CHAPTER II

LITERATURE REVIEWS

Quality control of herbal drugs

The quality control methods are plays an important role in traditional medicine which conserve as a tool for identification, authentication and quality control herbal drug [14]. There are numerous reports on the toxicity due to the misidentification and substitution of plant species. Pharmacognosy is the first step of quality control assessment which defined as a pharmaceutical discipline by Seider in 1815. The definition of pharmacognosy is mean "the science which has the task to learn everything about drugs originating from plants or animals in all aspects, except under the physiological effect, to describe the correctly and under a general vision connect this knowledge" [16-17]. WHO had published the *"Quality control methods for medicinal plant materials"* a recommended test procedure to evaluate the identity, purity and quality of the plant materials. The guidelines define that the quality and authenticity of the final botanical products is directly related to the proper identification and authenticity of the source materials. The majority of the information for quality assurance is based on the standardization parameters such as authentication, water content, loss on drying, total ash, acid-insoluble ash, extractive values and chemical fingerprint of medicinal plant materials. These standardization parameters are essential to publish in the pharmacopoeia [14].

Macroscopic and microscopic methods are the effective tool to establish the correct identity of the plant materials [18]. The macroscopic evaluation or the organoleptic characteristics of plant materials is based on the shape, size, colour, surface characteristics, texture, fracture and appearance of the cut surface either with the naked eye or with simple magnification such as with a hand lens or stereomicroscope. The same species of plant materials could be have similar morphological characteristics. Furthermore, the evaluation of this parameter can provide a qualitative assessment of adulterating species such as filth, organic and non-organic contaminants, and material degradation [5]. Microscopic examination gives a clear idea about the identification of crude plant material in the whole, fragmented or

powdered form. Microscopy of medicinal plants focused on the observation of the cellular structures and their content of plant material by use of a compound microscope. The powder of crude drugs can be identified the presence of absence of various cell types based on their cyto-morphological character such as parenchyma, collenchymas, fibers, stone cells, vessels, trichomes, secretary cells, epidermal cell. Botanical microscopy is an integral part of pharmacognosy training which the method requires high expertise because sometimes it may not provide unequivocal authentication based on the fact that the similar microscopic characteristics presented in related species [10].

Comparing to with other techniques, macroscopic and microscopic identification are very cost and time-efficient. The evaluations of these parameters provide the specific characteristics of crude drugs such as morphological or macroscopic characteristics, cyto-morphological and microscopic characteristic in both its entire and its powder form. Therefore, these authentication methods play an important role in the monographs on herbs in many pharmacopeias, including the Chinese, European and Thai herbal pharmacopoeia [10].

Physico-chemical parameters are important to identify the purity and quality of herbal drugs. The physico-chemical constants could be useful for detection of adulteration or improper handling of drugs, identification, authentication and also compilation of compilation of a suitable monograph [19]. The procedures normally adopted to get the qualitative information about the purity and standard of a crude drug include the determination of various parameters. The total ash is particularly important to determine the purity of crude drugs. Ash values are simply represents the total amount of material remaining after incineration which includes ash derived from the part of the plant itself and deliberately added to it crude drug as a form adulteration [19-21]. The extractive values give an idea about the chemical constituent of crude drugs which soluble in ethanol or water [22, 23]. Moisture is an unexpected component of crude drugs because an excess of moisture can result in the breakdown of important constituents by enzymatic activity and may encourage the growth of yeast and fungi during storage [24]. Methods of determination of moisture content include the loss on drying and the volumetric azeotropic distillation methods to

estimate the loss on drying after heating at 100 to 105 °C and measure of water content in crude drugs [5].

Preliminary phytochemical screening reveals the presence of a wide range of phytoconstituents from crude extract such as alkaloids, glycosides, saponins, carbohydrates, flavonoids, tannins, amino acids and steroids for supporting the reason for its wide range of biological activities [25].

The fingerprinting analysis is nowadays getting momentum for the quality control of multi-component herbal medicines and has been widely accepted as a useful tool to determine authenticity and reliability of chemical constituents of herbal drug and formulations [26]. Chromatographic techniques are the most versatile tools for the analysis of herbal medicines. A chromatographic fingerprint is a chromatogram representing the chemical characteristics of herbal materials [10]. Thin layer chromatography is the common method for herbal medicine analysis for over the past decades. Even nowadays, TLC is still frequently used to provide first characteristic fingerprints of herbal medicines and can be establish in various pharmacopoeias such as American Herbal Pharmacopoeias, Chinese drug monograph and analysis and Thai herbal pharmacopoeias [11]. The advantages of TLC method to perform the fingerprinting of herbal medicines are based on the simplicity, versatility, high velocity, specificity, sensitivity and ease to prepare the sample. High performance liquid chromatography is become popular method for the analysis of herbal medicines due to its superior precision, high resolution and capacity to separate a very wide range of organic compounds, from small-molecule drug metabolites to peptides and proteins, unlikely Gas chromatography technique which is suitable for volatile compounds [17]. The combination technique between High performance liquid chromatography and online UV spectrum detection *via* diode array detectors or tandem mass spectra to separated ingredient can be obtained simultaneously and identified these ingredients by comparing the spectra with the reference compounds [27].

Many plants contain unique sets of compounds in characteristic ratios that can allow for the differentiation of even closely related species and even different plant

part of the same part. TLC, GC, HPLC, CE and hyphenated techniques have already been used for the development of chromatographic fingerprints. The multiple chromatographic approaches can be extensively defines the authenticity and quality characteristics of a specific herbal extract containing the combination of multiple ingredients to regulated quality control of herbal medicines [17]. Several parameters could be evaluated qualitatively as well as quantitatively for determination of the quality control of medicinal plant materials such as authentication, identification, organoleptic characteristic, physicochemical analysis, chemical characterization and biological investigation as shown in figure 2.

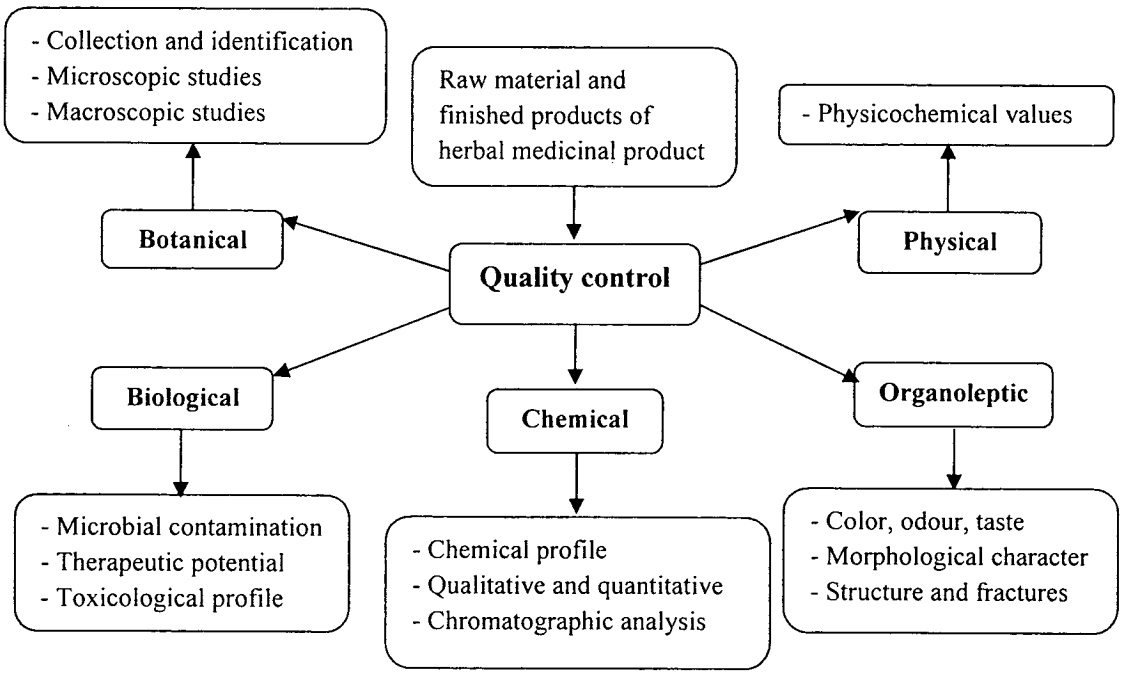

Figure 2 Quality control methods for medicinal plant materials

Safety and efficacy regulation

The safety and efficacy of herbal medicines are established through their long historical use to treat various health ailments and absence of evidence of harm, thus they are generally regarded as safe to consume. However, most herbal medicines still need to be studied scientifically in systemic toxicology and efficacy assessment. The WHO has published "Research guidelines for evaluating the safety and efficacy of herbal medicines" in order to determine the basic criteria for evaluating the safety and efficacy of herbal medicines aimed at assisting national regulatory authorities, scientific organizations and manufacturers in the particular area [1, 28]. Herbal medicine extracts contain a variety of molecules with potent biological activities which is difficult to analyze the biological activities of these extracts because of their complex nature and the possible synergistic effects of their components [29]. Numerous advanced biological experimental techniques have been used as standard safety tests along with the efficacy studies [4].

Most preclinical development studies employ rodents, primarily mice. The animal model can study effects of toxicants under well-controlled conditions in a complete mammalian system. Mammals could be share many similar aspects of anatomy, physiology and biochemistry, thus making extrapolations to humans reasonably valid scientifically and most regulatory approvals cannot take place without *in vivo* testing. However, there are some disadvantages of *in vivo* studies which are time consuming, expensive and variation in structure and function from one species to another. In addition, the used of high doses in animals may not be predictive for a target human population. Based on the rationale of animal welfare which concern about animal used for *in vivo* testing, there is much debate about the replacement of experimental animals with *in vitro* toxicology models. It is possible that a wide range of *in vitro* techniques, using both transformed and unmodified human and rodent cells, could serve as useful media in various toxicity assays.

There are many advantages of *in vitro* studies which could be reduced the number of animals used for *in vivo* tests, relatively simple to perform and quantify, using human cells is possible and less expensive than animal test. However, there are

some disadvantages of *in vitro* studies such as the route of exposure for an *in vitro* test of chemical directly to cells in culture through their culture through their culture is not comparable with an *in vivo* exposure, the results may be highly variable between laboratories or cannot repeated, difficult to relate *in vitro* dosages to those that produced toxicity to whole animals and may not satisfy regulatory. demands or provide scientifically adequate information as evidence [30].

The safety of herbal medicines is a major concern for traditional medicine practitioners, pharmacists, doctors and other healthcare professional because the long historical usage of herbal medicines [31]. The importance of toxicity testing is to provide safety evaluations of these plants. The extensive traditional use of plants as medicines has enabled those medicines with acute and obvious signs of toxicity to be well recognized and their use avoided. The more subtle and chronic forms of toxicity, such as carcinogenicity, mutagenicity and hepatotoxicity, may well have been disregarded by previous generations and it is these types of toxicities that are of most concern when assessing the safety of herbal remedies [32]. However, many different side effects to herbs have been reported and recently reviewed, including effects from biologically active constituents from herbs, side effects caused by contaminants, and herb-drug interactions [33]. It is likely that many different *in vitro* cytotoxicity methods could be used to help select the *in vivo* starting dose for an acute lethality assay. Two decades of experience indicate that *in vitro* basal cytotoxicity data determined in various primary cells, as well as in various permanent non-differentiated finite or transformed cell lines, generally show comparable cytotoxic concentrations of the same xenobiotic, regardless of the type of toxic endpoints investigated [34]. The investigation of half-lethal dose (LD_{50}), the dose at which it has been proven to be lethal-causing death to 50% of the tested group of animals, has been criticized for both scientific and animal welfare reasons, and the test procedure has been modified in various ways to reduce the number of animal used [35].

Ben-Cha-Moon-Yai remedy

Ben-Cha-Moon-Yai remedy or Pikud Ben-Cha-Moon-Yai is one of the Thai ancient remedies which revealed in Tumra Paadsard Song Khor. Pikud is an herbal remedies that are compose of a set of ingredients in an equal part by weight. The components of Ben Cha Moon Yai Remedy are shown in **table 1**.

Table 1 Component of Ben Cha Moon Yai remedy [15]

Scientific name	**Thai name**	**Family name**	**Plant Part**
Oroxylum indicum (L.) Kurz	เพกา	BIGNONIACEAE	Root
Aegle marmelos (L.) Corr.	มะตูม	RUTACEAE	Root
Dimocarpus longan Lour.	ลำไย	SAPINDACEAE	Root
Walsura trichostemon Miq.	คัดลิ้น	MELIACEAE	Root
Dolichandrone serrulata (DC.) Seem.	แคแตร	BIGNONIACEAE	Root

Aegle marmelos (L.) Correa ex Roxb.

Aegle marmelos (L.) Correa ex Roxb., commonly known as "Bael tree" belonging to the family Rutaceae and known in Thai as "Matum". It is a deciduous tree with a large or medium sized tree, 12-15 m in height and has been naturalized in Thailand, India, Sri Lanka and various southeastern Asian countries [36]. The leaves, roots, bark, and fruits of this plant are widely used in Ayurvedic and ethnomedicine [37]. They are used to treat an inflammation, catarrh, diabetes, asthmatic complaints, diarrhea, dysentery, and weakness of heart [38].

Chemical constituents

The chemical literature survey of *A. marmelos* revealed that there are several chemical constituents isolated and identified from leaf, fruit, seed, bark and root such as alkaloids, coumarins, steroids and tannin.

Table 2 Chemical constituents of various parts of *A. marmelos* [39]

Part	Chemical constituent
Leaf	aegeline, skimmianine, rutin, γ-sitosterole, ß-sitosterol, lupeol, cineol, citral, *O*-isopentenyl, hallordiol, mameline, citronellal, cuminaldehyde, euginol, marmesinin, aegelin. anhydromarmeline, aegelinoside A, aegelinoside B, limonene, (Z)-*ß*-ocimene [40-43]
Fruit	marmelosin, luvangetin, aurapten, psoralen, marmelide marmesiline, 6-(4-acetoxy-3-methyl-2-butenyl)-7-hydroxycoumarin, 6-(2-hydroxymethyl-3-butenyl)-7-hydroxycoumarin, marmelonine, 8-hydroxysmyrindiol, imperatorin, valencic acid, 8-[(3"-methyl-2"oxo-3"-buten-1"-yl)oxy]-7H-furo[3,2-g]benzopyran-2-one, xanthotoxol, isogosferol, xanthotoxin, scoparone, (+)-decursinol, demethylsuberosin, 6-formylumbilliferone, (+)-marmesin, marmeline, isofraxidin, isophellodenol C, xanthoarnol [44]
Seed	imperatorin, *ß*-sitosterol, plumbagin, 1-methyl-2-(3'-methyl-but-2'-enyloxy)-anthraquinone, *ß*-sitosterol glucoside, stigmasterol, vanillin, salicin [36]

Bark	skimmianine, fagarine, marmin
Twig	marmesin, scopoletin, limonene, (Z)-ß-ocimene [43, 45]
Root	aeglemarmelosine, skimmianine, imperatorin, aurapten, epoxyauraten, marmin, xanthotoxin, aegeline, skimmianine, umbelliferone [40, 45, 46]

Pharmacological activities

Various crude extracts from various parts of this plant have shown their biological activities. The extracts from *A. marmelos* leaves showed significant analgesic activity on acetic acid-induced writhing and tail flick test in mice [47]. These extract also produced marked inhibition of the carrageenan-induced paw oedema and cotton-pellet granuloma in rats and caused a significant reduction in yeast-induced hyperpyrexia in mice [48]. Anhydroaegeline compound isolated from leaves exhibited the highest inhibitory effect on α-glucosidase inhibition assay whereas anhydromarmelin, aegelinosides A and B showed slightly weak inhibition activity [42]. Previous studies also reported that Aegeline 2, an alkaloid-amide lead, isolated from the leaves of this plant demonstrated the anti-hyperglycemic and antidyslipidemic activities in animal models. The activity may result from lowered blood glucose and decreased in lipid profile [49]. In accordance with the previous studied from different parts of this plant, the result showed that the aqueous seed extract at 250 mg/kg was also lowered total cholesterol (TC), triglyceride (TG) and low density lipoprotein (LDL) but enhanced the cardioprotective lipid (HDL) in diabetic animals [50].

A large number of compounds have been isolated from different parts of the plant and a few of them have been studied for their biological activity. A new anthraquinone, 1-methyl-2-(3'-methyl-but-2'-enyloxy)-anthraquinone, isolated from seed also displaying significant antifungal activity against *A. fumigates* and *C. albicans* by using disc diffusion assay with MIC values of 6.25 µg/disc, and MIC 31.25-62.5 µg/ml in microbroth dilution assay and 31.25 µg/ml in percent spore germination inhibition assay [36]. In addition, xanthoarnol compound isolated from

the acetone extract of the green fruits of *A. marmelos* exhibited the antibacterial activity against *E. faecalis* with the MIC values of 18. 75 μg/ml as same as vancomycin, which can be considered as a potential antibacterial agent [44].

The ethanolic extract from stem bark showed the cytotoxicity against brine shrimp lethality testing with presenting LD_{50} of 17.5±2.0 μg/ml and also possessed the cytotoxicity against leukemias and melanoma cell lines in MTT assay [51]. In addition, the stem bark extract and isolated compounds from *A. marmelos* were inhibited the *in vitro* proliferation of different human tumor cell lines and strongly inhibited human k562 cells. The result was comparable to some of the most commonly used antitumor agents such as cisplatin [52]. For *in vivo* toxicity testing, the methanolic extract from leaves indicated non-acute and subacute toxicities in rats after intraperitoneally of maximum doses up to 1000 mg/kg and 100 mg/kg with 14 consecutive days, respectively [53].

Figure 3 *Aegle marmelos* (L.) Correa. [54]

Dimocarpus longan* Lour. subsp. *longan* var. *longan

Dimocarpus longan Lour. is an evergreen tree which belongs to the Sapindaceae family. It is widely grown in China, Taiwan, and South East Asia including Thailand and Vietnam [55]. Longans commonly known as "Lamyai" in Thailand and has been widely cultivated in the northern Thailand [56]. Both longan flowers and fruits make a significant contribution in Thai food and herbal preparations. Longan fruits contain vitamins and minerals, such as iron, magnesium, phosphorus and potassium, and large amounts of vitamins A and C [57]. Previous study reported that longan fruit extract contained a significant amount of polyphenolic compounds which has been widely known for its antioxidant effects [58]. The content of polyphenolic compound is varied from different part of plant and cultivars [59]. These plant materials are considered to be a cheap source of herbal antioxidant used in neutraceutical products.

Chemical constituents

D. longan has been widely investigate, especially in the field of chemical constituents studies. Many types of chemical compounds, isolated from different parts of this plant, have been reported a significant amount of polyphenolic, polysaccharide, vitamin and essential oil compounds. The occurrences of chemical compounds in this plant were shown in table 3.

Table 3 Chemical constituents of various parts of *D. longan*

Part	Chemical constituent
Flower	(-)-epicatechin, proanthocyanidin A2 [60]
Inflorescences	caryophyllene, γ-elemene, ∂-caryophyllene, β-guaiene, Germacrene D [61]
Aril	lysophophatidyl choline, phosphatidyl choline, phosphatidyl inositol, phosphatidyl serine, phosphatidyl ethanol amine, phosphatidate, phosphatidic acid glycerol [62]
Fruit	corilagin, gallic acid, ellagic acid, ascorbic acid [63, 64]

Fruit peel	polyphenol oxidase (PPO) [65]
Fruit pericarp	acetonylgeraniin, (-)-epicatechin, 4-O-methylgallic acid, flavones glycosides, glycosides of quercetin, kaempferol, *L*-arabinofuranose, *D*-glucopyranose, *D*-galactopyranose, *D*-galacturonic acid, methylated Ara, methylated Glc, methylated Gal [66-68]
Seed	corilagin, gallic acid, ellagic acid, monogalloyl-diglucose, digalloyl-diglucose, penta-to heptagalloyl-glucose, ellagic acid-pentaose conjugate, galloyl-hexahydroxydiphenoyl-glucopyranose (HHDP), pentagalloy-HHDP, procyanidin A-type dimer, procyanidin B2, quercetin-3-*O*-rhamnoside, 1-*ß*-*O*-galloyl-*D*-glucopyranose, ethyl gallate, methyl brevifolin carboxylate, brevifolin, 4-*O*-α-*L*-rhamnopyranosyl-ellagic acid , methyl gallate, (-)-epicatechin, proanthocyanidin trimer, geraniin, isomallotinic acid, methyl brevifolin carboxylate, chebulagic acid [69, 70, 71, 75, 76]

Pharmacological activities

The biological activities of *D. longan* had been evaluated along with other plants. Phenolic compounds presented in different parts of this plant were reported to have antioxidant capacities in various models. The different extracts of *D. longan* seeds contain high amount of polyphenolic compound. The polyphenol-rich longan seed extract exhibited strong antioxidant capacities as effective as Japanese green tea extract on DPPH radical scavenging, superoxide radicals, ORAC, and anti-tyrosinase [72].

The methanolic extract also inhibited the xanthine oxidase in hypoxanthine/xanthine oxidase assay [71]. In addition, a polyphenol rich longan seed extract inhibited cell proliferation against three colorectal carcinoma cell lines by blocking cell cycle progression during the DNA synthesis phase and inducing apoptotic death [73]. Therefore, it was suggested that a polyphenol rich longan extract

could be employed as a potential novel treatment agent for cancer. The seed extract also possessed strong antifungal activity toward Candida species and *Cryptococcus neoformans* [74]. In addition, *D. longan* seed extract has low acute toxicity with LD_{50} higher than 5000 mg/kg in mice and revealed no toxic effects after repeated doses by oral administration [75].

The methanolic extract of *D. longan* flower demonstrated the highest antioxidative activity followed by ethyl acetate and n-hexane extract toward *in vitro* assays such as DPPH free radical scavenging, oxygen radical absorbance capacity, and the inhibition of LDL assays. The main active components in the inhibition of LDL inhibition oxidation were characterized as (-)-epicatechin and proanthocyanidin A2 [60].

The *D. longan* fruit pericarp extract obtained from high pressure-assisted and ultra high pressure-assisted extraction methods can gave a higher potential in antioxidant and anticancer activities than conventional extraction method through various *in vitro* models such as DPPH radical scavenging, total antioxidant capacity, superoxide anion radical scavenging, lipid peroxidation, anti-tyrosianase and cytotoxicity using MTT assays. The water extract from *D. longan* twigs also showed antimutagenic, antioxidant, antityrosinase activities and decreased lipid oxidative damage. [76]

Despite polyphenolic compounds, polysaccharide was also characterized from the seed and fruit pericarp of *D. longan.* This compound demonstrated a free radical scavenging activity in DPPH assay [77] and showed a good potential in anti-glycated activity [78].

Figure 4 ***Dimocarpus longan*** **Lour.** [79]

Dolichandrone serrulata (DC.) Seem

Dolichandrone serrulata (DC.) Seem is a deciduous tree belongs to Bignoniaceae family. The plant is widely distributed in South-east Asia known in Thai as "Kae Trae" or "Kae Pa". The flower is edible with a bitter taste and has been used as part of Thai cuisine. In addition, the bark of this plant has been used as Thai ancient remedy for antipyretic and anti-inflammatory [80].

Chemical constituents

The chemical composition of *D. serrulata* had been characterized from flower and branches.

Table 4 Chemical constituents of various parts of *D. serrulata*

Part	Chemical constituent
Flower	new cyclohexylethanoid, sitosterol-3-*O*-*β*-*D*-glucoside [81]
Branch	dolichandroside, decaffeoyl-verbascoside, verbascoside, isoverbascoside, markhamioside A, 2"-*O*-apiosylverbascoside, luteoside B, ixoside [80]

Pharmacological activities

Although this plant has been used as vegetables, only few studies on pharmacological activities of this plant were provided. Most researchers investigated the antioxidant capacities of this flower extract. The result revealed that the flower extract from this plant exhibited low antioxidant capacities in three different antioxidant assay such as DPPH free radical scavenging activity, trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP) and total phenolic content [82-83]. For the isolated compound, Cyclohexylethanoid isolated from flower of *D. serrulata* demonstrated moderate anti-inflammatory activity in ethyl phenylpropiolate-induced ear edema in rats [81].

Figure 5 ***Dolichandrone serrulata*** **(DC.) Seem** [84]

Oroxylum indicum (L.) Kurz

Oroxylum indicum (L.) Kurz is deciduous tree belonging to the Bignoniaceae family which commonly known as "Indian Trumpet tree". It is an edible plant which young shoots and unripe fruits are eaten as vegetables which are widely distributed in South East Asia, South Asia and China. *O. indicum* has been used as medicinal herb for thousands of year in many Asia countries and used in folk medicine as a cure of various diseases [85]. The fruits and flowers are consumed as a common part of the diet in the north and northeast of Thailand [86]. The plant has been used as a single drug or as a component of drug formulation in India. The root is used in preparation of well known Ayurvedic formulation such as "Chyavanprash" and "Dashmularistha" which used as an anti-inflammatory, anti-helmintic, anti-rheumatic, anti-bronchitic and astringent [87]. The seeds are active in chronic cough, abdominal pain and purgative [88].

Chemical constituents

Phytochemical investigations from various parts of *O. indicum* have been presented a number of secondary metabolites such as flavonoids, glycosides, alkaloids, tannins and terpenoids [88].

Table 5 Chemical constituents of various parts of *O. indicum*

Part	Chemical constituent
Leaf	chrysin, baicalein, baicalein-7-*O*-glucoside, baicalein-7-*O*-diglucoside, chrysin-7-*O*-glucoronide, baicalein-7-*O*-glucuronide, chrysin-diglucoside [89]
Seed	chrysin 6-C-*β*-*D*-glucopyranosyl-8-*O*-*β*-*D*-glucuronopyranoside, baicalein 7-*O*-*β*-*D*-glucuronopyranosyl-(1→3)[β-*D*-glucopyranosyl-(1→6)]-*β*-*D*-glucopyranoside, scutellarein 7-*O*-*β*-*D*-glucopyranosyl-(1→6)-*β*-*D*-glucopyranoside, chrysin-7-*O*-gentiobioside, baicalein-7-*O*-diglucoside, baicalein-7-*O*-glucoside, scutellarein-7-*O*-glucopyranoside, chrysin-7-*O*-glucuronide, baicalin, chrysin-6-C-*β*-*D*-

glucopyranosyl-8-C-α-*L*-arabinopyranoside, chrysin, baicalein, oroxylin A, pinocembrin, pinobanksin, 2-methyl-6-phenyl-4H-pyran-4-one, lupeol, 2-α-hydroxyllupeol, echinulin, adenosine, dimethyl sulfone, *β*-sitosterol, baicalein-7-*O*-*β*-gentiobiosid, chrysin-7-*O*- *β*-gentiobiosid, baicalein-7-*O*-glucosid, baicalein-7-*O*-glucuronid, chrysin-7-*O*-glucuronid, baicalein, chrysin, baicalein-7-*O*-diglucoside (Oroxylin B), chrysin-7-*O*-diglucoside, baicalein-7-*O*-glucoside, baicalein, chrysin [90-93]

Stem bark dihydrooroxylin A-7-*O*-methyl glucuronide, 5- hydroxyl-7-methoxy-2-(2-methoxy-6-(3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yoloxy)phenyl)-4H-chromen-4-one, dihydro-iso-a-lapachone, 7-*O*-methylchrysin, 5-hydroxy- 4′,7-di methoxy flavones, dihydrooroxylin A, oroxylin A, chrysin, baicalein, 5,7-dihydroxyflavone, 5,7-dihydroxy-3-methoxyflavone, 3,5,7-trihydroxyflavone, 5,7,4′-trihydroxy-3-methoxyflavone, 3,5,7,4′-tetrahydroxyflavone, 5,7,4′-trihydroxyflavone, lapachol, [94-96]

Root chrysin, oroxylin A, pthallate, lapachol, *β*-sitosterol [87, 96]

Root bark chrysin, baicalein, ellagic acid, biochanin-A [97]

Pharmacological activities

The ethanol and hexane extracts of *O. indicum* did not show a mutagenic effect toward *S. typhimurium* strains TA98 and TA100 but after nitrosation under acidic condition these extracts become strongly mutagenic effect in both strains [98] whereas the methanolic extract from fruits exhibited the strong antimutagenic effect against the food-derived mutagen toward *S. typhimurium* strain TA98 in Ames test. The bioactive compound for antimutagenic activity was identified as baicalein [86]. In addition, among five edible plants in Thailand, the ethanolic fruit extract of *O.*

indicum exhibited strongest antioxidant scavenging activity in DPPH assay and antimutagenicity toward *S. typhimurium* strains TA98 and TA100 [99].

The total phenolic and total flavonoid contents had varied among the different part of this plant in various solvent extract. The previous studied reported that the highest amount of total phenolic and total flavonoid contents were obtained from the methanolic extract from seeds and stem bark, respectively [100]. In the stem bark, the methanolic extract contained the highest amount of both phenolic and flavonoid contents followed by dichloromethane and petroleum ether in accordance with antioxidant and antimicrobial activities whereas petroleum ether extract caused maximum cytotoxic and apoptotic activities on Hela cells [101].

Different parts of *O. indicum* have been investigated for their antioxidant potential in various *in vitro* models. The results demonstrated that the leaves and stem bark extracts exhibited the highest free radical scavenging activity in DPPH, nitric oxide, superoxide anion and hydroxyl radical scavenging assays which may be due to presence of polyphenolic compounds [102]. Seven flavonoid glycosides isolated from the seed of *O. indicum* were quantified as the main ingredient in the methanolic extract of this plant and showed the antioxidant activities in DPPH and ORAC assays [90].

The root bark extract exhibited the protective effect against ethanol-induced gastric mucosa damage and lipid peroxidation determined by malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione levels (GSH) assays [103]. In addition, among crude hexane and acetone extract and nine flavonoid compounds isolated from the stem bark of *O. indicum*, chrysin displayed highest potential on gastroprotective effect against various gastric ulceration models in Wistar rats [95]. Chrysin isolated from the root of *O. indicum* produced a significant protective effect against cisplatin induced nephrotoxicity in rats and it was decreased in lipid peroxidation activity [104].

The *n*-butanol fraction from *O. indicum* root bark demonstrated the potential on immune regulation activity which might be due to the present of baicalein in root

bark. It was also presented the reduction effect in whole blood malondialdehyde (MDA) content along with a rise in the activity of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) [105].

The ethyl acetate extracts from stem bark showed the anticancer effect to inhibited NF-KB cancer cell line and also possessed anti-inflammatory effect on LPS-induced inflammatory testing by inhibited the release of IL-1B and PGE_2. The extract also gave the highest cytotoxicity against Hela cell and antioxidant activity in DPPH and lipid peroxidation assays [106]. In addition, the low-polarity crude extract from *O. indicum* stem bark demonstrated the cytotoxicity, apoptosis inducing ability and anti-metastatic potential in both human breast carcinoma and human liver embryonic cell lines [107]. Previous studies also reported that both root bark and stem bark decoction showed anti-inflammatory activity in carrageenan induced paw edema in rats [108].

The aqueous and ethanolic extracts from *O. indicum* root at 300 and 500 mg/kg produced a significant to decrease in plasma glucose levels when compared with diabetic control group in alloxan-induced diabetes and dexamethasone-incuded insulin resistance in rats [109]. The methanolic extract from root bark of *O. indicum* produced a significant wound healing in animal model and also exhibited antimicrobial activity against various microorganisms [110] in accordance with the dichloromethane extract and all isolated compounds from the stem bark and root of *O. indicum* that also exhibited the antimicrobial activities against gram positive and negative bacteria, fungi and yeast [96].

Figure 6 *Oroxylum indicum* (L.) Kurz [111]

***Walsura trichostemon* Miq.**

Walsura trichostemon Miq is an evergreen tree belong to the family of Meliaceae. The *Walsura* genus comprises 30 to 40 species and widely distributed in China, India, Indonesia and South East Asia. The plant is naturally distributed in the evergreen forest in north, northeast and southeastern of Thailand. It is a plant with edible fruits which known as "Kad-lin". The stem bark and root had been used in folk medicine to treat tendon disabilities and wound healing. However, there are very few information has been reported on chemical constituents of this plant.

Chemical constituents

Previous investigation on *W. trichostemon* reported that tetraacetylated apotirucallane triterpenoid compound was isolated from the root of this plant. Several tetranotriterpenoids and triterpenoids had been isolated from different species of *Walsura* genus [112].

Table 6 Chemical constituents of various parts of *W. trichostemon*

Part	**Chemical constituent**
Root	trichostemonate [113]

Pharmacological activities

Among 40 species of *Walsura* genus, there are very scarcely researches concerning the pharmacological activities of *W. trichostemon*. Trichostemonated, tetraacetylated apotirucallane triterpenoid, isolated from the root of this plant demonstrated the cytotoxicity against human cervical carcinoma (HeLa) and human epidermoid carcinoma (KB) cell lines with IC_{50} of 0.93 and 3.28 μg/ml [113].

In addition, the ethyl acetated extract from the stem bark of this plant also exhibited strongest antimycobacterial activity on *Mycobacterium tuberculosis* and cytotoxic activity against human mouth carcinoma (KB), human small cell lung cancer (NGT-H187) and breast cancer (MCF-7) cell lines whereas hexane and methanol extracts showed only cytotoxicity in those cell lines [114].

Figure 7 ***Walsura trichostemon*** **Miq.** [115]