CHAPTER 1

INTRODUCTION AND OBJECTIVES

The Southeast Asian tropical rain forests, also known as Indo-Malayan or Malesian rain forests, are one of the most extensive and species-rich terrestrial habitats of the world (Richards, 1952; Whitmore, 1984). The flora of Malesia is estimated to support 25,000 species of flowering plants (van Steenis, 1971) which is about ten percent of the world's flora, with the Southeast Asian tropical rain forests estimated to comprise 6,500 medicinal plant species (Samy *et al*., 2005).

In Malaysia, 19.12 million hectares of rain forest area occupying some 58.1 percent of the land area in this country is estimated to have 15,000 plant species (Saw, 2010), with ten percent of them reported to have medicinal value (Samy *et al*., 2005). This suggests that, Malaysia is exceedingly rich in the natural resources used in traditional medicine in Southeast Asia.

Polygonaceae, the buckwheat or knotweed family (Henderson, 1959) is a cosmopolitan family of perennial herbs, some shrubs, small trees, climbers or twining lianas characterized by alternate simple leaves with sheathing ocrea stipules, unilocular ovary and endospermic seeds (Brandbyge, 1993; Brummitt, 1992; Heywood *et al*., 2007; Hutchinson & Dalziel, 1954). This family generally includes 43 (Brandbyge, 1993) to 55 (Qaiser, 2001) genera, embracing about 1200 species, and occupying a wide range of habitats (Brandbyge, 1993; Brummitt, 1992; Heywood *et al.*, 2007; Mabberley, 2008). Most genera are restricted to the temperate Northern regions while the remaining are tropical or subtropical (Heywood *et al*., 2007; Mabberley, 2008).

The genus *Persicaria* (L.) Mill. was first established in 1754 by Miller without typification but later lectotypified by Britton and Brown (1913) based on *Polygonum persicaria* L. (Kantachot *et al*., 2010). This genus currently consists of about 150 species worldwide. *Persicaria* is a cosmopolitan genus found mainly in temperate regions but some species occur in the tropical and subtropical regions from lowland to higher montane elevations (Heywood *et al.*, 2007; Mabberley, 2008). This taxon commonly perennials, with some of the species being edible and widely cultivated, for example *Persicaria minor* (Huds) Opiz (syn = *Polygonum minus* Huds) or usually known as *daun kesum* in the Malay language.

The exact circumscription and infrageneric classification of the *Persicaria* and *Polygonum* L. are still open to debate (Heywood *et al.*, 2007; Ronse Decraene & Akeroyd, 1988; Steward, 1930; Wilson, 1990). Some authors categorize *Persicaria* as merely a section of *Polygonum sensu lato* (Kuo *et al.*, 1996; Li *et al.*, 2003; Ridley, 1924; Steward, 1930). However, many authors have proposed recognizing *Persicaria* as a separate genus based on the palynological and anatomical data (Haraldson, 1978; Hedberg, 1946), floral character and fruit morphology (Ronse Decraene & Akeroyd, 1988; Ronse Decraene *et al*., 2000, 2004) and phylogenetic analyses (Frye & Kron, 2003; Kantachot *et al*., 2010; Kim & Donoghue, 2008). Freeman and Reveal (2005), Hara (1982), Qaiser (2001) and Wilson (1990) have separated *Persicaria* as a distinct genus in their revision of the family Polygonaceae. *Persicaria chinensis* (L.) H. Gross var. *chinensis* is classified into the tribe *Persicarieae* under the subfamily Polygonoideae, which comprises three genera that are native to the temperate regions of Eurasia. They are commonly perennial and annual herbaceous species often with succulent stems, two whorls of five tepals and not usually winged or keeled (Heywood *et al.*, 2007).

The family Polygonaceae has not been revised in Peninsular Malaysia until Ridley (1924), who reported more than 600 species worldwide and recognized the genus *Polygonum* with nine species in the Malay Peninsula while Turner (1995) reported 14 species in this family and documented the genus *Persicaria* with 11 species in peninsular Malaysia and Singapore. In Thailand, Kantachot *et al.* (2010) has reported 21 species of *Persicaria*, and three varieties from the species *P. chinensis* have been recorded. In Malaysia, two varieties have been identified based on the differences in the leaf shape and size, namely *P. chinensis* var. *chinensis* and *P. chinensis* (L.) H. Gross var. *ovalifolia* (Meisn.) H. Hara (syn *Polygonum malaicum* Danser & *Polygonum auriculatum* Meisn.) (Turner, 1995). The *ovalifolia* variety is found growing side by side with the *chinensis* variety in the wild population.

The medicinal plants from the family Polygonaceae, namely *Persicaria chinensis* (L.) H.Gross var. *chinensis* (syn *Polygonum chinense* L.), has been chosen as the target plant as this plant is being used as folk medicine in the Malaysian Chinese community (Henderson, 1959). *P. chinensis* var. *chinensis*, a perennial or scrambling herb, is native to tropical and subtropical eastern Asia (Wagner *et al.*, 1990). Ridley (1924) reports that this species is commonly found in hilly areas at around 1300 m in Perak, Pahang and Kedah. *P. chinensis* var. *chinensis* is found growing abundantly in the wet valleys and on grassy slopes in China from sea level up to altitude of 2400 m (Li *et al.*, 2003) and widely distributed in Taiwan, Japan, Philippines, India, Sri Lanka, Himalaya mountain region and Southeast Asian countries (Li *et al.*, 2003; Qaiser, 2001). This plant has capitate inflorescence, glabrous ocrea and persistent perianth present even in the fleshy fruit. The leaf shape varies from ovate to elliptic or lanceolate. The morphology of *P. chinensis* var. *chinensis* is highly variable, especially the leaf shape and size. This species has four varieties (Kanthachot *et al*., 2007, 2010) and they differ in the leaf shape, size, texture and stem indumentums.

In Malaysia, the wild variety of *P. chinensis* var. *chinensis* is commonly found in the montane forests up to 1200 m in the Main Range in Perak and Pahang, such as in Cameron Highlands and Gunung Ulu Kali. This plant is also found cultivated in the lowlands especially among the Chinese community as it is one of the main ingredients used to treat lung ailments in traditional medicine. However, the wild variety of this plant is rarely used in traditional medicine because the morphology of the wild variety, especially the growth habit, leaf shape and size are generally different from the cultivated variety. According to Turner (1995), the wild variety of this scrambling herb is normally found in damp waste ground in lowlands and mountains and is a native species in Malaya (Henderson, 1959). The wild variety can be propagated by seeds and cuttings but the cultivated variety hardly produce any mature fruits and it is commonly propagated by cuttings.

Persicaria chinensis var. *chinensis,* also known as Mountain knotweed, Chinese knotweed, or hill buckwheat is used medicinally in China where it is known as *huo tan mu, ch'ih ti li,* and *shan ch'iao mai* (Chinese) (Wiart, 2006). In China, the whole plant is used traditionally to clear heat and eliminate toxins, to treat dysentery, inflammatory skin disease, eczema and corneal nebula (Wan *et al*., 2009). The roots of *Polygonum chinense* are used to treat fluxes, to remove intestinal worms, and to counteract scorpion poisoning (Wiart, 2006). This plant is found to contain 25-R-spirost-4-ene-3,12-dione, stigmast-4-ene-3,6-ione, stigmastane-3,6-dione, hecogenin, and aurantiamide, which are anti-inflammatory and anti-allergic (Tsai *et al*., 1998; Wang *et al*., 2005; Xie *et al*., 2007). In Taiwan, *Polygonum chinense* is used in its entirety as a folk medicine to treat many infectious diseases (Jiangsu, 1986). Recently reported by Galloway & Lepper (2010), *Persicaria chinensis* has been introduced to New Zealand, possibly as a Chinese medicine ingredient but this plant has been found growing rampantly along the boundary of several properties in Auckland and its invasiveness has been investigated.

The pharmacological property of this herb is still unexplored (Wiart, 2006). Hence, this project is undertaken to evaluate the cytotoxic activity of the crude methanol, dichloromethane and water extracts of the leaves, stems and whole plant of both the cultivated and wild variety of *P. chinensis* var. *chinensis* by an *in vitro* growth inhibition assay system against selected cancer cell lines of CaSki, SKOV3, HT29, A549, MCF-7 and normal human lung fibroblasts cell MRC5.

As knowledge on the reproductive biology of both the cultivated and wild varieties is extremely lacking, this project also aims to elucidate and enhance the basic knowledge pertaining to the reproductive biology of this variety including its phenology, embryology, pollen morphology, pollination and fruit formation. This information which is generally lacking and incomplete, particularly in Malaysia, will therefore help in establishing the breeding system of this medicinal plant.

CHAPTER 2

LITERATURE REVIEW

2.1 The family Polygonaceae, genus *Persicaria*

2.1.1 Taxonomic history

The Polygonaceae is a large cosmopolitan family consisting mostly of herbs, some shrubs, trees (Tribe Triplareae including *Triplaris* Loefl., *Ruprechtia* C.A.Mey), or clambering, climbing (*Coccoloba* L., *Brunnichia* Banks ex Gaertn.), twining lianas (tribe Coccolobeae including *Muehlenbeckia* Meisn., *Fallopia* Adans.) (Brandbyge, 1993; Freeman & Reveal, 2005; Heywood *et al.*, 2007; Mabberley, 2008; Qaiser, 2001). Members of the Polygonaceae are greatly varied, of diverse morphological clades and has been described to range from 43 (Brandbyge, 1993; Kuo *et al*., 1996) to 55 genera (Heywood *et al.*, 2007; Qaiser, 2001), and comprises 1100 species (Brandbyge, 1993; Kuo *et al.*, 1996; Li *et al.*, 2003) to 1200 species (Heywood *et al.*, 2007; Mabberley, 2008; Qaiser, 2001).

Since this family was recognized in 1789 by Jussieu, the taxonomy of Polygonaceae has been continuously revised, depending on the morphological characters chosen to describe the subgroups with respect to the circumscription of subfamilies, tribes and genera (Brandbyge, 1993; Freeman & Reveal, 2005; Hara, 1982; Haraldson, 1978; Kim & Donoghue, 2008; Kuo *et al.*, 1996; Li *et al.*, 2003; Qaiser, 2001; Ronse Decraene & Akeroyd, 1988; Sanchez & Kron, 2008; Sanchez *et al*., 2011; Van Leeuwen *et al*., 1988; Wodehouse, 1931; Wood & Graham, 1965).

The family Polygonaceae has been classified into two subfamilies, Eriogonoideae and Polygonoideae (Brandbyge, 1993; Freeman & Reveal, 2005; Haraldson, 1978; Heywood *et al.*, 2007; Mabberley, 2008). These subfamilies can be readily distinguished because members of the subfamily Eriogonoideae are often woody, the leaves are whorled or opposite and the nodes are without ocrea; the inflorescences are sympodially branched with cymose and trimerous flowers (Brandbyge, 1993; Heywood *et al.*, 2007; Mabberley, 2008). Reveal (1989) divided the subfamily Eriogonoideae into two tribes, i.e. Eriogoneae and Pterostegieae, which consist of 15 genera and 2 genera respectively. On the other hand, members of the subfamily Polygonoideae have alternate leaves with sheathing ocrea stipules; they grow monopodially and the inflorescences are racemose with cymose branches. The subfamily Polygonoideae has five tribes, i.e. Triplareae, Coccolobeae, Rumiceae, Polygoneae and Persicarieae (Brandbyge, 1993; Heywood *et al.*, 2007; Mabberley, 2008). Between these two subfamilies, Eriogonoideae is rather well delimited as justified by some studies (Ronse Decraene & Akeroyd, 1988; Ronse Decraene *et al*., 2000, 2004; Ronse Decraene & Smets, 1991). The delimitation of the tribes Polygoneae and Persicarieae also seems to be justified but some of the genera still need to be further investigated (Brandbyge, 1993).

Since Linnaeus established the genus *Polygonum* at 1754, it has posed a great challenge to taxonomists who have tried to subdivide the genus into more natural units at generic rank, subgeneric and sectional. As it is, there is still a large number of controversies that need to be resolved before a convincing conclusion could be reached (Ronse Decraene & Akeroyd, 1988). Furthermore, individual authors are using different treatments and characters in different orders of importance resulting in the overlapping of numerous synonyms (Freeman & Reveal, 2005; Li *et al.*, 2003; Qaiser, 2001). In 1930, Steward had opined that, among the genera in Polygonaceae, the genus *Polygonum* was of outstanding difficulty and was one of the most troublesome groups to the systematic botanist. Wilson (1990) also states that the genus *Persicaria* is a segregate from *Polygonum sensu lato* and the debate continues as to its exact circumscription and infrageneric divisions. Therefore, the classification of

Polygonaceae has always been a point of disagreement among botanists, particularly in the genus *Polygonum*. *Polygonum* has been divided into as many as nine sections or 15 genera (Haraldson, 1978; Hedberg, 1946; Ronse Decraene & Akeroyd, 1988; Wood & Graham, 1965).

Steward (1930) has divided the genus *Polygonum* into eight sections: *Avicularia*, *Bistorta*, *Persicaria*, *Cephalophilon*, *Echinocaulon*, *Tiniaria*, *Aconogonon* and *Fagopyrum*. The species *Polygonum chinense* has been placed under section *Cephalophilon*. The genera *Polygonum sensu lato* have a number of different pollen types prompting Hedberg (1946) to propose the segregation of some sections into a number of genera and the genus *Persicaria* comprises the sections *Cephalophilon*, *Persicaria*, *Amblygonon*, *Tovara,* and *Echinocaulon*. Haraldson (1978) believes that the genus *Persicaria* is highly advanced in tribe Persicarieae and has recognized the genus *Persicaria* with four sections: *Persicaria*, *Cephalophilon*, *Echinocaulon*, and *Tovara*. The above conclusions are based mainly on her studies of the trichome structure, pollen morphology as described by Hedberg (1946), pollination behaviour and floral morphology. *Persicaria chinensis* is placed under the section *Cephalophilon*. Since then, Ronse Decraene and Akeroyd (1988) have also disagreed with uniting *Persicaria* with *Polygonum sensu stricto* and suggest maintaining *Persicaria* as a distinct genus (same as Haraldson, 1978) based on the significant floral characters (the morphology of the tepals, the structure of the nectaries and the morphology of the filaments) and classified genus *Persicaria* with four sections: *Persicaria*, *Bistorta*, *Tovara* and *Aconogonon*. The genus *Persicaria* section *Persicaria* (Ronse Decraene & Akeroyd, 1988) including section *Cephalophilon*, section *Echinocaulon* and section *Amblygonon* and *Persicaria chinensis* have been recognized in this section. Most recently, phylogenetic work has proposed recognizing *Persicaria* as a separate genus derived from the tribe Persicarieae and subfamily Polygonoideae (Frye & Kron, 2003; Kim &

Donoghue, 2008; Sanchez *et al.*, 2009, 2011) and *Persicaria chinensis* has been categorized in the section *Cephalophilon*.

However, some authors (Kuo *et al.*, 1996; Li *et al.*, 2003) still include *Persicaria* as one of the sections in the genus *Polygonum*. In the Flora of China, Li *et al.* (2003) have recognized the genus *Polygonum* with six sections: *Polygonum*, *Persicaria*, *Bistorta*, *Cephalophilon*, *Aconogonon*, and *Echinocaulon*. *Polygonum* section *Polygonum* represents *Polygonum* in the narrow sense (*Polygonum sensu stricto*) and the remaining five sections of *Polygonum* in the broad sense (*Polygonum sensu lato*) with wider circumscription (Li *et al.*, 2003). *Polygonum chinense* has been included in the section *Cephalophilon* and four varieties have been recorded.

This study adopts the classification of the most recent findings based on the phylogenetic works done by several researchers as mentioned above and earlier proposed classifications (mostly by Haraldson, 1978) and some ideas of Ronse Decraene and Akeroyd (1988). Hence, *Persicaria chinensis* has been adopted as the name of this species.

2.1.2 Distribution and ecology

This family has a worldwide distribution and is adapted to a wide range of different habitats ranging from tropical to arctic regions. The subfamily Polygonoideae is mainly found in the temperate regions of North America (Freeman & Reveal, 2005) and the tribe Persicarieae is north circumpolar with an occasional representation in the southern hemisphere. The genus *Persicaria* with about 100–150 (Freeman & Reveal, 2005; Qaiser, 2001) species, are widely distributed in both hemispheres but are mainly found in the north temperate regions (Qaiser, 2001). The genus *Polygonum* presents 4 species native to Peninsular Malaysia and Singapore (Henderson, 1959) and later Turner (1995) reports 11 species for *Persicaria* in Peninsular Malaysia and Singapore.

Persicaria chinensis var. *chinensis* is distributed in east Asian countries such as India, Bhutan, Myanmar, Japan, Philippines, Peninsular Malaysia, Singapore, Thailand, Vietnam, Sri Lanka and is introduced into Pakistan. This variety also grows in thickets or shaded places throughout Taiwan and is found abundantly in wet valleys or grassy slopes in China (Kuo *et al.*, 1996; Li *et al.*, 2003; Qaiser, 2001). In Peninsular Malaysia, this species is found growing naturally in Bukit Fraser, Pahang; Gunung Berimbun near Telom, Perak; Tapah, Perak and Gunung Kerbau, Kedah (Ridley, 1924). Turner (1995) describes *Persicaria chinensis* var *chinensis* as a widespread scrambling herb found at damp waste sites in lowlands and mountains.

2.1.3 Embryology and anatomy

Embryological information of the family Polygonaceae is very scanty and restricted only to a few species while the systematic position of the family is controversial (Maheswari Devi & Manorama, 1985). A few studies have been attempted to discuss the systematic position of the family using embryological evidences (Maheswari Devi & Manorama, 1984, 1985).

In this family, the embryological studies are characterized by the presence of 3-celled pollen grains; orthotropus, bitegmic and crassinucellate ovule on basal placentation with nucellar beak; periclinal divisions of the nucellar epidermis, hypotase; nuclear type of endosperm development with a small endosperm pouch and Asterad type of embryogeny (Agoram & Krishnamurthy, 1980; Doida, 1960; Dudgeon, 1918; Fink, 1899; Hofmeister, 1849; Lonay, 1922; Maheswari Devi & Manorama, 1984, 1985; Mahony, 1935, 1936; Mukherjee, 1972; Neubauer, 1971; Pausheva, 1977; Periasamy, 1964; Rao & Mukherjee, 1973; Rao, 1936; Soueges, 1919a, 1919b, 1920a, 1920b; Stevens, 1912; Strasburger, 1879; Woodcock, 1914) .

Maheswari Devi and Manorama (1984, 1985) reported that the anther wall development of *Polygonum chinense*, *P. alatum* Dulac, *P. capitatum* Buch.-Ham. *ex* D. Don, *P. molle* Wight, *P. flaccidum* Roxb. and *P. strigosum* R.Br. follow the Monocotyledonous type, with secretory tapetum. The dicotyledonous type of anther wall development has been observed in *Antigonon leptopus* Hook. & Arn. (Agoram and Krishnamurty, 1980), a herbaceous and perennial vine climbing by tendrils. Simultaneous cytokinesis during microsporogenesis results in tetrahedral tetrads in *Polygonum chinense*, *P. alatum*, *P. capitatum*, *P. molle*, *P. strigosum* and both tetrahedral and decussate tetrads in *P. flaccidum* (Maheswari Devi & Manorama, 1984, 1985). In *P. strigosum*, different sizes of tetrads and mature pollen grains are observed even within the same anther locule. Pollen polymorphism is quite common in this species (Maheswari Devi & Manorama, 1984).

The development of the female gametophyte is of the *Polygonum* type (Johri *et al.*, 1992). The first clear account of the *Polygonum* type of embryo sac development was reported in *Polygonum divaricatum* L. (Strasburger, 1879). In addition, fertilization is porogamous, and the pollen tube is persistent and unbranched. The synergids remain intact during the entry of the pollen tube (Mahony, 1935). Wall formation is initiated at the micropylar end of the globular proembryo in *Polygonum pensylvanicum* L. (Neubauer, 1971). A meristematic layer differentiates at the periphery in the cellular endosperm. The cell in the central region becomes multinucleate. After 10 days of its formation, the meristematic layer develops into an aleurone layer. The nucellus persists as a thin covering. The chalazal region of the endosperm remains free-nuclear in *Fagopyrum esculentum* Moench (Davis, 1966) and this aspect requires further study (Johri *et al.*, 1992).

The fruit anatomy of the Polygonaceae is relatively simple and basically similar (Brandbyge, 1993; Dammer, 1893; Graham & Wood, 1965) where two or three carpels enclose an orthotropous ovule with three dorsal traces running into the styles which sometimes are interrupted by a non-lignified zone at the level of the hypotase. The ovule will develop into a seed with the carpel wall differentiating into the pericarp, a mostly heavily sclerified exocarp or epidermis with several layers of parenchymatous mesocarp cells, and an endocarp. The seed develops an outer layer of rectangular cells (exotesta) surrounding a mealy or horny endosperm and embryo which the embryo is mostly straight with incumbent cotyledons and is positioned eccentrically in one corner of the seed (Ronse Decraene *et al.,* 2000). However, seed and fruit development are little known in Polygonaceae (Corner, 1976) expect for detailed studies on *Persicaria pennsylvanica* (L.) M.Gómez (Neubauer, 1971) and *Polygonum aviculare* L. (Lonay, 1922).

Heterostyly is almost always associated with reciprocal anther stigma positions. Although it had been discovered earlier, heterostyly achieved prominence only after (Darwin, 1864, 1865, 1892) described its functional significance as a mechanism facilitating the transfer of cross-fertilizing pollen between anthers and stigmas of the same height. This floral polymorphism is usually accompanied firstly, by a sporophytically controlled, diallelic self-incompatibility system that prevents self, intramorph fertilizations, and secondly by several other supporting floral features, particularly stigmas and pollen floral morphs (Barrett, 1990; Ganders, 1979).

Heterostyly occurs in some 24 families and over 124 genera of flowering plants (Ganders, 1979). In Polygonaceae, heterostylous flowers were first described in *Fagopyrum esculentum* over a century ago (Darwin, 1892). This species was known as one of the best studied heterostylous species from a genetic view point (Morris, 1951; Samborska-Ciania *et al*., 1989). Subsequently, heterostyly was also reported for the genus, *Oxygonum* Burch. (Graham, 1957; Hong, 1999), *Polygonum* (Reddy *et al*., 1977;

Chen & Zhang, 2010), *Aconogonon* (Meisn.) Rchb. (Hong, 1991), and *Persicaria* (Nishihiro & Washitani, 1998a).

2.1.4 Pollen studies

The acetolysis method introduced by Erdtman (1954) half a century ago is still a very popular and highly successful technique in palynology (Hesse & Waha, 1989). It is widely considered as the common basis for the comparison of pollen grains (Coetzee & Van der Schijff, 1979). Acetolysis is very useful technique for pollen grains with thick and stable exines; resulting in very clean pollen surfaces and thus gives excellent topographic information. However this method has numerous disadvantages (Hesse & Waha, 1989). The acetolysis procedure is known to cause collapse, damage, shrinking, contortion, twisting or warping (Coetzee & Van der Schijff, 1979; Halbritter, 1998) to the pollen grains with thin and/or fragile exines. It also often makes bilaterally symmetrical grains appear radially symmetrical and may result in incorrect measurements being taken for the polar and equatorial axis. Consequently, pollen grains might be classified in wrong symmetry and size classes (Smith & Tiedt, 1991).

Modern palynology with its many morphological and functional applications must consider all pollen characteristics, especially sporoderm characters and non-sporopollenin features (Halbritter, 1998). However, the non-sporopollenin parts of the pollen cannot be observed in acetolysis samples because the mixture of sulfuric acid and anhydrous acetic acid destroys all non-sporopollenin substances in the acetolysis process (Hesse & Waha, 1989). This problem can be overcome by using the scanning electron microscope and transmission electron microscope technique (Halbritter, 1998; Heslop-Harrison *et al*., 1986; Rowley & Skvarla, 1987).

Polygonaceae is considered a eurypalynous (multipalynous) family by various workers (Hedberg, 1946; Nowicke & Skvarla, 1977; Wodehouse, 1931). Pollen morphology characters of *Polygonum* section *Persicaria* were previously studied by several researchers (Hedberg, 1946; Wang & Feng, 1994; Zhang & Zhou, 1998; Zhou *et al*., 1999). However, the *Persicaria* as a separated genus was studied by Wodehouse (1931), Hong and Hedberg (1990) and Yasmin *et al*. (2010).

An extensive study on the pollen morphology of the Polygonaceae was published by Wodehouse (1931). He described several species from a range of North American genera with an explanation of "developmental tendencies" and also provided a key to the species. In his studies, four species from the genus *Polygonum* have been identified, including *Polygonum chinense*. However, most of the species, described, do not occur in the tropical and subtropical regions of eastern Asia. Hedberg (1946), in his classic paper on the pollen morphology of the genus *Polygonum*, described ten main pollen types and a number of aberrant types. He proposed a new taxonomic classification based on pollen morphology and constructed a key to the pollen type. Yasmin *et al.* (2010) summarized the pollen morphological characters of the genus *Persicaria* into two groups; tricolpate pollen (Capitata type pollen) and pantaporate pollen (Persicaria type pollen). Persicaria type pollen were observed in most of the *Persicaria* species while the other 5 species of *Persicaria*, i.e. *P. capitata* (Buch.-Ham. *ex* D.Don) H. Gross, *P. nepalensis* (Meisn.) H. Gross, *P. chinensis*, *P. posumbu* (Buch.-Ham. *ex* D.Don) H. Gross and *P. tenella* (Blume) H. Hara, fall into the Capitata type (comparable to Cephalophilon type suggested by Wang & Feng, 1994; Zhang & Zhou, 1998).

2.1.5 Pollination biology

Corlett (2004) provided an overview of flower visitors and pollination in the entire Oriental (or Indo-Malayan) Region. Burkill (1919) observed the pollination of some flowers in the Singapore Botanic Gardens and other parts of the Malay Peninsula. The ecology of insect pollination of some Malaysian dipterocarps and understorey trees further adds valuable information to the pollination ecology in tropical forests (Appanah, 1981, 1985, 1990; Appanah & Chan, 1981; Bawa, 1990; Chan & Appanah, 1980).

The pollination studies of the *Persicaria* in Malaysia have not been investigated so far and foreign researchers have studied some other species including *Polygonum thunbergii* Siebold & Zucc. (Momose & Inoue, 1993), *Antigonon leptopus* (Raju *et al*., 2001), and *Persicaria japonica* (Meisn.) Nakai (Nishihiro & Washitani, 1998b).

2.2 *In vitro* cytotoxic activity of the family Polygonaceae against human cancer cell lines

Nature has been a source of medical treatment as our ancestors had used plants as a source of medicine thousands of years ago. Currently, plant based systems are providing many effective anticancer agents and will continue to play an essential role in the primary health care of 80% of the world's population (Das & Yadav, 1998; Nakanishi, 1999). As summarized recently, a total of 187 plant species belonging to 102 genera and 61 families have been identified as an active or promising source of phytochemicals with antitumor properties, corresponding to a 41% increase during the last five years. Among them, only 15 species belonging to ten genera and nine families have been utilized in cancer chemotherapy at the clinical level, whereas the rest of the identified species are either active against cancer cell lines or exhibit chemotherapeutic properties in tumor-bearing animals under experimental conditions (Kintzios, 2006).

Plant metabolites with antitumor and anticancer properties are primarily cytotoxic; probably due to their evolution-driven development as natural pesticides for the self-defence of plant organisms (Kintzios, 2006). However, some plant metabolites exert cytotoxic effects in a less direct way. For example, flavanoids can inhibit cancer cell proliferation by modulating the activity of cyclin-dependent kinases (Chang *et al*., 2004; Dai & Grant, 2003), but also demonstrate a cytotoxic estrogen-like activity in high concentrations (Nair *et al.,* 2004; Oh & Chung, 2004; Woo *et al*., 2005). In other words, some plant metabolites can act as chemotherapeutic agents due to their growth-regulatory properties (Kintzios, 2006).

Preliminary *in vitro* test is an initial phytochemical screening for the discovery of novel anticancer drugs. The first step is the selection of starting materials, mainly based on ethnobotanical information and followed by the second step which is the identification of the biological activity (in the case of cancer chemotherapy and certainly includes selective cytotoxicity tests) of the extracts derived from the plant materials (Kintzios, 2006). A cytotoxicity test determines whether a product or compound will have any toxic effect on living cells, and is also generally used as a screening tool for raw materials or component products before they are put into the design of a medical device.

Subsequently, extracts are prefractionated by means of chromatography and these fractions are then screened for biological activity *in vitro* (Constant & Beecher, 1995). Thus, preclinical tests usually evaluate the cytotoxicity of a candidate antitumor agent *in vitro*, that is, on cells cultured on a specific nutrient medium under controlled conditions (Kintzios, 2006).

The *Polygonum* genus is well known for producing a variety of secondary metabolites including flavonoids (López *et al*., 2006; Peng *et al.*, 2003; Yagi *et al.*, 1994), triterpenoids (Duwiejua *et al*., 1999), anthraquinones (Beerling *et al*., 1994; Matsuda *et al*., 2001; Yim *et al*., 1998), coumarins (Sun & Sneden, 1999), phenylpropanoids (Murai *et al.*, 2001; Takasaki *et al*., 2001), lignans (Kim *et al*., 1994), stilbenoids (Nonaka *et al*., 1982), tannins (Wang *et al*., 2005), and drimane-type sesquiterpene dialdehyde polygodial (Alves *et al*., 2001; Asakawa & Aratani, 1976; Derita *et al*., 2008; Fukuyama *et al*., 1980; Hagendoorn *et al.*, 1994).

Five chemical constituents have been derived from *Polygonum chinense* from Taiwan (Tsai *et al*., 1998) included one new compound, 25R-spirost-4-ene-3,12-dione, and four known compounds, i.e. stigmast-4-ene-3,6-dione, stigmastane-3,6-dione, hecogenin and aurantiamide acetate. Tsai *et al*. (1998) also recorded that these five compounds exhibited anti-inflammatory and anti-allergic activities. In addtition, twelve chemical constituents have been reported from *Polygonum chinense* from China. They were identified as syringic acid, apigenin, 3,3'-di-O-methylellagic acid, gallic acid, protocatechuic acid, isorhamnetin, caffeic acid, quercetin, luteolin, gallicin, avicularin (quercetin-3-O-α-L-arabinofuranoside) and 3,4,8,9,10-pentahydroxy-dibenzo [b,d] pyran-6-one. All these compounds except gallic acid and quercetin, were reported for the first time in this species (Xie *et al*., 2007).