# CHAPTER 2
# LITERATURE REVIEW

## 2.1 *Tacca chantrieri* Andre.

*Tacca chantrieri* Andre. is the scientific name of Bat flower which is locally called “Nerapusee Thai” in Thailand. *T. chantrieri* belongs to the Taccaceae family and it is indigenous to the north and southeast regions of Thailand. The rhizomes of *T. chantrieri* are used in Thai and Chinese traditional medicines for the treatment of fever and inflammation. The rhizome has a thick subcylindric shape. The petiole is around 10–30 cm long. The leaf blade is oblong to oblong-elliptic about 20–50 × 7–14 cm long, glabrous or abaxially pubescent. The leaf base is rounded-cuneate to cuneate shape, apex shortly caudate. The scape is long and has 4 dark purple involucral bracts which consist of 2 outer ovate-lanceolate, 2 broad inner ovate with umbels with 5–7 flowers. The perianth lobes are purplish brown with lanceolate shape. The stigma has 3 lobes. The berry is purplish brown, ellipsoid, 3 cm, fleshy, 6-ridged, with persistent perianth lobes. Seeds are reniform [4-6].

**Figure 2.1** *Tacca chantrieri* Andre.

Many studies have shown that *T. chantrieri* was used in traditional medicine such that all parts of *T. chantrieri* can use for treatment of fatigue and body stimulant. Moreover, it can be boiled or preserved with alcohol for stimulating blood pressure, maintain sexual power and providing vitamins to nourish pregnant women. In addition, all parts of *T. chantrieri* can be boiled for treating body rash [7]. Moreover, Thai folk medicine mentioned that a decoction of *T. chantrieri* rhizomes or leaves can be used to relieve body and stomach pains, and as an antidote for food poisoning. In thai traditional formulation named Tamrab Ya Kewng, *T. chantrieri* is used for the treatment of fever, thirst and solve the toxic poison from the measles and the chickenpox [8].

Recent report of thai traditional formulation named Tamrab Ya Kewng and Tamrab Ya Jantaleera indicated that *T. chantrieri* may have the effect on fever, pain and inflammation. The comparison between Tamrab Ya Jantaleera and Tamrab Ya Kewng was carried out. The results show that both formulations are able to cure pain, fever and inflammation, but Tamrab Ya Kewng which had higher *T. chantrieri* composition showed more effect than Tamrab Ya Jantaleera [9].

The rhizomes of *T. chantrieri* have been used in Chinese medicines for the treatment of various diseases including high blood pressure, burns, gastric ulcers, enteritis, and hepatitis. The rhizomes of *T. chantrieri* have been analyzed to contain steroidal saponin constituents, resulting in the isolation of four new spirostanol saponins, along with one known saponin; their structures were elucidated on the basis of extensive spectroscopic analysis, including 2D NMR, and the results of hydrolytic cleavage. The isolated compounds were evaluated for their cytotoxic activities against HL-60 leukemia cells as shown in Figure 2.2. Compounds 1 and 5 showed considerable cytotoxicity with respective $IC_{50}$ values of 1.80 and 2.10 mM, whereas etoposide used as positive control gave an $IC_{50}$ value of 0.37 mM. For compounds 2 and 4, the corresponding C-24 hydroxy derivatives of 1, 5, and 3, which are structurally related to 1 with a terminal rhamnosyl group linked to C-2 of the inner glucosyl residue absent from 1, did not show any cell growth inhibitory activity at the sample concentration of 10.00 mg/ml, suggesting that the structures of both the aglycone and sugar moieties contribute to the cytotoxicity [10-12].

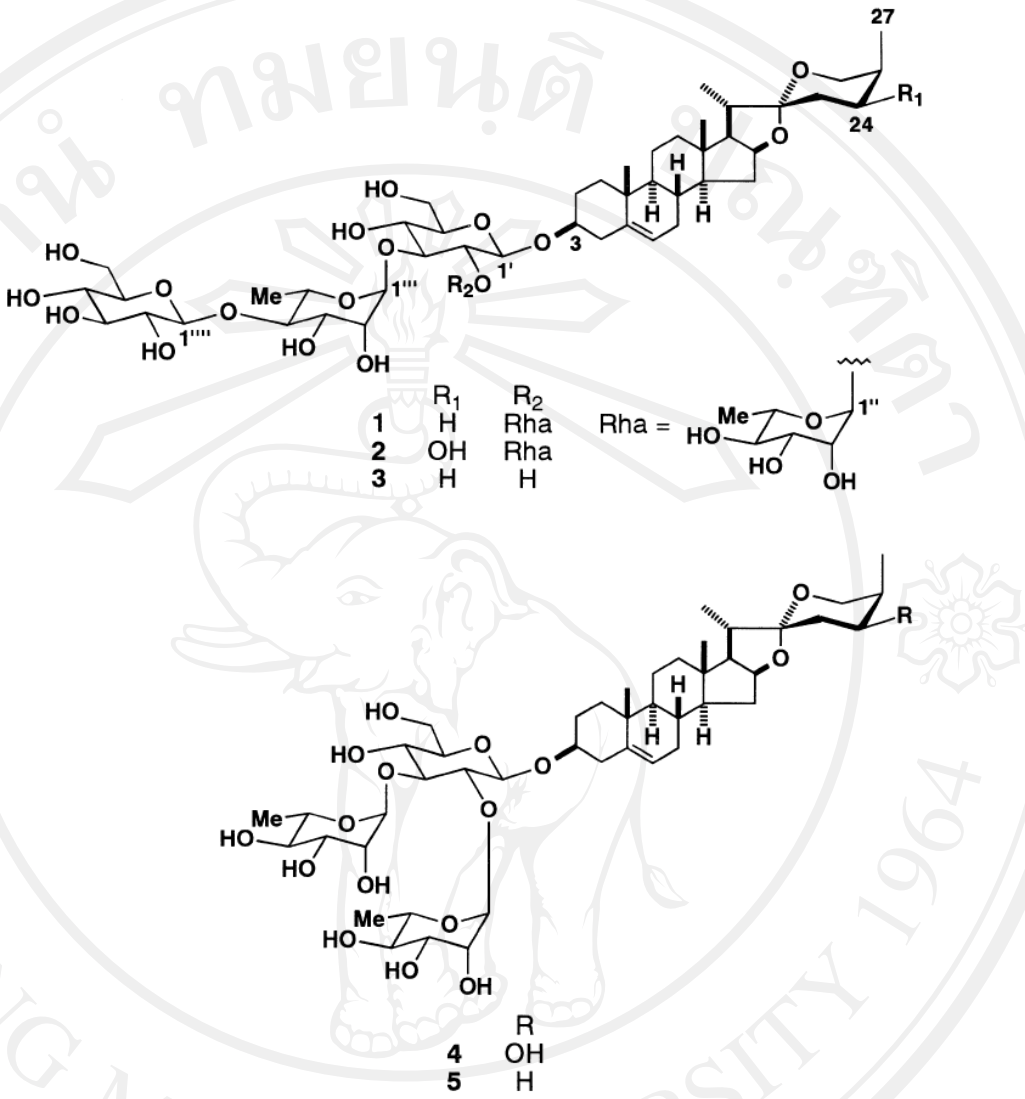


**Figure 2.2** The structure of steroidal glycosides found in *T. chantrieri*

Recent studies have documented that ethanol extract of the rhizomes of *T. chantrieri* showed the inhibitory effect against the tested bacteria, both Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram negative (*Escherichia coli*, *Enterobacter cloaceae*, *Aeromonas hydrophila* and *Serratia marcescens*), as well as yeast (*Candida albicans*). However, the extract at the concentration of 250 mg/ml had no bactericidal effect against *C. albicans*. This extract was further partitioned into non polar and polar groups by the mixture of ethyl acetate and water (2:1). The non-polar group in the ethyl acetate fraction exhibited the inhibitory and bactericidal effects at the concentration of 6.25 mg/ml, while the polar group in the water fraction at the

concentration of 250 mg/ml did not show the inhibitory and bactericidal effects against *B. subtilis* and *S. marcescens*. Moreover, the extract obtained from boiling fresh rhizomes in water for 2 hr had the inhibitory and bactericidal effects against the tested bacteria and yeast, except for *S. marcescens* and *C. albicans*, at the concentration of 160 mg/ml [13].

There was a study to compare the efficiency of crude extract from *T. chantrieri* leaf and rhizome in controlling tobacco cutworm. Different solvent: water, boiled water (2 hr), methanol and acetone were used for extraction. The result revealed that crude extract by water had no antifeedant efficiency. Crude extracts from boiling rhizome in water for 2 hr showed promising efficiency as well as those with methanol and acetone. Extraction from rhizome with acetone gave the best result even at the very low concentration of only 1 % [14].

From previous studies, the reports were investigating the hypotensive activity of the ethanol extract (TCE) and purified saponin extract of *T. chantrieri* (TCS), and characterize the hypotensive principles. TCE and TCS at the dose of 5 mg/kg caused hypotension and bradycardia in normotensive rats under pentobarbital anesthesia. Both TCE and TCS caused a decrease in the force and rate of contractions in isolated rat atria. The vasorelaxant activity of TCE and TCS could be demonstrated when tested on the aortic ring with endothelium intact. Therefore, it is implied that TCE and TCS exert both hypotensive effect and bradycardia by affecting the heart as well as blood vessels [15].

*T. chantrieri* is an indigenous perennial in the tropics which is used by local healers to relieve body and stomach pains, and as an antidote for food poisoning. The previous study was undertaken to investigate the analgesic, antipyretic and anti-inflammatory activities of *T. chantrieri* as claimed in the traditional medicine. The ethanolic extract of the plant's rhizome was prepared and tested in experimental animals. It was found that the extract significantly inhibited pain caused by acetic acid injection in the writhing response test in mice and the tail flick test in rats. This finding suggests that the extract exerts analgesic effect through both peripheral and central mechanisms. The analgesic effect was not antagonized by pretreatment with naloxone, an opiod antagonist and this signifies that a mechanism, other than the opioid system, was utilized. The extract also significantly decreased the yeast-induced

hyperthermia in rats. Anti-inflammatory effect of *T. chantrieri* extract was demonstrated in ethylphenylpropiolate-induced ear edema and formalin tests in mice. These findings indicated that the ethanol extract of *T. chantrieri* possessed analgesic, antipyretic and anti-inflammatory effects, which agrees with its use in traditional medicine [16].

Moreover, there is a report that the rhizome of *T. chantrieri* (Figure 2.3) can prevent the gastric ulcer. Ethanolic extract of *T. chantrieri* rhizomes was tested in experimental gastric ulcers induced by indomethacin, HCl/EtOH and water immersion stress and also in pylorus-ligated rats. It was found that the extract at the doses of 125 and 250 mg/kg significantly ($p < 0.05$) inhibited ulcer formation in rats and partly inhibited gastric secretion. Furthermore, in HCl/EtOH-induced ulcerated rats, gastric wall mucus and hexosamine content were markedly preserved by the extract pretreatment. These findings indicated that the *T. chantrieri* extract possessed gastroprotective potential and thus substantiates the use of *T. chantrieri* in traditional medicine [15]. Some studies found that rhizome of *T. chantrieri* had the antiplasmodial activity at 13 % and 32 % inhibition with the concentration 10 µg/ml of ethanol extract and cyclohexane extract respectively [18].

The active compound of *T. chantrieri* is a saponin compound. The stability report of the saponin compound revealed that saponins contain a free carboxylic group in the molecule that can be transformed into esters when stored, for a long time, in alcoholic solutions. Consequently, saponins are not stable and they can have chemical reaction [19] especially in alcohol which is often used as a solvent in the extraction of substances and the excipient in the pharmaceutical products. Therefore, it may be a major problem in the development in the form of extracts. The need to develop and study the techniques to prepare *T. chantrieri* extract in various forms, using advanced technology to increase the stability of the extracts and the efficiency of treatment.

**Figure 2.3** The rhizome of *T. chantrieri*

One method that can solve the problem of the stability of extracts and the efficiency of treatment is the advanced technologies such as nanotechnology [20-22] application. Nanotechnology is widely seen as having a great potential to bring benefits to many areas of research and applications because it is easy and it is the method for storage substance. Several polymers have been evaluated as carriers for drugs in the nanoparticulate forms. The application of nanotechnology is raising new challenges in the safety, stability of drugs, ability to target and control the drug released. On the other hand, the reduction or prevention of side effects can also be achieved by controlled release. Polymers have shown different properties and advantages but the useful polymers are polymers with biocompatible and biodegradable properties such as chitosan and alginate [23-25]. The chitosan-alginate nanoparticles are widely used and the nanoparticles are produced by ionotropic gelation method. Alginate is applied with chitosan as copolymer that the complex can be formed from the reaction between the carboxyl group of alginate and amine group of chitosan using calcium chloride as the cross-link agent. Sermento et. al. [26, 27] suggested that the size of chitosan-alginate nanoparticles is influenced by the mass ratio of alginate:chitosan and the pH during nanoparticle production. The results indicated that the alginate : chitosan mass ratio at 1:4.3 with the pH at 4.7 are the appropriate conditions for preparing the chitosan-alginate nanoparticles. Moreover, the chitosan-alginate nanoparticles are a promising carrier for insulin oral delivery because of its potential bioactivity and its ability to preserve insulin structure.

## 2.2 Chitosan

Chitosan is the derivative of chitin (Figure 2.4) from the deacetylation method that the acetamide group of chitin is changed to amino group ($-NH_2$). Chitin and chitosan are found together as copolymer. Chitosan is not soluble in water but it is soluble in weak acid. Chitosan is a polycationic polymer which is biocompatible and biodegradable. Hence, it is a non-toxic polyelectrolyte. Moreover, there are many advantages in the production, especially the product of skin, the cationic of the ammonium group ($-NH_3^+$) that are orderly structured in the structure of chitosan can adhere to the skin layer by establishing of electrostatic interactions with mucopolysaccharides, protein and lipid of skin that are anionic. From this property, chitosan is useful in many areas such as in food industry, medicine, cosmetic and agriculture. Chitin and chitosan are carbohydrate that similar to cellulose. Chitin is the polymer of β-1,4-linked N-acethylglucosamine whereas chitosan is the corresponding polymer of glucosamine. However, neither chitin nor chitosan is homopolymers, as both contain varying fractions of β-1,4-linked N-acethylglucosamine and glucosamine [28-30].

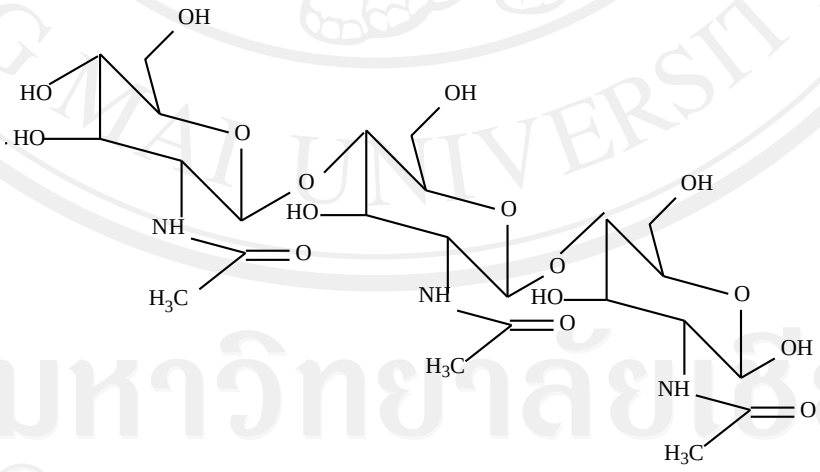


**Figure 2.4** Structure of chitin

Chemical reaction as shown in Figure 2.5 can change chitin to chitosan by deacetylation method. N-acetyl glucosamine is decreased when glucosamine is increased in the same amount, meaning that chitin is changed to chitosan. The rate of deacetylation is shown as percentage of degree of deacetylation (%DD). The distribution of chitosan in acid solution will increase because the increase of glucosamine that increases the solubility from the increase of cationic ion. Therefore, chitosan will be more soluble when it is in the acid solution such as acetic acid, lactic acid.

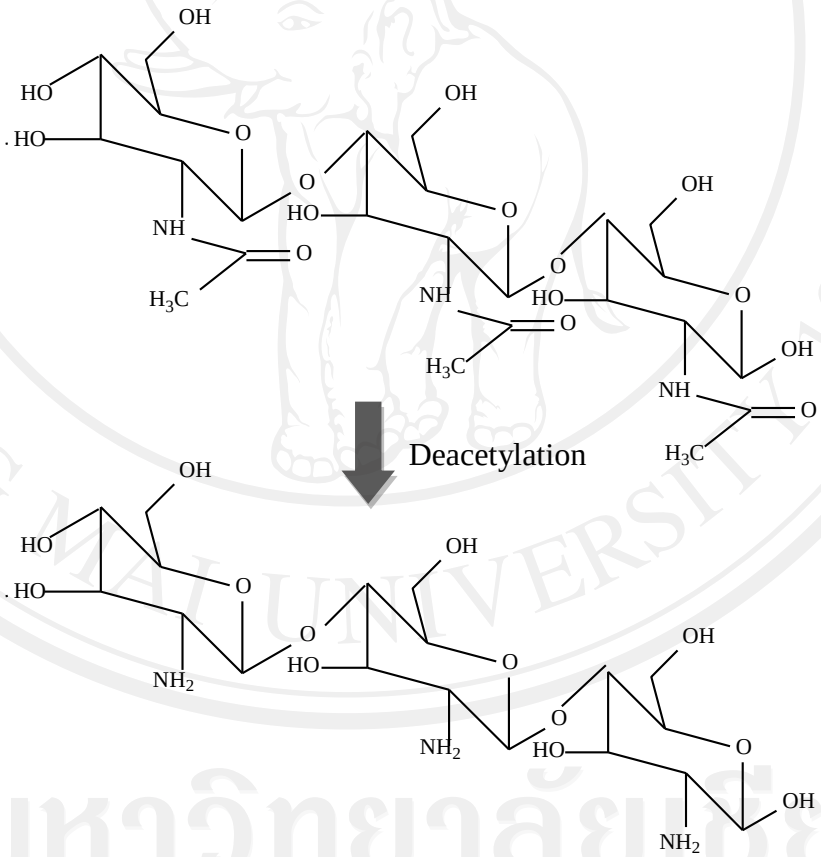


**Figure 2.5** The deacetylation of chitin

## 2.3 Alginate

Alginate is an anionic polysaccharide copolymer which is distributed widely in the cell walls of brown algae. Alginate had to be regarded as a binary copolymer composed of β-D-mannuronic acid and α-L-guluronic acid residues of widely varying composition and sequence as present in Figure 2.6-2.7. Its colour ranges from white to yellowish-brown. A very rapid and irreversible binding reaction of multivalent cations is typical for alginates. Therefore, a direct mix of these two components rarely produces homogeneous gels. The result of such mix is likely to be a dispersion of gel lumps ("fish-eyes"). The cation salts such as calcium gluconate, calcium tartrate, and calcium chloride can be dissolved and give $Ca^{2+}$ that can be used to form insoluble gel. These gels cannot dissolve in the acidic solution, but can be dissolved the neutral and alkali solutions. On the other hand, sodium alginate is the biocompatible and biodegradable polymer as same as chitosan. Moreover, alginate can be applied as a drug delivery system with chitosan. The problem of alginate is that it absorbs moisture easily and can react with metal. For the storage of alginate, alginate must be kept in dry place and avoid storing in metal containers [30, 31].


**Figure 2.6** The structure of alginate monomers : β-D-mannuronate (M) and *a*-L-guluronate (G)

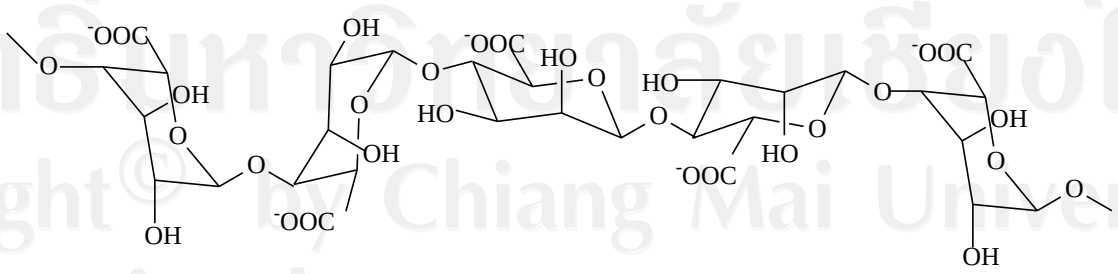


**Figure 2.7** The structure of chain conformation of alginate

**2.4 Inflammation**

Inflammation is the body's reaction to infectious agents invasion, antigen challenge or even just physical, chemical or traumatic damage. Major signs of inflammations are pain, edema, redness, heat and loss of function. Inflammation is divided into acute and chronic patterns. Acute inflammation has relatively short duration, lasting for minutes, several hours, or a few days, and its main characteristics are the exudation of fluid and plasma proteins (edema) and the emigration of leukocytes and predominantly neutrophils. Chronic inflammation is of longer duration and it is associated histologically with the presence of lymphocytes and macrophages, the proliferation of blood vessels, fibrosis, and tissue necrosis. Many factors can change the course and histologic appearance of both acute and chronic inflammation.

Inflammation occurred from many pathways but the major pathway is arachidonic acid metabolites. Arachidonic acid is derived directly from dietary sources or by conversion from the essential fatty acid, *linoleic acid.* This pathway does not occur freely in the cell but is normally esterified in membrane phospholipids. It is released from membrane phospholipids through the activation of cellular phospholipases (e.g., phospholipase $A_2$) by mechanical, chemical, and physical stimuli or by other mediators (e.g., C5a). Arachidonic acid metabolites, also called *eicosanoids,* are synthesized by two major classes of enzymes: cyclooxygenases (prostaglandins and thromboxanes) and lipoxygenases (leukotrienes and lipoxins). Eicosanoids can mediate virtually every step of inflammation. They can be found in inflammatory exudates, and synthesis is increased at sites of inflammation as shown in Table 2.1 and Figure 2.8

**Table 2.1** Inflammatory actions of eicosanoids

| Action | Eicosanoids |
|---|---|
| Vasoconstriction | Thromboxane $A_2$, Leukotrienes $C_4$, $D_4$, $E_4$ |
| Vasodilation | Prostacyclin ($PGI_2$), Prostaglandins $E_1$, $E_2$, $D_2$ |
| Increased vascular permeability | Leukotrienes $C_4$, $D_4$, $E_4$ |
| Chemotaxis and Leukocyte adhesion | Leukotrienes $B_4$, HETE, lipoxins |

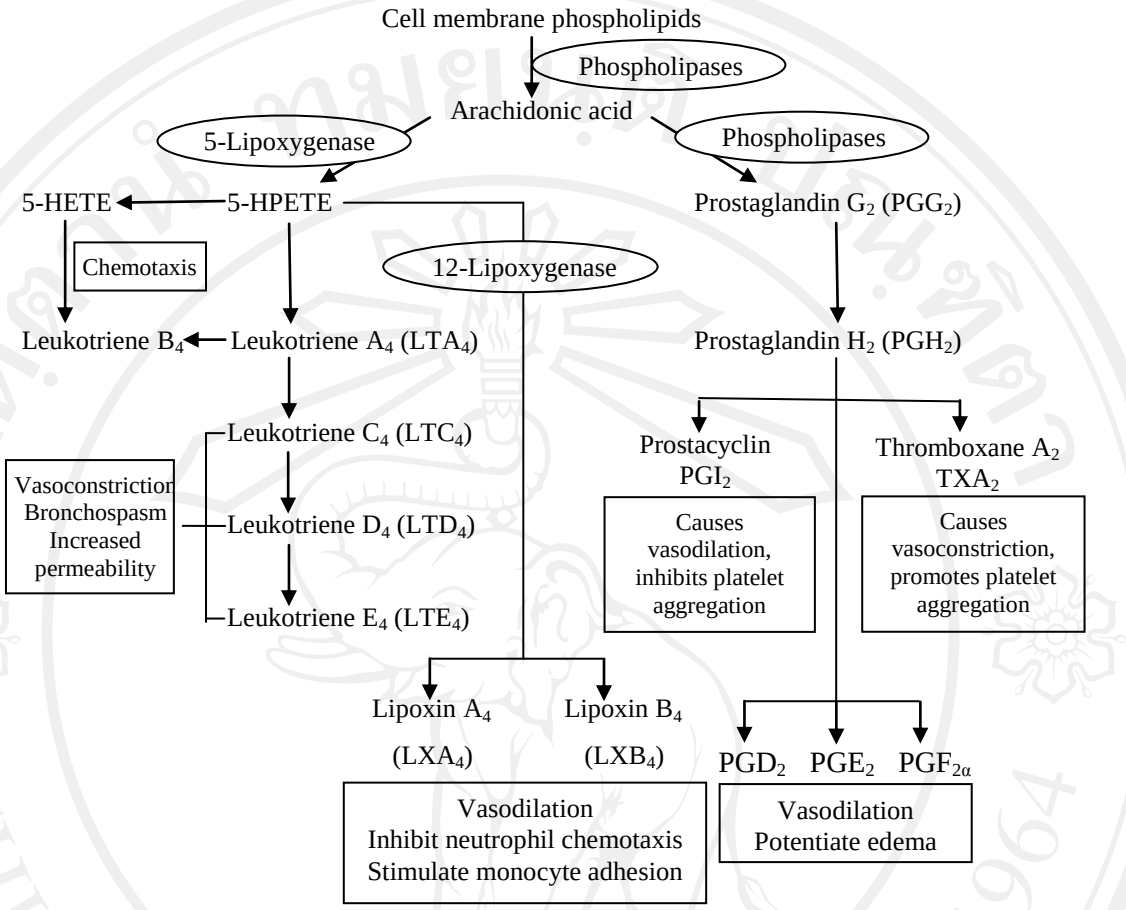


**Figure 2.8** Generation of arachidonic acid metabolites and their roles in inflammation

Cyclooxygenase pathway is one in the pathway of arachidonic acid metabolites. This pathway is mediated by two different enzymes (COX-1 and COX-2), and leads to the generation of prostaglandins. Prostaglandins are divided into series, based on structural features as coded by a letter (PGD, PGE, PGF, PGG, and PGH) and a subscript numeral, which indicates the number of double bonds in compound. The most important ones in inflammation are $PGE_2$, $PGD_2$, $PGF_{2\alpha}$, $PGI_2$ (prostacyclin), and $TXA_2$ (Thromboxane), each of which is derived by the action of a specific enzyme. Some of these enzymes have restricted tissue distribution. The prostaglandins are also involved in the pathogenesis of pain and fever in inflammation. $PGE_2$ cause hyperalgesia by making the skin hypersensitive to painful stimuli. It causes a marked increase in pain produced by intradermal injection of subobtimal concentrations of histamine and bradykinin and interacts with cytokines in

causing fever during infections. $PGD_2$ is the major metabolite of the cyclooxygenase pathway in mast cell; along with $PGE_2$ and $PGF_{2\alpha,}$ it causes vasodilation and potentiates edema formation [32-33].

There are many targets along the eicosanoid biosynthetic pathways at which anti-inflammatory therapy can be directed. Aspirin and NSAIDs, such as indomethacin or ibuprofen, inhibit cyclooxygenase and thus inhibit prostaglandin synthesis as shown in Table 2.2. Glucocorticoids, which are powerful anti-inflammatory agent, may act by down-regulating the expression of specific target genes, including COX-2, genes encoding proinflammatory cytokines (such as IL-1 ang TNF-α), and nitric oxide synthase (iNOS). Glucocorticoids also up-regulate genes that encode potent anti-inflammatory proteins, such as lipocortin 1. Lipocortin 1 inhibits release of AA from membrane phospholipids [33, 34].

**Table 2.2** Drugs used to inhibit cyclooxygenase (COX)

| Selectivity | Drug |
|---|---|
| Weak COX inhibitors | Acetaminophen |
| Inhibitor of COX-1 and COX-2 | Acetylsalicylic acid, Piroxicam, Indomethacin, Sulindac, Ibuprofen, Naproxen, Mefenamic acid, Diflunisal, Ketoprofen, Diclofenac, Etodolac, Nabumetone, Oxaprozin |
| Preferential COX-2 inhibitors | Nimesulide, Meloxicam |
| Selective COX-2 inhibitors | Celecoxib, Valdecoxib, Etoricoxib |

The studies of anti-inflammation were investigated by many methods such as carrageenan-induced rat paw edema, cotton-pellet induced granuloma [35-38] which induced for inflammation by the different pathway. Colorimetric COX (ovine) inhibitor screening assay kit is one of the screening methods for anti-inflammatory effect. COX (also called Prostaglandin H Synthase or PGHS) is a bifunctional enzyme exhibiting both COX and peroxidase activities. The COX component converts

arachidonic acid to a hydroperoxy endoperoxide ($PGG_2$), and the peroxidase component reduces the endoperoxide to the corresponding alcohol ($PGH_2$), the precursor of PGs, thromboxanes, and prostacyclins. Colorimetric COX (ovine) Inhibitor Screening Assay measures the peroxidase component of COXs. The peroxidase activity is assayed colorimetrically by monitoring the appearance of oxidized N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) at 590 nm. The scheme of color reaction occurred in Colorimetric COX inhibitor test was presented in Figure 2.9 [39].

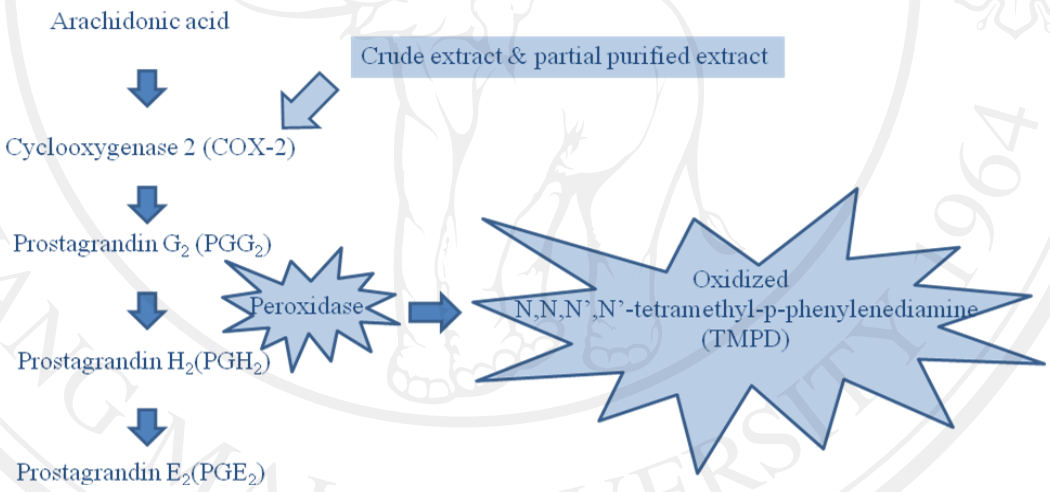


**Figure 2.9** Scheme of color reaction occurred in Colorimetric COX inhibitor test