CHAPTER II

REVIEW OF RELATED LITERATURE AND RESEARCH

Asparagus racemosus Willd.

Asparagus racemosus Willd. (AR) known as Shatavari is one of the most commonly used herbs in traditional medicine. This herb is in the family of Asparagaceae. It is locally known in Thai as "Samsib". It has an extensively scandent, much-brached, spinous under-shrub, with tuberous root. The flower of AR is white while the fruit is globular in shape and will turn to blackish-purple colour when it is ripe. The rootstock is in fusiform shape. The length of the succulent tuberous roots is about 30-100 cm and the diameter of the root is around 1-2 cm (Figure 1). AR is grown throughout tropical and subtropical countries.

Figure 1 The characterictic of *Asparagus racemosus* Willd

Source: http://thaiforestherb.blogspot.com

http://www.moac-info.net/modules/news/news_view.php

This herb is known to produce steroidal saponins called Shatavarin that is presented in the roots. Shatavarin is a glycoside of sarsasapogenin having two molecules of rhamnose and one molecule of glucose (Figure 2). Other active compounds such as quercetin, rutin and hyperoside are found in the flowers and fruits. The active compounds in the leaves are diosgenin and quercetin-3 glucuronide (Bopana and Saxena, 2007).

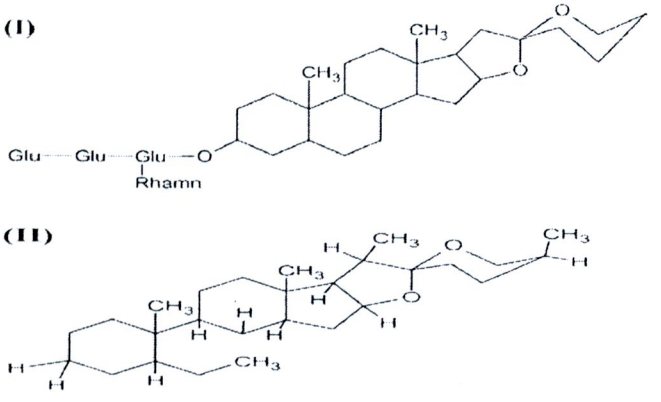

Figure 2 The main active compounds of *Asparagus racemosus* Willd (I) Shatavarin, (II) Sarsasapogenin

Source: Bopana and Saxena, 2007

The presence of sarsasapogenin in natural plants of AR was found as well as in *in vitro* cultures (Asmari, et al., 2004). Synthesis of sarsasapogenin in the callus cultures of AR was also reported earlier by Kar and Sen (1985).

α,α-diphenyl-ß-picrylhydrazyl (DPPH) autography-directed separation resulted in the identification of a new antioxidant compound from AR named "racemofuran" (Wiboonpun, et al., 2004). Previously, the isolation and spectral data of a new isoflavone, 8-methoxy-5, 6, 4'-trihydroxyisoflavone 7-*o*-ß-D-glucopyranoside, was reported from the roots of the plant (Saxena and Chourasia, 2001).

A new 9, 1-dihydrophenenthrene derivative named "Racemosol" was isolated from the ethanolic extract of roots (Sekine, et al., 1997). Its structure was elucidated by spectroscopic analysis as 9, 10-dihydro-1, 5-dimethoxy-8-methyl-2, 7-

phenenthrenediol. Previously, the isolation and characterization of a polycyclic alkaloid called "Asparagamine" from AR that exhibited a structural and remarkable anti-oxytocic activity (Sekine, et al., 1994).

Pharmacological properties of AR root

The entire plant is reported to contain Shatavarin, however, the fasciculate roots of AR are considered to be the richest source of Shatavarin (Mashitha, et al., 2010). Shatavarin, the major bioactive component in the root, is being used in aphrodisiac, immunomodulant, galactogogue, dyspepsia, antitussive, anticarcinogen, antidiarrhoeal and general tonic for both sexes etc. Several studies (Khanna, et al., 1991; Kulkarni and Verma, 1993; Mitra, et al., 1999; Nevrekar, et al., 2003; Gopumadhavan, et al., 2005) were reported that the root of AR demonstrated in many Ayurvedic and compound formulations as an important gradient. In present times, the formulations have been extensively studied for their pharmacological activities. Antibacteria, anticancer, antioxidant and immune-modulator properties have been found in experimental models. The pharmacological activitities are discussed below.

Effects of AR root extract on reproductive system

The root is a part of the herb believed to have the medicinal values to enhance fertility (Potduang, et al., 2008) and sexual function (Boonyapraphatsorn, 2000, p.173) in Thai folk medicine. Phytoestrogenic properties have been indicated to use as a hormone modulator. The pharmacological action of AR and other plant constituents in polyherbal formulations has been observed in a number of laboratory studies. The AR in the form of "U-3107 or EveCare®" as an aqueous suspension at a dose 1000 mg/kg B.W./day for 21 days to OVX rats was shown to increase in wet and dry uterine weight (Mitra, et al., 1999). The estrogenic activity of this herb has been reported that AR as it in the form of tablets "Menosan" at an oral dosage of 500 mg/kg B.W./day for 21 consecutive days to immature and OVX rats increased the uterine weight and showed the proliferation of endometrium (Gopumadhavan, et al., 2005). "EveCare" capsules are proved to be effective in the treatment of dysfunctional uterine bleeding (DUB) in women (Nevrekar, et al., 2003). The combination preferably includes approximately 85% of AR and approximately 5% each of other herbs (*Withania somnifera, Pedalium murex* and *Tinospora cordifolia*). It has been reported to be

effective in the treatment of pre-menstrual syndrome (PMS) in human females (Dhaliwal, 2003). A number of studies showing the galactogogue effects of AR both in humans and animals have appeared a long ago. It has been reported that the oral administration of alcoholic extract of AR root at a dose of 300 mg/kg B.W./day, for 15 days to adult pregnant female albino rats had an estrogenic activity on mammary gland and genital organs (Pandey, et al., 2005). Also, an increase in milk secretion was found in women suffering from deficient milk secretion after administration of AR in the form of tablets "Ricalex®" (containing 40 mg AR root extract per tablet) (Joglekar, et al., 1967). Moreover, in inhibitory effect of AR was shown to reduce in mammary tumorigenesis in rats from AR on 7, 12-dimethylbenzyne[α]anthracene (DMBA)-induced mammary tumors (Raɔ, 1981). Interestingly, AR has been studied for its influence on the male reproductive system. Animals fed with AR root powder at a dose of 500 mg/kg B.W./day, daily for 21 consecutive days exhibited significantly high testes weights as compared to control rats (Ghumare, et al., 2004).

Toxicological studies of AR root extract on reproductive system

In Thai folk medicine, AR root is commonly preserved in syrup or molded to ball of medicine to use in pregnancy for preventing abortion (Boonyapraphatsorn, 2000, p. 173). In Ayurveda, AR root extract has been considered as absolutely safe for long term use, even during pregnancy and lactation (Sabnis and Gaitonde, 1968). LD50 of herbal galactogogue containing AR roots was 64 g/kg oral dose. Nevertheless, there is a study (Goel, et al., 2006) indicated that the pre-and post-natal studies did indicate some teratogenic effects with methanolic extract of AR root in dose of 1000 mg/kg B.W/day orally for 60 days. Furthermore, Asparagus genus like, *Asparagus adscendens* seeds has shown to have an abortificient effect in rats and mark malformations were seen in neonates. Asparagus root extract has been reported to inhibit fetal implantation in animals and significantly change the weight and length of the fetuses (Sethi, Nath and Sing, 1990).

Effects of AR root extract on anti-bacteria

There is a study (Mandal, et al., 2000) indicates that different concentrations (50, 100 and 150 mg/mL) of the methanolic extract of the AR root showed a considerable antibacterial effect under *in vitro* conditions.

Effects of AR root extract on anti-ulcerogenic activity

Anti-ulcerogenic activity of ayurvedic herbomineral formulations, the main ingredient of which is the root extract of AR has been traditionally used in the treatment of peptic ulcers since ancient times. Increasing of the mucosal defensive factors like mucus secretion was found to be 250 mg/kg B.W./day of fresh root of AR given for 5 days (Sairam, et al., 2002). Recently, inhibitory effect on release of gastric hydrochloric acid and protects gastric mucosal damage was observed in the treatment with crude extract of AR at a dose of 100 mg/kg B.W./day orally for a period of 15 days (Bhatanagar, et al., 2005).

Effects of AR root extract on immunostimulant activity

Since macrophages play an important role in the development of intraperitoneal adhesions, the modulation of macrophage activity would provide a new approach for the prevention of adhesions. AR being reported to be an immunostimulant, significantly decreased the adhesions by increasing macrophage phagocytosis in animals treated with AR extract (200 mg/kg), orally for 15 days (Rege, et al., 1989). Furthermore, it was found that a combination of crude extract of AR (100 mg/kg) and other constituents of plant extracts (*Withania somnifera* (100 mg/kg), *Tinospora cordifolia* (100 mg/kg) and *Picrorhiza kurrooa* (100 mg/kg)) prepared in distilled water were administered orally to mice, for 17 weeks inhibited carcinogen ochratoxin-induced suppression of chemotactic activity (Dhuley, 1997).

Effects of AR root extract on hypolipidaemic activity

"Abana", an Indian herbomineral preparation, containing 10 mg AR root extract per tablet showed hypolipidaemic activity in rats. It has been observed that long term treatment of suspension of Abana (50 mg/kg B.W/day., daily, orally) to male adult rats demonstrated a potential for use as a cardio-protective agent (Khanna, et al., 1991). Dried root powder of AR has also been found for the reduction of cholesterol levels in hypercholesteremic rats (Visavadiya and Narasimhacharya, 2005).

Effects of AR root extract on neurological system

"Mentat", a herbal psychotropic formulation containing AR has been found to be effective in the treatment of alcohol abstinence induced withdrawal symptoms such as body tremor, convulsion and anxiety in ethanol fed rats (Kulkarni and Verma, 1993). In recent years, oral administration of dietary supplement of AR at a dose of 18

mg/kg B.W./day for a period of 2 weeks protected mice against kianic acid induced neuronal damage (Parihar and Hemnani, 2004). Several reviews (Nandagopal, Muralidharan and Thirumurugan, 2011; Ashwlayan and Singh, 2011) have been published on the potential benefits of AR in cerebroprotective effects. It has been suggested that oral administration of methanolic extract of AR at 400 mg/kg B.W./day protected rats from ischemia-induced brain injury may be due to reduction of oxidative stress which occurs by alteration in levels of antioxidants, neurotransmitters and the AR extract had the potential to use in treatment of ischemia (Nandagopal, Muralidharan and Thirumurugan, 2011). Besides, the administration of methanolic root extract of AR at 150 mg/kg B.W./day for 7 days improved learning and memory in memory deficits mice (Ashwlayan and Singh, 2011).

Table 2 Conclusion of the experiments evaluated on the pharmacological actions of AR

Property	Model	Extract/Preparation	Action	References
Lactogogue	Adult pregnant female rat	Ethanolic extract of AR at 300 mg/kg B.W./day daily for 15 days	Estrogenic effect of AR stimulates on the female mammary gland and genital organs	Pandey et al. (2005)
	Humans	AR in form of "Ricalex" tablet containing 40 mg AR per tablet	Increases milk secretion	Joglekar et al. (1967)
Uterotrophic effect	Rat uterus (*in vitro*)	Polyherbal preparation "U3107" or "EveCare®" ccontaining 32 mg AR per 5 ml syrup at 1000 mg/kg B.W./day for 21 days	Increases serum estrogen level but mechanism is unclear	Mitra et al. (1999)
Dysfuntional bleeding (DUB)	Humans	AR in form of "EveCare®" capsule	Healing of the endometrium by stimulated microvascular thrombosis	Nevrekar et al. (2002)
Pre-menstrual syndrome (PMS)	Humans	85% of AR and 5% each of *Withania somnifera*, *Pedalium murex* and *Tinospora cordifolia*	Effective in treatment of symptoms	Dhaliwal (2003)

Table 2 (CONT.)

Property	Model	Extract/Preparation	Action	References
Breast cancer	Female rats	AR root powder	Inhibitory to DMBA-induced mammary tumorigenesis	Rao (1981)
Testes weight	Male rats	AR root powder at 500 mg/kg B.W./day for 21 days	Stimulation of diameter of all germ cells to increase	Ghumare et al. (2004)
Teratogenecity	Female rats	Methanolic extract of AR root at 100 mg/kg B.W./day for 60 days	Pre- and Post-natal developmental disorder were found	Goel, Prabha, and Kumar (2006)
Anti-bacterial action	Nutrient agar plates seeded with bacteria (*in vitro*)	Methanolic extract of AR root at various doses (50, 100 and 150 µg/mL)	Possess bacteria inhibitory	Mandal et al. (2000)
Anti-ulcerogenic action	Male and female rats	Methanolic extract of AR root at various doses (25, 50 and 100 mg/kg B.W./day, twice daily, orally) for 5 days	Stimulation of mucosal defensive factors	Sairam et al. (2002)

Table 2 (CONT.)

Property	Model	Extract/Preparation	Action	References
	Adult male and female rats	Extract of fresh AR root at 100 mg/kg B.W./day for 15 days	Effective in reducing gastric ulcer	Bhatnagar, Sisodia and Bhatnagar (2005)
Immunostimulant	Male and female rats	Exthanolic extract of AR root at 200 mg/kg B.W./day for 15 days	Increases in secretory activities of macrophages	Rege et al. (1989)
	Male mice	Crude extract of 100 mg/kg and 100 mg/kg each of Withania somnifera, Tinospora cordifolia and Picrorhiza kurrooa	Stimulation of chemotaxis and modulate cytokine production in macrophages	Dhuley (1997)
Hypolipidaemic action	Adult male rats	Herbomineral preparation	Stimulation in hypocholesterolaemic action	Khanna, Chander and Kapoor (1991)
	Adult male rats	AR dried root powder supplement	Stimulation in reducing cholesterol levels	Visavadiya and Narasimhacharya (2005)

Phytoestrogens

The interest in plant derived estrogens has recently been increased by the realization that several studies concluded that HRT increases the risk of endometrial and ovarian cancer. Interest in phytoestrogens has increased in the recent years after a wealth of scientific data have shown that phytoestrogens possess potent and wide-ranging biological activities.

Phytoestrogens may protect against development of certain diseases including cancer, cardiovascular disease and osteoporosis as well as other hormone-dependent conditions like menopausal symptoms. Phytoestrogens are popular natural compounds consumed for the treatment of menopausal symptoms (Cherdshewasart, Sriwatcharakul and Malaivijinond, 2008).

Phytoestrogens are so named becaused they originate from plants and have estrogenic activity. More specifically, they are defined as any plant substance or metabolite that induces biological responses in vertebrates and can modulate the actions of endogenous estrogens by binding to estrogen receptors. Structurally, they are similar to naturally occurring most potent mammalian estrogen 17ß-estradiol, synthetic estrogen, and anti-estrogens. Phytoestrogens can be divided into three main classes: isoflavones, coumestans, and lignans (Thompson, et al., 2006). Epidemiological studies provide evidence for a protective role of isoflavones, and to a lesser extent coumestans and lignans, against the development of numerous chronic diseases, including several cancers, cardiovascular disease and osteoporosis (Duncan, Phipps and Kurzer, 2003).

Isoflavones are the most extensively studied phytoestrogens. These compounds are found predominately in soybeans and soybean products. Some *in vivo* experiments used soybeans and soybean products as a source of isoflavones to study their beneficial effects on bone. Ovariectomized rodent models have been widely used to induce bone loss and to test whether isoflavones can prevent bone loss. Many reports have indicated that natural food sources with high concentrations of isoflavonoids have effectiveness in preserving bone mass due to ovarian hormone deficiency (Yamazaki, 1986; Yamazaki and Kinoshita, 1986; Arjmandi, 1996; Ye, 2003).

Coumestans was first isolated from alfalfa, strawberry, and ladino clover by (Bickoff, et al., 1950). Coumestans has a close structural relationship to estradiol and binds to estrogen receptors (ERs) alpha (α) and beta (β) (Scarlata and Miksicek, 1995). Several studies have shown that coumestans prevent bone loss in OVX rats (Ye, et al., 2003) with increase in uterine weight, increase in embryo degeneration, and inhibit ovarian cycles (Moon, et al., 2009).

The highest known sources of lignans found in flaxseed, pumpkin seeds, soybeans, broccoli, sesame seeds and some berries. In a study published in the British Journal of Nutritionin 2001, Canadian researchers found that purified lignans from flaxseed protect bone strength by preventing bone loss in female rats (Ward, et al., 2001).

Moreover, the lignans are powerful antioxidants and potent free radical scavengers to enhance the immune system functioning being effective against many different diseases such as protection against or supportive treatment in breast, gastrointestinal, and prostate cancer (Mathern, 2010).

Menopause

Menopause is derived from the Greek word menos (month) and pause (to stop) refers to the final menstrual period. The cessation of menstrual periods is due to the declining of estrogen and progesterone production by the ovaries. Menopause is generally considered to have occurred after 12 months of amenorrhea (Birkhauser, et al., n.d.). The menopause can be divided into 4 stages: premenopause, perimenopause, menopause and postmenopause. The graph below shows the stages of menopause in relation to age (Figure 3).

1. **Premenopause**

The premenopause often referred to as perimenopause, is the phase before the beginning of menopause. The average age for a woman to begin experiencing premenopausal symptoms is 45 years.

2. **Perimenopause**

The perimenopause is the time leading up to menopause. The perimenopause is characterized by hormonal changes which often lead to menopausal symptoms and usually occurs between 45 and 60 years of age.

3. Menopause

The menopause is the final menstrual bleed and it is generally considered to have occurred after 12 months of amenorrhea. Menopause has a wide starting range, but can usually be expected in the age range of 45 to 55 years. In the Western countries, the most typical age range for menopause is between the ages of 50 to 52 years (Kenemans, 2003). The average age for the menopause in Western women is 50.4 years (Soules, et al., 2001). Most Thai women go through the menopause between 49 years and 52 years (Charoensiri, 2011) and the average age at menopause in Thai women is 49.5 years (Chompootweep, et al., 1993).

4. Postmenopause

The postmenopause is the time after the last episode of menstrual bleeding.

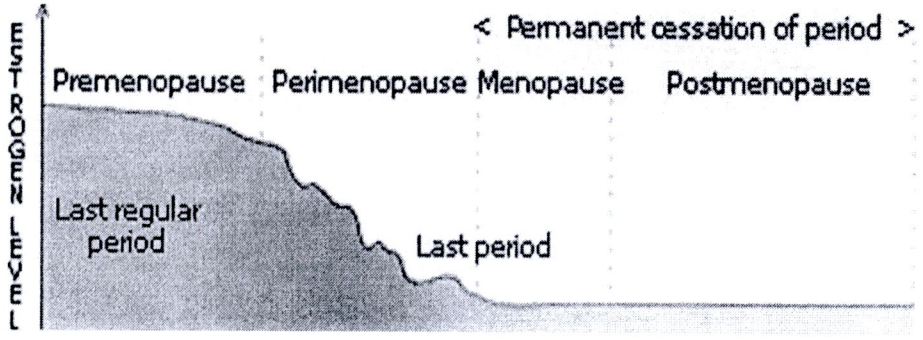

Figure 3 Menopausal stages

Source: http://www.yousaytoo.com/natural-menopause-stages/609041

The physical and emotional changes of menopause

The ovaries are the main source of female hormones which control the development of female body characteristics such as breast, body shape and body hair. The hormone also regulates the menstrual cycle and bone. Therefore, a woman can develop osteoporosis later in life when the ovaries do not adequate estrogen. The hormone levels slowly decrease and the body undergoes many physical and emotional changes. The hormonal change can produce symptoms that range from subtle to very

intense. The following conditions may result when hormone levels rise and/or fall (Table 3).

Table 3 Symptoms of estrogen deficiency

Physical	Emotional
Hot flashes	Depression
Fatigue	Minor anxiety
Headaches/migraines	Emotional instability
Night sweats	Feelings of despair
Vaginal and/or bladder infections	Crying easily
Difficulty falling asleep	Memory lapses
Poor concentration	Lack of concentration
Irregular bleeding	
Osteoporosis	

Source: http://www.jacemedical.com/articles/Hormonal

Bone loss

Bones are rigid organs that form part of the endoskeleton of vertebrates. They move, support, and protect the various organs of the body, produce red and white blood cells and store minerals. Bone tissue is a type of dense connective tissue. Bones come in a variety of shapes and have a complex internal and external structure.

The structure of a long bone is divided into several regions; epiphysis, metaphysis and diaphysis (Figure 4). Metaphyseal region of long bones in human has been attributed to the rapid bone turnover due to extensive bone remodeling. The metaphysis is the junctional region between the epiphyseal plate and the diaphysis. The metaphysis contains abundant trabecular bone, but the cortical or compact bone is thinner than the diaphysis.

There are two types of bone tissue: cortical (compact) and cancellous (spongy). Cortical bone, the hard outer layer of bones, is called due to its minimal gaps

appearance, and accounts for 80% of the total bone mass of an adult skeleton. Cortical bone may also be referred to as dense bone. Cancellous or spongy bone has a higher surface area but less dense, softer, weaker, and less stiff compared to cortical bone. It typically occurs at the ends of long bones, proximal to joints and within the interior of vertebrae. Cancellous bone is highly vascular and frequently contains red bone marrow where hematopoiesis, the production of blood cells, occurs.

Figure 5 shows the histology of cortical bone and cancellous bone. Cortical bone is denser than cancellous bone. Its spaces are much reduced in size. Cancellous bone actually looks like a sponge. It has large marrow spaces. Cancellous bone is composed of a network of rod-and plate-like elements named trabecular bone. Trabecular bone is the primary anatomical and functional unit of cancellous bone.

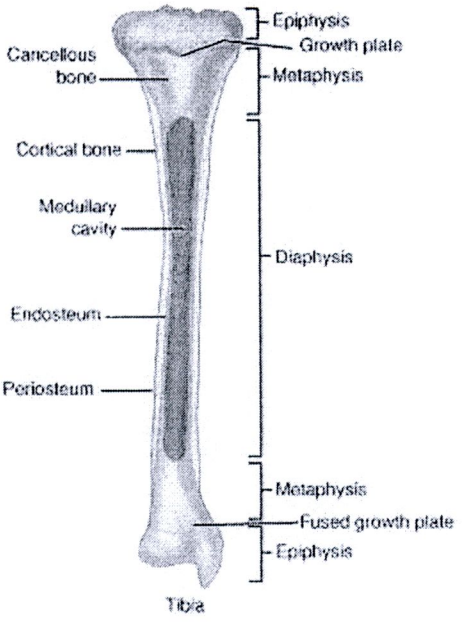

Figure 4 Diagram of a long bone

Source: http://bellespics.eu/keyword/long%20bone/

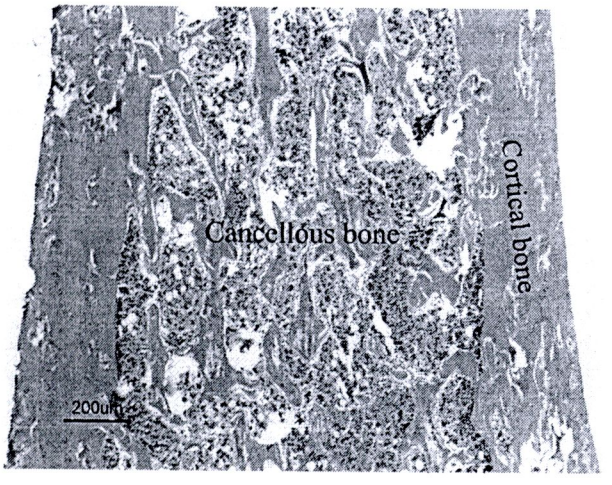

Figure 5 Histological section (stained with H&E) of the long bone at diaphysis in longitudinal plane of rat showing two types of bone tissue: compact and cancellous bone. Scale bars = 200 μm

Bone is a metabolically active tissue that undergoes constant remodeling throughout life by cells known as osteoblasts and osteoclasts. Under normal conditions, bone resorption and bone formation are coupled to maintain a stable bone mass. The osteoblast cells are located on the bone surfaces that produce alkaline phosphatase (ALP), type I collagen, osteocalcin, osteonectin, and N- and C-terminal propeptides of type I procollagen (P_1NP and P_1CP) etc. This process is known as bone formation by responsible for the synthesis and mineralization of bone matrices. The osteoclast is a type of bone cell that removes bone tissue by removing its mineralized matrix and breaking up the organic bone. This process is known as bone resorption. The osteoclast cells secrete the proteins which can be measured in urine or serum as markers such as hydroxyproline, Aminoterminal cross-linking telopeptide of bone collagen (Ostex), tartrate-resistance acid phosphatase (TRAP) and β-crosslap (β-CTx). Below is a summary of the process of bone breakdown and renewal.

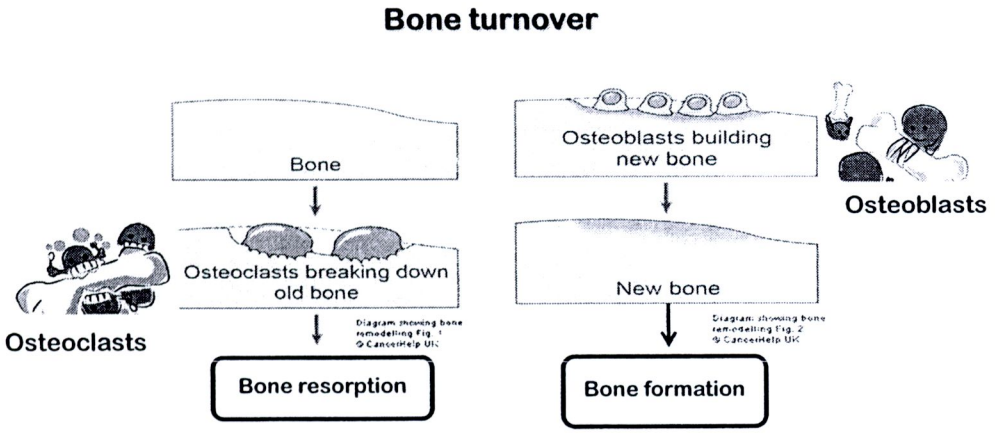

Figure 6 Turnover process

Source: http://cancerhelp.cancerresearchuk.org

The loss of bone has been attributed to an imbalance between bone formation and bone resorption, leading to osteoporosis. Osteoporosis is a disease of the skeleton characterized by bone fragility due to a reduction in bone mass and alteration in bone architecture.

Factors that regulate the rate of bone loss are heredity, dietary calcium intake, vitamin D levels, physical inactivity, low body weight, smoking, alcohol intake and hormones, inparticular sex steroids. Both sexes show initiation of bone loss between 35 and 40 years but in female, advancing age and menopausal decline of estrogen accelerates the loss of bone and increased the risk of osteoporosis (Riggs and Melton, 1986).

As a consequence of ovarian hormone deficiency, the rate of bone turnover increases and the imbalance between resorption and formation widens. Bone is progressively weakened with cancellous bone affected first by thinning and loss of trabeculae. The number of trabeculae, the trabecular thickness and the connectedness of the trabecular network decrease (Mohsen, 2006).

There are a variety of different techniques to diagnose osteoporosis by measuring a patient's bone mineral density (BMD). The methods to measure BMD include quantitative computer tomography (QCT) of the spine or proximal quantitative

computer tomography (pQCT) of the forearm, radiographic absorptiometry (RA) of the middle finger, peripheral Dual-energy X-ray absorptiometry (pDXA) of the forearm or heel, and ultrasound (US) of the heel or shinbone. The most standardized methods to diagnose osteoporosis by measuring a patient's BMD is called dual x-ray absorptiometry (DXA) of the hip and spine. The BMD test is a test that measures the density or thickness of the bones. It measures how much calcium and other types of minerals are present in a specific area of bone.

The World Health Organization (WHO) uses T-scores to define normal bone mass, low bone mass (or osteopenia), and osteoporosis. The T-scores compares the bone density to the average bone density of the healthy adults of the same gender. By using the diagram in Table 4 that can see how T-scores are used to define the status of the bone health.

Low bone mass is often called osteopenia. It is not a disease but a condition in which the bone density or bone thickness is lower than the average bone density of young healthy adults of the same gender. Low bone mass is diagnosed when the T-score is between -1 and -2.5.Osteoporosis is a disease that causes bones to become thin and weak often resulting in fractures. A bone density test can diagnose osteoporosis when the T-score is -2.5 or below.

Table 4 WHO classification for diagnosis of osteoporosis using BMD measurement

Osteoporosis	Low bone mass	Normal bone mass	High normal bone mass
-4.0 and -2.5	-2.5 and -1.0	-1.0 and +1.0	+1.0 and +4.0

Note: Using T-scores to define bone health. T-scores are based on statistical measurements called standard deviations (SD) that reflect the difference between one's bone density and normal bone density in the reference population.

Source: http://www.health.state.ny.us/diseases/conditions/osteoporosis/tests.htm

The development of new biochemical markers of bone metabolism has greatly enriched the array of analysis used in the assessment of skeletal pathologies. Bone marker measurements have the advantage that changes in bone marker concentrations can also be seen earlier than changes in BMD.

Bone is composed of approximately 70% mineral (mainly hydroxyapatite crystals) and 30% organic matter (mostly collagen and non-collagen proteins) which is synthesized by osteoblasts. Ninety percent of the organic extracellular matrix of bone consists of type I collagen. Of the non-collagenous proteins, osteocalcin, bone sialoprotein, and osteopontin are the most abundant. In addition, several enzymes (e.g. phosphatase, metalloproteinases) play an important role in skeletal metabolism.

Table 5 Markers of bone formation and resorption

Bone formation markers	Bone resorption markers
Serum total alkaline phosphatase (ALP)	Urine Calcium
Serum bone-specific alkaline phosphatase (SALP)	Urine hydroxyproline (Hyp)
Serum osteocalcin	Serum amino terminal cross-linking telopeptide of bone collagen (Ostex)
Serum osteonectin	Serum tartrate-resistance acid phosphatase (TRAP)
Serum N- and C-terminal propeptides of type I procollagen (P_1NP / P_1CP)	Serum β-crosslaps (β-CTx)

Source: www.brianet.com/bonemarker.pdf

Recent advances in characterization of the biochemical components constituting bone have enabled to detect disturbances of bone turnover by measuring

specific bone-derived molecules in body fluids such as serum or urine. For clinical purposes, these biochemical markers of bone turnover are usually classified into markers of bone formation or bone resorption. A summary of the currently available marker components is provided in Table 5.

Plants, medicinal herbs and herbal supplements of osteoporosis

Osteoporosis is more prevalent in women who have gone through menopause and have lower levels of bone protecting estrogen in the bodies. Treatment available like HRT protects against bone loss but associated with the risk of breast and endometrial cancers. Therefore, other natural estrogens have been investigated as an alternative treatment. The use of plants, medicinal herbs and herbal supplements to treat the symptoms of osteoporosis is becoming increasingly popular as an alternative treatment.

Many studies have evaluated the potential of plants, medicinal herbs and herbal supplements as osteoprotective agents such as, *Epimedium sagittatum*, *Tinospora cordifolia*, *Pueraria mirifica*, *Pueraria lobata,* Osteocare and blueberry.

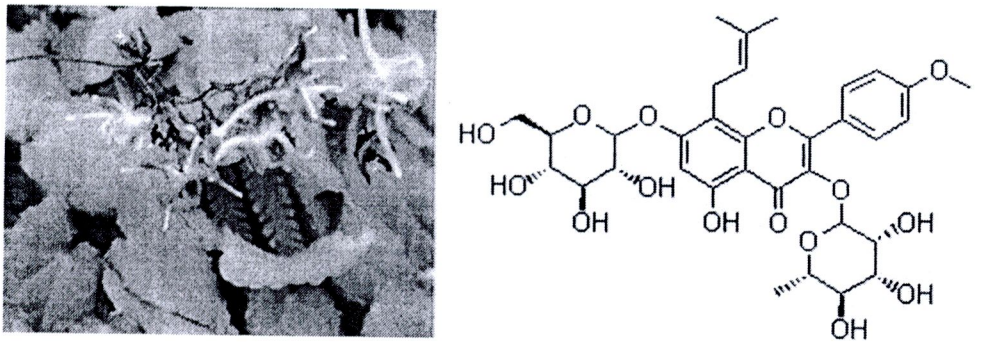

Figure 7 The characterictic of *Epimedium sagittatum* (left figure) and the chemical structure of icariin (right figure)

Source: http://www.shaman.co.nz/epimedium.html
http://commons.wikimedia.org/wiki/File:Icariin.svg

Epimedium sagittatum (Figure 7) is an important traditional Chinese herbal medicine used widely as a tonic, aphrodisiac, and antirheumatic. Pharmacological

studies have been showed that it has potential activity against osteoporosis (Li, et al., 1996; Ma, et al., 2002). Icariin ($C_{33}H_{40}O_{15}$; molecular weight: 676.67) (Figure 6) is a flavonoid isolated from *E. sagittatum*. It was reported that icariin stimulated osteoblast proliferation *in vitro* and that was the active chemical constituent stimulating osteoblasts (Li, et al., 1996; Ma, et al., 2002). Furthermore, icariin may significantly improve the bone density and is effective in preventing bone loss and estrogen deficiency for treatment of osteoporosis (Nian, et al., 2009).

Tinospora cordifolia has been used in ayurvedic preparations. The plant stem of *T. cordifolia* is the main constituent of herbal preparations that are being used in general debility, dyspepsia, fever, urinary disease, antispasmodic, anti-inflammatory , anti-arthritic, anti-allergic and anti-diabetic agent (Mathew and Kuttan, 1997). The organic molecules with diverse structures such as alkaloids, terpenoids (Figure 8), glycosides, sterols (Figure 8), lactones, and fatty acids have been reported from this herb (Ahmed, et al., 2006). *T. cordifolia* ethanolic stem extract in OVX rat model exhibited estrogen-like effects in bone and serum markers without causing cancer in reproductive organs (Kapur, et al., 2008).

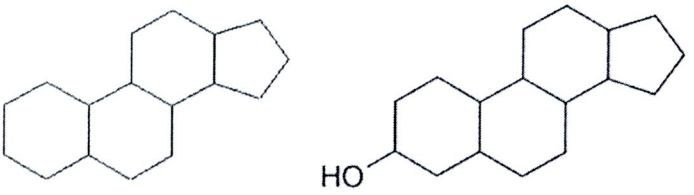

Figure 8 Terpenoid and sterol chemical structure

Source: http://www.mobot.org/mobot/research/apweb/top/glossaryq_z.html
http://en.wikipedia.org/wiki/Sterol

Pueraria mirifica (Kwao Khruea), an indigenous Thai medicinal plant is traditionally consumed for the treatment of menopausal symptoms. Its tuberous root was found to contain at least 13 known phytoestrogens. The majority of the phytoestrogens analyzed in *P. mirifica* are puerarin, daidzin (Figure 9), daidzein,

genistin (Figure 8), and genistein (Malaivijitnond, Kiatthaipipat and Cherdshewasart, 2004). *P. mirifica* has been thoroughly examined for its estrogenic activity on female reproductive organs. It induces a vaginal cornification and increased uterine weight in OVX rats (Malaivijitnond S, et al., 2006). The long-term administration of *P. mirifica* prolongs the menstrual cycle length and suppresses ovulation in adult female monkeys (Trisomboon, Malaivijitnond and Watanabe, 2004) and decreases serum luteinizing hormone and follicle stimulating hormone levels (Trisomboon, Malaivijitnond and Watanabe, 2005). The estrogenic activity of *P. mirifica* on bone loss was observed in male rats. *P. mirifica* significantly increases the bone density at the higher dose treatment (1000 mg/kg B.W./day) without affecting male reproductive organs (seminal vesicle, ventral prostate and accessory sex organs) (Urasopon, et al., 2007). Recently, the administration of the highest dose (1000 mg/kg B.W./day) of *P. mirifica* increases the weight of uterus, the undesirable side effects on the reproductive organs even it completely prevents the bone loss both in all bone types (Urasopon, et al., 2008).

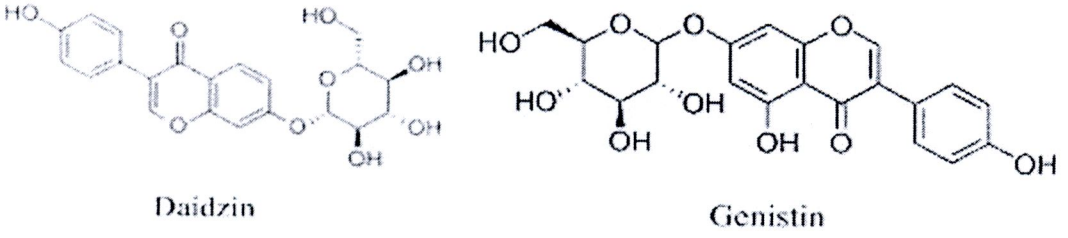

Figure 9 Daidzin and Genistin chemical structure

Source: http://www.chemicalbook.com/ChemicalProductProperty
http://www.pharmazie.uniduesseldorf.de/Institute/pharm_bio/

Pueraria lobata, an herbal tuberous plant is a candidate source for phytoestrogens. The plant tubers were analyzed for isoflavonoids including puerarin, daidzin, genistin, daidzein and genistein (Cherdshewasart, Sriwatcharakul and Malaivijitnond, 2008). One of the major phytoestrogens isolated from the root of *P. lobata* is puerarin ($C_{12}H_{20}C_9$) (Figure 10). It also has important uses on treatment of fever, liver and cardiovascular diseases (Wang, Zhao and Chai, 1994). Recently,

puerarin has been demonstrated to have effects on decreasing loss in bone density in OVX mice (Wang, et al., 2003).

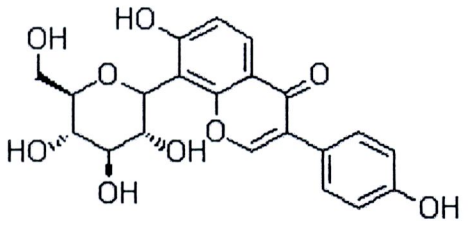

Figure 10 The chemical structure of puerarin

Source: http://www.21food.com/product/search_keys-Puerarin-p1.html

Osteocare (OST-6) is a herbomineral preparation comprising mainly *Termina liaarjuna* W&A, *Withania somnifera Dunal*, *Comniphora mukul* Hook Ex stock and *Praval bhasma* that are well known for their beneficial effects in rickets and osteoporosis. *P. bhasma* is a rich source of calcium obtained from oyster shell and due to appropriate ayurvedic processing has the advantage of easy absorption from the intestine. *T. arjuna* also contains significant amounts of calcium. At the same time *W. somnifera* is found to contain a number of phytosterols, alkaloids, 18 fatty acids, beta-sitosterol and polyphenols (Elsakka, 1990). Recently, the preventive effect of the treatment with herbal formulation OST-6 at a dose of 250 mg/kg B.W./day twice a day orally for a period of 16 weeks on the progress of bone loss in calcium deficient OVX rats significantly restored the femoral weight and density (Reddy, Lakshmana and Udapa, 2004).

Blueberry contains a group of phytochemicals that have been implicated as a mediator of cardiovascular protection. The phytochemicals presented in blueberry include phenolic acids and flavonoids (Sellappan, Akoh and Krewer, 2002). The composition and interaction of polyphenols presented in blueberry makes it an excellent source of stable free radical (Hakkinen, 1999). The bone protective role of blueberry in an OVX rat model explored that the blueberry is effective in preventing bone loss caused by ovarian hormone deficiency as seen by the increases in tibial and

femoral BMD and favorable changes in biomarkers of bone metabolism (Devareddy, et al., 2008).

Uterus

The uterus is a major female hormone-responsive reproductive sex organ of most mammals. In human, the uterus is a muscular organ and receives the right and left uterine tubes. The uterus can be devided into three parts: the fundus, the body and the cervix (Figure 11). In rat, the uterus is bicornuate with the uterine horns located on either side of the abdominal cavity. The horns extend to the lower pole of the kidneys. Blood is supplies to the ovaries, the uterus, and the cervix by the ovarian artery that run along the entire along length of the inner side of each uterine horn. The two ovaries are small, almost round and attached to the uterine horns via convulated oviduct (Figure 12).

The uterus is composed of three layers of wall; endometrium, myometrium and perimetrium (Figure 13). The perimetrium is the outer layer. At the light area of section, this is mostly "endometrium". The endometrium is the inner mucosal layer lining and is covered with columnar epithelium. The endometrium can be differentiated into two layers, a deep basal layer at the junction with the myometrium, and a superficial functional layer lining the lumen. It is the functional layer that is hormone responsive and undergoes the monthly cycle of proliferation, secretion, necrosis and shedding. The endometrium is sensitive to the fluctuating level of estrogen and progesterone secreted by the ovary, and the changes are known as the menstrual cycle. Surrounding the entire section is the location of the "myometrium". The myometrium is a very thick, muscular layer, consisting largely of bundles of smooth muscle fibers. Within the myometrim are prominent blood vessels, both arterial and venous, which undergo marked dilation and thickening of their walls during hormone fluctuation. With cessation of hormonal stimulation after the menopause, the myometrial cell atrophy and the uterus shrinks. The fibrocollagenous tissue between the muscle bundles, which is comparatively insignificant when the myometrial smooth muscle is prominent during sexual maturity, then becomes obvious (Stevens and Lowe, 2005, pp. 352-353). Several studies (Benita, 1984; Lin, et al., 1991) reported that the endometrium and myometrium thickens in response to

rising estrogen levels during the menstrual cycle and there was a study (William, et al., 1935) found that the endometrium and myometrium will become atrophic after the ovariectomy.

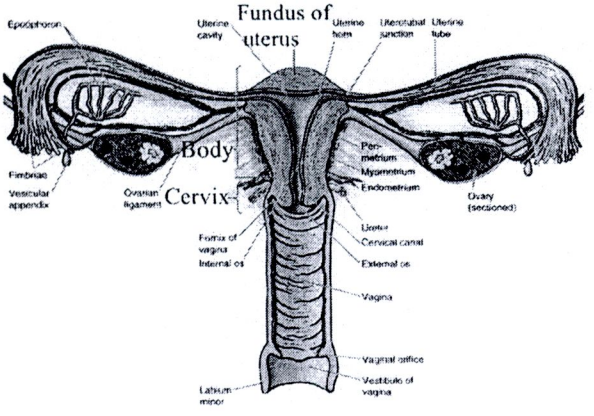

Figure 11 Anatomy of human uterus

Source: http://www.bcnlp.ac.th/Anatomy/page/apichat/reproductive/page/uterus.html

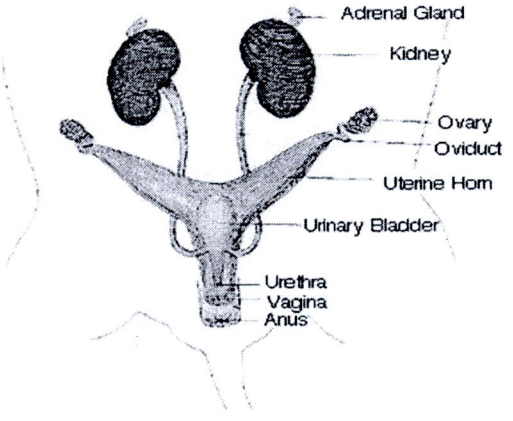

Figure 12 Anatomy of rat uterus

Source: http://www.biologycorner.com/worksheets/rat_urogenital.html

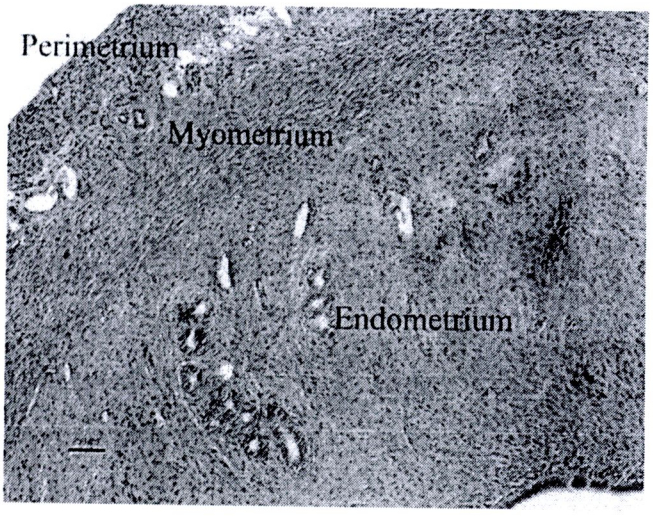

Figure 13 Histological section (stained with Hematoxylin & Eosin) of the uterus of rat in transverse plane. Scale bars = 50 μm.

Mammary gland

A mammary gland is an organ in mammals that produces milk to feed young offsprings. Mammary glands develop as downgrowths from the epidermis along a line (milk line or streak) which runs obliquely from the axilla toward the groin on each side. In humans normally only one breast develops on each side (Stevens and Lowe, 2005, p.388). The mammary glands of other mammals have more than two breasts, such as rats (Figure 14). In the rat, the mammary glands form extensive subcutaneous sheets of tissue that extend from the cervical to the inguinal regions as six ventrolateral pairs, each with its own nipple (Masso-Welch, et al., 2000). The mammary gland is composed of epithelium, adipose tissue and connective tissue stroma which are invested with blood vessels, nerves, smooth muscle fibers, lymph nodes and lymphatics. Mammary glands consist of ducts and lobuli. Each lobe of breast is a system of branching ducts that penetrate deep into the fibroadipose tissue of the breast. Each duct is surrounded by loose fibrocollagenous support tissue containing a rich capillary network (Figure 15). In the large ducts, these often form two layers whereas in the smaller ducts there will be a single layer of cells (Burkitt, et al., 1993, pp. 362).

Figure 14 Breeding female rats with an average of 12 mammary glands

Source: http://www.news.cornell.edu/chronicle/99/8.19.99/mole-rats.html

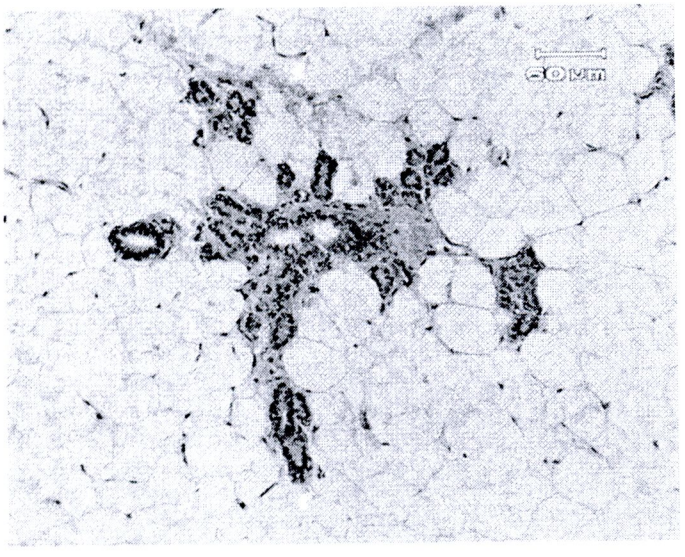

Figure 15 Histological features of H&E-stained paraffin section of the mammary glands of female Wistar rat showing the epithelium, duct, adipose tissue and connective tissue

Animal model of osteoporosis

Experimental animal models play an important role in improving the knowledge of the diagnosis as well as on preventive and therapeutic techniques regarding osteoporosis. Rodents, dogs, monkeys, and apes are the principal animals used model osteoporosis. The rat is the most frequently used laboratory animal for studying osteoporosis. Rats are chosen because they grow rapidly, have a well characterized skeleton, are widely available, and have proven to be excellent models for several of the most common risk factors for osteoporosis. It is considered to be the procedure that gives reliable model of osteoporosis (Russell, et al., 2001).

There are various methods of obtaining a standardized pattern of osteoporosis, for example, low calcium diet, alcohol abuse, parathyroidectomy, nerve resection, tendon resection or ovariectomy. The OVX rat model is most commonly used in research on postmenopausal osteoporosis. Ovariectomy of mature rats leads to a condition similar to menopause, in that surgery leads to cancellous and endocortical bone loss by increasing the overall rate of bone remodeling and by altering the balance between bone formation and bone resorption, such that resorption predominates at selected skeletal sites. Losses of endocortical as well as cancellous bone are the primary causes of bone loss (Lelovas, et al., 2008). Ovariectomized was followed with bone loss at several skeletal sites rich in trabecular bone, such as vertebral bodies, the proximal femur, and the metaphyses of long bones such as distal femur and proximal tibia (Venkatesan, et al., 2005).