CHAPTER 1

Introduction

1.1 Statement and significance of the problem

Herbs have been used in ethnomedicines for the remedy of many diseases, along with cold conditions, ache, and inflammation for thousands of years. Nowadays, these herbal medicines are still utilised worldwide as complementary or alternative medical ingredients with the hope of advocating hygiene and manipulating chronic or severe diseases such as cancer and HIV infection. According to a previous report, fourteen to sixteen percent of the American adults often use herbal supplements together with conventional medicines (Li et al., 2014). Since the efficacy and safety of conventional prescription drugs could be changed by co-administration with herbal medicines, there is a significant need to screen the potential herb-drug interactions. Even though the basis of mechanisms for most proclaimed herb-drug interactions have not been completely documented, the pharmacokinetic and pharmacodynamic mechanisms are accredited to be involved. The potentiality of diverse functional groups of chemicals that can cause interaction with different protein targets and alter the physiological surroundings leading to partly elucidate pharmacodynamic interactions, whilst pharmacokinetic interactions are generally caused by alterations the pharmacokinetic profiles of drugs. From previous *in vitro/vivo* studies they have indicated that enzymes which metabolise drugs in the liver and intestine and drug transporters contribute to the pharmacokinetic interactions (Li et al., 2014). The capability of the products from nature to result in interactions with therapeutics (food or herb-drug interactions) is found and conversed on the tier of CYP450 organisation or and pump like P-gp. Drug interactions are simultaneously supposed for minimally 150 herbs (Hayeshi et al., 2006) so an investigation of mechanisms of the emerging herb-drug interactions is very indispensable. Interactions among herbs and conventional drugs can arise and would result in serious clinical outcomes. Some other theoretical interactions are pointed out

by preclinical information. Mechanisms through pharmacokinetic and/or pharmacodynamic are regarded to display a duty in these interactions, even though the basis of mechanisms for the changed drug actions and/or levels of drugs in the body by accompanying herbal products are yet to be ascertained. The herb-drug interaction significance in clinical relies on various determinants participated with the specific herbs, drugs and patients. Herbal products ought to properly tagged to warn consumers to capability interactions when co-administered with medications, and to suggest a counsel with physicians and other medical staffs (Hu et al., 2005).

P-glycoprotein which is shortly called is P-gp or ABCB1 (ATP-binding cassette sub-family B member 1). It is a transmembrane protein with size 170 kilodaltons. P-gp is classified in the ATP-binding cassette transporter superfamily (another short name is ABC transporters). This transporter acts as a pump that repels its substrates from cytosol using energy by hydrolysis of an ATP molecule, leading to reduction of substrate's concentration in a cell. P-gp takes action like a multidrug resistance (MDR) determinant in various types of cancers due to it transports many anticancer drugs out of the cell. P-gp is not solely expressed in cell membrane of tumors and cancers, but likewise in the cell membrane of various types of regular tissues, particularly in the brain cell, hepatocyte, kidney cell, and intestinal epithelium cell (apical site), where P-gp takes an action to extrude xenobiotics and impedes with drug absorption (Ampasavate et al., 2010). This pump is an outstanding drug transporter existed in great amount in the cell membrane at apical site of several pharmacologically important epithelial barriers along with the liver, renal cell, intestine, and blood-brain barrier (BBB), thus, P-gp modulation would alter drug's pharmacokinetics (Ampasavate et al., 2010; Li et al., 2014) There are reports from some studies that P-gp modulation can influence oral bioavailability, biliary or renal clearance, and brain uptake of substrate drugs. In addition, ABCB1 serves as a key factor in granting the multi-drug resistance (or MDR) phenotype resulting in obstinate treatments of cancers. Thus, alteration/modulation of P-gp's function may lead to significant changes in the prescription drugs' pharmacokinetic profiles and increase potential risks in occurring of drug-drug interactions (DDIs) (Li et al., 2014).

Robust novel *in silico* approaches for an upward absolute protein dynamics assay and ligand binding were latterly informed. These techniques start along molecular

dynamics (MD) simulations out of that vicarious “non-redundant” structures are analysed utilising methods in statistics. These manners were utilised employing targeted molecular dynamics simulations of P-glycoprotein with 4 crystal structures of homologues of P-glycoprotein to be targets. In this technique, most recent post of every atom has steered to target coordinates gotten out of the crystal structures through a force vector appliance computed for reduction of the length betwixt instant and target posts. Targeted molecular dynamics provides sampling of great conformational alterations that may ordinarily be unapproachable by reason of the big energy hindrances that possibly place betwixt suchlike conformations and restrictions in time of calculation. The consequent non-redundant intermediate structures acquired from these targeted molecular dynamics computations were after that utilised in molecular docking computations to illustrate the ligand binding to the distinct structures. Targeted molecular dynamics simulations integrates the conformational info obtained from molecular dynamics along ligand docking and elucidates a dynamic motion of twain protein and ligand (Wise, 2012).

Regarding our research problem, the global raising in the favouring and consumption of alternative and complementary medicines has escalated renewed concerns about potential herb-drug interactions, which may be clinically significant and some may even be life-threatening. Herb-drug interactions are preferably significant for drugs with narrow therapeutic indices or high potencies and therefore need to be identified prior in the drug development procedure. Providing information on herb-drug interactions to the prescriber and consumer may contribute towards preventing harmful adverse effects (Tarirai et al., 2012).

Although a lot of information has been generated on P-gp related efflux inhibition, there is still relatively limited information available on certain botanicals and herbal products that may possibly decrease/increase the bioavailability of substrate drugs by means of their effects on P-gp related drug efflux. More information on herb-drug pharmacokinetic interactions is therefore needed to prevent potential adverse effects in sick people that are taking drugs with narrow therapeutic indices concomitantly with herbal products or botanicals commonly consumed in their normal diet. Clinicians or pharmacists need reliable and independent information resources about the herb-drug interaction instead of depend on the literature provided by

supplement manufacturers. Giving faultless and clinically reliable counsel to people about the likelihood of herb-drug interaction is a great challenge for healthcare professionals. The scientific information and optimised techniques used to screen potential interactions between herb and drugs generated through this basic research is useful in the fields of biopharmaceutics, ethnobotany, ethnopharmacology and other health sciences.

1.2 Literature review

1.2.1 Worldwide consumption of herbs

The traditional medicine, herbal remedies and alternative medicinal systems (e.g. Chinese medicines, Ayurvedic medicines) have roots in local traditions based on available natural resources and plants (Shoeb, 2006). Straight through the last century and particularly the last decades, escalate in travelling, commercials, advertising and internet have shirked the distances between people, exchanging information and products in a faster pace. Nowadays one can order herbal remedies from all over the world and receive them by mail few days later. Thus, some of the most common herbs used by US adults like Ginkgo (*Ginkgo biloba*), Garlic (*Allium sativum*), psyllium seed husks (*Plantago ovata*) does not originate from the US fauna and new herbs are continuously introduced to the growing market (Barnes et al., 2008; Eisenberg et al., 2001).

While the most frequently used herbs among Norwegian cancer patients were green tea (*Camelia sinensis*), garlic, ginger (*Zingiber officinale*), noni juice (*Morinda citrifolia*) and Aloe vera (*Aloe barbadensis)* (Engdal et al., 2008). The five most popular herbs among people in Jamaica were bitter melon (*Momordica charantia L.*), life plant (*Bryophyllum pinnatum* (Lam.)/ *Kalanchoe pinnata*), Aloe vera, common floss flower (*Eupatorium odoratum L.*), soursop (*Annona muricata L.*) and ginger (Picking et al., 2011). In comparison, pregnant women in Great Britain used ginger, cranberry and red raspberry leaf tea (*Rubus idaeus*) (Nordeng & Havnen, 2005) while older adults in USA bought Echinacea (*Echinacea purpurea*), garlic supplements and *Ginkgo biloba* (Bruno & Ellis, 2005). Thus, although the intercultural influence is increasing, still the herb used by people varies between countries, patients groups and age groups.

Studies from USA have reported extensive use (twenty to thirty-six percent) of herbs in the general population (Kuo et al., 2004; Martin et al., 2002). Wide variability of herbal use has, however, been shown for different ethnic groups in the US were fifty percent of Hispanics, fifty percent of Asians, forty-one percent of Whites, and twenty-two percent of African-Americans reported herbal use (Feldmann et al., 2008; Kuo et al., 2004). In comparison, the United Kingdom phone survey from 1999 reveals, a herbal medicine use of seven percent in the general population (Ernst & White, 2000). An Australian report shows an escalation in herbal treatment application from nearly ten percent in 1993 to above twenty percent in 2004 (MacLennan et al., 2006).

A cross-sectional study among the adult population in the USA showed that 41% utilised an herbal treatment occasionally or habitually to self-treat afore looking for medical care from a physician (Martin et al., 2002). The typical herb user in the USA population (The 2002 National Health Interview Survey) were female, aged 45 to 64 years with higher education (Kennedy, 2005). About forty percent of survey respondents from the United States had faith in that combination uses of conventional medicines and herbal remedies was more effectual than uses either alone (Kuo, Hawley, Weiss, Balkrishnan, & Volk, 2004). Fifteen percent of mature people cured their children with herbs and nearly all (86%) respondents reported that they found it to be helpful or very helpful (Martin et al., 2002).

1.2.2 Herbal consumption in Thailand

Eleven percent accretes in products from herbs consumption in Thailand from twenty-seven million US dollars to thirty-two million US dollars during 2001-2003. Also in Thailand, herbal products more than two thousand are registered. Various determinants advocate to raise herbal product applications including simple availability, insight that herbs is safe to consume, self-medication requirement and inexpensive of products. Anyway, an understanding of consumers that herbs are safe may be wrong (Saokaew et al., 2011). Now herbal medicines are available in a lot of modern-day hospitals but herbal specialists are lacking. Many physicians and other staff members lack essential knowledge about herbal medicine. The information toward efficacies and safeties of the utilisation of herbal medicines are insufficient, particularly about researches which support herbal usage (Chotchoungchatchai et al., 2012).

In 2013, Inta and co-workers studied traditional intellect of herbs utilisation of the Northern Thai people, in order to determine the important medicinal plant species and dominant use-categories in the Northern Province of Thailand. The results showed that the number of herb species is 93 consisting 82 genera and 49 families were found. The most important herb species were *Aloe vera* (L.) Burm.f. which is utilised topically for wound healing and various skin conditions, and administered by oral as a laxative, *Andrographis paniculata* Ness is used as an herbal supplement for health promotion, *Chromolaena odorata* (L.) R.M. King and H. Robinson (old name: *Eupatorium odoratum*), which is used for wound healing and as an antiseptic for local treatment, *Jatropha podagrica* Hook. is an ornamental plant which is likewise applied to remedy a variety of infections in traditional medicine, and *Thunbergia laurifolia* Lindl. that its leaves are widely used as antipyretic and antidote against poisonous agents which had the use values (UV) of 1.02, 1.01, 0.75, 0.71, and 0.65, respectively. High UV when there are numerous utilise-reports for an herb indicating that the herb is important (Inta et al., 2013).

The herbal product safety is a matter of concern in Thailand. Serious adverse effects including herb-drug interactions owing to herbal medicine utilisation have been apprised. Improvement about herb-drug interaction informing is needed. This can be used to notify healthcare professionals and then they can provide proper advices to consumers of herbal products to avoid or reduce unwanted health effects. Most herbs using is self-directed. Many patients generally buy herbal medicines from markets, groceries, shops or even community pharmacies. If adverse effects do not occur seriously, patients may not go to see physicians at hospitals or clinics. The belike way is that herbal product consumers can advise pharmacists in their communities who are practicable to expose to information and able to predict such effects. Many Thai herbs that cause adverse events are shown in Table 1.1 and 1.2 (Saokaew et al., 2011).

Table 1.1 The adverse event number related with herbal products submitted to the Health Product Vigilance Centre, Thai FDA (Saokaew et al., 2011)

Herbs	Reports [n (%)]	Source of reports [n (%)][a]		Adverse events[b] [n (%)]
		spontaneous	intensive	
1. *Curcuma longa* (Turmeric)	260 (43.8)	38 (14.6)	222 (85.4)	805 (43.1)
2. *Andrographis paniculata* (Andrographis)	60 (10.1)	27 (45.0)	33 (55.0)	131 (7.0)
3. *Cissus quadrangularis* (Veld grape)	56 (9.5)	1 (1.8)	55 (98.2)	181 (9.7)
4. *Centella asiatica* **(Asiatic pennywort)**	42 (7.1)	0 (0)	42 (100)	200 (10.8)
5. *Zingiber cassumunar* (Plai)	38 (6.4)	8 (21.1)	30 (78.9)	97 (5.2)
6. *Derris scandens* (Jewel vine)	35 (5.9)	0 (0)	35 (100)	152 (8.1)
7. *Momordica charantia* (Bitter melon)	30 (5.1)	1 (3.3)	29 (96.7)	105 (5.6)
8. *Clinacanthus nutans* (Snake plant)	29 (4.9)	3 (10.3)	26 (89.7)	81 (4.3)
9. *Cassia siamea* (Thai cassia)	7 (1.2)	7 (100)	0 (0)	30 (1.6)
10. *Arthrospira platensis* (Spirulina)	5 (0.8)	5 (100)	0 (0)	8 (0.4)
11. *Cassia alata* (Wild senna)	5 (0.8)	3 (60.0)	2 (40.0)	10 (0.5)
12. *Curcuma xanthorrhiza* (Giant curcuma)	5 (0.8)	5 (100)	0 (0)	12 (0.6)
13. *Morinda citrifolia* (Indian mulberry)	5 (0.8)	5 (100)	0 (0)	7 (0.4)
14. *Allium sativum* (Garlic)	3 (0.5)	3 (100)	0 (0)	8 (0.4)
15. *Ganoderma lucidum* (Ling zhi)	2 (0.3)	2 (100)	0 (0)	9 (0.5)
16. *Gingko biloba* (Gingko)	2 (0.3)	2 (100)	0 (0)	5 (0.3)
17. *Tinospora crispa* (Heart-leaved moonseed)	2 (0.3)	2 (100)	0 (0)	8 (0.4)
18. *Cassia angustifolia* (Indian senna)	1 (0.2)	1 (100)	0 (0)	3 (0.2)
19. *Oenothera biennis* (Evening primrose oil)	1 (0.2)	1 (100)	0 (0)	3 (0.2)
20. *Piper nigrum* (Pepper)	1 (0.2)	1 (100)	0 (0)	1 (0.1)
21. *Pueraria mirifica* (Kwao Krua Kao)	1 (0.2)	1 (100)	0 (0)	2 (0.1)
22. *Solanum trilobatum* (Indian nightshade)	1 (0.2)	1 (100)	0 (0)	2 (0.1)
23. *Talinum paniculata* (Ginseng)	1 (0.2)	1 (100)	0 (0)	2 (0.1)
24. *Zingiber officinale* (Ginger)	1 (0.2)	1 (100)	0 (0)	6 (0.3)
Total	**593 (100)**	**119 (20.1)**	**474 (79.9)**	**1868 (100)**

a Reports received from the spontaneous reporting or intensive monitoring system.

b Some reports have more than one event.

Table 1.2 Reports of adverse events caused from various herbal products (Saokaew et al., 2011)

Herbs [no. of events]	System Organ Class	n (%)[a]	Detail (n)
Curcuma longa[b] (Turmeric) [805]	Gastrointestinal system disorders	305 (37.9)	Abdominal pain (100), diarrhoea (60), eructation (48), nausea (45), vomiting (40), constipation (4), flatus (3), flatulence (2), cheilitis (1), constipation aggravated (1), gum hyperplasia (1)
	Body as a whole – general disorders	155 (19.3)	Fatigue (60), chest discomfort (45), therapeutic response increased (35), fever (7), chest tightness (3), back pain (2), leg pain (1), oedema mouth (1), pain burning (1)
	Psychiatric disorders	118 (14.7)	Anorexia (111), appetite increased (3), somnolence (3), insomnia (1)
	Central and peripheral nervous system disorders	104 (12.9)	Headache (94), dizziness (6), burning sensation (2), burning skin (2)
	Skin and appendages disorders	53 (6.6)	Pruritus (27), rash (13), rash erythematous (3), rash maculopapular (2), skin dry (2), angioedema (1), **exfoliative dermatitis (1)**, itching (1), macular rash (1), rash bullous (1), uticaria (1)
	Respiratory system disorders	49 (6.1)	Dyspnoea (31), coughing (18)
	Liver and biliary system disorders	6 (0.7)	Jaundice (6)
	Heart rate and rhythm disorders	4 (0.5)	Palpitation (4)
	Metabolic and nutritional disorders	4 (0.5)	Thirst (4)
	Urinary system disorders	4 (0.5)	Micturition frequent (2), urinary frequency (1), urine odour foul (1)
	Endocrine disorders	2 (0.2)	Gynaecomastia (2)
	Special senses other, disorders	1 (0.1)	Taste perversion (1)
Andrographis paniculata[b] (Andrographis) [131]	Skin and appendages disorders	51 (38.9)	Pruritus (13), rash (8), rash maculopapular (7), urticaria (6), sweating increased (4), erythema multiforme (3), angioedema (2), rash erythematous (2), skin exfoliation (2), **exfoliative dermatitis (1)**, dry lips (1), itching (1), **Stevens-Johnson syndrome (1)**
	Body as a whole – general disorders	18 (13.7)	Fatigue (6), oedema periorbital (3), eyelid oedema (2), fever (2), therapeutic response decreased (2), **anaphylactic shock (1)**, flank pain (1), oedema of extremities (1)
	Gastrointestinal system disorders	18 (13.7)	Vomiting (6), nausea (5), abdominal pain (4), diarrhoea (2), throat dry (1)
	Psychiatric disorders	15 (11.5)	Anorexia (12), sleepiness (2), insomnia (1)
	Respiratory system disorders	12 (9.2)	Dyspnoea (5), coughing (4), bronchospasm (2), sputum increased (1)
	Central and peripheral nervous system disorders	9 (6.9)	Headache (6), burning sensation (1), dizziness (1), faintness (1)
	Urinary system disorders	3 (2.3)	Face oedema (2), urinary frequency (1)
	Application site disorders	2 (1.5)	Anaesthesia local (2)
	Vascular (extracardiac) disorders	2 (1.5)	Vasculitis (2)
	Musculoskeletal system disorders	1 (0.8)	Muscle weakness (1)
Cissus quadrangularis (Veld grape) [181]	Gastrointestinal system disorders	100 (55.2)	Flatulence (30), constipation (14), nausea (14), diarrhoea (12), abdominal pain (10), vomiting (6), abdominal discomfort (4), mouth dry (4), constipation aggravated (1), dyspepsia (1), faecal abnormality (1), fullness abdominal (1), stool loose (1), throat dry (1)
	Central and peripheral nervous system disorders	21 (11.6)	Headache (8), dizziness (6), faintness (6), burning sensation (1)

Table 1.2 (continued)

Herbs [no. of events]	System Organ Class	n (%)[a]	Detail (n)
	Body as a whole – general disorders	14 (7.7)	Chest tightness (7), fatigue (4), eyelid oedema (2), oedema of extremities (1)
	Application site disorders	10 (5.5)	Anaesthesia local (10)
	Skin and appendages disorders	10 (5.5)	Rash erythematous (4), pruritus (3), urticaria (2), rash bullous (1)
	Urinary system disorders	10 (5.5)	Polyuria (3), urine abnormal (3), face oedema (2), urine discolouration (1), urine flow decreased (1)
	Psychiatric disorders	9 (5.0)	Anorexia (3), insomnia (3), hunger abnormal (1), sleepiness (1), somnolence (1)
	Heart rate and rhythm disorders	6 (3.3)	Palpitation (6)
	Vision disorders	1 (0.6)	Conjunctivitis (1)
Centella asiatica **(Asiatic pennywort)** [200]	Gastrointestinal system disorders	95 (47.5)	Abdominal pain (16), flatulence (16), constipation (12), mouth dry (10), nausea (10), vomiting (10), diarrhoea (8), throat dry (5), abdominal discomfort (2), dyspepsia (2), flatus (2), fullness abdominal (1), stool black (1)
	Psychiatric disorders	33 (16.5)	Anorexia (21), insomnia (5), appetite increased (3), sleepiness (2), bulimia (1), somnolence (1)
	Central and peripheral nervous system disorders	24 (12.0)	Dizziness (13), headache (6), faintness (3), burning sensation (2)
	Body as a whole – general disorders	14 (7.0)	Fatigue (8), syncope (3), abdominal distention gaseous (1), chest tightness (1), feeling cold (1)
	Heart rate and rhythm disorders	12 (6.0)	Palpitation (12)
	Urinary system disorders	10 (5.0)	Polyuria (7), micturition frequency (2), urine discolouration (1)
	Skin and appendages disorders	7 (3.5)	Pruritus (2), urticaria (2), **exfoliative dermatitis (1)**, hot dry skin (1), rash maculopapular (1)
	Application site disorders	4 (2.0)	Anaesthesia local (4)
	Reproductive disorders, female	1 (0.5)	Leukorrhoea (1)
Zingiber cassumunar[b] (Plai) [97]	Gastrointestinal system disorders	21 (21.6)	Abdominal pain (6), vomiting (6), nausea (5), diarrhoea (4)
	Body as a whole – general disorders	20 (20.6)	Therapeutic response decreased (15), fatigue (4), fever (1)
	Skin and appendages disorders	18 (18.6)	Pruritus (7), Rash (5), rash erythematous (2), dermatitis (1), **exfoliative dermatitis (1)**, erythema multiforme (1), papulovesicular rash (1)
	Psychiatric disorders	15 (15.5)	Anorexia (15)
	Central and peripheral nervous system disorders	14 (14.4)	Headache (12), burning sensation (1), burning skin (1)
	Respiratory system disorders	7 (7.2)	Dyspnoea (5), coughing (2)
	Urinary system disorders	1 (1.0)	Urinary frequency (1)
	Vision disorders	1 (1.0)	Conjunctival discolouration (1)
Derris scandens (Jewel vine) [152]	Gastrointestinal system disorders	78 (51.3)	Abdominal pain (16), constipation (10), mouth dry (10), diarrhoea (8), flatulence (8), Vomiting (8), throat dry (7), nausea (6), faeces discoloured (2), abdominal discomfort (1), faecal abnormality (1), fullness abdominal (1)
	Psychiatric disorders	17 (11.2)	Anorexia (6), sleepiness (4), appetite increased (3), insomnia (3), somnolence (1)
	Body as a whole – general disorders	15 (9.9)	Chest tightness (6), fatigue (3), syncope (3), back pain (2), feeling cold (1)

Table 1.2 (continued)

Herbs [no. of events]	System Organ Class	n (%)[a]	Detail (n)
	Central and peripheral nervous system disorders	15 (9.9)	Dizziness (6), headache (6), faintness (3)
	Urinary system disorders	9 (5.9)	Polyuria (6), urine discolouration (2), urine frequency (1)
	Heart rate and rhythm disorders	6 (3.9)	Palpitations (6)
	Skin and appendages disorders	5 (3.3)	Pruritus (2), rash (1), rash maculopapular (1), urticaria (1)
	Respiratory system disorders	3 (2.0)	Coughing (2), throat irritation (1)
	Application site disorders	2 (1.3)	Anaesthesia local (2)
	Reproductive disorders, female	1 (0.7)	Leukorrhoea (1)
	Special senses other, disorders	1 (0.7)	Taste loss (1)
Momordica charantia (Bitter melon) [105]	Gastrointestinal system disorders	50 (47.6)	Diarrhoea (22), nausea (8), flatulence (6), vomiting (6), abdominal pain (2), mouth dry (2), throat dry (2), abdominal distress (1), stool loose (1)
	Central and peripheral nervous system disorders	20 (19.0)	Dizziness (8), headache (4), faintness (3), anaesthesia mouth (1), anaesthesia tongue (1), burning sensation (1), numbness localized (1), paraesthesia (1)
	Heart rate and rhythm disorders	10 (9.5)	Palpitation (10)
	Skin and appendages disorders	7 (6.7)	Pruritus (3), rash erythematous (2), rash (1), rash bullous (1)
	Urinary system disorders	5 (4.8)	Face oedema (2), urine abnormal (2), urine discolouration (1)
	Application site disorders	4 (3.8)	Anaesthesia local (4)
	Body as a whole – general disorders	4 (3.8)	Fatigue (2), fever (1), oedema of extremities (1)
	Psychiatric disorders	4 (3.8)	Appetite increased (3), insomnia (1)
	Respiratory system disorders	1 (1.0)	Throat sore (1)
Clinacanthus nutans[b] (Snake plant) [81]	Skin and appendages disorders	24 (29.6)	Pruritus (14), rash (7), hot dry skin (2), pruritus aggravated (1)
	Gastrointestinal system disorders	16 (19.8)	Abdominal pain (4), diarrhoea (4), nausea (4), vomiting (4)
	Central and peripheral nervous system disorders	15 (18.5)	Headache (12), burning (3)
	Body as a whole – general disorders	13 (16.0)	Therapeutic response decreased (8), fatigue (4), fever (1)
	Psychiatric disorders	6 (7.4)	Anorexia (6)
	Respiratory system disorders	5 (6.2)	Dyspnoea (3), bronchospasm (2)
	Application site disorders	1 (1.2)	Anaesthesia local (1)
	Vision disorders	1 (1.2)	Conjunctival discolouration (1)
Cassia siamea[b] (Thai cassia) [30]	Liver and biliary system disorders	16 (53.3)	Jaundice (8), hepatic enzymes increased (3), hepatic function abnormal (2), hepatitis (2), hepatic pain (1)
	Psychiatric disorders	12 (40.0)	Anorexia (12)
	Body as a whole – general disorders	2 (6.7)	Fatigue (2)
Arthrospira platensis (Spirulina) [8]	Skin and appendages disorders	3 (37.5)	Rash (1), rash maculopapular (1), urticaria (1)
	Central and peripheral nervous system disorders	1 (12.5)	Numbness (1)
	Liver and biliary system disorders	1 (12.5)	Liver function test abnormality (1)
	Musculoskeletal system disorders	1 (12.5)	Myalgia (1)
	Reproductive disorders, female	1 (12.5)	Menstrual disorder (1)
	Respiratory system disorders	1 (12.5)	Coughing (1)

Table 1.2 (continued)

Herbs [no. of events]	System Organ Class	n (%)[a]	Detail (n)
Cassia alata (Wild senna) [10]	Body as a whole – general disorders	3 (30.0)	Therapeutic response decreased (2), eyelid oedema (1)
	Gastrointestinal system disorders	3 (30.0)	Vomiting (2), nausea (1)
	Central and peripheral nervous system disorders	2 (20.0)	Headache (2)
	Skin and appendages disorders	2 (20.0)	Urticaria (2)
Curcuma xanthorrhiza (Giant curcuma) [12]	Urinary system disorders	6 (50.0)	Face oedema (6)
	Gastrointestinal system disorders	2 (16.7)	Abdominal pain (2)
	Skin and appendages disorders	2 (16.7)	Erythema multiforme (1), urticaria (1)
	Body as a whole – general disorders	1 (8.3)	Eyelid oedema (1)
	Reproductive disorders, female	1 (8.3)	Leukorrhoea (1)
Morinda citrifolia (Indian mulberry) [7]	Urinary system disorders	5 (71.4)	Blood urea nitrogen increased (2), creatinine clearance decreased (1), renal failure acute (1), renal function abnormal (1)
	Skin and appendages disorders	2 (28.6)	Papular rash (1), **Stevens-Johnson syndrome (1)**
Allium sativum Linn. (Garlic) [8]	Body as a whole – general disorders	2 (25.0)	Back pain (2)
	Gastrointestinal system disorders	2 (25.0)	Eructation (2)
	Skin and appendages disorders	2 (25.0)	Pruritus (1), rash maculopapular (1)
	Central and peripheral nervous system disorders	1 (12.5)	Dizziness (1)
	Respiratory system disorders	1 (12.5)	Dyspnoea (1)
Ganoderma lucidum (Ling zhi) [9]	Gastrointestinal system disorders	4 (44.4)	Abdominal pain (2), diarrhoea (2)
	Central and peripheral nervous system disorders	2 (22.2)	Dizziness (1), faintness (1)
	Skin and appendages disorders	2 (22.2)	Photosensitivity toxic reaction (2)
	Liver and biliary system disorders	1 (11.1)	Hepatitis (1)
Gingko biloba (Gingko) [5]	Body as a whole – general disorders	3 (60.0)	Oedema legs (3)
	Liver and biliary system disorders	1 (20.0)	Hepatic enzymes increased (1)
	Skin and appendages disorders	1 (20.0)	Rash (1)
Tinospora crispa (Heart-leaved moonseed) [8]	Gastrointestinal system disorders	5 (62.5)	Abdominal pain (2), constipation (2), abdominal discomfort (1)
	Body as a whole – general disorders	2 (25.0)	Chest tightness (2)
	Liver and biliary system disorders	1 (12.5)	Hepatitis (1)
Cassia angustifolia (Indian senna) [3]	Cardiovascular disorders, general	2 (66.7)	Hypotension (2)
	Body as a whole – general disorders	1 (33.3)	Therapeutic response decreased (1)
Onethera Biennis (Evening primrose oil) [3]	Central and peripheral nervous system disorders	1 (33.3)	Dizziness (1)
	Psychiatric disorders	1 (33.3)	Insomnia (1)
	Respiratory system disorders	1 (33.3)	Dyspnoea (1)
Piper nigrum (Pepper) [1]	Skin and appendages disorders	1 (100.0)	Rash maculopapular (1)
Pueraria mirifica (Kwao Krua Kao) [2]	Reproductive disorders, female	1 (50.0)	Leukorrhoea (1)
	Skin and appendages disorders	1 (50.0)	Macular rash (1)

Table 1.2 (continued)

Herbs [no. of events]	System Organ Class	n (%)[a]	Detail (n)
Solanum trilobatum (Indian nightshade) [2]	Skin and appendages disorders	2 (100.0)	Pruritus (1), rash (1)
Talinum paniculata (Ginseng) [2]	Skin and appendages disorders	2 (100.0)	Rash acneiform (1), rash erythematous (1)
Zingiber officinale (Ginger) [6]	Psychiatric disorders	3 (50.0)	Appetite increased (3)
	Body as a whole – general disorders	1 (16.7)	Fatigue (1)
	Gastrointestinal system disorders	1 (16.7)	Nausea (1)
	Skin and appendages disorders	1 (16.7)	Pruritus (1)

a Percentage of each herbal product.

b Indications by the List of Herbal Medicine Products in Thailand[15] (*Curcuma longa* [Turmeric]: dyspepsia; *Andrographis paniculata* [Andrographis]: non-infectious diarrhoea, sore throat and common cold; *Zingiber cassummunar* [Plai]: muscle pain and inflammation; *Clinacanthus nutans* [Snake plant]: herpes simplex, herpes zoster, aphthous ulcer, urticaria and rash; *Cassia alata* [Wild senna]: constipation).

Bold indicates serious adverse event.

Patients with chronic diseases often use herbs including dietary supplements coupled with conventional medicines. Due to these patients must receive chronic treatments for their diseases that last a long time and some treatments require using of medications through their lives. Therefore, these patients trend to seek alternative themselves treatments concomitant with conventional medications that aims to provide a better living conditions and believes to have cured more quickly or may be intended to prevent or treat other participant diseases. So there may be result in that the conditions of diseases are better, but in another way, the diseases may be heal slowly or the patients may encounter adverse effects including herb-drug interactions from the used herbs/supplements directly. The effects of such interactions may be life-threatening. In 2012, Sornsuvit et al. (Sornsuvit et al., 2012) studied to define herbs and dietary supplements utilised in 56 patients with chronic diseases (Table 1.3) followed up at Changphuek Health Promoting Hospital, Muang district, Chiang Mai province by interview and evaluate the potential herb/dietary supplement-drug interactions. The patients with the ages ≥60 years old who have at least one of the chronic diseases including cardiovascular disease, bone and joint disease, diabetes, respiratory disease, and usually use herbal or dietary supplement with conventional medicines. 36.1% of patients bought herbal products and dietary supplements from drug store. The 33 herbal products and dietary supplements were apprised, 11 among them have potential to produce 56 interactions with conventional medicines. Among the herbal products and dietary supplements having potential for interactions with drugs (Table 1.4 and 1.5), *Moringa oleifera* Lam. (Thai name: Marum) was found the most frequent

(approximately 63%) followed by garlic/garlic oil (21.0%), *Curcuma longa* L. (Thai name: Khamin Chan) (13.8%), *Phyllanthus amarus* Schum & Thonn. (Thai name: Look-Tai-Bai) (10.8%), *Centella asiatica* (Thai name: Bua Bok) (7.2%), *Andrographis Paniculata* (Thai name: Fa Thalai Chon) (6.8%), and *Gynostemma pentaphyllum* (Thunb.) Makino (Thai name: Pan-Cha-Khan) (5.3%). 3 interactions per patient were discovered in the most patients.

Table 1.3 Herbal products and dietary supplements used in 56 patients with chronic diseases followed up at Changphuek Health Promoting Hospital (Sornsuvit et al., 2012)

Herbs	The number of patients (%)
Single herbs	50 (58.1)
Moringa oleifera Lam.	21 (24.4)
Carthamus tinctorius L.	4 (4.7)
Hibiscus sabdariffa	3 (3.5)
Boesenbergia rotunda (L.) Mansf.	2 (2.3)
Centella asiatica Urban.	2 (2.3)
Phyllanthus amarus Schum & Thonn.	2 (2.3)
Ganoderma lucidum (Fr.) Karst.	2 (2.3)
Gymnema inodorum (Lour.) Decne	2 (2.3)
Tiliacora triandra (Colebr.) Diels.	2 (2.3)
Tinospora crispa (L.) Miers ex Hook.f. & Thomson	1 (1.2)
Andrographis paniculata (Burm.f.) Wall. ex Nees.	1 (1.2)
Houttuynia cordata Thunb.	1 (1.2)
Pandanus amaryllifolius Roxb.	1 (1.2)
Phyllanthus emblica L.	1 (1.2)
Sauropus androgynus (Linn.) Merr.	1 (1.2)
Rhinacanthus nasutus (L.) Kurz.	1 (1.2)

Table 1.3 (continued)

Herbs	The number of patients (%)
Thumbergia laurifolia Lindl.	1 (1.2)
Orthosiphon aristatus (Blume) Miq.	1 (1.2)
Others	1 (1.2)
Herbal formulas	18 (20.9)
Gynostemma pentaphyllum (Thunb.) Makino	6 (6.9)
Curcuma longa L. capsule	5 (5.8)
Others	7 (14)
Dietary supplements	18 (20.9)
Allium sativum L. capsule	5 (5.8)
Calcium carbonate	4 (4.7)
Rice bran oil	3 (3.5)
Fish Oil	2 (2.3)
Vitamin C	1 (1.2)
Enzyme	1 (1.2)
Mangosteen's peel juice	1 (1.2)
Nano Oxy	1 (1.2)

Table 1.4 The number of patients with the potential herb/dietary supplement-drug interactions (Sornsuvit et al., 2012)

The number of interactions per one patient	The number of patients (%)
0	26 (46.5)
1	6 (10.7)
2	9 (16.1)
3	10 (17.8)
4	3 (5.3)
5	2 (3.6)
Total	56

Table 1.5 The number of patients with the potential herb/dietary supplement-drug interactions and possible mechanisms (Sornsuvit et al., 2012)

Herbs/dietary supplements	Conventional drugs	The number of patients (%)	Possible mechanisms
Moringa oleifera Lam.	Enalapril	14 (25.0%)	Pharmacokinetic interaction
	Amlodipine	13 (23.2%)	
	Simvastatin	6 (10.7%)	
	Omeprazole	1 (1.7%)	
	Cardesartan	1 (1.7%)	Pharmacodynamic interaction
Curcuma longa L.	Isosorbide mononitrate	2 (3.6%)	Pharmacokinetic interactions
	Diclofenac	1 (1.7%)	
	Simvastatin	1 (1.7%)	
	Enalapril	1 (1.7%)	
	Amlodipine	1 (1.7%)	
	Atorvastatin	1 (1.7%)	
	Acetaminophen	1 (1.7%)	
Andrographis paniculata (Burm.f.) Wall. ex Nees.	Theophylline	1 (1.7%)	Pharmacokinetic interactions
	Simvastatin	1 (1.7%)	
	Amlodipine	1 (1.7%)	
	Aspirin	1 (1.7%)	Pharmacodynamic interaction
Ganoderma lucidum (Fr.) Karst.	Aspirin	1 (1.7%)	Pharmacodynamic interaction
Orthosiphon aristatus (Blume) Miq.	Enalapril	1 (1.7%)	Pharmacodynamic interaction
	Amlodipine	1 (1.7%)	Pharmacokinetic interactions
Centella asiatica Urban.	Enalapril	2 (3.6%)	Pharmacokinetic interactions
	Amlodipine	2 (3.6%)	
Thumbergia laurifolia Lindl.	Theophylline	1 (1.7%)	Pharmacokinetic interaction
	Diazepam	1 (1.7%)	

Table 1.5 (continued)

Herbs/dietary supplements	Conventional drugs	The number of patients (%)	Possible mechanisms
Phyllanthus amarus Schumach. & Thonn.	Enalapril	2 (3.6%)	Pharmacokinetic interactions
	Amlodipine	2 (3.6%)	
	Fenofibrate	2 (3.6%)	
Rhinacanthus nasutus (L.) Kurz.	Diclofenac	1 (1.7%)	Pharmacokinetic interactions
	Acetaminophen	1 (1.7%)	
Garlic/Garlic oil (*Allium sativum* L.)	Enalapril	3 (5.3%)	Pharmacodynamic interaction
	Amlodipine	3 (5.3%)	
	Isosorbide mononitrate	1 (1.7%)	
	Hydrochlorothaizide	1 (1.7%)	
	Simvastatin	1 (1.7%)	Pharmacokinetic interaction
	Aspirin	2 (3.6%)	Pharmacodynamic interaction
Gynostemma pentaphyllum (Thunb.) Makino	Aspirin	3 (5.3%)	Pharmacodynamic interaction

Towards flavonoids consumption, many flavonoid-containing herbs are used for medical proposes and food, for example, Fabaceae plants (legume family) contain various flavonoids including amorphigenin and formononetin which are widely found in several species of Fabaceae (Wink et al., 2012), and rotenone. Amorphigenin is present in *Amorpha fruticosa* (false indigo) isolated from the seedlings, seeds, and leaves and exhibited to have significant anti-proliferative activity, anti-cancer activity in many cell types, hepatoprotective activity and neuraminidase inhibition activity (Liang et al., 2015). Formononetin is isolated from *Astragalus membranaceus* (huang qi). Its root is a largely utilised Chinese medicinal plant that is famous for its vital-energy tonifying, skin reinforcing, tissue generative, abscess draining, diuretic, and actions. Formononetin is also found in the root of *Glycyrrhiza glabra* or *G. uralensis* (licorice), which have been largely utilised in China, Japan and the Western countries. The licorice extracts have generally been recognised as safe and are also used as flavouring and sweetening agents for chewing gums, candies, toothpaste, and beverages. Furthermore, formononetin is a main isoflavone ingredient of *Radix Astragali* (*Astragalus*

membranaceus dried root). It is traditionally used for the treatment of diabetes, wound healing and strengthening the immune system (Auyeung et al., 2007; Dong et al., 2007; Hoo et al., 2010). Rotenone is commonly found in *Derris elliptica*, *Derris scandens* and *Pachyrhizus erosus* which are the well-known Thai indigenous Fabaceae herbs (Bullangpoti, 2009; Estrella-Parra et al., 2014; Sreelatha et al., 2010). Extracts from *D. elliptica* (Wall.) Benth. (Thai name; hang lai daeng) stems and roots is utilised as an insecticide, fish poison and molluscicide. This plant also possesses some pharmacological activities like antitumor, antihypertension, and antifungal. Hang lai daeng is mostly raised in many tropical areas for its roots as a fountainhead of the natural insecticide; rotenone. With the market for natural insecticides bit by bit enlarging on account of food safety longings at this moment, the necessity for this compound has escalated evidently (Li & Geng, 2015). *D. scandens* is a medicinal plant ordinarily known in Thai as Thao-Wan-Priang. Its dried stem is utilised in Thai folk medicine as an antidysentery, expectorant, antitussive, diuretic, and treatment for muscle ache. It is verified that *D. scandens* can be utilised for health promotion in postmenopausal women, patients with cardiovascular diseases, and cancer prevention (Kuptniratsaikul et al., 2011; Sreelatha et al., 2010). *P. erosus* [English name: yam bean, Thai name: Man Kaeo, Huapaekua, Man Lao (Lim, 2016)], a horticultural crop, is used as food and cosmetic materials (Damayanti et al., 2008).

Apart from legume family, chrysin was accounted to possess extensive spectrum biological activities. It was picked as marker for standardisation such as to ensure a consistent and acceptable quality some herbal products including *Oroxylum indicum* (L.) Vent (Bignoniaceae). *O. indicum* (Thai name; phe kaa, litmai, lin faa) have been utilised as a single remedy or constitutive of famous Ayurvedic formulations. The root bark and stem bark of this plant have anti-allergic properties and are utilised in treatments of urticaria, asthma, allergic disorders, jaundice, sore throat, hoarseness, laryngitis, gastralgia, diarrhoea, dysentery, erythema and measles. This herb contains flavonoids including chrysin as active compound. Chrysin contents in *O. indicum* from root, stem, and leaf are 0.014, 0.004, and 0.007 percent respectively (Srinivas & Aparna, 2012). Moreover, chrysin is also found in propolis that has been utilised largely in traditional medicines for many years on account of the complex chemical constituents, and there is an attestation to instruct that propolis has various

pharmaceutical properties along with antibacterial, antiviral, antitumor, anticancer, anti-inflammatory, and immunomodulatory. The presence of some flavonoids including chrysin can be utilised as a marker to differentiate propolis from other bee products. The content of chrysin has been utilised as a parameter for propolis quality. The amount of chrysin is nearly 15% (Zhou et al., 2008). *Scutellaria baicalensis* Georgi (Lamiaceae) is one of the most largely utilised folk herbal remedies. Its roots (called huang qin) have been utilised for anti-inflammation, anticancer, curing bacterial and viral infections of the respiratory and gastrointestinal tracts, clearing away heat, purging fire, moistening dryness, detoxifying toxicity, lowering blood pressure and total cholesterol level. Chrysin is likewise identified in underground and aerial parts of this plant (Li et al., 2004). Epigallocatechin is one of major catechins of green tea, brewed from the dried leaves of *Camellia sinensis* L. (Theaceae), Thai name; Cha kheaw, which is one of the most largely consumed beverages in the world (Velayutham et al., 2008). This compound possesses a high antioxidant activity (Almajano et al., 2008).

Tagetes erecta L. (Asteraceae), Thai name; Daao rueang, it possesses some pharmacological activities used for treatments of wounds and burns, skin complaints, inflammation, conjunctivitis and poor eyesight, menstrual regularities, and antiviral. *Cosmos sulphureus* Cav. (Asteraceae), Thai name; Daao kajay, it possesses some pharmacological activities as antioxidant, antigenotoxic, anti-inflammatory, and antimicrobial activities, and utilised for treatments of Jaundice, intermittent fever, and splenomegaly. *Antigonon leptopus* Hook. & Arn. (Polygonaceae), Thai name; puang chompoo, it possesses some pharmacological activities as anti-diabetic, anti-inflammatory, analgesic, anti-thrombin, and lipid peroxidation inhibitory activities. *Bougainvillea glabra* Choisy (Nyctaginaceae), Thai name; fueang fa, it possesses some pharmacological activities as antidiabetic and anti-inflammatory. These flowers are edible flowers habitually utilised for salad and flower tea garnishing by the ethnic population who live in northern Thailand. The major ingredients of ethanol extracts of the flowers were phenolic acids and flavonoids. Flavonoids found in the lyophilised hydrophilic extracts (means±standard deviation with mg/100 g dry weight unit) of these four edible flowers are the following. Regarding *T. erecta*; rutin, myricetin, quercetin, apigenin, kaempferol were detected in 5.09±0.49, 54.81±1.44, 13.57±0.71, 8.41±0.24, 83.42±3.51 mg/100g respectively. For *C. sulphureus*, the flavonoids were detected in

19.67±1.27, 59.99±2.3, 9.45±0.41, 7.00±0.21, 25.6±1.6 mg/100g respectively. For *A. leptopus*, the flavonoids were detected in 21.95±2.43, 47.54±1.4, 11.08±0.43, 0.83±0.02, 75.86±1.39 mg/100g respectively. Towards *B. glabra*, the flavonoids were found in 1.3±0.22, 61.52±3.12, 14.17±1.15, 8.89±0.41, 87.18±7.59 mg/100g respectively (Kaisoon et al., 2012).

Other examples of flavonoid-containing herbs such as Ginkgo products, one of the most popular dietary supplements have been illustrated to possess antioxidant and free radical scavenging activities. The flavonoids present in *G. biloba* leaves include quercetin, kaempferol, and isorhamnetin (Zhao et al., 2007). Approximately 65 % of patients who have some liver diseases in Europe and the US take herbal preparations that milk thistle is the most common. The active complex obtained from the seeds of this herb is called silymarin. It is mostly constituted of 3 isomer flavonolignans: silybin, silychristin, and silydianin. Silybin is speculated as the most biologically active compound and fabricate 50 to 70 % of silymarin. Silymarin has been encouraged for the treatment of non-alcoholic/alcoholic liver disease, chronic hepatitis, cirrhosis, and environmental toxin exposure (Wei et al., 2013).

1.2.3 Safety issues of herbal medicines

While herbal products are increasingly being used, safety issuances and the keeping track of adverse effects have not been emphasised. This favouring of herbal products energises the critical appraisal of their safety and exigent requisite. The reasons for adverse reactions of herbal medicines include direct toxicity of herbal materials, allergic reactions, and toxic effects from contaminants, adulterations of other herb or synthetic chemicals, and interactions with drugs or other herbs. The safety of herbal medicines and herbal related supplements deserve deep consideration, similar to drugs. Among them, herb-drug interactions, one of the important safety issues, are often overlooked and sometimes simply categorised as toxic effects of consumed drugs.

1) Herb use and concomitant use amongst general practice patients

Notwithstanding the hulking informed using of herbal products and co-use of herbs and regular medicines in patient clusters, there are few studies within primary care and general practice. General practitioners (GPs, family doctors) provide the main health care to the general population in the society. They have the long term follow up on regular medication/chronic unhealthiness and common illnesses (i.e. diabetes,

hypertension, arthritis) with a large degree of variety in the patient population (age, gender, illnesses, socioeconomic status etc.) (Gillam et al., 1989). Thus the GP is the physician to whom the patients are expected to disclose their herb use and the GP is on the other hand, expected to ask for this information in the medical history taking.

A study from Israel reported a prevalence of herbal use in family medicine practices of 36% and nearly 50 % of these co-used natural and conventional drugs usually or sometimes (Giveon et al., 2004). Another study showed herb use of 35% and a concomitantly use of CAM and conventional drugs of nearly 80% among the CAM users in Scotland (Featherstone et al., 2003). Due to the low number of studies from general practice as such, it is relevant to look at use among typical patient groups in general practice, elderly patients and patients with chronic diseases.

The elderly patients tend to go more often to their GP, have more polypharmacy issuances and they are increasingly weakened to interactions due to reduced health in general (hearth failure, liver failure, kidney failure etc.) (Loya et al., 2009). Considering thirteen to forty-seven percent of elderly patients reports to utilise herbs in the general population (Bruno et al., 2005) and as many as thirty-one to seventy-five percent of the elderly concomitant using herbs and prescription medications (Gonzalez-Stuart, 2011; Loya et al., 2009), the risk of severe interactions are high. For instance, it is reported herbal interaction with various cardiovascular drugs, which may result in adverse alterations in drug efficacy and/or toxicity (Cohen & Ernst, 2010; Gurley et al., 2005). In addition, approximately fifty percent of the populaces has 1 or upward chronic conditions and have, like the aged, an elevated care rate and polypharmacy (fifty percent) (Schoen et al., 2007). They likewise incline to utilise increasingly herbal treatments, which raise the feasibility of herb-drug interactions (Ravven et al., 2011). Thus, the risk of harmful concomitant use in the GP practice is present and needs to be addressed.

Only 20-45% of the population informs their physician of herb use (Davis et al., 2012; Giveon et al., 2003; Wheaton et al., 2005). Apart from that, health care professionals seldom asked patients regarding the using of herbs (Giveon et al., 2003). “The doctor didn’t ask” is the commonplace words explicating the communicative deficiency (Saw et al., 2006). The physicians likewise tended to underrate the using

(Giveon et al., 2003). Thus, a lack of disclosure from the patient about herbal use is to some degree documented, particularly so among GP patients.

2) Co-use of herbs with conventional drugs

Herbs are perceived as safe and "natural" and are often marketed without mentioning any potential for harm (van den Berg et al., 2011). It is reported a concomitant using of herbal treatments and drugs up to 50% in different patients groups (Nordeng et al., 2011; Smith et al., 2010; Zhang et al., 2011). An US study identified that 40% of liable adverseness of herb-drug interactions among the herbal users in outpatient clinics (Bush et al., 2007). Pregnant women reports to use herbal remedies (9-40%) and about 86% of these used conventional drugs concomitantly (Moussally et al., 2009; Nordeng et al., 2011). The using of herbal treatments amongst adults with cancer is informed to be 35.9% (Molassiotis et al., 2005) and 1 study discovered that nearly 40% concomitant using herbal treatments and chemotherapy (Engdal et al., 2008). Elderly are another group at risk and in a study were 31.5% of the participants defined to be at riskiness of owning at least 1 probable herb-drug interaction (Loya et al., 2009). Smith and co-workers reported of warfarin interactions with herbs or vitamins for 7% of the patients (Smith et al., 2010). Thus, adverse effects and interactions between drugs and common herbs are a challenge today (Zhou et al., 2003). Combined use of herbal products and therapeutic drugs could raise or reduce the effects of each constituent, especially for narrow therapeutic index drugs (for instance, digoxin, warfarin and midazolam). The resultant herb-drug interactions sometimes lead to clinically significant risks (Liu et al., 2015; Ruschitzka et al., 2000).

Herbs contain numerous, frequently unknown, pharmacological active or inactive compounds. It is therefore more likely that an interaction between a one drug and a sophisticated herbal formula takes place, than toward another one drug. Moreover, the merged activity of all compounds jointly in an herb could be disparate from the expected sum activity appraised from single separated compounds (van den Berg et al., 2011). This emphasises the significance regarding taking action to study interactions along crude herbal extracts as an essential enlargement for the exploration about separated herbal fractions.

In the most countries, regulations of herbal remedies do not demand the display of therapeutic efficacies, safeties, or qualities like herbs are encouraged as natural and innocuous. It is relevant anyway, that herbs are not bare from side effects as some herbs have been demonstrated their toxicities. Up-to-date study has illustrated familiar scheme of attendant intake of herbal and prescription medications. This sophistication raises the riskiness of clinical drug interactions. Below forty percent of patients reveal their herbal supplement applications to caregivers coupled with a lot of medics are inattentive of the latent riskinesses of herb-drug interactions which are one of the most essential clinical concerns. Remedies with polypharmacy afterward likewise increase the herb–drug interaction risk in patients (Fasinu et al., 2012).

Clinical presentations of herb–drug interactions differ extensively relying on the herbs and prescription medications involved. Some examples of clinical presentations of herb–drug interactions consist the enhancing of the oral corticosteroids actions when liquorice (*Glycyrrhiza glabra*) is co-used; enhancing of warfarin actions with resulting in bleeding in the possession of danshen (*Salvia miltiorrhiza*), dong quai (*Angelica sinensis*), or garlic (Thai name is Kra Tium.) (*Allium sativum*); ginseng (*Panax ginseng*)-influenced mania in cases on antidepressants; produce of extrapyramidal effects in consequence of the combining of neuroleptic drugs with betel nut (Thai name is Mak.) (*Areca catechu*); Tricyclic antidepressants (TCAs) when they are co-used with *Pausinystalia yohimbe*, raised blood pressure can be observed, co-administration of Ayurvedic syrup shankhapushpi and phenytoin raises clearance and hourly seizures of this conventional drug. These clinical illustrations rely on the herb–drug interaction mechanisms (Fasinu et al., 2012).

Although many herbal medicines are safe, it must be remembered in mind that herbs are sometimes intended to be used over a long duration, which implies the increased chance for enzyme inhibition or induction and other mechanisms of herb-drug interactions. The potential herb-drug interactions riskiness has been a great concern for the safety evaluation of herbal medicines. Nowadays, more than 150 herbal medicines have been recognised to have potential herb-drug interactions (Hayeshi et al., 2006). Clinicians or pharmacists need reliable and independent information resources about the herb-drug interaction instead of depend on the literature provided by supplement

manufacturers. Giving faultless and clinically reliable counsel to people about the likelihood of herb-drug interaction is a great challenge for healthcare professionals.

1.2.4 Type of herb-drug interaction and their clinical implication

Herb-drug interactions can present serious threats to human health, although the relevant cause and effect relationships have not been well determined. Synergistic or additive therapeutic actions would result in unanticipated toxicities and will disturb the dosage regimen of long-term drug therapy. On the other hand, antagonistic herb-drug interactions will decrease efficacy or result in therapeutic failure for both of herbal medicines and drugs.

A lot of herbal products are treated as 'food supplements' for regulatory aims, even though rigorously speech they should be considered as medicines based on a strict classification. They act as drugs following modern-day pharmacological theories. Herb-drug interactions which are relied on the identical theories like drug-drug interactions should not come to be a surprise (Izzo et al., 2002). Mechanisms of herb-drug interactions are intrinsically complex and usually classified into pharmacokinetic or pharmacodynamic interactions. Pharmacokinetic interactions include absorption, distribution, metabolism and elimination, while pharmacodynamics interactions are those where the actions of a compound are borne upon with the possession of second compound at the site of act. Pharmacodynamic interactions are lesser predictable and rather tough to identify. Moreover, one should not lose sight on the fact that they also may interact via multiple mechanisms *in vivo*, e.g., drug-metabolising enzyme inhibition and/or induction, protein-binding interactions, and/or absorption effects.

The significant cause for food/herb/drug–drug interactions involves the interlacing substrate specificness for the body's pathways of biotransformation. "Pharmacodynamic drug interactions" involve the capability of various chemical moieties to have interactions against receptor sites and physiologically change surroundings while pharmacokinetic interactions occur out of changed absorption, intervention in distribution scheme along with modifications and competing in the pathways of metabolisms and excretions. The key fundamental mechanism of "pharmacokinetic herb–drug interactions", as drug–drug interaction, is likewise the inducing or inhibiting of enzymes which play roles in metabolisms in the liver and intestine especially the

CYP enzyme family. In addition, identical action on drug transporters and pumps that flow out substances especially the intestinal P-gp's liable in most other instances. The pre-systemic action of CYP and pumps flowing out any molecules frequently bear upon bioavailability of compounds taken orally, therefore the modulatory effect of concomitant used herbs has been demonstrated to lead to notable decrease or raised in the blood levels of the touched on drugs (Fasinu et al., 2012).

1) Pharmacodynamic interactions

Pharmacodynamic herb-drug interactions are able to emerge via the competition of drug transporters and binding sites on plasma proteins. Additive, synergistic or antagonistic effects are possible, which resulted from the combinations of drugs with some herbal products (Comelli et al., 2007). For example, ginkgo strengthens the aspirin's antiplatelet action (Rosenblatt & Mindel, 1997). Contrarily, a herb could directly be antagonistic to the effect of a drug (for instance, green tea antagonises the warfarin's anticoagulation activity (Taylor & Wilt, 1999). In order to predict this type of herb-drug interactions, a certain amount of basic pharmacological knowledge is needed about the relevant bioactivities varying based on the combination of the drugs and herbal medicines. Unfortunately, here often lacks such kind of information. The importance of pharmacodynamic herb-drug interactions in clinical practice has not been properly evaluated. Since information on mechanisms involved in herb-drug interactions is incomplete. Some of the pharmacologically active herbal extracts are related with different degrees of toxicity in their own right (Izzo & Ernst, 2001). The reported cases often do not provide an obvious distinctness betwixt adverse events by reason of the intrinsic toxicity and those incurred by herb-drug interactions.

2) Pharmacokinetic interactions

Pharmacokinetic interactions interfere with drug absorption and disposition processes such as drug absorption out of the GI tract after oral administration, metabolism, distribution and/or elimination of co-administered drugs via different mechanisms (Sinxadi & Blockman, 2008; Williamson, 2003). As mentioned before, herb-drug pharmacokinetic interactions turn into clinically important when substantial changes come about to pharmacokinetic parameters of the co-administered drug. These

pharmacokinetic parameters are directly related to the drug's efficacy and toxicity and include the AUC, C_{max} and so on (Zhou et al., 2005).

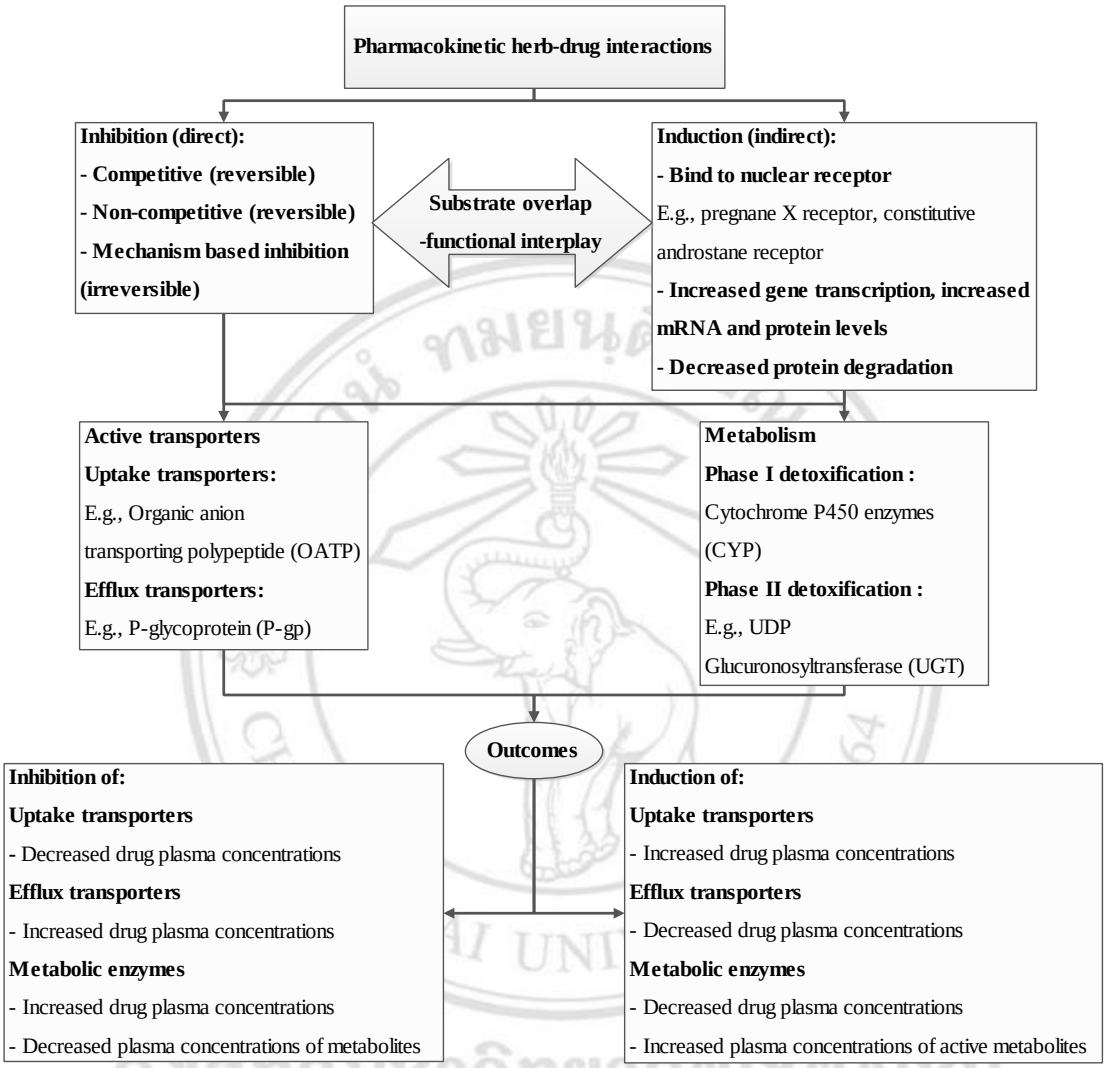

Figure 1.1 Mechanisms and outcomes of some enzymes and drug transporters mediated interactions among herbs and conventional drugs (Gouws et al., 2012)

A number of herbs interacting to cytochrome P450 likewise have identical actions on transporters shown in Table 1.6 (Fasinu et al., 2012).

Table 1.6 Some herbs that interact with CYP and efflux proteins

Medicinal Plant and parts used	Scientific name	Major constituents	Mechanism of drug interactions	Candidates for interactions
Cranberry (fruit extract)	*Vaccinium macrocarpon*	Anthocyanins, flavonoids	Inhibition of CYP enzymes and P-gp	Warfarin, CYP1A2, 2C9, and 3A4 substrates
Dong quai (root)	*Angelica sinensis*	Flavonoids, coumarins	Inhibition of CYP1A2, 3A4, and P-gp	CYP substrates
Gan cao (root)	*Glycyrrhiza uralensis*	Glycyrrhizin	CYP2C9 and 3A4 induction	Warfarin, Lidocaine, CYP2C9, and 3A4 substrates
Garlic (bulb)	*Allium sativum*	Allicin, phytoncide	CYP 3A4 and P-gp induction	Saquinavir, warfarin, CYP2D6, and 3A4 substrates
Germander (leaves)	*Teucrium chamaedrys*	Saponins, flavonoids, diterpenoids	Production of toxic CYP3A4-induced metabolites	CYP3A4 inducers like Phenobarbital, rifampicin
Ginseng (root)	*Panax ginseng*	Ginsenosides	Inhibition and induction of CYP2C9, 2C19, 2D6, and 3A4 activity	Imatinib, CYP2E1, and 2D6 substrates
Grape seed (seed oil)	*Vitis vinifera*	Proanthocyanidin, resveratrol	Decreased CYP2C19, 2D6, and 3A4 activity	CYP2C19, 2D6, and 3A4 substrates
Kava kava (root)	*Piper methysticum*	Kavalactones	Decreased CYP1A2, 2D6, 2E1, and 3A4 activity	CYP substrates
Liquorice (root)	*Glycyrrhiza glabra*	Inhalant	Inhibition of CYP2B6, 2C9 and 3A4	CYP2B6, 2C9 and 3A4 substrates
St John's wort (aerial parts)	*Hypericum perforatum*	Hyperforin, hypericin, flavonoids	Inhibition and induction of CYP and P-gp	Orally administered CYP substrates

1.2.5 Effects of herbs on drug efflux transporters

Most human drug transporters are under 2 super families namely the adenosine triphosphate cassette binding (shortly called ABC) efflux transporters and solute linked carrier (shortly called SLC) uptake proteins expressed on the apical or basal side of epithelial cells in a lot of organs (Takano et al., 2006; Zhang et al., 2006). Several reports have indicated that human drug efflux transporters, operating alone or together along drug metabolising enzymes, act an essential incumbency in oral drug absorption and bioavailability (Hellum & Nilsen, 2008; Pal & Mitra, 2006; Varma et al., 2003; Zhang et al., 2007). Outflow of drugs versus a precipitous concentration gradient is ordinarily mediated by the ABC efflux transporters like ABCB1 and multidrug resistance-associated protein-2 (MRP2) mostly placed in the canalicular membrane of the human liver or intestinal epithelium (Mandava et al., 2010; Pal & Mitra, 2006).

1) Herb-drug interactions via ABC transporters

Since the discovery of the permeability glycoprotein (P-gp) about forty-two years ago the research on ATP-binding cassette transporters (ABC transporters) have shed light on their roles in cytotoxic drug efflux in human cells and drug resistance in cancer cells (Szakacs et al., 2006). The ABC transporter plays major functions regarding drug absorption, distribution, and elimination (Fasinu et al., 2012). The ABC pumps are

situated in the cell-membrane and contains typically 2 transmembrane domains (TMDs) and 2 nucleotide (ATP)-binding domains (NBDs) (Szakacs et al., 2006; Taipalensuu et al., 2001). The ABC transporters are greatly expressed in key pharmacological barriers as in the intestines, liver, kidneys and in the blood–brain barrier (BBB) affecting drug absorption and elimination. These transporters protect the body against toxic substances including drugs, and have in general a low substrate affinity, effluxing both chemotherapeutics and naturally occurring biological compounds (Szakacs et al., 2006). The ABC transporters are comparatively simply modulated by determinants like therapeutic drugs, herbal medicines, natural foods and beverages. These transporters play a duty in constraining influx to guard the intracellular storing of their own substrates and may become inhibited leading to toxic blood drug concentrations. Conversely, their induction may cause subtherapeutic plasma levels of substrate drugs bringing about treatment failure (Takano et al., 2006).

The human genome consists forty-nine genes for these pumps within 7 subfamilies (designated A to G), ≥18 of which, when mutated, incur disease (Licht & Schneider, 2011). P-glycoprotein is sorted as adenosine triphosphate-binding cassette subfamily B member1 (other names; ABCB1, P-gp) and is the most well-known member of the ABC-transporters, other well-known ABC pumps including with the breast cancer resistance protein (other names; BCRP, ABCG2), multidrug resistant protein 2 (other names; MRP2, ABCC2) and the bile salt exporting pump (other names; BSEP, ABCB11) (Taipalensuu et al., 2001). A summary of the various ABCs is shown in Table 1.7 (Sosnik, 2013) and the affectation regarding some herbs on ABC transporters is shown in Table 1.8 (Fasinu et al., 2012)

Table 1.7 Identified ATP-binding cassette (ABC) transporters

Subfamily	Subtype	Other name	Expression site	Main substrates	Observations
ABCA (ABC1)[a]	ABCA1	ABC1	Ubiquitous	Phospholipids, cholesterol	–Lipid trafficking –Cholesterol export to HDL pathway –Mutations are associated with Alzheimer disease
	ABCA2	ABC2	Brain, kidney, lung, heart	–Lipid transport –Estramustine, mitoxantrone	Drug resistance
	ABCA3		Alveolar type II cells	Anthracyclines	Associated with poor response to chemotherapy in cancer
	ABCA4	ABCR	Retina	N-retinylidene-phosphatidylethanolamine	
	ABCA5		Skeletal muscle, kidney, liver, placenta		Lysosomal trafficking (?)
	ABCA6		Ubiquitous with higher levels in liver, lung, heart and brain	Lipids	–Macrophage lipid homeosthasis –Associated with MDR in cancer
	ABCA7		Myelolymphatic tissues, keratinocytes	Lipids, cholesterol	–Lipid trafficking –Cholesterol export to HDL pathway
	ABCA8		Heart, skeletal muscle, liver	Some lipophilic drugs (?)	
	ABCA9		Diverse	Macrophage lipid homeostasis (?)	
	ABCA10		Diverse	Lipids, cholesterol	Lipid trafficking
	ABCA12		Keratinocytes, stomach	Lipids, cholesterol	Lipid trafficking
	ABCA13		Trachea, testis, bone marrow	Unknown	
ABCB (MDR)	ABCB1	P-gp	–Blood–brain, blood–testis and blood–nerve, fetal–maternal barriers, bile canalicular membrane of hepatocytes, intestinal epithelium, apical membrane of proximal tubules,	–Organic hydrophobic and amphipathic molecules (molecular weight between 200 and 1900 g/mol) –Anthracyclines, vinca alkaloids, Dox, docetaxel, paclitaxel, etoposide, mitoxantrone, methotrexate, phenytoin, saquinavir, ritonavir, nelfinavir, indinavir, saquinavir, atazanavir, lopinavir, amprenavir, nelfinavir, erythromycin, fexofenadine, hypericin, ivermectine, lansoprazol, loperamide, losartan, lovastatin, mibefradil, morphine, gramicidin D, valinomycin, digoxin, digitoxin, grepafloxacin, octreotide, phenytoin, tacrolimus, talinolol, terfenadine, vecuronium, colchicine, bisantrene, actinomycin D, verapamil[b], cyclosporine A[b]	Substrates usually enter cells by passive diffusion –Decreases oral absorption and bioavailability of substrates and increases biliary excretion –Associated with MDR in cancer and infectious diseases
	ABCB2	TAP1	Ubiquitous, endoplasmatic reticulum	Peptides	Associated with defective antigen presentation
	ABCB3	TAP2	Ubiquitous, endoplasmatic reticulum	Peptides	
	ABCB4		Apical membrane of hepatocytes	Anthracyclines, vinca alkaloids, taxanes, epipodophyllotoxins and mitoxantrone, phosphatidylcholine	Associated with liver disease
	ABCB5		Skin	Anthracyclines, camptothecins and thiopurines	Human malignant melanoma, colorectal cancer
	ABCB6		Ubiquitous, mitochondria	Iron	
	ABCB7		Ubiquitous, mitochondria	Fe/S cluster	
	ABCB8		Mitochondria	Peptides, phospholipids	
	ABCB9		Heart, brain		Found in lysosomes
	ABCB10		Mitochondria	Peptides, heme	Intermediates in heme/ biosynthesis (?)
	ABCB11		Apical plasma membrana of hepatocytes	–Taxones, bile salts –Paclitaxel	–Associated with liver disease –Drug resistance is low
ABCC (MRP)	ABCC1	MRP-1	Ubiquitous, basolateral membrane of epithelia	–Co-transports amphipathic organic anions and hydrophobic non-anionic molecules conjugated or complexed with reduced glutathione, glucuronic acid or sulfate –Anthracyclines, vinca alkaloids, etoposide, teniposide, topotecan, SN-38, melphalan, chlorambucil, methotrexate, non-organic heavy metal oxyanions	–Associated with drug resistance in cancer and infectious diseases –In non-small cell lung cancer

Table 1.7 (continued)

Subfamily	Subtype	Other name	Expression site	Main substrates	Observations
ABCC (MRP)	ABCC2	MRP-2	Canalicular apical membrane of hepatocyte, luminal membrane of small intestine (maximal in duodenum and decreases to colon), luminal membrane of the proximal tubules of kidney, placenta, blood–brain barrier	–Organic anions –Olmesartan, valsartan, vinblastine, vincristine, etoposide and their glucuronic acid and sulfate conjugates, ezetimibe glucuronide, methotrexate, cisplatin, rifampicin, sulfinpyrazone, ceftriaxone, camptothecins, mitoxantrone, saquinivir	–Distribution similar to P-gp –Partial overlapping of substrates with P-gp –Decreases oral absorption and bioavailability of substrates and increases biliary excretion –Associated with drug resistance in cancer
	ABCC3	MRP-3	Liver (basolateral membrane of hepatocytes, intra-hepatic bile duct epithelial cells), gallbladder epithelium, small and large intestine, adrenal gland, and to a lesser extent in pancreas and kidney (distal convoluted tubules, ascending loops of Henle)	–Organic anionic molecules –Partial overlapping with MRP-1 and MRP-2 –Methotrexate, etoposide,	–Inefficient transporter of glutathione conjugates. –Associated with Dox resistance in lung cancer cell lines
	ABCC4	MRP-4	Lung, kidney (proximal tubule), bladder, gallbladder, tonsil, prostate (basolateral membrane of acinar cells), lung, skeletal muscle, pancreas, spleen, thymus, testis, ovary, small intestine	–Organic anionic molecules –Cyclic nucleotides and nucleotide analogues, zidovudine, lamivudine, 6-mercaptopurine, thioguanine, camptothecins	–Thiopurines would be converted into the corresponding nucleotide before efflux –Potential role in cancer and antiviral therapy resistance
	ABCC5	MRP-5	Ubiquitous, skeletal muscle, brain	–Organic anionic molecules –Cyclic nucleotides and nucleotide analogues, 6-mercaptopurine, thioguanine, methotrexate, cisplatin, zidovudine	
	ABCC6	MRP-6	Liver, kidney	–Organic ions –Anthracyclines, cisplatin, epipodophyllotoxins	
	ABCC7	CFTR	Exocrine tissues	Chloride ion	–Chloride channel –Associated with cystic fibrosis
	ABCC8	SUR	Pancreas	Sulfonyl urea	–Sulfonyl urea receptor –ATP-sensing units of a complex K^+ channel
	ABCC9	SUR2	Heart, muscle		
	ABCC10	MRP-7	Ubiquitous	Vinca alkaloids, paclitaxel, docetaxel, Ara-C, gemcitabine, epothilone-B	Associated with MDR in cancer
	ABCC11	MRP-8	Ubiquitous in low concentration, mainly in testis, breast	Thiopurines	Drug resistance in breast cancer
	ABCC12	MRP-9	Ubiquitous in low concentration, mainly in testis, breast, ovary, brain, skeletal muscle		Associated with MDR in cancer
ABCD (ALD)	ABCD1	ALDP	Many	Very-long-chain fatty acyl-CoA esters	–Translocation of the acyl-CoA esters across the peroxisomal membrane –Mutations result in X-linked adrenoleukodystrophy
	ABCD2	ALDR		Very-long-chain fatty acyl-CoA esters (?)	
	ABCD3	PMP70			
	ABCD4	PMP69			
ABCE (OABP)	ABCE1	OABP	Ovary, testis, spleen	Binds organic anions	–Lacks transmembrane domain –Oligoadenylate binding protein –Promotes interferon activity –Not associated with disease
ABCF (GGN20)	ABCF1	ABC50	Ubiquitous		–Lack transmembrane domain –Bind ATP –Not associated with disease
	ABCF2				
	ABCF3			Cholesterol binding?	
	ABCF4				
ABCG (White)	ABCG1	White	Adipocyte, macrophage, liver, spleen, lung	Cholesterol, phospholipids, sphingomyelin, phosphatidylcholine	–Cholesterol export to HDL pathway –Heterodimerizes with ABCG4

Table 1.7 (continued)

Subfamily	Subtype	Other name	Expression site	Main substrates	Observations
ABCG (White)	ABCG2	BCRP	Hepatocyte, intestinal epithelium in small and large intestine, mammary gland, placenta, brain and blood–brain barrier endothelium, testis, blood cells	Toxic ions (Hoechst 33342, mitoxantrone, daunomycin, doxorubicin, 9-aminocamptothecin, topotecan, irinotecan, SN-38, erythromycin, methothrexate, etoposide, tetracycline, ofloxacin, norfloxacin, ciprofloxacin, nitrofurantoin, zidovudine, lamivudine, abacavir, zalcitabine, stavudine, efavirenz, imatinib, gefitinib, erlotinib, sterols, rosuvastatin, pitavastatin, pravastatin, cerivastatin, antihelmintic benzimidazoles	–Associated with MDR –Homodimerizes to form the functional pump –Associated with drug resistance in cancer and infectious diseases –MDR in leukaemia (controversial data), cancers of gastrointestinal tract, endometrium, lung, melanoma, breast (low)
	ABCG4	White2	Nervous system	Cholesterol	Heterodimerizes with ABCG1
	ABCG5 ABCG8	White3, Sterolin 1 Sterolin 2	Hepatocyte, enterocyte in small intestine and colon	Sitosterol, cholesterol (remains to be tested)	–Heterodimerize to form the functional pump –Limitation of sitosterol (and cholesterol) absorption in intestine and increased hepatobiliary excretion

[a] The nomenclature of ABCA pumps is inconsistent because a functional human ABCA11 gene does not exists [268].
[b] Competitive inhibitor of the same pump.

Table 1.8 Effect of herbs on transporters

Drug transporter	Anti-cancer substrates	Interacting herbal products
P-glycoprotein (ABCB-1, MDR-1)	Actinomycin D, daunorubicin, docetaxel, doxorubicin, etoposide, irinotecan, mitoxantrone, paclitaxel, teniposide, topotecan, vinblastine, vincristine, tamoxifen, mitomycin C, tipifarnib, epirubicin, bisantrene	*Rosmarinus officinalis*
MRP-1 (ABCC-1)	Etoposide, teniposide, vincristine, vinblastine, doxorubicin, daunorubicin, epirubicin, idarubicin, topotecan, irinotecan, mitoxantrone, chlorambucil, methotrexate, melphalan	*Curcuma longa*
MRP-2 (ABCC-2)	SN-38G (metabolite of irinotecan), methotrexate, sulfinpyrazone, vinblastine	Inchin-ko-to
BCRP (ABCG-2, MXR)	9-Aminocamptothecin, daunorubicin, epirubicin, etoposide, lurtotecan, mitoxantrone, SN-38, topotecan	Flavonoid-containing herbs such as *Glycine max* (soybean), *Gymnema sylvestre*, and *Cimicifuga racemosa* (black cohosh)

P-gp localised in the intestine acts a significant duty in limitation of xenobiotic absorption thus it leads to the low bioavailability of drugs in many diverse types of molecular structures, for example, cytotoxic agents, cyclosporine, antiviral drugs including protease inhibitors, and so on (Ampasavate et al., 2010). It restricts permeability through the GI tract by powerful pumping these potential toxic molecules back into the lumen of intestine (Hayeshi et al., 2006). Therefore, escapade of the deportation in the intestine using P-gp inhibitors may reduce the indispensable doses of these medications, then consequently the remedy expenses (Ampasavate et al., 2010).

This pump is expressed in other sites like the liver and blood-brain barrier where P-gp likewise acts as an essential function for excretion. Thus, when P-gp is inhibited that could result in adverse drug interactions at these spots since raised concentrations in cells or changed pharmacokinetics (Hayeshi et al., 2006). It has been discovered that many dietary compounds could modulate P-gp activity, guiding that these compounds could capably result in food-drug interaction. Nowadays, food-drug interactions, along with herb-drug interactions, have come into being a major concern (Ampasavate et al., 2010). Food-drug interactions, herb-drug interactions, as well as drug-drug interactions maybe mediated via either competitive inhibition by P-gp substrates or via non-competitive inhibition. Escalating of oral bioavailability of a digitalis like digoxin, and the selective beta-blocker talinolol which are P-gp substrates by some substances which acts P-gp inhibitors include quinidine, and verapamil have been studied. An opioid drug like loperamide does not affect the central nervous system at regular doses, but loperamide depressed respiration when administered in combination with an antimalarial drug, namely, quinidine, possibly by the inhibition of P-glycoprotein function (at the BBB) by quinidine. Examples of drug interactions caused by herbs, phytochemicals and compounds from food such as grapefruit and Seville orange juice, they have been illustrated their actions to raise the oral bioavailability of some agents, probably by inhibiting P-gp mediated drug delivery or CYP3A4 mediated drug metabolism or both. Quercetin is a flavonoid that caused a fatal interaction in pigs when it was taken orally in combination with digoxin. Presumably inhibition of P-gp at the apical site of epithelial lipid bilayer at lumen of the intestine by quercetin resulting in enhanced this digitalis absorption and consequently cardiotoxicity. The potential adverse events/interactions of combination of consumptions of herbs, food and drugs maybe henceforward aggravated because it is explicit that patients use more than one of these remedies and the results are unknown in most cases. Certain schemes have been proposed for the application of products from nature to particularly increase absorption in human intestine of low penetrable substances by standardised inhibition of P-gp by apricot extracts, hence operating positive application of food–drug interactions (Hayeshi et al., 2006). P-gp can likewise adhere another type of drugs frequently called multidrug resistance modulators that prefer the noticeable capacity of restraining or inducing P-gp action in pumping out a drug molecule. Because these MDR-modulatory agents could

command the deportation of therapeutic cytotoxic agents, these agents are consequently popular medicinal destinations (Jara et al., 2013).

1.1) P-glycoprotein-mediated drug efflux

Using ATP as an energy source, P-gp actively pumps compounds from within the epithelial cells returning into the intestinal lumen, and from the endothelial cells in the CNS like the brain back into the blood (Mandava et al., 2010; Pal & Mitra, 2006). Within hepatocytes in the liver, P-gp pumps mostly hydrophobic (neutral or positively charged) substances into the bile and this efflux occurs in many other organs as well (Pal & Mitra, 2006; Sadeque et al., 2000).

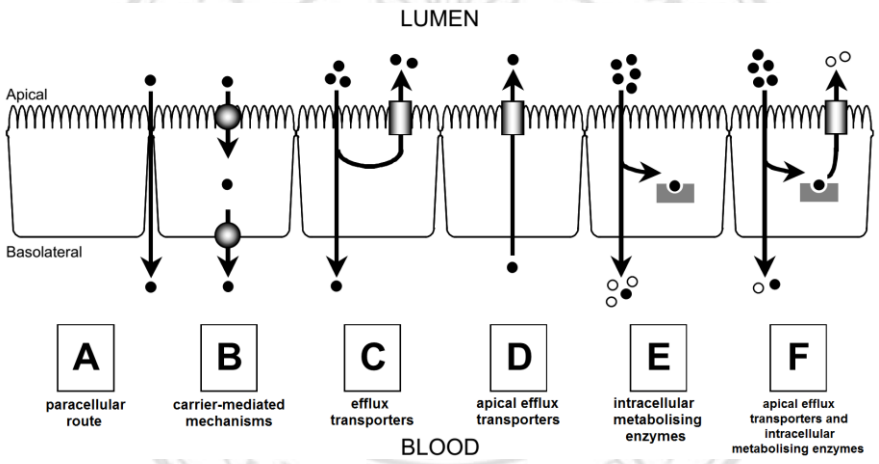

Figure 1.2 Schematic illustration of the intestinal epithelium and pathways of drug transport across it in both directions. C illustrates efflux of compounds reverting into the intestinal lumen thus restricting absorption of drug into the blood (Chan et al., 2004)

A possible mechanism for the action of P-gp on xenobiotics is that the transporter protein binds substrates via a *substrate-induced-fit* mechanism. *Substrate-induced fit* is when an appearance and size of the substrate compound alters the placing or cross-linking geometry of the TM segments within the P-gp molecule thus promoting structural accommodation for the same substrate (Chan et al., 2004; Loo et al., 2003). Inhibition of P-gp by compounds or phytochemicals may thus involve their direct interaction against one or more binding sites on this transporter through competitive, non-competitive or allosteric inhibition and/or induction. Phytochemicals may also inhibit binding of ATP against P-gp, hydrolysis or coupling of ATP hydrolysed molecules therefore depleting the energy which drives the translocation of P-gp-bound

substrate drugs (Salama et al., 2004). Phytoconstituents that induce P-gp could potentially increase the *substrate-induced-fit* of drugs and/or phytochemicals resulting in higher efflux of these P-gp substrates (Sun et al., 2004; Venkataramanan et al., 2006).

1.2) Inhibition of P-glycoprotein by herbal compounds

P-gp recognises various structurally and pharmacologically unlinked zero and positively charged; lipophilic molecules while MRP2 transports relative hydrophilic molecules, along with glucuronide, glutathione and sulphate conjugates of endogenous and exogenous molecules (Chan et al., 2004). Breast cancer resistance protein (BCRP) recognises relatively hydrophilic anticancer agents (Doyle & Ross, 2003). P-glycoprotein is the major explored amongst ABC transporters. It is expressed in a plenty of tissues as a constitutive transporter and intensified on the surfaces of epithelium at the apical side of the ductal cells of pancreas, hepatic bile canaliculi, renal proximal tubules, and columnar cells of mucosae of the small intestine, colon, and the suprarenal glands. It is energetically associated in the processed of drug pharmacokinetics including absorption and elimination from the intestinal, hepatic, renal and CNS systems particularly in the systems of hepatobiliary, straight drugs and their metabolites excretion from the intestine and in urine. Therefore, the inhibiting of P-gp by concomitant used herbal products prefers a potential for modification in the drug's pharmacokinetics. Pharmacokinetic interaction comes about when herbs or its ingredients restrain or reduce the drug transporters' regular action level via a competitive or noncompetitive implement. Interactions can likewise take place via the inducting toward transporters by way of the raise of the messenger RNA of the pertinent peptide. Research works have analysed some key P-glycoprotein inhibitors which have clinical effects along with chemicals from plants – Vinca alkaloids as vincristine and vinblastine, Cinchona alkaloids as quinidine, Indole alkaloids as reserpine and yohimbine, flavonoids, furanocoumarins, and so on. The transporters are energetically associated in the anticancer agents' pharmacokinetic profiles and expect for one of the notable ploys of cancer cells' multiple resistance to chemotherapeutic molecules (Fasinu et al., 2012). The examples of some narrow therapeutic index drugs that interact with herbal compounds by inhibit P-gp function resulting in increase of drug level in the body are shown in Table 1.9 and 1.10.

Table 1.9 *In vivo* effects of phytochemicals on pharmacokinetics of some nonmetabolised P-gp substrates (Li et al., 2010)

Substrate	Route of administration	inhibitor/inducer*	Route of administration	Species	Pharmacokinetic effect
Digoxin	Oral	Monoterpenoids	Oral	Rat	Increased bioavailability to 90%
Digoxin	Oral	Quercetin	Oral	Pig	Increased C_{max} and AUC 4.3- and 1.7-fold, respectively
Digoxin	Oral	Grapefruit juice	Oral	Human	9% increase in the digoxin AUC from time zero to 4 hours and from time zero to 24 hours; no change in C_{max}
Digoxin	Oral	St. John's wort*	Oral	Human	0.72-fold decrease in C_{max}
Paclitaxel	Oral	Quercetin, flavone	Oral	Rat	The relative bioavailability increased by 2.4–3.1-fold
Irinotecan	Oral	Quercetin	Oral	Rat	Increased C_{max} and AUC, with a concomitant decrease in T_{max}, plasma clearance, and volume of distribution

Table 1.10 P-gp mediated herb interactions with anticancer drugs (Bansal et al., 2009)

Source	Herbal Constituents	Anti-cancer drug	Model	Pharmacological outcome
Curcuma longa	Curcumin	Rhodamine-123	Primary cultures of rat hepatocytes	Curcumin inhibited Rhodamine-123 efflux
		Vinblastine	Drug-resistant KB-V1 cells	Increased sensitivity to vinblastine
Allium sativum **(Garlic)**	Sulfur-containing compounds, numerous flavonoids/isoflavonoids (such as nobiletin, quercetin, rutin, and tangeretin)	NA	Recombinant human P-gp membranes	Inhibit the activities of Pgp
Panax ginseng **(Ginseng)**	Ginsenosides	Rhodamine 123, vinblastine, doxorubicin	Multidrug-resistant KBV20C cells	Ginsenoside Rg3 promoted accumulation of rhodamine 123, inhibited vinblastine efflux, and reversed the resistance to doxorubicin and vincristine in
Grapefruit Juice	Flavonoids	Rhodamine 123, vinblastine, doxorubicin	Caco-2 cells	Inhibit drug uptake
Camellia sinensis **(Green tea)**	Catechins (flavanols)	Doxorubicin	Mice bearing doxorubicin-resistant P388 leukemia	Increased the efficacy
		Vinblastine	$CH^{R}C5$ cells	Potentiated the cytotoxicity
Sylibum marianum **(Milk thistle)**	Flavonolignans (Silymarin)	Daunomycin	P-gp positive cells	Increased daunomycin accumulation
Piper nigrum	Piperine	Cyclosporine	Caco-2 cells	Inhibited drug transport
		Cyclosporin	LS-180 intestinal carcinoma cells; rats; healthy volunteers	Induces intestinal Pgp in vitro and in vivo
Hypericum perforatum **(St. John's wort)**	Hyperforin and hypericin	Irinotecan	Healthy volunteers	Increased SN-38 plasma concentrations
		Irinotecan	Rats	Ameliorated the gastrointestinal and hematological toxicities of irinotecan along with significant alterations in pharmacokinetics of irinotecan and SN-38
Rosmarinus officinalis **(Rosemary)**	Methanol-extracted fraction	Doxorubicin and vinblastine	Drug-resistant P-gp expressing MCF-7 cells	Increased intracellular drug accumulation

1.2.6 Interactions of Thai herbs with P-gp through inhibition

Thai herbs that have been reported to inhibit function of P-gp for drug extrusion are listed in Table 1.11.

Table 1.11 List of P-gp inhibition of pharmacokinetic interactions between Thai herbs and drugs

Herb	Thai name	Type of study	Isolated constituent	Result	Ref.
Kaempferia parviflora	Krachaidum	*In vitro* using P-gp expression cell line	Ethyl alcohol and water extracts of rhizome and including 6 flavone derivatives from rhizome; 3,5,7,3′,4′-pentamethoxyflavone and 5,7-dimethoxyflavone	Enhanced substrates as rhodamine 123 and anticancer drug; daunorubicin accumulation	a
Curcuma longa Linn	Khaminchan	*In vitro* using P-glycoprotein-mediated [^{3}H]-digoxin transport in human multidrug resistance 1 gene expressed cell lines	Curcumin, demethoxycurcumin and bisdemethoxycurcumin (They are classified into curcuminoids.)	- Only curcumin and demethoxycurcumin may restrain P-glycoprotein efflux action. - Curcuminoids inhibit rhodamine 123 (P-gp substrate) outflow from serosal to mucosal surfaces of the rat ileum	b, c
Curcuma sp.	Khamin-oi				
		Rat	Curcumin	AUC↑, C_{max}↓, CL_{oral}↓ of celiprolol	d
Stemona burkillii	Non Tai Yak	*In vitro* using cells multidrug resistance human cervical carcinoma with P-glycoprotein expression (KB-V1)	Stemofoline (*Stemona* alkaloid) from root	raise sensitiveness of anticancer agents as Vinca alkaloid like vinblastine, paclitaxel and doxorubicin in either dose and time-dependent features within cell line	e
Stemona curtisii	Non Tai Yak	*In vitro* using MDR human cervical carcinoma with high P-glycoprotein expression (KB-V1 cells)	Ethanol root extract	- The extract reversed the vinblastine, paclitaxel and colchicine resistances of cells dose-dependently. - The extract raised the intracellular uptake and retaining of [^{3}H]-vinblastine inside the cells in a dose dependent manner.	f
Allium sativum	Kra Tium.	*In vitro* colourmetric ATPase assay	Crude extracts and oils	Very low to moderate inhibition of P-gp as compared with verapamil (positive control)	g
Ginseng	Som	various *in vitro* experiments	- Ginsenoside Rg1, Rc, Rd, and Re - Rg3 (only in red ginseng)	- Moderate P-gp inhibition - Rg3 restrain vinblastine outflow and recede MDR to doxorubicin and VP-16 in the cultured cell. - Blocking of drug efflux by Rg3 via direct binding to P-gp	d

Table 1.11 (continued)

Herb	Thai name	Type of study	Isolated constituent	Result	Ref.
Ginkgo biloba	Pae-Gouy	Healthy volunteers	- Crude extract - Flavone glycosides (quercetin, kaempferol, isorhamnetin) and Terpene lactones (ginkgolide A, ginkgolide B, ginkgolide C and bilobalides)	- Increased in the C_{max} and bioavailability of raltegravir - Chronic use of *ginkgo biloba* extract increases of the C_{max} by 33% and the AUC by 21% of talinolol minus any remarkable changes in the T_{max} and $T_{1/2}$	h, i
Piper nigrum and Piper longum	Prik-thai and Di pli	Patients	Piperine	- ↑AUC, ↑C_{max} and - ↑k_a of Phenytoin - C_{max}↓ of Rifampin	d
Camellia sinensis	Cha	*In vitro*: MDR cell CH^R C5 and human Caco-2 cells	Polyphenols/catechins: (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-catechin gallate (CG)	- Increase the accumulation of rhodamine-123 3-fold - EGCG potentiates the cytotoxicity of vinblastine. - EGCG also inhibits P-gp in human Caco-2 cells.	j
		Sprague–Dawley rats	(-)-epigallocatechin-3-gallate (EGCG)	- The increases of AUCs were observed by 57.7% (irinotecan) and 18.3% (its metabolite SN-38). - AUC in bile were reduced by 15.8 and 46.8%, successively. - The decrease of blood to bile distribution ratio (AUC_{bile}/AUC_{blood}) was observed.	k
Zingiber officinale	Khing	*In vitro* using human multidrug-resistant carcinoma KB-C2 cells with daunorubicin and rhodamine 123 (fluorescent substrates)	[6]-gingerol	The accumulations of daunorubicin and rhodamine 123 inside the cells increased in the possession of [6]-gingerol in a concentration-dependent manner.	l
Psidium guajava L.	Farang	*In vitro* using - the outflow transport of rhodamine 123 in Caco-2 cells - transport across everted rat ileum	Dried leaves macerated with 95% ethanol	- P-glycoprotein mediated rhodamine 123 outflow in cells was inhibited. - Rhodamine 123 outflow from serosal to mucosal surfaces of the rat ileum was inhibited.	c
Andrographis paniculata	Fa Thalai Chon	*In vitro* using the outflow transport of rhodamine 123 in Caco-2 cells	Dried leaves macerated with 95% ethanol	P-glycoprotein mediated rhodamine 123 outflow in cells was inhibited.	c
Phyllanthus emblica L.	Ma Kham Pom	*In vitro* using the outflow transport of rhodamine 123 in Caco-2 cells	Dried fruits macerated with 80% ethanol	P-glycoprotein mediated rhodamine 123 outflow in cells was inhibited.	c
Solanum trilobatum L.	Mawaeng Krueo	*In vitro* using the outflow transport of rhodamine 123 in Caco-2 cells	Dried fruits macerated with 95% ethanol	P-glycoprotein mediated rhodamine 123 outflow in cells was inhibited.	c

Table 1.11 (continued)

Herb	Thai name	Type of study	Isolated constituent	Result	Ref.
Momordica charantia L.	Mara	- *In vitro* using multidrug resistance cervical carcinoma cell line (KB-V1) - Caco-2 cells	- Kuguacin J - 1-monopalmitin	- The ethanol leaf extracts raised the accumulating of [^{3}H]-vinblastine inside the KB-V1 cells dose-dependently. - Kuguacin J raisesd sensitivity to anticancer drug like vinblastine and paclitaxel of KB-V1 cells. - Kuguacin J inhibits the P-gp action resulting in raising the rhodamine 123 and calcein AM accumulating inside the cells. - Kuguacin J raises [^{3}H]-vinblastine accumulating inside the cells and reduced the [^{3}H]-vinblastine outflow inside the cells. - Kuguacin J concentration-dependently restrained the incorporation of [^{125}I]-iodoarylazidoprazosin into P-glycoprotein. - 40% methanol extract raised the rhodamine-123 accumulating in Caco-2 cells. - 1-monopalmitin and some related compounds increased the [^{3}H]-daunomycin accumulating in Caco-2 cells.	m, n, o
Ganoderma lucidum	Lingzhi	- Adriamycin (ADM)-resistant leukemic cell line (K562/ADM) - HepG2/ADM cells - ABCB1-overexpressing MCF-7/ADR cells	- Polysaccharides - A lanostane-type triterpene; Ganoderenic acid B	- *Ganoderma lucidum* polysaccharides clearly reversed the doxorubicin resistance of K562/ADM. - Ganoderenic acid B exhibited potent reversal effect on ABCB1-mediated multidrug resistance of HepG2/ADM cells to doxorubicin, vincristine and paclitaxel. - Ganoderenic acid B could reverse the resistance of ABCB1-overexpressing MCF-7/ADR cells to doxorubicin. - Ganoderenic acid B enhanced intracellular rhodamine-123 accumulation in HepG2/ADM cells through inhibition of its efflux. - Ganoderenic acid B didn't alter the expression level of ABCB1 and the activity of ABCB1 ATPase. - Molecular docking study revealed that the positions of ganoderenic acid B binding to ABCB1 were different from the region of verapamil interacted with ABCB1.	p, q

Table 1.11 (continued)

Herb	Thai name	Type of study	Isolated constituent	Result	Ref.
Phyllanthus amarus	Luk Tai Bai	*In vitro* using Caco-2 cells	Lignans; phyllanthin and hypophyllanthin	- Hypophyllanthin and phyllanthin directly inhibited P-glycoprotein action with comparable potencies resulting in increasing intracellular accumulating of substrates including calcein acetoxymethyl ester and 5(6)-carboxy-2′,7′-dichlorofluorescein diacetate.	r
Gynostemma pentaphyllum	Pan Cha Khan	P-gp-overexpressed CEM/VLB_{100} cells	Total gypenoside extract	- Total gypenoside preparation ~15-fold reversed colchicine (COL) resistance . - A purified gypenoside sample ~42-fold reversed COL resistance.	s
Tiliacora triandra	Yanang	Multidrug resistance human non-small cell lung carcinoma cell line with a high P-glycoprotein expression level (A549RT-eto)	Mixture of hexadecanoic: octadecanoic acid: (Z)-6-octadecenoic acids	- Sensitisation to anticancer drug; etoposide - The raising of the relative rate of rhodamine-123 accumulating was observed.	t
Rhinacanthus nasutus (L.) Kurz	Tong Pan Chang	Caco-2 cells	Rhinacanthin-C	- Rhinacanthin-C increased the calcein and CDCF accumulation in Caco-2 cells, guiding the inhibitory activity to P-glycoprotein function of the compound.	u

a=(Patanasethanont et al., 2007), b=(Ampasavate et al., 2010), c=(Junyaprasert et al., 2006), d=(An & Morris, 2010), e=(Chanmahasathien et al., 2011), f=(Limtrakul et al., 2007), g=(Foster et al., 2001), h=(Blonk et al., 2012), i=(Kudolo et al., 2006), j=(Jodoin et al., 2002), k=(Lin et al., 2008), l=(Nabekura et al., 2005), m=(Pitchakarn et al., 2012), n=(Limtrakul et al., 2004), o=(Konishi et al., 2004), p=(Li et al., 2008), q=(Liu et al., 2015), r=(Sukhaphirom et al., 2013), s=(Huang et al., 2007), t=(Kaewpiboon et al., 2014), u=(Wongwanakul et al., 2013)

From Table 1.11, many frequently used herbs in Thai traditional medicines have been illustrated their P-gp inhibitory activity in *in vitro, in vivo* and including clinical studies. However, the proofs of their safety by clinical studies are not sufficient. Herb-drug interaction warning is very important for patient who takes Thai traditional medicines with conventional drug particularly very narrow therapeutic index drugs which are P-glycoprotein substrates. At the present day, the information from *in vitro* and *in vivo* studies is enough to point out that herb-drug interactions are able to emerge and may result in serious adverse drug events or other clinical outcomes. The future study should be performed to identify these herb-drug interactions but in the experimental studies including *in vitro, in vivo* studies demand a lot of money and time consuming, especially the studies in humans that must be approved by ethics committees with many complicated procedures. Thus the computation study for

prediction of herb-drug interaction has become an interesting remark with the faster, saver expenses, lesser time consuming than conventional methods and requirement of facilities that is convenient to obtain information and latter provide proper advices to patients on their utilising of herbs.

1.2.7 P-glycoprotein

The efflux pump P-glycoprotein (other names; P-gp, ABCB1, and MDR1), Juliano and Ling are the first researchers who discovered MDR1 in 1976 as a surface glycoprotein in the plasma membrane of Chinese hamster ovary cells expressing the multi-drug resistance (MDR) phenotype which is selected in culture for colchicines resistance (Linardi & Natalini, 2006; Marchetti et al., 2007). P-gp is enciphered by the multi-drug resistance (MDR1) gene, likewise recognised as ABCB1 gene. Multi-drug resistance is a glossary that explains the cross-resistance of cells versus various groups of drug with distinct types of structure and targets and a function of MDR1 constrains a broad variety of drugs from crossing lipid bilayer and placing them into the extracellular space (Linardi & Natalini, 2006). ABCB1 is a 170 kilodaltons transmembrane glycoprotein. The human ABCB1 gene that is located on chromosome 7q21 enciphered P-gp pump (Xu et al., 2012). The MDR1 genome carries 29 exons that express a 3843 base pairs sequence of transcripts and after that in the protein translation process, the 1280 amino acids P-gp protein will be encoded (Yang et al., 2008). P-gp was the 1st identified eukaryotic ABC pump and it grants multidrug resistance (MDR) to cancer cells that is clinically important (Sauna & Ambudkar, 2007).

1) Tissue localisation and physiological role

The anatomic localisation of ABCB1 in divers tumors (it entails the MDR phenotype by transporting molecules for instance anticancer agents out of tumor cells to achieve sub-lethal drug levels). Furthermore, ABCB1 is localised at the polarised cell's apical (luminal) membrane in various plain human organs along excretory (kidney, suprarenal gland, liver) and barrier (placenta, blood–brain barrier, intestine, blood–ovarian barriers and blood–testis) roles, suggesting that it may have a physiological function in the process of removing toxic substances, elimination and safeguarding of the body versus noxious xenobiotics and metabolites by expelling such substances into

the intestinal lumen, urine, and bile and by keeping out storing of these substances in the brain, testis, and fetus (Marchetti et al., 2007).

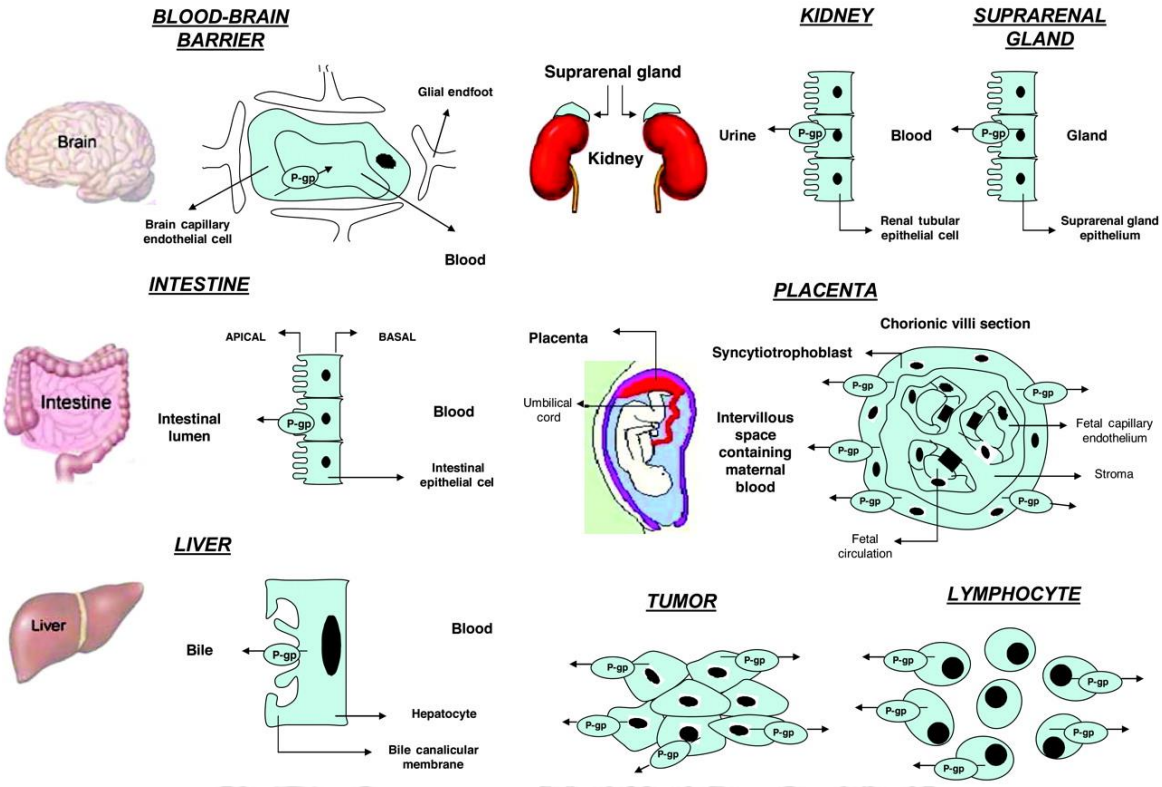

Figure 1.3 Drawing of the major locations of P-glycoprotein in the body (Marchetti et al., 2007)

The ABCB1's role presented in the placenta of the mice and in the adrenal cortex of suprarenal gland may involve in steroid secretion of the body. This pump on the cell membrane of endothelium at blood-tissue barrier sites, for example, the blood-brain barrier, it could guard the brain from circulatory xenobiotics, along with anti-cancer agents. Moreover, this transporter has been discovered to be working in human hematopoietic stem cells, pointing out its support to the resistance to chemotherapeutic agents of these cells. Expression of P-gp in certain peripheral blood mononuclear cells (PBMCs), for example, cytotoxic lymphocytes; including cytolytic T cells and natural killer cells, indicates their involvement in cell mediated cytotoxicity. ABCB1 is also shown its localisation in columnar cells of epithelium of lower gastrointestinal tract (GIT), canalicular surface of hepatocytes, capillary cells of endothelium of brain and testis and apical surface of renal proximal tubule by immunohistochemical staining method. Because of selective pervading of ABCB1 at the drug entree and leave

accesses, this is presumed that, in a wide variety of ordinary tissues, ABCB1 could act an important physiological part in absorption, distribution and excretion of xenobiotics and substrates produced inside the body (Bansal et al., 2009). ABCB1 mediated efflux influences each step which a drug encounter during its stay in the body. It affects absorption through intestinal carriers, which oust drug molecules rearward into the lumen; distribution, by shielding drug entree into tissues like brain; metabolism, like it takes action synergistically with cytochrome P450 3A4 (CYP 3A); excretion, by influencing twain biliary and renal tubular function. Like this, ABCB1 acts as a barrier which prevents entry of xenobiotics in the body and expelled out them once they have entered and protect the cell or tissue from cytotoxic substances, hold toxic substances in blood circulation (Gupta et al., 2014).

P-gp expression is regularly elevated and constitutive in tumors. According to it associates in physiological phenomenon in normal cell, protect cells from harmful substances and expelled out toxic substances which have entered earlier. But in cancerous cell, this P-gp transporter expelled out cytotoxic drugs from cytoplasm leading to development of resistance. In fact, expression of P-gp in tumors is moderate but its expression increases only when chemotherapy is started particularly in case of breast tumors, acute myeloid leukemias, lymphomas and myelomas. In this manner, this P-gp acts as a barrier in cancer chemotherapy. Positiveness of ABCB1 in certain cancers is participated along raised degrees of other drug resistance markers, for example, expression of another drug transporter; multidrug resistance-associated protein (MRP) and glutathione-S-transferase (π class). A robust correlation of raised degrees of P-gp expression with relapse has been premised in acute lymphoblastic leukemia, neuroblastoma and pediatric soft tissue sarcomas (Gupta et al., 2014).

2) P-gp molecular structure

Models describing the MDR1/P-gp function are convinced on biochemical experiments, mutagenesis studies, X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, bioinformatics-based approaches, and the atomic level structures of many other ABC proteins that can help in elucidation of the functional mechanism of a protein (Sarkadi et al., 2006).

2.1) Topology of P-gp

P-gp is one of the most significant pump superfamily member of fifty-one human genes that encipher ATP-binding cassette (ABC) transporters, utilising the energy from hydrolysis of adenosine triphosphate to actively eliminate molecules athwart a lipid membrane (Desai et al., 2013). ABCB1 is a single chain polypeptide with 1280 amino acid residues stowing 2 homologous portions of equal length, comprising 2 six-helix trans-membrane domains (TMDs) and 2 cytoplasmic nucleotide-binding domains (NBDs) or ATP-binding regions in a N-TMD1-NBD1-TMD2-NBD2-C topology (Figure 1.4). The approximately sixty amino acids form a cytoplasmic linker sequence that links the 2 halves of ABCB1 protein betwixt NBD1 and TMD2. Each transmembrane domain comprises 6 transmembrane helices. It responses to bind substrates and to release carried substrates. Transported substrates including drugs bind to their binding sites organised by interplaying between transmembrane helices. Some binding sites may be big enough to facilitate more than one substrate molecule in one cycle. ATP hydrolysis is aroused in the existence of drug that is delivered, pointing out straight pairing of molecule transport and ATP hydrolysis. ATP hydrolysis most possibly arises by way of a changing site mechanism. Erection and demolition of the catalytic transition state would be straight paired with drug transportation through lipid bilayer of cell. The nucleotide binding domains partake high sequence and structural conservation betwixt members of ATP-binding cassette transporter family essential to achieve their roles as adenosine triphosphate-hydrolysing machines. The true nucleotide binding sites of the transporter are partaken betwixt the nucleotide binding domains and therefore are configured merely upon solid association of the nucleotide binding domains. These sites are liable for binding and hydrolysis of adenosine triphosphate and are constituted of various high conserved consensus binding/hydrolysis motifs. These consist the Walker-A and LSGGQ signature motifs (that sandwich nucleotide), the Walker-B, A-loop, D-loop, Q-loop and switch motifs. Hydrolysis of adenosine triphosphate is strongly coupled to and provoked by binding of substrate and is believed to emerge at alternate sites in 2 different phases. A conceivable conformational asymmetry at the NBDs is suggested by this mechanism (Ma & Biggin, 2013; Wise, 2012)

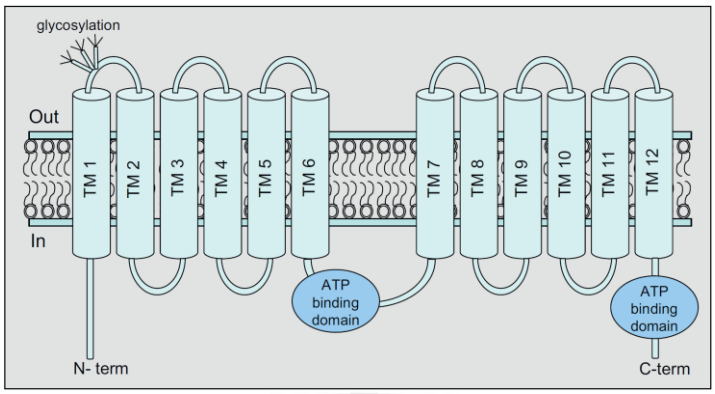

Figure 1.4 P-gp molecular structure model (TM: transmembrane domain) (Fu & Arias, 2012)

2.2) Homology models of P-glycoprotein

In the lack of data about the high resolution structure of P-gp, an option is to create a protein homology model that gives a three-dimensional map of a protein. The availableness of a target protein structures at the molecular level is a crucial factor for the approach of structure-based drug design. Protein homology models are relied on the presumption of structural homology betwixt a structurally resolved protein, the template and a protein of unidentified conformation, the target. The important factors that must be considered for the selection of templates for modelling of membrane proteins are sequence homology and the identical number of predicted transmembrane spanning helices (Ecker et al., 2008).

3) The molecular mechanism of P-gp action

It is believed that twain the amino and carboxyl terminal adenosine triphosphate-binding domains can hydrolyse adenosine triphosphate. Nevertheless, there is no confirmation that ATP can be hydrolysed at the same time by both sites. Adenosine triphosphate-binding domain(s) located in the cytosol side are likewise known as nucleotide-binding folds (NBFs); they shift the energy to transpose the substrates athwart the membranes. Each adenosine triphosphate-binding domain fills 3 regions that are Walker A, B, and signature C motifs. High conserved lysine residue inside the walker A motif of histidine permease is straight associated with the adenosine triphosphate binding and a high conserved aspartic acid residue inside the walker B motif operates to bind the Mg^{2+} ion. *Homo sapiens* ABCB1 demands dual (Mg^{2+})-

adenosine triphosphate-binding and hydrolysis to operate its drug transporter function. Mg^{2+} that could act a part in stabilising the adenosine triphosphate-binding site also has been suggestive. Signature C motifs presumably take part to accelerate adenosine triphosphate hydrolysis by way of chemical transition state interaction and are likewise guided to be associated in the energy transduction of adenosine triphosphate hydrolysis to the conformational alterations in the membrane integral domains needed for the substrate translocation. Distinct the adenosine triphosphate-binding sites that are constrained to Walker A motifs of adenosine triphosphate-binding domains, several substrate binding sites have been explored all over the transmembrane domain (TMDs) of ABCB1. The key drug binding sites dwell in or close TM6 and TM12. Besides this, TM1, TM4, TM10, and TM11 have drug-binding sites. Amino acids in TM1 are associated with the binding pocket formation that acts a part in defining the proper substrate size for P-glycoprotein, whereas glycine residues in TM 2 and 3 are essential in defining specificity of P-gp substrate. The close proximity of TM2/TM11 and TM5/TM8 points out that these regions betwixt the 2 halves must surround the drug-binding pocket at the cytoplasmic side of ABCB1 transporter. They may produce the ‘‘hinges’’ needed for conformational alterations throughout the duration of the transportation cycle. Beyond the TMDs, intracellular loops and even adenosine triphosphate-binding domains possess drug-binding sites (Gupta et al., 2014).

The initial step in deportation of drug out of a cell is recognition of drug molecule by P-glycoprotein come after by binding and hydrolysis of adenosine triphosphate. The key drug binding sites dwell in or close TM1, TM4, TM6, TM10, TM11, and TM12. There is the setting up of a binding pocket which acts a part in determination of the appropriate substrate drug size for this transporter and, consequently, substrate specificity. The energy unleashed in this physiological process is utilised to extrude substrate outwardly the plasma membrane via centric pore. 2 adenosine triphosphate molecules are hydrolysed for the transportation of each substrate molecule, one of proposed model is that 1 adenosine triphosphate molecule in the transportation of substrate and another adenosine triphosphate molecule in making alterations of protein conformations of the transporter to retune the transporter for the next catalytic cycle (Gupta et al., 2014). The mechanism by which P-gp operates its efflux transporter action is not fully understood even there are many models to elucidate this process

(Lopez & Martinez-Luis, 2014). There are 3 models of P-gp-mediated drug efflux overrun in the present, for example “the classical pore pump model,” “flippase model,” and “hydrophobic vacuum cleaner (HVC) model”. In the “classical pump model,” or “pore model,” the transporter carries substrates inwardly to the cytoplasm and then expels substrate molecules outside the cell via a water protein channel into extracellular space. On the contrary, in the flippase model (Figure 1.5), hydrophobic substrates must be implanted in the inner leaflet of the cell membrane, consequently binding to the protein inside the membrane plane and afterwards flipping the substrates to the outer leaflet of the lipid bilayer (Li et al., 2010). This mechanism is on the basis of the ascription that drug molecules in the cell membrane’s external and internal medium ought to be at equilibrium with the plasma membrane’s outer and inner leaflet. Depending on this equilibrium, P-glycoprotein swaps drug molecules from the cell membrane’s inner leaflet to the outer leaflet (Lopez & Martinez-Luis, 2014). The largely recognised “hydrophobic vacuum cleaner model” (Figure 1.6) compiles the main specialties of the 2 prior models and proposes that P-gp which places 2 portals inside the cavity of ABCB1 transporter that link to the inner leaflet of the cell membrane can gather lipophilic substrates from any parts of the cell membrane and move them to another place through the cavity (proffered central channel). ABCB1 therefore drives its substrates from the inner leaflet of the cell membrane (lipid bilayer) and carries these molecules out to the extracellular bulk aqueous phase (Li et al., 2010; Lopez & Martinez-Luis, 2014). A specific important aspect for consideration in the mechanism of ABCB1 efflux action is the part of adenosine triphosphate in stimulation of the transporter protein. The proffered mechanism is that 2 ATP molecules are needed in the first step of protein stimulation. The suggested mechanism from the another previous study is that it likewise associates 2 ATP molecules, but 1 molecule of ATP is tightly bound and another molecule mildly bound to the protein, providing 2 particular substrate-binding domains, 1 with high affinity and another with low affinity. Another proposed model is that 1 ATP molecule activates the P-gp efflux transporter to expel drugs away the transporter protein, and a 2nd ATP molecule converts P-glycoprotein to the inward-facing (initial) conformation where it anew binds with substrate and nucleotide to begin the coming cycle (Lopez & Martinez-Luis, 2014).

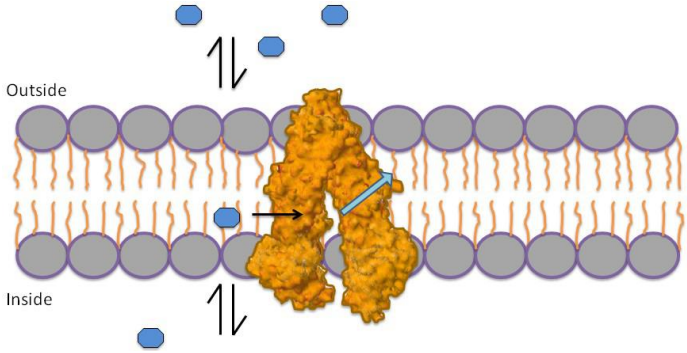

Figure 1.5 Flippase model. P-gp reciprocates substrate molecules from the cell membrane's inner leaflet to the outer leaflet, so that it keeps a concentration balance of the substrate on both sides (Lopez & Martinez-Luis, 2014)

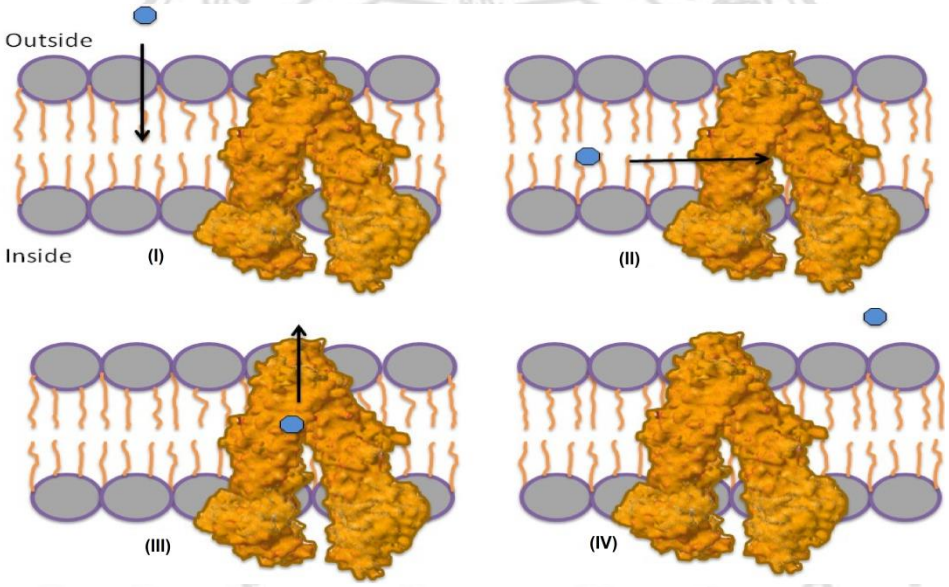

Figure 1.6 Hydrophobic vacuum cleaner mechanism. (I) Substrate molecule penetrate the cell membrane to the lipid bilayer; (II) the substrate molecule can get in the P-gp via portals that impart its substrate from the lipid bilayer into the internal cavity of P-gp; (III) the substrate molecule bind to their binding site; (IV) P-gp transposes the substrate molecule to the extracellular space (Lopez & Martinez-Luis, 2014)

P-gp owns an intrinsic ATPase activity in the lack of exogenous substrates. Furthermore, substrates of P-glycoprotein are capable to positively or negatively change ATPase action and, hence, the rate of adenosine triphosphate hydrolysis. The P-gp ATPase activity modulating compounds (compounds that increase or decrease ATPase activity of P-gp) have been classified into 3 groups: *I. the first group* includes

compounds that provoke basal ATPase activity of P-gp at low concentrations and inhibit the activity at high concentrations (for instance, calcium channel blocker verapamil, paclitaxel, and vinblastine); *II the second group* includes compounds that raise ATPase activity of P-gp in a dose-dependent feature (for instance, valinomycin, tetraphenylphosphonium and bisantrene); and *III the third group* encloses compounds that inhibit twain basal and compound-triggered ATPase activity of P-gp (for instance, gramicidin D, cyclosporin A, and rapamycin) (Lopez & Martinez-Luis, 2014).

4) Integrated action of P-gp and CYP3A4

Beyond oxidative metabolism, conjugation reactions may function a major role in the xenobiotic detoxifications from small intestine that activated xenobiotic metabolites are conjugated with charged species such as glucuronic acid, glutathione (GSH), glycine, or sulphate. Many drug molecules are extruded into intestinal lumen after these molecules are conjugated. The transportation system liable for the expulsion of the conjugated metabolites and organic anions from intracellular to extracellular has just been characterised. Herbs can take action as inhibitors or inducers that affect pharmacokinetics of drugs, when some conventional drugs are concurrently administrated. A realisation of the raised/reduced bioavailability of one drug in the possession of herbs may highly succour in the design of proper drug regimen. Co-administration of herbs and drugs may result in increasing absorption by reason of P-gp-mediated efflux and CYP-mediated metabolism restraining resulting in potential toxicities. The effects of some drugs on P-gp and CYP3A4 are summarised in Table 1.12 (Pal & Mitra, 2006).

Table 1.12 P-gp and CYP-mediated herb-drug interaction

P-gp- and CYP-medicated drug–herbal interaction		
Drug/herbal	**CYP(s) substrate**	**P-gp substrate**
Irinotecan(anticancer drug)	3A4, 3A5	+
Fexofenadine	3A4	+
Indinavir, ritonavir, saquinavir, lopinavir (anti-HIV agents)	3A4	+
Cyclosporine(immunosuppressant)	3A4	+
Simvastatin, pravastatin (cardiovascular drug)	3A4	+

A striking coincidence of the substrates of CYP3A and P-glycoprotein, including a wide variety of lipophilic therapeutic compounds (and many anticancer drugs), and

their tissue distributions has been observed. These observations led us to guide that CYP3A and P-gp might act complementary parts in drug disposition by biotransformation (phase I) and antitransport (phase III), particularly in the villi of the small intestine, where CYP3A and P-gp take action synergistically as an oral drug bioavailability barrier. Several pharmacokinetic studies using CYP3A and/or ABCB1 inhibitors illustrated the importance of CYP3A and ABCB1 in drug absorption and disposition (Zhang et al., 1998).

In the intestine during a process of absorption, a molecule of drug confronts P-gp, before placing contact with the CYP3A4 (Benet et al., 2004).

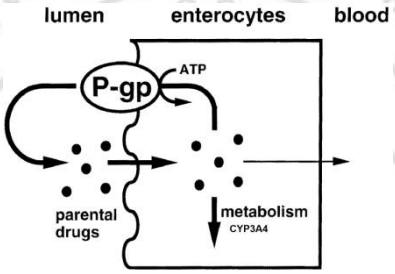

Figure 1.7 The showing the synergistic role of CYP3A enzymes and the efflux transporter P-glycoprotein (P-gp). After being uptaken by enterocytes, some of the drug molecules are metabolised by CYP3A4. Drug molecules which get away metabolic conversion are disposed from the cells into the lumen via P-gp (Suzuki & Sugiyama, 2000)

5) Importance of P-gp in therapy

P-glycoprotein has an important clinical relevance by reason of its role in pharmacokinetic of drugs. P-gp influences the drug absorption, distribution and secretion, and it possesses a significant part in malignant neoplasm's multidrug resistance (MDR). This pump deducts the clinical efficacy of various drugs (antihistamines, HIV protease inhibitors, antineoplastic, antiarrhythmics, Ca^{2+} channel blockers, antibiotics, hypocholesterolaemiants, antidepressants, antiepileptics, steroids, cardiac glycosides, antihypertensives and immunosuppressants) by alteration pharmacokinetics including the absorption and distribution of these drugs in tissues. P-gp's overexpressed in many human tumors and it's a significant obstacle to accomplish in remedies of cancer. Noticeably, ABCB1 in tumors likewise presents to

impart resistance to apoptosis triggered by distinct stimuli, like tumor necrosis factor, serum starvation, ultraviolet B- and gamma-irradiation, and Fas. However, the mechanism that ABCB1 inhibits apoptosis is still ambiguous. In acquired immune efficiency syndrome patients, ABCB1 advocates to resist to protease inhibitors, like saquinavir, nelfinavir, ritonavir and indinavir. ABCB1 is likewise related to multidrug resistance in certain human parasitic infections such as *Entamoeba histolytica, Trypanosoma cruzi*, *Leishmania tropica, Leishmania amazonensis* and *Plasmodium falciparum* infections (Lopez & Martinez-Luis, 2014).

6) Substrates of P-gp

Like CYP3A4, P-gp is non-specific for broad various drugs from a lot of distinct indication fields (Table 1). Furthermore, there is an interpose of the specificity of substrate especially of cytostatics with other ATP-binding cassette transporters like ABCC1, ABCC2, and ABCG2 and a wide interpose with the drug-metabolising enzyme cytochrome P450 3A4, producing ABCB1 and cytochrome P450 3A4 a synergistic defense mechanism versus the encroachment of xenobiotics. Since twain genes are regulated together by the nuclear receptor PXR, they are twain provoked by PXR/CAR ligands as rifampicin or St. John's wort. High-affinity substrates likewise function like inhibitors, along the most notable Ca^{2+} channel antagonist verapamil used also in a plenty of experimental settings. The inhibition of P-glycoprotein at a variety of barriers especially at the blood–brain barrier by verapamil could result in effects to CNS of loperamide, an opioid utilised versus diarrhoea which normally has no systemic effects. Likewise a class I antiarrhythmic agent quinidine is a potent P-glycoprotein inhibitor; the results from previous study in healthy volunteers illustrated that loperamide plasma concentrations were approximately twice as high after co-administration of quinidine, and pupil size reduced, pointing out raised concentration in the central spinal fluid (Table 1.13) (Cascorbi, 2011).

Table 1.13 Substrates of P-glycoprotein (ABCB1) (Cascorbi, 2011)

Class	Drug
Substrate	
Anticancer drugs	Docetacel, doxorubicin, etoposide, imatinib, paclitaxel, teniposide, vinblastine, vincristine
Steroids	Dexamethasone, methylprednisolone
Immunosuppressants	Cyclosporine, sirolimus, tacrolimus
HIV protease inhibitors	Amprenavir, indinavir, nelfinavir, saquinavir, ritonavir
Antibiotics	Erythromycin, levofloxacin, ofloxacin
β-blockers	Bunitrolol, carvedilol, celiprolol, tanilolol
Ca^{2+}-channel blockers	Diltiazem, verapamil
Cardiac drugs	Digoxin, digitoxin, quinidine
HMG-CoA inhibitors	Artovastatin, lovastatin
H_1-antihistamins	Fexofenadine, terfenadine
Antiemetics	Ondansetron
Diverse	Amitryptiline, colchicine, itraconazole, lansoprazole, loperamide, losartan, morphine, phenytoin, rifampicin
Fluorescent dyes	Rhodamine 123
Inducers	
Anticonvulsants	Carbamazepine, phenytoin, phenobarbital, primidon
Tuberculostatics	Rifampicin
Herbals	Hyperforin (constituent of St. John's wort)
Inhibitors	
Calcium channel antagonisten	Verapamil
Makrolide antibiotics	Erythromycin, clarythromycin, *not* azithromycin
HIV protease inhibitors	Ritonavir
Immunosuppressents	Cyclosporin
Antiarrhythmics	Chinidin, propafenon

7) Inhibitors of P-gp

All models of protein activation by ATP predict that inhibition of the ATPase activity of protein can interrupt P-gp efflux action. Nevertheless, this is not a universal principle, since certain P-gp inhibitors raise the action of ATPase of protein. Moreover, first step of studies have guided that inhibitors of ABCB1 ought to bind to the nucleotide binding site (NBS) of protein then resulting in the inhibition of ATPase activity, but there is the controversial result from another study regarding a flavonoid-type inhibitor that showed an activation of P-glycoprotein ATPase activity by the compound minus binding to the protein NBS (Lopez & Martinez-Luis, 2014).

Ordinarily, ABCB1 inhibitors function in one of the following three mechanisms: I. Encouragement an alteration of conformation in ABCB1 that obstructs the adenosine triphosphate binding site and, subsequently, ATPase function; II. Encouragement an alteration of conformation in ABCB1 that enhances adenosine triphosphate binding, but simultaneously obstructs the substrate binding site; III. Inactivation the substrate

binding site minus induction any conformational alterations, for instance, QZ59-SSS and QZ59-RRR which are the stereoisomers of cyclic hexapeptide inhibitors (Lopez & Martinez-Luis, 2014).

For several years, analysis of exiguous chemicals that interrupt with the action of P-gp has held importance as these molecules can block the pump function of P-gp that causes the reduction of effective concentration of drugs which are given in remedies for HIV, cancer, parasitic diseases and others. Based on toxicity, specificity, and affinity of these compounds, P-glycoprotein inhibitors are classified into 3 generations (Table 1.14). The first generation of P-glycoprotein inhibitors are compounds that have been utilised for clinical treatments of diseases already, for instance, Ca^{2+} channel blocker drug like verapamil and immunosuppressant drug like cyclosporine A. After that, these drugs were assayed versus P-glycoprotein and discovered to restrain the enzyme involving drug metabolism. These drugs desired high concentrations to restrain ABCB1, therefore, they were not endorsed to use them for medical proposes like P-gp inhibiting drugs (Lopez & Martinez-Luis, 2014).

The second generation inhibitors are compounds minus prior medical application for therapy and these compounds possess an upper affinity than the 1st generation inhibitors in order to bind with P-glycoprotein. The issuance with these 2nd generation inhibitors is that they're rapidly metabolised by the CYPA4 enzyme, result in modification of their pharmacokinetics and reduction of their efficacies. It is essential to indicate that the second generation inhibitors are designed to have lower toxicity than the first generation inhibitors, in spite of holding some undesirable toxicity characters that constrain their utilisation as drugs (Lopez & Martinez-Luis, 2014).

The third generation inhibitors are compounds gotten from combinatorial chemistry and following structure-activity relationship (SAR) studies to analyse molecules that exhibit P-glycoprotein inhibitory activity by properties including advanced specificity for P-gp binding and minor toxicity. These inhibitors of P-glycoprotein possess a higher potency approximately ten-fold than the 1st and 2nd generation inhibitors, and are not restrained by the CYPA4 enzyme, and consequently, their pharmacokinetic profiles are not modified (Lopez & Martinez-Luis, 2014).

Table 1.14 The instance of classical P-glycoprotein inhibitors by generation (Lopez & Martinez-Luis, 2014)

First Generation	Second generation	Third Generation
Verapamil Cyclosporine A Vincristine Reserpine Quinidine Tamoxifen Trifluoperazine	(*R*)-verapamil Valspodar (PSC-833) Dexniguldipine Elacridar (GF120918) Biricodar Dofequidar	Tariquidar (XR9576) Zosuquidar (LY335979) Laniquidar (R101933) ONT-093 (OC-144-093) Mitotane (NSC-38721) Annamycin

Compounds which are a member with all three generations exert their P-gp inhibitory activities by one of the ensuing mechanisms (Table 1.15): I. breaking the adenosine triphosphate hydrolysis; II. changing expression of P-gp; and III. reversible restraint or competing at a P-gp binding site, that can be shown by photoaffinity labelling (Lopez & Martinez-Luis, 2014).

Table 1.15 Cellular mechanism of classical inhibitors of P-glycoprotein (Lopez & Martinez-Luis, 2014)

ATPase Activity		P-gp Expression		Competition for Binding Site
Inhibitor	Stimulator	Down Regulator	Up Regulator	
Valspodar Tariquidar Elacridar ONT-093	Verapamil Cyclosporine A Vincristine Quinidine Tamoxifen Toremifene Trifluoperazine Dexverapamil Biricodar	Verapamil Cyclosporine A Reserpine Toremifene Trifluoperazine Dexverapamil Valspodar	Vincristine	Verapamil Cyclosporine A Vincristine Reserpine Quinidine Valspodar Dexniguldipine Biricodar Elacridar Dofequidar Tariquidar Zosuquidar

One of the most ordinary mechanisms of classical inhibitors of P-gp is competition at drug binding sites. Nevertheless, ABC1 transporter naturally owns numerous binding sites, resulting in difficulty for target-based inhibitor design. The inferior selectivity appeared by inhibitors with this mechanism may be owing to that ABC1 has numerous binding sites for a large number of diverse molecules. It's likewise hard to find out a common functional group of compound that affect inhibitory effect to

this transporter; anyway, particular pharmacophores can provide conceivable active functional groups to interact with P-gp (Lopez & Martinez-Luis, 2014).

8) P-gp inhibitors from natural resources

The interactions of grape fruit with many drugs provide the first confirmation of herbal products in P-gp inhibition. Many herbal products and compounds listed in Table 1.11 were identified as potent P-gp inhibitors. Besides those inhibitors in the table, ginsenoside Rg3, a red ginseng saponin was discovered to be a competitive ABCB1 inhibitor (Srivalli & Lakshmi, 2012). Antineoplastic lamellarin D, a new pro-apoptotic alkaloid compound of marine source exhibited insensitivity to ABCB1 mediated drug efflux (Srivalli & Lakshmi, 2012). Gomisin A, a dibenzocyclooctadiene compound isolated from *Schisandra chinensis*, appeared a confirmation regarding inhibition of P-gp substrate interaction non-competitively and therefore reversing MDR. This compound is not a P-gp substrate by itself and can bind concurrently to both P-gp and substrate. It restrains the basal P-gp involved ATPase action. CBT-1 is the bisbenzylisoquinoline plant alkaloid which exhibits a P-gp inhibitory activity. Laulimalide is a macrolide obtained from *Hyatella* species. It is a microtubule stabilising agent and also identified as a P-gp inhibitor. It was shown that its antitumor activity is more potent than taxol (a 100-fold) in MDR cell lines (Srivalli & Lakshmi, 2012).

Table 1.16 List of natural constituents identified as P-gp inhibitors (Srivalli & Lakshmi, 2012)

CATEGORY	EXAMPLES
Herbs	Garlic, green tea, marine resources.
Peptides	Kendarimide A, a novel peptide from a marine sponge of *Haliclona oculata.*
Fruits	Citrus fruit, grape, orange.
Herbal constituents	
Glycosides	Iridoid and polyethanoid flavonoids, picroside II, acteoside.
Curcumin	*Curcuma longa*
Ginsenosides	Ginseng (*Panax ginseng*)
Piperine	*Piper nigrum.*
Hyperforin and Hypericin	St. John's wort.
Bitter melon leaf extracts	*Momordica charantia.*
Flavonoids	Diosmin from citrus fruit, quercetin from tea, ginkgo and St. John's wort, naringin, biochanin, silymarin.
Terpenoids	
Monoterpenoid	(R)-(+)-citronellal, (S)-(-)-betacitronellol and others from *Zanthoxyli fructus* extracts.
Sesquiterpenes	Extracts from *Zinowiewia costaricensis.*
Diterpenoids	Lathyrane from the seeds of caper spurge (*Euphorbia lathyris*).
Triterpenoids	Derived from the red sea sponge, *Siphonachalina siphonella.*
Others	Root extracts of *Stemona curtisii.*

1.2.8 Computational (*in silico)* approaches to herb–P-gp interaction study

Despite these compelling findings, the interaction profile between P-gp and bioactive herbal constituents was limited, which can effectively enter human systemic circulation after oral administration (Li et al., 2014). A principal obstacle in the drug discovery procedure, P-gp has encouraged an advancement of many tests intended to P-gp's substrate identification. To add to experimental analyses, diversified *in silico* approaches have been emerged for P-gp binding prediction. Amongst these methods, pharmacophore modelling has been created. Other methods enclose quantitative structure activity relationship (QSAR) modelling. Although several methods inform

sensitivity of ≥80%, the distinctive chemical variety of the P-gp substrates, resounded by plenty pharmacophores and descriptors, has discouraged endeavours to be appropriate of the chemical info. Many tenets considering the number, sizes, and localities of the binding pockets/sites have henceforward made difficult the issuance.

1) Introduction to ligand-based studies for P-gp

Toward the significant role of ABCB1 on multidrug resistance and pharmacokinetics, broad researches have been managed to analyse substrates of ABCB1 or develop upward specificity, selectivity and potency of inhibitors of P-gp. The polyspecificity (that is a promiscuity) of P-glycoprotein in recognition of its substrate and inhibitor produces a tough design of capable candidate compounds. Regarding the traditional experimental assays, they were utilised to investigate the transportation and interactions of novel molecule identities with this transporter but these experimental tests are time-consuming, inconvenient and expensive. Thus, computational models that allow fast and inexpensive screening platforms for ABCB1 inhibitors or substrates identification have been recognised to be useful instruments. Widespread used computational models are usually on the basis of quantitative structure–activity relationship (QSAR) modelling such as two-dimension-QSAR and three-dimension-QSAR, pharmacophore modelling and molecular docking methods that are advanced for prediction or estimation of the P-gp modulatory activity of compounds or identification of inhibitors or substrates of P-glycoprotein. The theoretic models apprised for prediction of ABCB1 inhibitors or substrates are summarised in Table 1.17. Furthermore, various statistical methods like machine learning approaches, including multiple linear regression (MLR), support vector machine (SVM), Kohonen self-organising map (SOM), decision tree (DT), Bayesian classifier, linear discriminant analysis (LDA), and partial least square discriminant analysis (PLSD), have been operated in the theoretical model development (Chen et al., 2012).

Table 1.17 The theoretic models for prediction of P-gp substrates and inhibitors (Chen et al., 2012)

Method	Model	Descriptors	Dataset		Performance
			Training	Test	
CONAN	Classification	Pharmacophore-based descriptors	144	45	Training[a]: accuracy = 80%; test[b]: accuracy = 63%
LDA	Classification	Electrotopological state values, shape indices and molecular properties	95	58	Training: SE[c] = 100%, *SP*[d] = 90.6% test: accuracy = 86.2%
SVM	Classification	159 descriptors	74	25	SE = 84.2%, SP = 66.7%, accuracy = 80%
PLSD	Classification	Volsurf descriptors	53	272	Training: accuracy = 88.7%; test: accuracy=72.4%
Bayes	Classification	Atom typing descriptors and fingerprints	424	185	Test: accuracy=82.2%
TOPS-MODE	Classification	TOPS-MODE descriptors	163	40	Training: SE = 82.4%, SP = 79.17%, accuracy = 80.9%; test: accuracy = 77.5%
BRNN	Correlation	249 descriptors	43	14	Training: r^2 = 0.756, test: r^2 = 0.728
SVM, *k*NN, DT, binary QSAR	Classification	MolconnZ, AP, VolSurf and MOE Descriptors	144	51	Training: accuracy = 94%; test: accuracy = 81%
SVM, PS	Classification	79 descriptors	163	40	Training: 95.5%; test: 90%
PLS	Correlation	CoMFA and CoMSIA descriptors	28	30	Training: r^2 = 0.82; test: r^2 = 0.6
MLR, SVM	Correlation	423 CODESSA descriptors	56	14	Training: r^2 = 0.85; test: r^2 = 0.81
RP, NBC	Classification	Fingerprints and molecular properties	973	300	Training: SE = 84.7%, SP = 82.1%, accuracy = 88.9%; test: SE = 79.2%, SP = 83.8%, accuracy = 81%
PLS	Correlation	Almond and Volsurf descriptors	109	20	Training: r^2 = 0.83, LOO q^2 = 0.75; test: r^2=0.72
	Pharmacophore		27	19	Training: r^2 = 0.77; test: Spearman r = 0.68
			21	19	Training: r^2 = 0.88; test: Spearman r = 0.7
			17	19	Training: r^2 = 0.86; test: Spearman r = 0.46
DT	Classification	Pharmacophore models	163	97	Training: accuracy = 87.7%; test: accuracy = 87.6%
SVM	Classification	ADRIANA.Code, MOE and ECFP4 fingerprints	212	120	Training: LOO accuracy = 75%; test: accuracy = 88%

[a] Training represents training set.
[b] Test represents test set.
[c] SE represents sensitivity.
[d] SP represents specificity.

The instigations on the advancement of appropriate computational molecular models used to predict of P-glycoprotein inhibitors in the initial phase of the drug discovery procedure and in herbal products to identify potential safety concerns. In order to estimate P-gp inhibitory activities of compounds, several groups have developed the computational approaches lying on ligand-based manners like quantitative structure–activity relationship (QSAR) modelling, rule-based modelling and pharmacophore modelling. Freshly, likewise machine-learning approaches were prosperously utilised for estimation of P-gp substrates and inhibitors. Moreover, grid-based approaches, for example, FLAP (fingerprints for ligands and proteins) were

victoriously employed to the set of 1,200 P-glycoprotein inhibitors and non-inhibitors by a successful rate of eight-six percent of using an external test set (Klepsch et al., 2014). After which, these ligand-based models were employed such virtual screening machinery to analyse novel P-glycoprotein ligands. Likewise unsupervised machine learning techniques ["Kohonen self-organizing map (Kohonen SOM)" which is the Self-Organising Map was developed by professor Kohonen] were applied for substrates and non-substrates prediction by the data group consisting of 206 molecules. The best model obtained from this study can provide a right prediction eighty-three percent of substrates and eighty-one percent of inhibitors. Freshly, recursive partitioning and naive Bayes based classification toward the group containing 1,273 molecules were reported (Klepsch et al., 2014). Herein, the best model could provide a prediction accurate eighty-one percent of the molecules of the test set. By reason of an inadequacy of info of structures, upcoming predictive models utilising structure-based techniques has not been yet enthusiastically chased. Nevertheless, lately, the number of obtainable three-dimension molecular structures of ABC transporters and the execution of experimental approaches laid the direction as the appliance of structure-based techniques to forecast/estimate interactions versus drug molecules and transporters. For this issue, in the latest 2 years, not much structure-based predictive models have been explicated. A free web-server was advanced on the basis of a support vector machine (SVM) classification model that is utility to use it as a tool to predict compounds online and binding modes of P-gp substrates can be analysed (Klepsch et al., 2014).

2) Experimental chemical datasets used for computational model constructions in ligand-based approaches

The arrangement of implicated sets of data with advanced quality and quantity is the 1st step in regard to computational model construction with advanced confidence. Customarily, the public datasets consist a very constrained amount of chemicals (lesser than or near 200). Gombar et al. collected a set consisting ninety-eight molecules, which encloses of thirty-two non-substrates and sixty-six substrates tested by monolayer efflux tests which were done *in vitro* (Gombar et al., 2004); Penzotti et al. apprised a dataset of 195 chemicals, there are 108 ABCB1 substrates and 87 nonsubstrates (Penzotti et al., 2002). Xue et al. gathered a set of 201 chemicals comprising 116 substrates and 85 nonsubstrates of ABCB1 (Xue et al., 2004). Sun informed a broad validated set of 609

chemicals in 2005, offered by Dr. Klopman (in the publicly available "Klopman set"). The reversal factor (RF) was employed to determine the efficacy for reversion of multidrug resistance. Regarding the 609 chemicals in the set, 378 chemicals were active, along the RF value > 2.0, and the remaining chemicals were inactive (Sun, 2005). Wang et al. apprised a big set of data in 2011 that consisted 332 chemicals as 206 substrates and 126 non-substrates of ABCB1 (Wang et al., 2011).

In 2011, Chen et al. apprised the biggest set for inhibitors and non-inhibitors of P-glycoprotein. This set contained 1273 structurally diverse compounds, comprising 797 inhibitors and 476 non-inhibitors of P-glycoprotein (Chen et al., 2012). The data containing 609 chemicals that exhibited the multiple drug resistance reversal (MDRR) activity was obtained from the experiment of Bakken and Jurs (Bakken & Jurs, 2000), and also from the experiment of Ramu and Ramu (Ramu & Ramu, 1992, 1994) that provided the data containing 347 chemicals, these were 2 most essential chemical sources. The MDRR ratio obtained from the experiment was utilised as a criterion to measure whether a chemical is the P-gp inhibitor or not: if the MDRR ratio was <4 the chemical was categorised into the non-inhibitor class; if the MDRR ratio was >5 the chemical was categorised into the inhibitor class; if the MDRR ratio was ≥4 and ≤5 the chemical was regarded to be moderate active inhibitor and then that chemical wasn't put in the chemical set (Chen et al., 2012).

3) Quantitative structure-activity relationship (QSAR) studies

QSAR models are regressive or classifying models that are applied in the chemistry, biology, and engineering, likewise a three-dimensional QSAR model comprises an arithmetical equation discoursing potency or activity or efficacy as a function of three-dimensional interaction fields on all sides of aligned compounds in a set that is used as a template called a training set. The relationship betwixt the three-dimensional spatial change of interaction field values and the experimentally remarked variations in the target property is created using multivariate statistical analyses that refer to multiple advanced techniques for examination of relationships amongst multiple variables simultaneously. This method is frequently employed to aid the design of increasingly potent inhibitors or even substrates of any protein receptors with superior

affinity or even to screen the ligands that have the potential to cause herb-drug-interaction through inhibition of drug metabolism enzymes or transporters.

The computational techniques based on molecular level transporter models may be ustilised for prediction of inhibitor binding modes, therefore standing for an expedient tool in the screening herb-drug interaction or discovery of novel transporter ligands. The techniques that don't demand realisation of transporter structure, like 3D-QSAR modeling, can be applied. They are employed to correlate biological activity with molecular descriptors or features that stand for the interaction between the ligand and the protein. The obvious assumption of this approach is that the biologically active compounds have specific components represented by chemical features or electrostatic and steric fields around the molecules that lead to the activity. Various commercial tools are obtainable to evoke 3D-QSAR models. Depending on the number of publications, the most extensively utilised programmes are somewhat comparative molecular field analysis (CoMFA) (Cramer et al., 1988) and with this method, researchers can create statistical and graphical models that associate the chemical and biological properties of molecules to their three-dimension structures and the three-dimension steric and electrostatic properties, the next favourite method is comparative molecular similarity index analysis (CoMSIA) (Klebe et al., 1994), and ADRIANA.Code (ADRIANA.Code, 2006).

In 2004, Gombar et al. used LDA to build a predictive model applied against substrates of P-glycoprotein (Gombar et al., 2004). The training set comprising ninety-five chemicals was classified as sixty-three substrates and thirty-two non-substrates on the basis of the results that were obtained from *in vitro* monolayer efflux assays. The LDA classification model against twenty-seven descriptors provided 100% sensitivity and 90.6% specificity in the cross-validation testing; furthermore, 86.2% prediction accuracy was gained on the external test set containing fifty-eight chemicals. The analysis of these twenty-seven descriptors in the ultimate classifying model guided that the essential structural characters of P-gp substrates were the capability to partition into membranes, molecular bulkiness and the counts and electrotopological values of certain isolated and bonded hydrides. Anyway, the training set utilised by Gombar et al. wasn't broad sufficient; thus, the chemical space overspread by this model may be constrained.

The linear discriminant model against a set of one hundred and sixty-three compounds which comprises ninety-one substrates and seventy-two non-substrates was constructed in 2005 (Chen et al., 2012). Regarding the training set, 82.42% sensitivity and 79.17% specificity were obtained from the ultimate model on the basis of 9 TOPS-MODE descriptors. For the external validation set which has forty compounds (twenty-two substrates and eighteen non-substrates), 77.5% prediction accuracy was gained from the model. When descriptors in the model were analysed. The results demonstrated that the Gasteiger–Marsilli atomic charge, standard bond distance, and polarisability influenced the molecular interaction betwixt ABCB1 and its substrates.

The application of PLSD analysis for categorisation of twenty-two ABCB1 substrates and thirty-one ABCB1 non-substrates on the basis of the VolSurf descriptors was done in 2006 (Chen et al., 2012). The model provided 88.7% accuracy of the training set, but it only reached 72.4% accuracy of the external set of two hundred and seventy-two compounds. Afterwards, the researchers employed PLSD analysis to create the classification model to separate ABCB1 substrates from ABCB1 inhibitors on the basis of the GRIND descriptors. It identified the set of compounds providing sixty-nine substrates and fifty-six inhibitors along a mean accuracy of eighty-two percent.

The three-dimensional quantitative structure-activity relationship (3D-QSAR) model development utilising the Almond and VolSurf descriptors for a multitudinous dataset containing one hundred and twenty-nine chemicals, that were assessed for ABCB1 inhibition utilising the calcein-AM method test was done in 2005 (Chen et al., 2012). The compounds from this set were separated into a training set containing one hundred and nine chemicals and a test set containing twenty chemical. For variable selection for the created model, Fractional factorial design (FFD) fractional selection were used, then the partial least square discriminant model comprising 3 potential variables were obtained. The values including 0.8252 of r^2, 0.7459 of leave-one-out (LOO) q^2, 0.7456 of leave-two-out (LTO) q^2, and 0.7400 of random grouping (RG) q^2 were obtained from the best prediction of the training set. It was supporting that this partial least square discriminant model reached 0.7160 of correlation coefficient (r^2). when the tested compounds were predicted. Regarding analysis of the Almond descriptors that are variables in this model, the researchers proffered the ensuing

pharmacophore hypothesis: 2 lipophylic groups 16.5 Å apart and 2 H-bond acceptor groups 11.5 Å apart.

The building of a naive Bayesian classifying model for categorisation of multidrug resistance reversal compounds becoming 2 categories that were active and inactive groups of compounds on the basis of atom type-based molecular descriptors and fingerprints was performed in 2005 (Sun, 2005). The complete dataset was portioned to be a training set of four hundred and forty-two chemicals and a test set of one hundred and eight-five compounds. A success rate of 82.2% was obtained from the prediction of the multidrug resistance reversal activities of the tested compounds by the classifier created from the training set. The researcher assumed that the model on the basis of atom typing descriptors and naive Bayesian classifying presented extraordinary info for the theoretical design of multidrug resistance reversal compounds.

In 2006, Lima et al. (Lima et al., 2006) created and used a set of predictive models to classify a chemical group which contains one hundred and ninety-five multitudinous substrates and non-substrates using a variety of combining of optimisation processes and descriptor types (Lima et al., 2006; Penzotti et al., 2002). Regarding the modelling procedure, 4 descriptor sets were applied, involving three hundred and eight-one molecular connectivity indices, one hundred and seventy-three atom pair (AP) descriptors, seventy-two VolSurf descriptors and one hundred and eighty-nine descriptors computed utilising Molecular Operating Environment (MOE), and 4 modelling methods were employed, comprising, *k*-nearest neighbors (*k*NN) classification QSAR, binary QSAR, DT and support vector machine. Every QSAR modelling technique was merged with each descriptor type to build sixteen (four methods × four descriptors) combinatorial QSAR models. The best models on the basis of support vector machine and either atom pair or VolSurf descriptors reached magnificent faultless predictive rates which were ninety-four percent for the training set and eighty-one percent for the test set.

The 3D-QSAR models constructions by application of Comparative Molecular Field Analysis (CoMFA) and Comparative Molecular Similarity Index Analysis (CoMSIA) methods against twenty-eight P-glycoprotein inhibitors were done by Muller et al. in 2008, involving twenty-four chemicals, tariquidar derivatives and 4 XR, which

has a structural relationship (Müller et al., 2008). ≥0.8 of internal predictive squared correlation coefficient was obtained from the best three-dimension-quantitative structure-activity and relationship models. The models were afterwards checked their validities utilising the external test set which contains thirty XR chemicals. 0.6 of predictive squared correlation coefficient was obtained from the best CoMSIA model. It ought to be remarked that the CoMFA and CoMSIA 3D-QSAR models were advanced on the basis of a series of homologs; thus, these models don't have general predictive efficacy.

In 2009, the computational study using the ligand-based approach was performed using multiple linear regression and support vector machine techniques to develop the model for prediction of P-gp modulators. The hybrid QSAR models were created to analyse multidrug resistance modulating effects for seventy chemicals (Wu et al., 2009). 1st, the heuristic method (HM) was employed to pick the descriptors utilising the CODESSATM programme, and after that the predictive models were constructed by multiple linear regression, support vector machine and hybrid QSAR modelling. When the best hybrid was used against the training set, then 0.33 root-mean-square (RMS) error is obtained. In addition, 0.47 RMS errors were obtained when the test set was predicted. The 0.36 RMS errors also was obtained from the entire set. 0.85, 0.81 and 0.84 of the consistent correlation coefficients (r^2) were obtained successively.

Wang et al. developed various predictive models to classify P-glycoprotein substrates on the basis of a big dataset with three hundred and thirty-two different molecules (Wang et al., 2011). They used ADRIANA.Code, MOE and ECFP_4 fingerprints to calculate three sets of molecular descriptors of each molecule. The models were built by support vector machine on the basis of a training set, which encloses one hundred and thirty-one P-glycoprotein substrates and eighty-one P-glycoprotein non-substrates. 0.73 of Matthews correlation coefficient was obtained from the best model. Regarding validation of this model utilising the test set, the 0.88 prediction accuracy was obtained. The test of the model on the basis of ECFP_4 fingerprints disclosed various substructures having signification in categorising substrates and non-substrates.

In 2011, Chen et al. (Chen et al., 2011) built the models used to classify the 1273 compounds dataset which were then separated to be the training set with nine hundred and seventy-three chemicals and test set with three hundred chemicals by random sampling. 1st, The recursive partitioning (RP) technique was utilised to constructed DT models and the test set were utilised to validate the models. The best DT gave the right prediction of the test set about 83.5% of the P-glycoprotein inhibitors and sixty-seven percent P-glycoprotein non-inhibitors. 2nd, the naive Bayesian categorisation modelling was utilised to develop classification models that could be applied for predicting ABCB1 inhibitors and noninhibitors. The Bayesian classification model could correctly predict 81.7% of the training set along LOO cross-validation method and for the test set, this model could correctly predict 81.2% of 300 molecules.

From the recent study of Sousa et al. in 2013, they applied QSAR methodology in order to identify the most relevant molecular features of macrocyclic diterpenes (lathyrane and jatrophane-type diterpenes) from Euphorbia species with P-gp inhibitory activity obtained by a standard functional test that determines the rhodamine-123 accumulation in the cells as the fluorescence activity ratio; FAR (compounds with FAR values higher than 1 were considered to be active as P-gp inhibitors and those with FAR values higher than 10 were regarded as strong inhibitors) and to determine which structural modifications can be performed to improve their activity (Sousa et al., 2013). Using experimental biological data at two concentrations (4 and 40 µg/ml) of compounds, they developed a QSAR model for a set of 51 bioactive diterpenic compounds which includes lathyrane and jatrophane-type diterpenes and another model just for jatrophanes. The molecular structures of chemicals were optimised utilising the molecular mechanics (MM^+) force field enclosed in HyperChem Release 7.5. Thermodynamic and quantum descriptors were calculated using MOPAC included in VEGA ZZ 2.2.0. HyperChem structure files, MOPAC output files and added descriptors (logP and the number of H-bond donors and H-bond acceptors) computed utilising E-DRAGON were used as input of the CODESSA programme for the computation of a whole of 306 structural descriptors. 5 classes of structural descriptors were computed utilising CODESSA programme including topological, quantum-chemical constitutional, electrostatic, and geometrical. The Heuristic Method, achieved by CODESSA, was utilised to operate the selecting of the molecular descriptors correlated

with the biological effect of molecules. Regarding the training and test set selecting, the first QSAR model includes all the diterpenic compounds under study which is called the general model; and the second QSAR model includes only jatrophane-type compounds under study which is called the jatrophane model. The set of compounds was distinguished into several subsets according to their biological activity range; the division was performed in 10 subsets for the general model at concentration of 4 µg/ml and in 8 subsets at concentration of 40 µg/ml. For jatrophane model at concentration of 4 and 40 µg/ml, the set of compounds was divided in 7 and 5 subsets, respectively. The compounds integrating the training and test sets were chosen randomly from each subset; the selection of training set compounds was performed in order to ensure the diversity and cover the chemical space of the structures under study and to guarantee that test set compounds were representative of dataset. The compounds were selected taking into account the training/test set ratio of 4:1. Multiple linear regression (MLR) with anterior model selecting was utilised to create quantitative structure–activity relationship equations. The F value was used for the analysis of variance and R^2 and RMSE of the training set as criteria for selecting models. The internal validation of the QSAR models was always operated utilising the leave one out (LOO) method. The value of this LOO the cross-validation correlation coefficient is given by q^2. The size of descriptors subset used taken into account that the number of molecules in the training set shouldn't be lesser than 5 times the number of descriptors. The cross-validation correlation values for all diterpenes QSAR models developed for biological activities at compound concentrations of 4 and 40 µg/ml were 0.758 and 0.729, respectively. Regarding the prediction ability, they get R^2_{pred} values of 0.765 and 0.534 for biological activities at compound concentrations of 4 and 40 µg/ml, respectively. Applying the cross-validation test to jatrophanes QSAR models, they obtained 0.680 and 0.787 for biological activities at compound concentrations of 4 and 40 µg/ml concentrations, respectively. For the same concentrations, the obtained R^2_{pred} values for jatrophanes models were 0.541 and 0.534, successively. The obtained models were statistically valid and showed high prediction ability.

4) Structure-based approaches

If data of three-dimension structures for a target protein of drug can be reached, regarding drug design, researchers can use various structure-based approaches in the

procedure. Generally, structure-based methods provide distinctive particulars regarding protein-ligand interactions and therefore are worth accessories for screening of herb-drug interactions, and lead discovery and optimisation in a drug development process (Waszkowycz, 2008).

Structure-based method mainly depended on the availableness of crystal structures of the target protein at level of atom. P-gp and its homologues being membrane embedded proteins are mostly reluctant to form diffracting crystals which complicates the crystallisation procedure of such proteins. Thus, protein homology modeling based on templates of bacterial homologues or mouse P-gp, standing for distinct catalytic states, is the method of choice for studies in structure-based approaches. Table 1.18 provides a summary of available templates and homology models of P-glycoprotein at the present time.

Table 1.18 Available crystal structures of ATP-binding cassette transporters along with P-glycoprotein (ABCB1) (Klepsch et al., 2010)

ABC Transporter	Organsim	Catalytic State	Resolution [Å]	PDB Code
ABCB1	Mouse	Apo	3. 80	3G5U
ABCB1	Mouse	Apo	4. 40	3G60
ABCB1	Mouse	Apo	4. 35	3G61
ABCB1	Hamster	Apo	~20	-
ABCB1	Hamster	AMP-PNP	~20	-
ABCB1	Hamster	ATP	8	-
ABCC1	Human	ATP	~22	-
ABCG2	Insect	ATP	~18	-
SAV1866	Staphylococcus aureus	ADP	3. 00	2HYD
SAV1866	Staphylococcus aureus	AMP-PNP	3. 40	2ONJ
MsbA	Escherichia coli	Apo	5. 30	3B5W
MsbA	Vibrio cholerae	Apo	5. 50	3B5X
MsbA	Salmonella typhimurium	AMP-PNP	4. 50	3B5Y
MsbA	Salmonella typhimurium	ADP-OV	4. 20	3B5Z
MsbA	Salmonella typhimurium	AMP-PNP	3. 70	3B60

The 1st X-ray structure of a eukaryotic ATP-binding cassette efflux pump, P-glycoprotein (PDB accession code 3G5U, *M. musculus*, resolution: 3.8 Å), was published in 2009 by Aller et al. (Aller et al., 2009). In addition, the other structures were published along with 2 co-crystallised QZ59-RRR/QZ59-SSS which are the

enantiomeric cyclic peptide inhibitors (CPPIs, resolution: 4.40 Å /4.35 Å). The first crystal structure of mouse ABCB1 represents a large step forward for structure-based studies on this transporter, but it's still tough to determine the exact orientation of many side chain residues at this (3.8 Å) resolution. However, having 87% sequence identity to human ABCB1 it may serve as a nice template for homology modelling.

The P-gp crystal structure obtained from mouse with amino acid sequence identity that is 87% compared to human P-gp has been recently published. It offers a prospect to emerge new prediction techniques which evoke an advantage of the molecular structural data of receptor to not merely predicate compounds which attach to P-gp but likewise to instruct optimisation of molecules, for example, to search for modulating interactions with the transporter. Amongst the three published P-gp structures, two were crystallised using QZ59 which is a cyclic inhibitor and the molecular structure are stereoisomers that specifies the binding groove of drug molecule. Each transporter protein is in an inward-facing structural form which is postulated to be interrelated for beginning of substrate recognition (Dolghih et al., 2011).

Even though high-resolution P-gp crystal structures from a human are not obtainable yet, however, the mouse P-gp crystal structures and various homology models on the basis of bacterial transporters have been proposed (Jara et al., 2013; Wise, 2012). Considering *Mus musculus* P-gp crystal structural model, the catalytic glutamates that possibly stimulate the H_2O molecules utilised when ATP is hydrolysed are disported about more than 30 Å, revealing of a faultless extrication of the 2 NBDs. The extensively opened NBDs in the models formed a 9 Å exposure for the purpose of entry to the drug binding sites in an intracellular portion of lipid bilayer organised per transmembrane (TM) helices (TM4 with TM6 and TM10 with TM12). It has been entrapped in the outward facing-like form (that is to say upward probably to be the conformation after ATP is hydrolysed companion with expulsion of the drug). Nevertheless, the scientists have modelled the P-gp to hand other 2 conformations, both of these conformations cooperative with a drug binding proficient state (Jara et al., 2013; Wise, 2012). New crystal structure of P-gp (code 4Q9H) is presented. It is remarked that the binding of certain ligands, along with an ATP-hydrolysis stimulating agent, results in a great conformational alteration in the fourth transmembrane helix, which is postured to potentially transmit a signal to the NBDs (Szewczyk et al., 2015).

The crystal structures of SAV1866 illustrate wholly commissioned and dimerised NBDs along catalytic glutamyl residues split almost 14 Å. These molecular models' transmembrane domains are shaped in the outwards-facing arranging along a relative polar cavity widened to the area outside a cell. This shaping has been equalised versus a drug unleash structural form. ATP hydrolysis and leaving of ADP and phosphate would lead to an alteration to an inbound shaping (open toward to intracellular) that discloses high-affinity drug binding areas. 4 models of lipid flippase of bacteria, MsbA, illustrate rousing structural formational divergences that have been equalised with variant spans of the transportation implement. These cover the transporter models by means of TMDs opened inward with a little detached NBDs (PDB accession code 3B5X), and models along an opened outward DBS and in full occupied, dimerised NBDs (PDB accession code 3B5Z, 3B5Y, and 3B60). One among all models is notable in possessing ADP-Vi, a transition state counterpart, attached in 1 of the NBDs (PDB accession code 3B5Z). Moreover, 1 MsbA model illustrated in full released NBDs along catalytic glutamates split about >65 Å like the *Mus musculus* P-gp discoursed aforementioned (Wise, 2012). Afterwards, the first crystallographic P-gp structure from the mammal was gained. This accomplishment evokes a distinctive chance to appraise the interactions of novel along with well-known inhibitors inside an analytic P-gp configuration. The *Mus musculus*'s P-gp amino acid sequence has an advantage of homology approximately eighty-six percent amino acid identity along the *Homo sapiens* P-glycoprotein and with plausibly proffered for standing for a resting state form, there is no nucleotide binding to nucleotide binding domains (nucleotide free) that is a conformation along a full ability to bind a drug molecule. Freshly, the crystallographic P-glycoprotein model from *Caenorhabditis elegans* was gained by a resolution of 3.4˚ Å. The transporter from *C. elegans* is 46% identical to the human one (Jara et al., 2013).

4.1) Molecular docking studies

Protein-ligand docking is one of the most common structure-based studies. Molecular docking is utilised for computational methods that seek to look for the best matches betwixt a receptor and ligand. It associates the prediction of conformations and orientation (or posing) of the ligand within a binding site and seeks to fill the ligand into the binding site in configurations and conformations suitable to interact with the receptor (Kitchen et al., 2004). The 2 main steps of the protein-ligand docking

procedure are; the exact placing of the ligand at the protein binding-site and the estimation of ligand affinity utilising a scoring function (Kirchmair et al., 2008).

Docking requires a 3D structure of the protein as input. Typically, the software will generate 3D conformations of the ligands and optimise their interactions with the protein by computing the binding affinity (scoring) between the two. In most docking programs used today, the ligand is treated as a flexible structure but the conformation of the protein is treated as being (mostly) rigid, and water molecules are typically not considered at all. Obviously, both of these approximations constitute major simplifications of the real environment in which ligands and proteins interact. Still they are useful because of the immense amount of computation that would be necessary to accurately model the effects of water and protein flexibility – imagine the difficulty of modelling a lock and key that are constantly changing shape, in aqueous solution, and trying to measure the interactions between the two. However, these simplifications are thought to be the two most important reasons why docking fails to correctly predict the affinity of a ligand for a protein, and the pose the ligand will adopt on binding (Verkhivker et al., 2000), and this failure has plagued docking since its birth in the 1980s.

In tenet, the searched space comprises all probable conformations and orientations of the protein-ligand complex. Anyway, pragmatically, it's infeasible to thoroughly identify the searched space with present computational resources. Most docking programmes elucidate for flexibility of ligand, and many endeavours to shape a flexible protein receptor model. Each snapshot of the couple of molecule and protein receptor is adverted to be a pose. Various conformational searching strategies have been employed to the protein receptor and ligand. These strategies enclose systematic or stochastic torsional searches regarding rotatable bonds, molecular dynamics simulations, and genetic algorithms to emit novel conformations with low energy (Dias & de Azevedo, 2008).

The evaluation and ranking of the conformations of ligand which are predicted based on the search algorithm is a critical manner of every docking protocol (Huang et al., 2010). The ability to produce the exact conformation is not enough. It's likewise essential to be capable of recognise it. The scoring function should capacitate the

distinctness betwixt the genuine binding modes and all of the other alternative modes identified, or betwixt active and random compounds. However, a very accurate scoring function may be very costly for computation and would thus act the analysis of the various binding modes impractical. For this reason, a number of ascriptions and simplifications are utilised to decrease the complicatedness of the scoring functions, along a natural cost in terms of accuracy. Wherefore, the penury of a proper scoring function, twain in terms of accuracy and speed, is the key bottleneck determinant in docking simulations (Kitchen et al., 2004).

The accuracy of docking software packages is often tested by so-called redocking experiments. These involve docking one or a set of ligands back into the binding site of the native three-dimension structure of the protein; the docking is judged to be successful if the software is able to reproduce the experimentally-observed ligand pose. Redocking results are commonly evaluated by calculating the root mean square deviations (RMSD) between the native ligand conformation (as observed in the x-ray structure) and the ligand conformation suggested by the docking software (docking poses). RMSD is a measure of the average deviation in the positions of the heavy atoms of the ligand between the two complexes. The native pose is typically judged to have been "successfully" reproduced if the RMSD is below 2.0 Å (Gohlke et al., 2000; Jones et al., 1997; Kramer et al., 1999), although such fixed limits should be treated with caution in some cases.

Briefly, molecular docking is expedient for discriminatory active compounds from inactive chemicals and to analyse conformations of ligand same as the ones remarked in the crystal structures of protein ligand complexes. Anyway, the sorting of chemicals in the meaning of binding affinities is challenging (Warren et al., 2006). Some favourite molecular docking programmes are MOEDock, AutoDock, DOCK, Fred, GOLD, FlexX, eHiTS, Glide, and Surflex (Huang et al., 2010).

In the early periods, the QSAR and pharmacophore modelling approaches were the regular manners utilised for predicting the ABCB1 inhibitors or substrates since the penury of obtainable ABCB1 crystal structures onward to 2009 when the mouse ABCB1 X-ray structures were discovered by Aller et al. (Aller et al., 2009). The ABCB1 crystal structures allow fine initiation regarding molecular docking researches.

In various researches, the homology models were emerged to identify the assumed ligand-binding sites or search for the plausible P-glycoprotein conformations in distinct states. Nevertheless, mere a few published papers presented the ABCB1 models were utilised for docking of molecules inside the assumed ligand-binding sites (Chen et al., 2012).

Becker et al. proposed 4 three-dimension models of P-glycoprotein in 2009, illustrating 2 distinct states in accordance with the catalytic cycle employing the Sav1866 and MsbA crystal structures to be the templates in homology modelling (Becker et al., 2009). The lengths between residues of the theoretic models correlated nicely against lengths obtained from cross-linking info. 1 among the nucleotide-free three-dimension models was employed to dock 4 distinct ligands, involving calcium channel blocker as verapamil, rhodamine B, alkaloid as colchicines and vinca alkaloid as vinblastine, inside the centric binding pocket harboured by the transmembrane domains. The interplaying between each ligand and amino acids has been discovered from the docked poses that have been already analysed in the experiments for their binding to a particular ligand. Approaches of molecular docking point out that no enter way is big adequate to grant the entrance of 1 adenosine triphosphate molecule inside the catalytic site of the ATP-bound forms guiding that these structures ought to get by modifications to facilitate their ligands. Nevertheless, the studied ligands' binding poses provided with theoretic predictions could not be proved the validity with robust experimental attestation (Chen et al., 2012).

Crystal structures of *Mus musculus* P-gp from the Protein Data Bank and human P-gp created using a homology modelling technique were applied to create plausible protein-ligand complexes in molecular docking approaches as well. Anyway, molecular docking technique was not applied to classify chemicals. From the previous study, the researchers utilised induced fit docking within the *Mus musculus*'s P-glycoprotein crystal structure for splitting of P-glycoprotein binders from nonbinders based on calculated docking scores analysed from these molecules. Even though the data sets were substantially exiguous (126 and 64 molecules), the AUC of 0.93 and 0.90 in turn, could be noticed. Recently likewise the researchers utilised a group of 245 P-glycoprotein substrates and nonsubstrates for evaluation of the docking predictive efficacy. Anyway, no obvious categorisation of the substrates and nonsubstrates could

be noticed when the Glide docking scores SP and XP were employed (Klepsch et al., 2014).

In 2013, Jara et al. (Jara et al., 2013) studied the P-gp/inhibitor or P-gp/substrate complexes that were constructed with docking the compound (molecular docking technique) utilising the Autodock 3.0.5 programme. From the study, a commonplace binding site for Rhodamine123 and inhibitory agents along with propafenone (3-phenylpropiophenone nucleus) derivatives and XR9576 (tariquidar) with different modulation activity was discovered to be a lipophilic pocket associating transmembrane domains 4, 5 and 6, tagged site P1, with molecular docking through the mouse ABCB1 crystal structure. Site P1 holds important residues, for instance: S218(S222, transmembrane domain 4), F299 (F303, transmembrane domain 5), V334(V338, transmembrane domain 6), L335 (L339, transmembrane domain 6), F339 (F343, transmembrane domain 6), which have been proffered to act an important part that are used to bind substrates and shown in the experiments. The major interactions against the substrates or modulatory agents in this part are hydrophobic. The binding to this area may decrease the movement of transmembrane domains (particularly transmembrane domain 6) which could influence the posterior adenosine triphosphate hydrolysis. From docking outcome, a 2nd site, near to P1 one, was discovered, which associated transmembrane domain 12 (for instance V982). The relevancy of this area is not forsaken, because in XR9576's best docking conformation interplays not mere against site P1 but likewise against transmembrane domain 12. Their results illustrated that the graceful correlation between the effects in the experiments and the relative binding energies gauged by docking can be observed.

In 2014, the study of Li et al.(Li et al., 2014) was designed to examine the inhibitory activities of 50 major herbal constituents present in 25 commonly used traditional Chinese medicines (TCM) on ABCB1 *in vitro* and *in vivo* as well as related inhibitory mechanisms. Molecular docking analysis with the Discovery Studio 3.0 programme with CDOCKER protocol was also used to elucidate the mechanism for structure–inhibition relationships of herbal constituents against the crystal structure of mouse P-glycoprotein (PDB: 3G60) which was selected to be the receptor for molecular analysis in the their study because BLAST sequence alignment revealed that there was 87% whole sequence identity and nearly 100% identity inside the binding pocket with

the exemption of mSer725/hAla729 betwixt mouse and human P-glycoprotein. The active site of P-gp was defined according to the internal ligand's binding. After refinement with CHARMm force field, the herbal constituents were docked into the transporter using default options. A docking devoid any output pose was regarded a setback. The conformation synonymous to the lowest CDOCKER Energy was chosen as the most probable binding conformation. A computational docking model (CDOCKER) was used to investigate the molecular binding modes of the herbal constituents with P-gp. The results of the binding modes suggested that different amino acid residues in the internal cavity of this pump were responsible for the binding of herbal inhibitors. Their results showed that amino acid residues including Phe974, Phe728, Val978, Ser975, Gln191, Gln721, and Tyr949 in the internal cavity of this pump could be liable for the binding of herbal compounds investigated in the present study. Emodin had four potential Pi interactions with Phe974 and Phe728, while chrysophanol exhibited four potential Pi interactions with Phe974, Phe728 and Val978. In particular, 3-hydroxyl of emodin could form a hydrogen bond with Ser975 (1.839 Å), which may be critical for the stronger inhibition of emodin. Although the 18-hydrogen atom of glycyrrhetic acid (GA) did not interact with any amino acid residues of P-gp, different binding conformations were observed for 18β-GA and 18α-GA. Furthermore, 18β-GA interacted with Gln191 via two strong hydrogen bonds, while there was no potential interaction except Van der Waals between 18α-GA and P-gp. DAG could form a hydrogen bond potentially with Gln721, but no potential interaction existed between AG and P-gp except Van der Waals. The hydroxyls of 20(S)-GF1 interacted with Gln721 and Gln191 via three hydrogen bonds, which was different from Rh1 binding with Tyr949 via a hydrogen bond. Apart from that, verapamil (a positive inhibitor of P-gp) formed a Pi interaction with Phe974 in the study. In conclusion of this study is that some phytochemicals displayed P-gp inhibitory activity to particular realms *in vitro* and *in vivo*. Altogether, their consequences provisioned the rudiment for the trustful appraisal of the potential riskiness of herb–drug interactions in humans.

4.2) Structure-based pharmacophore modelling

The spatial info about the target protein is utilised in structure-based pharmacophore modelling to generate a topological description of interactions between a ligand and protein receptor (Leach et al., 2010). Beginning from the three-dimension

coordinates of a ligand which binds to a macromolecular target, conceivable molecular interactions betwixt the binding couple are appraised. It's important to assure the reliability of the binding-site residues and ligand coordinates by visual inspection of their degree of fitness to the coincident electron density map available, for instance, at the Uppsala Electron Density Server (Kleywegt et al., 2004). The step after that is the manual or automatic analysis of molecular interactions betwixt the compound and protein. On the basis of confronting chemical functionalities and their geometrical arrangement toward each other, pharmacophoric features are filled on the compound side where molecular interactions are remarked. Excluded volume spheres can be put on atoms of binding site to point out sterically unfavourable areas for a mapped ligand conformation. Instances for the creation and optimisation of structure-based pharmacophoric models can be seen in the publications (Barreca et al., 2007; Markt et al., 2007). Softwares that provide for the manual pharmacophoric generation from protein-ligand complexes, for example, are Schrodinger's Phase, Accelrys' Discovery Studio, IntelLigand's LigandScout and MOE by the Chemical Computing Group.

Salam et al. described a new method for creating structure-based pharmacophores utilising energetic analysis (Salam et al., 2009). This technique combines pharmacophore insight and screening of database by protein-ligand energetic terms calculated with a docking scoring function (i.e., Glide XP) to sort the importance of pharmacophore features. The combining the energy terms and speed of a ligand-based pharmacophore search from a structure-based analysis leads to a method that yanks the solidities of both approaches to generate high enrichments with a fine variety of active molecules.

4.3) Molecular dynamics studies

Molecular dynamics (MD) simulations have turned into more gainful in studying biological systems relevant to drug discovery (Perez-Sanchez & Wenzel, 2011); Salsbury Jr (2010). In some cases, the protein structure derived from the experiment may not be suitable for structure-based study. For instance, the structure would stand for the protein with a closed conformation in which the movement of a hinge region impedes the entry to the ligand binding pocket. Regarding molecular docking-based study, a target protein must be in an open conformation and then be predicted. Suchlike

a prediction of protein conformations can be operated using molecular dynamics simulation (Marco & Gago, 2007). Beyond determination of the open conformation of proteins, conformations induced by co-factor binding can be predicted by molecular dynamics simulations (Amaro & Li, 2010). With regard to structure-based study, molecular dynamics simulations act an essential role in realisation the features that are essential for ligand-binding affinity. This info could be applied to choose supereminent-affinity ligands from screening procedures.

Protein-mediated transportation of a molecule is a dynamic process associating various intermediate target protein conformations. Therefore, a static molecular structure of protein receptor may not be sufficient to analyse the molecular transport mechanisms and molecular dynamics simulations may overpower these constraints. For instance, to define the catalytic mechanism of the nucleotide binding domains of ATP-binding cassette efflux pumps, Jones and workmates operated a 390 bps MD simulation of the nucleotide binding domain of the histidine permease (HisP) of bacteria (Jones & George, 2002). The authors discovered that the peptide bond betwixt Phe99 and Gln100 serves as the hinge spot in ATP-binding cassette pumps, which moves the conserved glutamine in and out of the catalytic site. They also identified major interfaces associated in TMD and NBD communication that, along with data from the previous experiment, could be paraphrased to a delicate catalytic cycle of the ATP-binding cassette pump superfamily.

A meaning of MD simulations is for verification a stability order that a molecular docking study provides it and making clarification of molecular interactions that a ligand interplays with a protein target for binding (Jara et al., 2013).

In 2013, Jara et al. used classical molecular dynamic techniques using mouse P-gp to study the P-gp inhibitors involved of propafenone derivatives (3-phenylpropiophenone nucleus) including Gp240 and the best inhibitor XR9576 (tariquidar). 1of 3 steps pertains the classic molecular dynamics (MD) simulations along with dynamic characterisation of the binding mode, dissection of energy of molecular interactions and exploration of the amino acids associated that were used to study for these compounds focusing on the conformational alterations of protein at the P-glycoprotein binding site. Toward molecule binding, the results could be elucidated

with analysing the resetting, mainly because of alterations in the placement of the side chains of protein, including the existence of the interplay with major amino acids. Therefore, terse MD simulations were operated for describing those placing and evading any feasible unfolding because of the nonexistence of the lipid bilayer (Jara et al., 2013).

The energies approximate 5 and 2 kcal/mol were used for XR9576 and Gp240 respectively in order to bind to P-gp at P1 site and indicated that were more stable than rhodamine. The first major encouragement to $\Delta G_{binding}$ is van der Waals (hydrophobic type). The lipophilicity of the P1 site relates with this force. Moreover, XR9576 is more consistent than Gp240 because of the van der Waals. The second, which's a hydrophobic interaction too, was the alteration in nonpolar solvation. The hydrophobic interaction likewise was discovered. The major unique imparting inhibitor activity was due to the hydrogen acceptors capability. These were the obtained results from energetic decomposition elucidation.

Nevertheless and notwithstanding the restrictions and the incumbrances for straightforwardly comparison free energies versus EC_{50}s (in place of K_i's), the free energies of binding gained from the molecular dynamic energetic exploration are discreetly in the order of the observe EC_{50} for twain Gp240 (K_i = 0.97 µM approximately versus 17.61 µM observed EC_{50}) and XR9576 (K_i = 2.1 nM approximately versus 16.3 nM observe EC_{50}).

Their observations suggested that the main characters associated with the inhibitory activity are the lipophilicity and flexibility of molecule. The latter determinant may raise the capacity of P-gp inhibitory agents for fitting of aromatic rings within the P-gp's transmembrane domain.

1.2.9 *In vitro* assays

There are many *in vitro* assays available to investigate the interaction of a test compound with P-gp, each with advantages and disadvantages.

1) Transcellular Transport

Transport studies can identify P-gp substrates, modulators or inhibitors. With monolayer efflux assays, where the ratio or basolateral-to-apical (B→A) permeability

against apical-to-basolateral (A→B) permeability is considered like the standard for identification of P-gp substrates as it measures the efflux of drugs in the most direct method (Polli et al., 2001; Rautio et al., 2006).

The major disadvantage of these assays is they are labour intensive by reason of cell culture and analytical techniques, limiting throughput.

In brief, cells are seeded on a membrane surface and the tested chemical is filled to the apical and/or basolateral compartment. At each pre-determined time point, the tested compound concentration is measured in each compartment. In the case of P-gp which is apically located, the basolateral to apical flux will domineer (Szakács et al., 2008). As this technique requires identification of the compound, it is not suitable for complementary products as the composition is often unknown and is highly variable.

In these studies different cell types may be employed including porcine kidney epithelial (LLC-PK1), Madin-Darby canine kidney (MDCK) cells, and human colonic adenocarcinoma (Caco-2). Caco-2 cells are similar to intestinal epithelium, and are the broadest characterised model for examination of the permeability of drugs (Balimane et al., 2006; Elsby et al., 2008).

Membranes isolated from the cells mentioned above, can be isolated. They contain high levels of transporters suitable for the characterisation. Membrane-based assays characterisation of the transporter may be according to (1) the catalytic activity, (2) the binding of the compound to the membrane transporter, (3) the veritable substrate transportation. An assay based on inside-out membrane vesicles is a clinically relevant method (Kharasch et al., 2005).

2) ATPase Expression

As P-gp is ATP dependent (see section 3.3), monitoring ATPase activity is a useful tool to determine P-gp interactions. In the absence of a test compound, P-gp will express a basal ATPase activity level. If a test compound binds to P-gp then an increase in ATPase activity will be detected. Whilst monitoring ATPase activity can be scaled to a high-throughput assay (Garrigues et al., 2002).

One important advantage of ATPase assays that is the key when working with unknown tests compounds such as complementary products is that these assays do not

require analytical techniques to identify the product. ATPase assays have been developed for a variety of systems including enriched and crude plasma membranes (Garrigues et al., 2002; Kokubu et al., 1997), and enriched microsomal membranes (Litman et al., 1997). Purified P-gp can also be used (Ambudkar et al., 1997; Lu et al., 2001).

3) Cellular Accumulation

The accumulation of fluorescent dyes within the cell can be used to identify inhibition of P-gp. These assays are easily adapted for high-throughput screening, though identification of inhibitor or substrates can be difficult as there are multiple binding sites on P-gp (Schwab et al., 2003).

Measuring the cellular accumulation of these dyes in the presence of a test compound may be done via flow cytometry, as in the case of Rhodamine 123 (a cationic dye) or by standard fluorometric measurements as with Calcein. Calcein AM is a non-fluorescent, high lipid soluble dye that expeditiously penetrates the plasma membrane of cells. In addition to this, Calcein AM is a fine substrate for the efflux pumps for both P-glycoprotein and multidrug resistance protein (MRP). When Calcein AM enters the cells it is metabolised by the cytosolic esterase's into calcein, which is highly hydrophilic and fluorescent in Figure 1.8, schematic representation of the calcein AM extrusion assay. a) The lipid soluble calcein AM is able to cross the cell membrane. In the presence of esterase's the calcein AM is hydrolyses to the fluorescent calcein which is also hydrophilic. b) If inhibition of the P-gp pump occurs then calcein cannot be extruded and the fluorescence is recorded (Sarkadi et al., 2006).

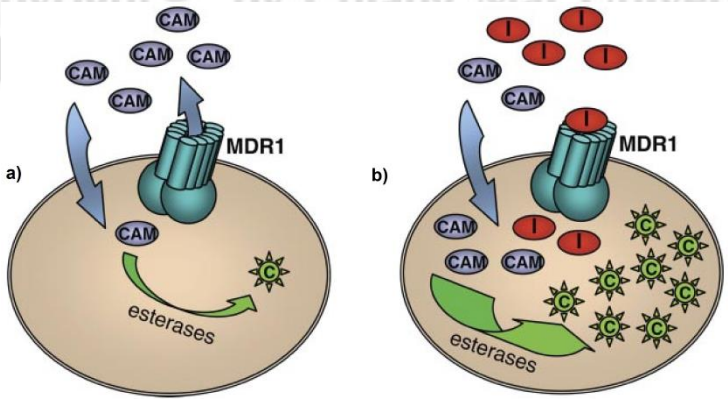

Figure 1.8 Schematic representation of the calcein AM extrusion assay.

1.3 Hypothesis

There are increasing evidences that P-gp inhibition is a major cause of a number of herb-drug interactions. However, there is a little knowledge about the interaction of ligands (inhibitors) with P-gp at molecular levels. It remains unclear how activities of this drug transporter are influenced by the presence of most herbal medicines, particular flavonoid-containing herbs, in body system. When different compounds (e.g., a drug and flavonoids) are co-administered, they may compete at the identical binding site of P-gp, lead to potential inhibition. We hypothesise that the atom-atom interactions between the ligands and the residues at the P-gp binding site determine affinities of ligands and that relates to inhibitory activity of ligands and these interaction features can be used as the templates to predict P-gp inhibitory activity of other ligands.

According to our hypothesis, we conduct a series of computational experiments to determine inhibitory actions of flavonoids on P-gp, *in silico* studies to explore the flavonoid–P-gp interactions using QSAR, molecular docking, pharmacophore modelling and molecular dynamics simulation methods.

1.4 Purpose of the study

This work aims to: 1) develop a QSAR model for prediction of a potential inhibitory activity on P-gp function of flavonoids using the molecular descriptors; 2) investigate the location of possible binding sites, molecular interactions, orientation and binding affinities of flavonoids in the P-gp binding cavity by molecular pharmacophore modelling and docking methods; 3) investigate of P-gp–flavonoid molecular dynamic interactions using a molecular dynamics (MD) simulation approach; 4) the developed computational approaches will proffer future directions to conduct the computational models more accurate, reliable, and publicly accessible for predicting the pharmacokinetic interactions of Thai herbs and their products through inhibition of P-gp.

1.5 Research design

An overview of this investigation of herbal constituents on P-gp inhibitory activity is shown in Figure 1.9.

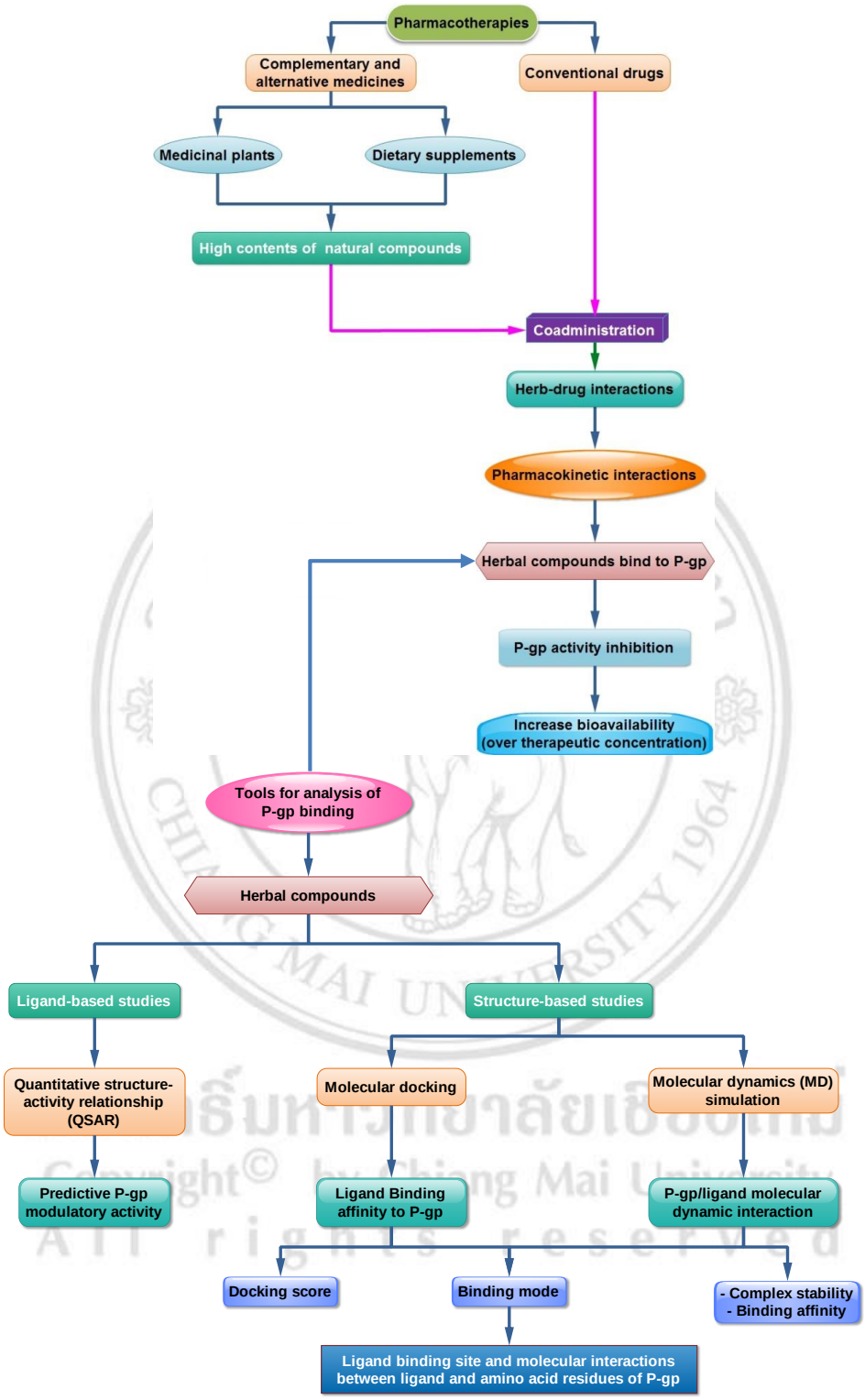

Figure 1.9 An overview of the research