D-glucose	Khopde *et al*., 2001; Ghosal *et al*., 1996
		1,6-di-*O*- galloyl-ß-D-glucose	Zhang *et al*., 2003; El-Mekkawy *et al*., 1995
		1-ß-2,3,6-tetra-galloyl-glucose	Zhang *et al*., 2001(a)
		3,6-di-*O*-galloyl-D-glucose	Zhang *et al*., 2003
		kaempherol-3-*O*- ß-D-glucoside	Khopde *et al*., 2001; Ghosal *et al*., 1996
		quercein-3-*O*- ß-D-glucoside	Khopde *et al*., 2001; Ghosal *et al*., 1996
		geraniin	Kumaran and Karunakaran, 2006
		L-malic acid 2-*O*-gallate	Zhang *et al*., 2004; Zhang *et al*., 2001
		mallonin	Zhang *et al*., 2001(a)
		mucic acid	THP, 2000
		mucic acid-1,4-lactone-2-*O*-gallate	Zhang *et al*., 2001
		mucic acid-1,4-lactone-3,5-di-*O*-gallate	Zhang *et al*., 2001

Table 1-1 (Continued)

Botanical name	Part of plant used	Chemical constituents	References
Phyllanthus emblica Linn.	Fruit	mucic acid-1,4-lactone-3,5-di-*O*-gallate	Zhang *et al*., 2001
		mucic acid-1,4-lactone-3-*O*-gallate	Zhang *et al*., 2001
		mucic acid-1,4-lactone-5-*O*-gallate	Zhang *et al*., 2001
		mucic acid-1,4-lactone-6-methylester-2-*O*-gallate	Zhang *et al*., 2001
		mucic acid-1,4-lactone-6-methylester-5-*O*-gallate	Zhang *et al*., 2001
		mucic acid-1-methylester-2-*O*-gallate	Zhang *et al*., 2001
		mucic acid-2-*O*-gallate	Zhang *et al*., 2004; Zhang *et al*., 2001
		mucic acid-6-methylester-2-*O*-gallate	Zhang *et al*., 2001
		mucic acid-dimethylester-2-*O*-gallate	Zhang *et al*., 2001
		nicotinic acid	Zhang *et al*., 2004
		pectin	Scartezzini and Speroni, 2000
		pedunculagin	Ghosal *et al*., 1996; Bhattacharya *et al*., 2002
		phyllanemblinin A	Zhang *et al*., 2001(a)

Table 1-1 (Continued)

Botanical name	Part of plant used	Chemical constituents	References
Phyllanthus emblica Linn.	Fruit	phyllemblic acid	THP, 2000; Scartezzini and Speroni, 2000
		phyllemblin	Scartezzini and Speroni, 2000
		punigluconin	Ghosal *et al*., 1996; Bhattacharya *et al*., 2002
		putranjivain A	Zhang *et al*., 2001(a); El-Mekkawy *et al*., 1995
		pyrogallol	Khan *et al*., 2002
		quercetin	Zhang *et al*., 2003
		iso-quercetin	El-Mekkawy *et al*., 1995
		rutin	THP, 2000; Bhattacharya *et al*., 2002
		isostrictiniin	Zhang *et al*., 2003
		tannic acid	Kumar *et al*., 2006

Table 1-2 Chemical constituents found in *Terminalia chebula* Retz.

Botanical name	Part of plant used	Chemical constituents	References
Terminalia chebula Retz.	Fruit	ascorbic acid	Naik *et al*., 2004
		casuarinin	Cheng *et al*., 2003
		chebulagic acid	Lee *et al*., 1995; Hamada *et al*., 1997
		chebulic acid	THP, 2000
		chebuloside II	Tasduq *et al*., 2006
		2,4-chebulyl- ß-D-glucopyranose	Saleem *et al*., 2002
		cheblagic acid	Xie *et al*., 2006
		chebulinic acid	THP, 2000; Saleem *et al*., 2002; Cheng *et al*., 2003
		chebulanin	Cheng *et al*., 2003
		ellagic acid	Saleem *et al*., 2002; Naik *et al*., 2004
		gallic acid	THP, 2000; Kaur *et al*., 1998; Chase, 2002; Saleem *et al*., 2002; Naik *et al*., 2004; Hamada *et al*., 1997

Table 1-2 (Continued)

Botanical name	Part of plant used	Chemical constituents	References
Terminalia chebula Retz.	Fruit	1,2,3,4,6-penta-*O*-galloyl-ß-D-glucopyranose	Lee *et al*., 1995
		1,6-di-*O*-galloyl-ß-D-glucose	Cheng *et al*., 2003
		saponins	THP, 2000
		ß-sitosterol	THP, 2000
		tannic acid	THP, 2000
		linoleic acid	THP, 2000
		oleic acid	THP, 2000
		palmitic acid	THP, 2000

1.5 Biological activities of the investigated species

Previous investigations on biological activity of these twelve plants are shown in Table 1-4.

Table 1-4 Biological activities of the investigated species

Botanical name	Part of plant used	Activities	Results of biological activities	References
Phyllanthus emblica Linn.	Fruit	Antioxidant	Butanolic extract of the water fraction showed cytoprotective action on the stomach wall from lesion by increased secretion of hexosamine and gastric mucus in the indomethacin induced ulceration of rats and also decreased the level of malonaldehyde (MDA) and increased superoxide dismutase (SOD)	Bandyopadhyay *et al*., 2000

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Phyllanthus emblica Linn.	Fruit	Antioxidant	Aqueous extract showed antioxidant activity against γ-radiation-induced lipid peroxidaion (LPO) in rat liver microsomes and superoxide dismutase (SOD) damage in rat liver mitochondria	Khopde *et al*., 2001
		Antioxidant	An emblicanin-A and emblicanin-B of fresh fruit juice showed antioxidant activity against ischemia-reperfusion (IRI) -induced oxidative stress in rat heart	Bhattacharya *et al*., 2002
		Antioxidant	A standardized extract (Merck) showed a long-lasting and broad spectrum antioxidant activity in cosmetic formulations and no pro-oxidation activity induced by iron and/or copper	Chaudhuri, 2002

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Phyllanthus emblica Linn.	Fruit	Antioxidant	Flavonoid from methanol extract increased free radical scavenging enzymes and decreased lipid peroxide content in hypercholesterolemic rats	Anila and Vijayalakshmi, 2003
		Antioxidant	The dehydrated powder showed the ability to detoxify the dimethyl hydrazine (DMH) partly by enhancing the multicomponent antioxidant system in the rat	Anilakumar *et al*., 2004
		Antioxidant	Ascorbic acid content in extract showed antioxidant activity by DPPH and ABTS tests, when the concentration was higher than 0.25 mg/ml, the bleaching power of the solutions was too high	Scartezzini *et al*., 2006

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Phyllanthus emblica Linn.	Fruit	Antioxidant	The free and bound phenolics of extract showed antioxidant activity by DPPH assay (IC_{50} = 0.65±0.05, 0.85±0.04 μg/ml, respectively)	Kumar *et al*., 2006
		Antioxidant	Ethyl acetate extract and five pure compounds from ethyl acetate extract (gallic acid, methyl gallate, corilagin, furosin and geraniin) showed strong NO scavenging activity in vitro, when compared with water and hexane extracts	Kumaran and Karunakaran, 2006
		Analgesic	Ethanolic and aqueous extracts showed inhibitory effect on acetic acid-induced writhing response in mice and no activity in the tail-immersion test in mice	Perianayagam *et al*., 2004

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Phyllanthus emblica Linn.	Fruit	Antioxidant	Methanolic extract (75%) inhibited lipid peroxide formation, scavenged hydroxyl and superoxide radicals in vitro and reduced serum glucose concentrations in the normal and diabetic rats within 4 hours after administration	Chase, 2002
		Antioxidant	Methanolic extract (75%) inhibited lipid peroxidation induced with Fe^{2+}/ascorbate and to scavenge hydroxyl and superoxide radicals in vitro and IC_{50} of lipid peroxidation, hydroxyl radical scavenging and superoxide scavenging were found to be 74.0±5.8, 155.0±8.1 and 6.5±1.5 μg/ml, respectively	Sabu and Kuttan, 2002

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Phyllanthus emblica Linn.	Fruit	Antiulcerogenic	Methanolic extract showed ulcer protective in different acute gastric ulcer models in rats induced by aspirin, ethanol, cold restraint stress and pyloric ligation and healing effect in chronic gastric ulcers induced by acetic acid in rats	Sairam *et al*., 2002
		Antitussive	Ethanolic extract showed that the cough suppressive activity was dose-dependent and less effective than the classical narcotic antitussive drug codeine but more effective than the non-narcotic antitussive agent dropropizine	Nosál'ova *et al*., 2003

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Phyllanthus emblica Linn.	Fruit	Hypolipidemia	Fresh juice showed lipid lowering and antiatherosclerotic effects in cholesterol-fed rabbits lowered the level of serum cholesterol, TG, phospholipid and LDL by 82%, 66%, 77% and 90%, respectively	Mathur *et al*., 1996
		Hypolipidemia	Flavonoid from methanol extract inhibited hepatic HMG CoA reductase activity and elevated level of plasma LCAT, the mechanism of hypolipidemic action is by the concerted action of inhibition of synthesis and enhancement of degradation	Anila and Vijayalakshmi, 2002
		Antipyretic	Ethanolic and aqueous extracts showed reduction in brewer's yeast induced hyperthermia in rats	Perianayagam *et al*., 2004

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Phyllanthus emblica Linn.	Fruit	Immunomodulatory	The extract resulted in enhanced cyto-protection, apoptosis and DNA fragmentation induced by chromium and relieved the immunosuppressive effects of Cr on lymphocyte proliferation and even restored the IL-2 and γ-IFN production considerably	Ram *et al*., 2002
		Immunomodulatory	*Phyllanthus emblica* had been found to enhance natural killer (NK) cell activity and antibody dependent cellular cytotoxicity (ADCC) in syngeneic BALB/c mice, bearing Dalton's lymphoma ascites (DLA) tumor	Suresh and Vasudevan, 1994

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Phyllanthus emblica Linn.	Fruit	Hepatoprotective	Ethanol extract (50%) exhibited increased cell viability of rat primary cultured hepatocytes being with ethanol by increasing %MTT and decreasing the release of transaminase	Pramyothin *et al*., 2006
		Hepatoprotective	Water extract inhibited the hepatotoxicity produced by acute and chronic CCl_4 administration as seen from the decreased levels of serum and liver lipid peroxides (LPO), glutamate-pyruvate transaminase (GPT) and alkaline phosphatase (ALP)	Jeena and Kuttan, 2000
		Hepatoprotective	Hydroalcoholic extract (50%) against antituberculosis drugs-induced hepatic injury	Tasduq *et al*., 2005

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Phyllanthus emblica Linn.	Fruit	Cytotoxicity	Ethanol extract showed fully suppress chronic myelogenous leukemic K562 cell growth at 5-500 ng/ml and ethanol extract and n-butanol fraction showed increased amounts of extract on cell proliferation of K562, B-lymphoid Raji, T-lymphoid Jurkat and erythroleukemia HEL human cell lines and the IC_{50} on K562, Raji, Jurkat and HEL cells were 0.4, 8.0, 2.5 and 10 ng/ml, respectively and the IC_{50} of pyrogallol on K562, Jurkat, Raji and HEL cells was found to be in range of 10-30 μM	Khan *et al*., 2002

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Phyllanthus emblica Linn.	Fruit	Cytotoxicity	L-malic acid 2-*O*-gallate, mucic acid 2-*O*-gallate, 1-*O*-galloyl-ß-D-glucose and corilagin showed stronger inhibition against B16F10 (murine melanoma) cell growth than against HeLa (human uterine carcinoma) and MK-1 (human gastric adrenocarcinoma) cell growth by MTT method	Zhang *et al*., 2004
		Antitumor	The extract inhibited tumor incidences on two-stage process of skin carcinogenesis in Swiss albino mice, induced by 7,12-dimethyabenz(a)anthrecene (DMBA)	Sancheti *et al*., 2005

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Phyllanthus emblica Linn.	Fruit	Antitumor	Aqueous extract showed cytotoxic to L 929 cells in a dose dependent manner (IC_{50} = 16.5 μg/ml) and reduced ascites and solid tumours in mice induced by DLA (Daltons lymphoma ascites) cells, increased life span of tumour bearing animals (20%) and inhibited cell cycle regulating enzymes cdc 25 phosphatase in a dose dependent manner (IC_{50} = 5 μg/ml)	Jeena *et al*., 2001
		Anticancer	Aqueous extract (10%) inhibited hepatocarcinogenesis induced by *N*-nitrosodiethylamine (NDEA) in a dose dependent manner	Jeena *et al*., 1999

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Phyllanthus emblica Linn.	Fruit	Antimutagenicity	Ethanolic extract showed protection against genotoxicity induced by the rodent carcinogent, 7,12-dimethyabenz(a) anthrecene (DMBA)	Banu *et al*., 2004
		Antimutagenicity	Acetone extract against indirect acting mutagen, 2-aminofluorene, in both TA98 and TA100 tester strains of *Salmonella typhimurium* than against the direct acting mutagens	Arora *et al*., 2003
		Anti-diabetic	Methanolic extract (75%) at dose 100 mg/kg body weight reduced the blood sugar level in normal and in alloxan (120 mg/kg) diabetic rats within 4 hours	Sabu and Kuttan, 2002

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Phyllanthus emblica Linn.	Fruit	Anti-sugar cataract	Aqueous extract and tannoids of extract inhibited rat lens with IC_{50} values 0.72 mg/ml and 6 μg/ml, respectively and human aldose reductase (AR) 0.88 mg/ml and 10 μg/ml, respectively	Suryanarayana *et al*., 2004
		Antidiarrheal	Methanolic extract showed inhibitory effect on rats with diarrhea induced by castor oil and magnesium sulfate, produced reduction in gastrointestinal motility in charcoal meal tests in rats and inhibited PGE(2)-induced enteropooling as compared to control animals	Perianayagam *et al*., 2005

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Phyllanthus emblica Linn.	Fruit	Antibacterial	Alcoholic and aqueous extracts exhibited antibacterial activity against *Bacillus subtilis*, *Proteus vulgaris*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Esherichia coli* and *Staphylococcus aureus* by using agar well diffusion method at concentration of 200 mg/ml and concluded that alcoholic extract showed greater activity than aqueous and hexane extracts	Ahmad *et al*., 1998
Terminalia chebula Retz.	Fruit	Antioxidant	Aqueous extract inhibited γ-radiation-induced lipid peroxidation in rat liver microsomes and damage to superoxide dismutase enzyme in rat liver mitochondria and also scavenger of DPPH radicals	Naik *et al*., 2004

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Terminalia chebula Retz.	Fruit	Antioxidant	Aqueous extract showed inhibition in the thiobarbituric acid reactive substances (TBARS) formation and restored antioxidant enzyme superoxide dismutase (SOD) from the radiation induced damage and also evaluated in terms of ascorbate equivalents by different methods such as cyclic voltammetry, decay of $ABS^{\bullet -}$ radical by pulse radiolysis and decreased in the absorbance of DPPH radicals	Naik *et al*., 2003
		Antioxidant	Methanolic extract (75%) inhibited lipid peroxide formation, scavenged hydroxyl and superoxide radicals and reduced serum glucose concentrations in the normal and diabetic rats within 4 h after administration	Chase, 2002

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Terminalia chebula Retz.	Fruit	Antioxidant	MeOH, $CHCl_3$, EtOAc, *n*-BuOH, organic aqueous, water extracts, casuarinin, chebulanin, chebulinic acid and 1,6-di-*O*-galloyl-ß-D-glucose showed anti-lipid peroxidation activity (IC_{50} < 9.00 mg/ml, except casuarinin, IC_{50} = 29.67 mg/ml), anti-superoxide radical formation activity (IC_{50} values in the range of 0.04-2.42 mg/ml), free radical scavenging activity (chebulinic acid had the strongest activity, IC_{50} = 2 µg/ml, casuarinin and all extracts, except organic aqueous extract possessed free radical scavenging activity with IC_{50} values in the range of 4-9 µg/ml)	Cheng *et al*., 2003

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Terminalia chebula Retz.	Fruit	Antioxidant	Ethanolic extract inhibited oxidative stress induced by UVB but in the peroxidation model using *t*-buOOH showed a notable cytoprotective effect on the human epidermal keratinocytes-Neonatal/Foreskin (HEK-N/F) cells with 60.5±3.8% at a concentration of 50 µg/ml and the age-dependent shortening of the telomeric DNA length by the southern blots of the terminal restriction fragments (TRFs) of DNA extracted from subculture passage	Na *et al*., 2004
		Antioxidant	Methanolic extract (75%) inhibited lipid peroxidation and scavenge hydroxyl and superoxide radicals and IC_{50} were 85.5±6.5, 165.5±8.5 and 20.5±3.2 µg/ml, respectively	Sabu and Kuttan, 2002

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Terminalia chebula Retz.	Fruit	Antioxidant	Aqueous extract reversed the tert-butyl hydroperoxide (t-BHP)-induced cell cytotoxicity and lactate dehydrogenase leakage and exhibited in vitro ferric-reducing antioxidant activity and DPPH free radical-scavenging activities and also lowered the serum levels of the hepatic enzyme markers aspartate and alanine aminotransferases and reduced the indicators of oxidative stress in the liver, such as the glutathione disulfide content and lipid peroxidation, in a dose-dependent manner	Lee *et al*., 2005

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Terminalia chebula Retz.	Fruit	Antioxidant	Methanolic extract showed down regulation of glutathione content, glutathione-S-transferase, glutathione reductase, lipid peroxidation, H_2O_2 generation, blood urea nitrogen, serum creatinine, DNA synthesis and ornithine decarboxylase activity with concomitant restoration of glutathione peroxidase activity by $NiCl_2$ induced renal oxidative stress, toxicity and cell proliferation response in male Wistar rats	Prasad *et al*., 2006
		Anti-diabetic	Methanolic extract (75%) at dose 100 mg/kg body weight reduced the blood sugar level in normal and in alloxan (120 mg/kg) diabetic rats within 4 hours	Sabu and Kuttan, 2002

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Terminalia chebula Retz.	Fruit	Antiproliferation	Methanolic extract (70%) decreased cell viability, inhibited cell proliferation and induced cell death in a dose dependent manner on all cell lines studied (human (MCF-7) and mouse (S115) breast cancer cell line, human osteosarcoma cell line (HOS-1), human prostate cancer cell line (PC3) and a non-tumorigenic, immortalized human prostate cell line (PNTIA)) and also induced apoptosis at lower concentrations, but at higher concentrations, the major mechanism of cell death was necrosis and growth inhibitory, chebulinic acid (IC_{50} = 53.2 μM ± 0.16) and ellagic acid (IC_{50} = 78.5 μM ± 0.24), on HOS-1 cell line	Saleem *et al*., 2002

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Terminalia chebula Retz.	Fruit	Cytotoxicity	Methanolic extract (90%) inhibited the melanin production of mouse B16 melanoma cells by using MTT method	Jin *et al*., 2006
		Hepatoprotective	Ethanolic extract showed hepatoprotective activity against anti-tuberculosis (anti-TB) drug-induced toxicity	Tasduq *et al*., 2006
		Immunosuppressive	Gallic acid and chebulagic acid that blocked the cytotoxic T lymphocyte (CTL)-mediated cytotoxicity and inhibited the killing activity of CD8+ CTL clone at IC_{50} values of 30 and 50 μM, respectively and also blocked granule exocytosis in response to anti-CD3 stimulation at the equivalent concentrations	Hamada *et al*., 1997

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Terminalia chebula Retz.	Fruit	Antiviral	Water extract showed anti-herpes simplex virus (HSV) activity in vivo and inhibited replication of human cytomegalovirus (CMV) and murine CMV (MCMV) in vitro and suppressed MCMV yields in lungs of treated mice compared with water treatment	Yukaya *et al*., 1996; Kurokawa *et al*., 1995
		Antimutagenic	Aqueous extract inhibited γ-radiation-induced strand breaks formation in plasmid pBR322 DNA	Naik *et al*., 2004
		Antimutagenic	A tannin fraction showed effective against S9-dependent mutagen, 2-aminofluorene, 4-nitro-*o*-phynylenediamine but not at all effective against 4-nitroquinoline-*N*-oxide	Kaur *et al*., 1998

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Terminalia chebula Retz.	Fruit	Antimutagenic	Acetone extract against indirect acting mutagen, 2-aminofluorene, in both TA98 and TA100 tester strains of *Salmonella typhimurium* than against the direct acting mutagens	Arora *et al*., 2003
		Anti-anaphylactic	Water soluble fraction inhibited compound 48/80-induced anaphylactic shock 100% with doses of 0.01-1.0 g/kg and also inhibited histamine release from rat peritoneal mast cells (RPMC) and reduced the serum histamine levels at concentration ranging from 0.005 to 1.0 g/kg in a dose-dependent manner and increasing effect on anti-dinitrophenyl IgE-induced tumor necrosis factor-α production from RPMC	Shin *et al*., 2001

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Terminalia chebula Retz.	Fruit	Anticaries	Aqueous extract inhibited the growth, sucrose induced adherence and glucan induced aggregation of *Streptococcus mutans*, 10% solution of the extract inhibited the salivary bacterial count and salivary glycolysis and mouthrinsing with the extract reduced total bacterial counts and the total streptococcal counts in the saliva samples obtained up to and including 3 hours after rinsing	Jagtap and Karkera, 1999
		Hypolipidemia	Not showed potent hypolipidemic agent and induced partial inhibition of rabbit atheroma Alcoholic and water extracts showed	Shaila *et al*., 1998

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Terminalia chebula Retz.	Fruit	Antibacterial	Alcoholic and water extracts showed antibacterial effect against *Helicobacter pylori* by using agar diffusion method and the water extract showed MIC and MBC of 125 and 150 mg/l, respectively and at concentration of 1-2.5 mg/ml inhibited urease activity of *H. pylori*	Malekzadeh *et al*., 2001
		Antibacterial	Alcoholic and aqueous extracts against *Bacillus subtilis*, *Proteus vulgaris*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Esherichia coli* and *Staphylococcus aureus* by using agar well diffusion method at concentration of 200 mg/ml and alcoholic extract showed greater activity than aqueous and hexane extracts	Ahmad *et al*., 1998

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Terminalia chebula Retz.	Seed	Antibacterial	Methanolic extract against *S.aureus* by agar well-diffusion bioassay and diameter of inhibition zone were 18 mm in unripe seed and 21 mm in ripe seed	Bonjar, 2004
		Antifungal	Methanolic extract at 20 mg/ml concentration against Clotrimazole-resistant *Candida albicans* by agar well-diffusion bioassay and the MIC were 1.25 mg/ml in unripe seed and 0.62 mg/ml in ripe seed	Bonjar, 2004(a)
Terminalia bellerica Gaertn.	Fruit	Antioxidant	Methanolic extract (75%) inhibited lipid peroxide formation, scavenged hydroxyl and superoxide radicals in vitro and reduced serum glucose concentrations in the normal and diabetic rats within 4 hours after administration	Chase, 2002

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Terminalia bellerica Gaertn.	Fruit	Antioxidant	Ethanolic extract (200, 400 and 800 mg/kg, p.o.) and gallic acid (50, 100 and 200 mg/kg, p.o.) showed dose-dependent recovery in aspartate aminotransferase, serum alanine aminotransferase, serum alkaline phosphatase, lipid peroxidation and glutathione level but the effect was more pronounced with gallic acid and at 200 mg/kg dose of gallic acid most effective against carbon tetrachloride induced liver and kidney damage	Jadon *et al*., 2007

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Terminalia bellerica Gaertn.	Fruit	Antioxidant	Methanolic extract (75%) inhibited lipid peroxidation induced with Fe^{2+}/ascorbate and to scavenge hydroxyl and superoxide radicals in vitro and IC_{50} of lipid peroxidation, hydroxyl radical scavenging and superoxide scavenging were found to be 27.0±3.2, 71.0±2.7 and 40.5±6.5 μg/ml, respectively	Sabu and Kuttan, 2002
		Antimutagenic	Acetone extract against indirect acting mutagen, 2-aminofluorene, in both TA98 and TA100 tester strains of *Salmonella typhimurium* than against the direct acting mutagens	Arora *et al*., 2003

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Terminalia bellerica Gaertn.	Fruit	Anti-diabetic	Methanolic extract (75%) at dose 100 mg/kg body weight reduced the blood sugar level in normal and in alloxan (120 mg/kg) diabetic rats within 4 hours	Sabu and Kuttan, 2002
		Antibacterial	Alcoholic extracts exhibited antibacterial activity against *Bacillus subtilis*, *Proteus vulgaris*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Esherichia coli* and *Staphylococcus aureus* by using agar well diffusion method at sample concentration of 200 mg/ml and concluded that alcoholic extract showed greater activity than aqueous and hexane extract	Ahmad *et al*., 1998

Table 1-4 (Continuted)

Botanical name	Part of plant used	Activities	Results of biological activities	References
Terminalia bellerica Gaertn.	Fruit	Antimicrobial	Methanol extract was more effective than crude aqueous tested by extract against most of 9 human microbial pathogens except *E. coli* (enteropathogent) and *P. aeruginosa* and highly effective against *S. aureus* with lower MIC values.	Elizabeth, 2005
		Hypolipidemia	Not showed potent hypolipidemic agent and induced partial inhibition of rabbit atheroma	Shaila *et al*., 1998

1.6 Objectives

This thesis was planned to study:

1. Antioxidant and cytotoxic activity against three types of human cancer cells; breast, cervical, prostrate and normal human lung cell of Triphala formula and its ingredients.

2. Chemical constituents of the active plant extracts which show free radical scavenging activity and/or cytotoxic activity against cancer cell lines.

3. Antioxidant activity and/or cytotoxic activity against cancer cells of the isolated compounds.