CHAPTER 2
LITERATURE REVIEWS

2.1 Inflammation and diseases (12-14)

Inflammation is a defense mechanism of the body to any injury and to death cells or death tissues. The response is to destroy and eliminate the injury and lead to repairment and regeneration at the defected tissues area. This process helps the tissues and organs to function again. In case that the tissue is not be able to function during inflammation process, the inflammatory response will lead to scar at that area. The inflammatory response is associated with the change in local area including vascular change and influx of inflammatory cells. The inflammatory response develops in blood capillary around the tissue.

There are two types of inflammation that are acute inflammatory (the immediately response after triggered by the stimuli) and chronic inflammation (the response develops following acute inflammation when the cause is not completely removed). The causes of inflammation are:

1. Mechanical such as external injury
2. Physical such as temperature and radiation
3. Chemical such as toxin and toxic agent
4. Nutritive such as lacking of oxygen, vitamin and nutrition
5. Biological such as any infection

When the body is stimulated, the acute inflammation response develops immediately. The changes mainly are vasodilatation to increase cappillary permeability and infiltration of white blood cells. The response leads to pain, edema, redness and warmth. The inflammatory response is shown in the Figure 2.1.

After the body eliminates the causes of injury, the inflammatory response gets decreased. However if the body cannot eliminate the cause of injury, the inflammatory response still persists and then the chronic inflammation may develop

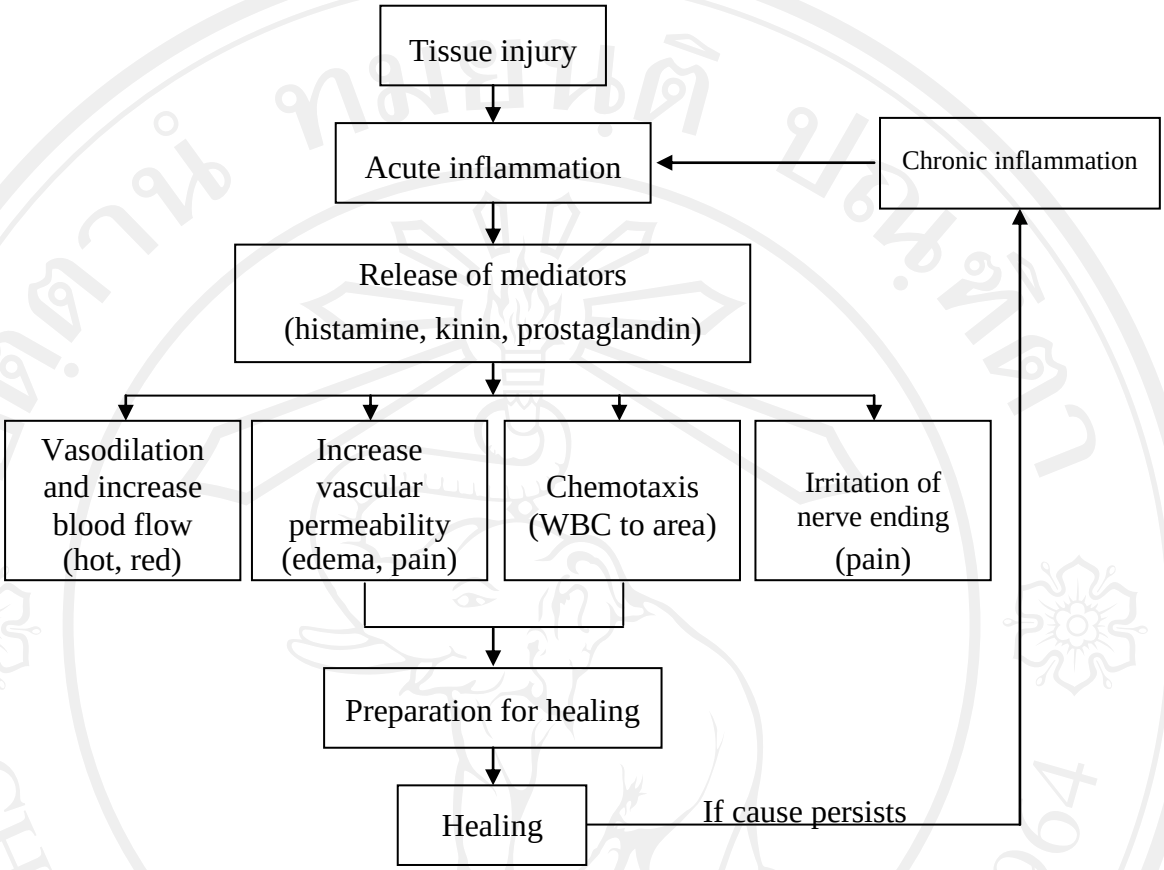


Figure 2.1 Cycle of inflammatory response (15)

from the acute inflammation. Chronic inflammatory response is one factor that can lead to several diseases such as arthritis. Initially, the inflammation of joint proceeds gradually, there is no any symptom but if there is a factor can trigger to the inflamed joint such as having activity using joint and consumption some food, the acute inflammation will occurs, called "exacerbation". This situation affects patients' daily life and they can not temporary use the joint until the symptom is relieved. However to relieve the symptom may last much time and the symptom is also on and off. While the inflammation is going on, the joint may be deformed.

Both acute and chronic inflammation responses are involved with cellular response, vascular response and cytokines. All systems are to promote elimination the cause of the inflammation and tissue repair. The relation of the inflammation response is shown in the Figure 2.2.

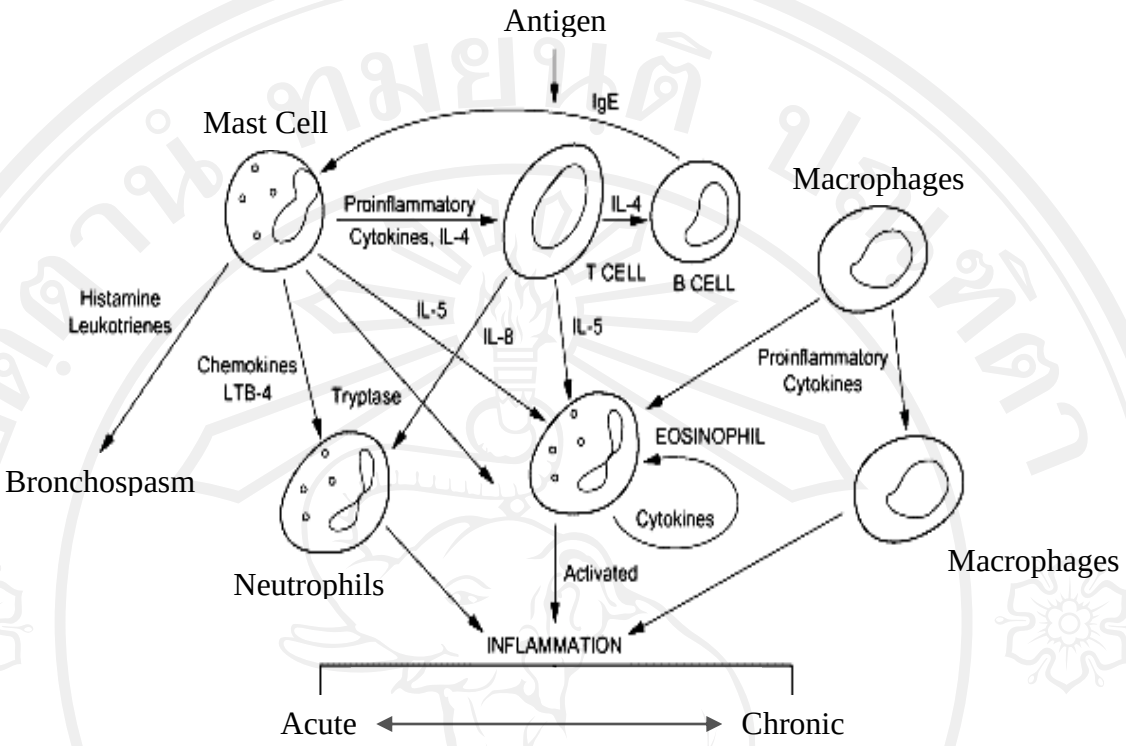


Figure 2.2 The relation of cellular stage and cytokines in inflammatory response in respiratory system (16)

Beside the cytokines which extends the inflammatory response, there is another group of inflammatory mediators including vasoactive amines (histamine, serotonin as vasodilator), plasma protein (activation the complement system, kinin system, clotting system and fibrinolytic system to promote white blood cells function), platelet activating factor, lysosomal constituents of leukocytes, arachidonic acid metabolites (stimulation and inhibition of the inflammatory response), nitric oxide and oxygen-derived free radicals.

Nitric oxide and oxygen-derived free radicals are the radicals generated by white blood cells to help elimination of the cause and activate white blood cells function. They also activate another pathway to get more inflammatory mediators such as arachidonic acid pathway. The overall result is extension of inflammatory response. Moreover, the radicals are one factor can lead to several diseases as follows: arthritis, rheumatoid arthritis, dermatitis, cancer, diabetes and Alzheimer's disease.

Although the radicals can be eliminated by body enzyme including superoxide dismutase, catalase and gluthathione peroxidase, the excess radicals can occur. This stage called “oxidative stress” and chronic disease may be developed. The arthritis is one disease occurs with high concentration of reactive oxygen species ($O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$ และ $NO^{\cdot}$) at the joint area. These generated radicals can destroy hyaluronic acid of joint and activate lipid peroxidation. The overall processes are to increase inflammation and cartilage destruction (17). Especially hydroxyl radical ($OH^{\cdot}$) is highly active radicals and it is the most important in inflammation.

Dermatitis is a chronic disease involved with the gene role in dry skin or contact to some chemicals and matters. The symptom of dermatitis is due to histamine released from mast cells and lead to inflammation on skin. The radicals are also high at the inflamed skin. Moreover antioxidant can relieve inflammation of dermatitis (18).

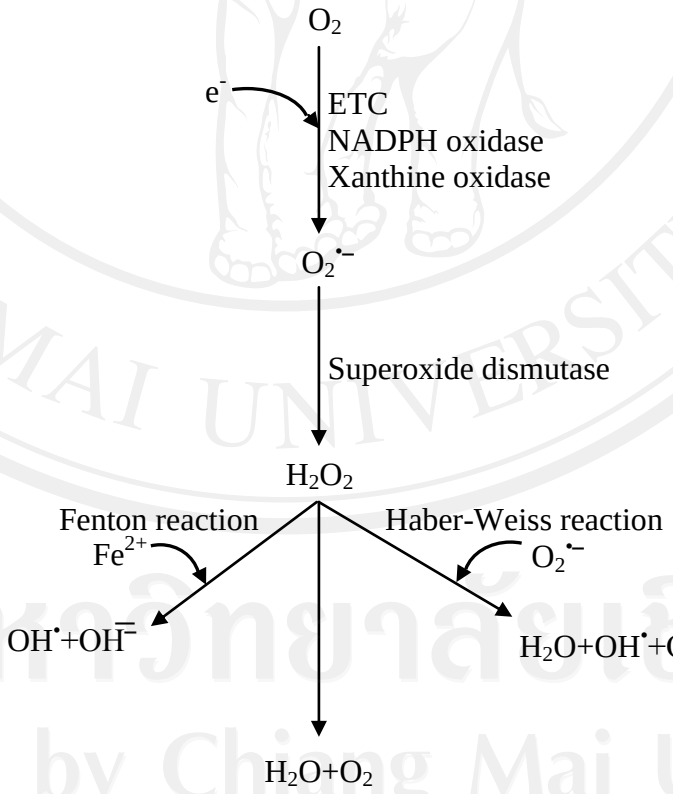


Figure 2.3 The defense mechanism of body in radicals elimination (19)

2.2 *Terminalia catappa* Linn. (Hu-Kwang) (20)

Scienctific name: *Terminalia catappa* Linn.

Common names: Indian almond, Bengal almond, Ketapang, Hu-Kwang, Kobateishi, Lanren (欖仁), Singapore almond, Tropical almond

Source: India, Malay Peninsula , Thailand and tropical zone

Botanical descriptions: large tropical and cultivated tree with 10-25 meters high, smooth bark, obovate leaf with 10 centimeters wide and 20 centimeters long, inflorescence flower with 10 centrimeters long, fruit is a drupe, two leaf-fall peroids during February to April and August to October.

Medical uses:

The bark: astringent, carminative, antidiarrhea, for apthous ulcer, leucorrhea and gonorrhea

Leaves: perspiration, tonsillitis, arthritis, gastro intestinal disease and liver disease

Red leaves: anthelmintic

Nut: as topical preparation; nut oil with red leaves used for leprosy, relieve chest pain, local anesthesia and skin infection

Fruit: Laxative

Arial part: astringent, fever, dysentery, diarrhea, galactagogue, yaws

The indication recorded in Thai herbal book specifies that bark is used for diarrhea and dysentery. Leaf is used for skin iching and perspiration. Fruit is used as laxative. Furthermore another country: Taiwanese uses the fallen leaves for liver disease. Surinamese uses as fermented tea for diarrhea and dysentery.

According to ethnobotanical treatment of India, Indonesia, Pakistan and Samoa, *T. catappa* Linn. leaf is used as compresstion for arthritis and dermatitis to relieve pain and inflammation. Crushed leaf is also used for skin emolient and inflammation. Furthermore there are researches which are related to ethnobotanical usage:

In 1997, Dunstan and colleagues found that 70% ethanol extract of *T. catappa* Linn. leaf and bark inhibited prostaglandin synthesis by 26% and 73%, respectively (21).

In 2004, Fan and colleagues studied on ethanol leaf extract and 5 partitioned extracts (petroleum ether, chloroform, ethyl acetate, n-butanol and water). They found that there was ursolic acid in chloroform fraction. The mouse was tested with chloroform fraction 1 mg/ear, ursolic acid 0.3 mg/ear compared to indomethacin 0.3 mg/ear as topical. The percentage of edema inhibition were 60.2%, 53.1-57.0% and 50% respectively (7).

There were several phytochemical researches found chemicals in *T. catappa* Linn. leaf which possess anti-inflammation. The phytochemicals found in *T. catappa* Linn. leaf are:

1. Punicalagin (2,3-*(S)*-hexahydroxydiphenoyl-4,6-*(S,S)*-gallagyl-D-glucose) (22, 23)

It is hydrolysable tannins, ellagitannins type. Punicalagin is the main active compound in *T. catappa* Linn. leaf and found 0.48% per dried leaf.

Physicochemical property

Molecular formular: $C_{48}H_{28}O_{30}$

Molecular weight: 1084.72

Solubility: water, ethanol

Decomposition: hydrolysis

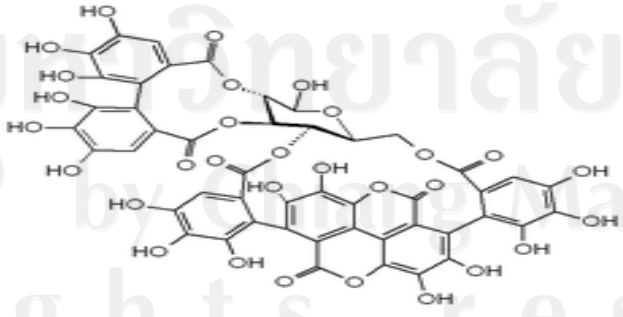


Figure 2.4 Punicalagin structure (24)

Anti-inflammatory activity of punicalagin and punicalin has been reported as following:

In 1999, Lin and colleagues studied on carrageenan-induced hind paw edema in rats, at the dose of 10 mg/kg of punicalagin inhibited inflammation about 58.15% and punicalin gave a lower effect (25).

In 2006, Adam and colleagues found that punicalagin at 50 mg/L inhibited COX-2 protein expression in HT-29 cells 48% (26).

2. Corilagin (beta-1-*O*-galloyl-3,6-*(R)*-hexahydroxydiphenoyl-D-glucose) (27-29)

Corilagin is hydrolysable tannins, gallotannins type. It was found in *T. catappa* Linn. about 2.09 mg/g of dried red leaves.

Physicochemical property

Molecular formular: $C_{27}H_{24}O_{18}$

Molecular weight: 636.46

Melting point: >200°C (Decompisition)

Solubility: water, methanol, ethanol, acetone, DMSO

Decompisition: Hydrolysis

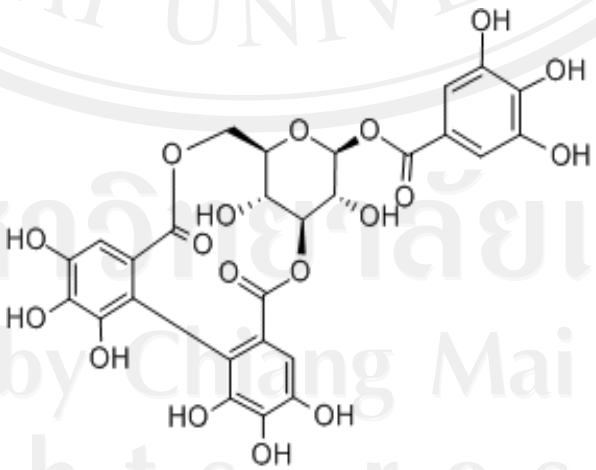


Figure 2.5 Corilagin structure (24)

Anti-inflammatory activity of corilagin has been reported as following:

In 2008, Zhao and colleagues found that corilagin possessed anti-inflammatory activity via antioxidant activity, inhibition COX-2 enzyme and inhibition of inflammatory mediators (27).

In 2008, Shin and colleagues studied on corilagin extracted from *Euphorbia helioscopia* that was used for rheumatoid arthritis. It was found that corilagin inhibited infiltration of white blood cells, decreased inflammatory mediator release and reduced inflammation in collagen-induced arthritis using mice (30).

In 2010, Guo and colleagues studied on anti-inflammatory activity of corilagin to *in vitro* HSV-1 encephalitis. It was found that anti-inflammatory activity of corilagin was to decrease TNF-α, IL and NO (29).

3. Chebulagic acid

Physicochemical property

Molecular formular: $C_{41}H_{30}O_{27}$

Molecular weight: 954.66

Solubility: water, methanol, ethanol, acetone, DMSO

Decomposition: hydrolysis

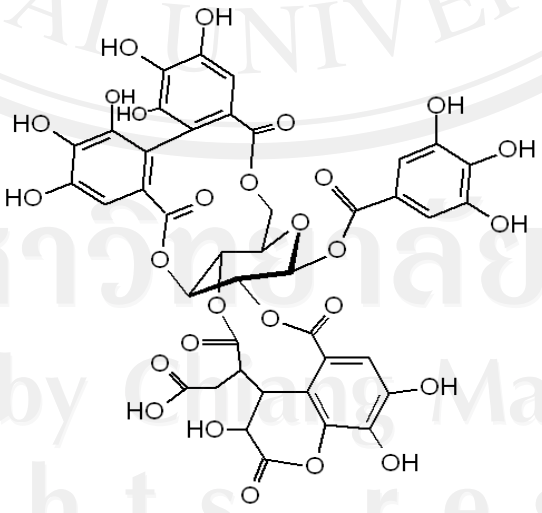


Figure 2.6 Chebulagic acid structure (24)

Anti-inflammatory activity of chebulagic acid has been reported as following:

In 2005, Lee and colleagues studied on collagen-induced arthritis in mice and found that chebulagic acid reduced inflammation via T-cell (31).

4. Gallic acid (3,4,5-trihydroxybenzoic acid) (32)

Gallic acid is phenolic acid found in *T. catappa* Linn. leaf about 5.1% of water extract. Gallic acid can penetrate into skin.

Physicochemical property

Molecular formular: $C_7H_6O_5$

Molecular weight: 170.12

Melting point: 250 °C

Solubility: water, ethanol

Decomposition: heat

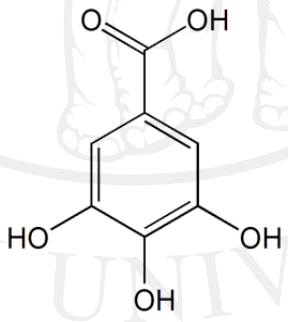


Figure 2.7 Gallic acid structure (24)

Anti-inflammatory activity of gallic acid has been reported as following:

In 2006, Kim and colleagues found that gallic acid reduced inflammation and allergic reaction by inhibition of histamine release and pro-inflammatory cytokine synthesis(33).

In 2007, Madlener and colleagues found that gallic acid significantly inhibited COX-1 and COX-2 enzymes (34).

5. Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one)

Quercetin is flavonoids.

Physicochemical property

Molecular formular: $C_{15}H_{10}O_7$

Molecular weight: 302.236

Melting point: 316 °C

Solubility: water, ethanol

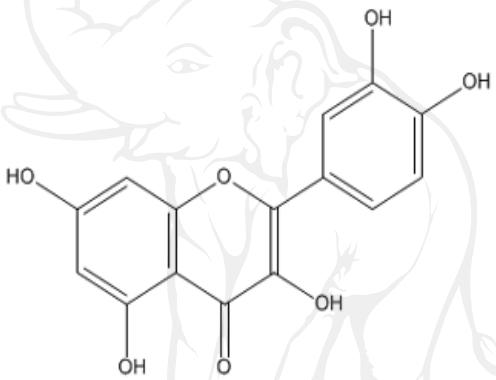


Figure 2.8 Quercetin structure (24)

Anti-inflammatory activity of quercetin has been reported as following:

In 2003, Morikawa and colleagues studied on carrageenan-induced inflammation in rat by SC injection quercein 10 mg/kg. It was found that quercetin reduced inflammation by inflammatory mediator inhibition and reducing edema (35).

In 2006, Matsuda and colleagues studied on anti-arthritis mechanism of quercetin. The results showed that quercetin inhibited joint inflammation by inhibition of inflammatory mediators synthesis and nitric oxide from macrophage (36).

In 2005, Liu and colleagues found that one mechanism of quercetin to reduce inflammation was inhibition of IL-6 synthesis from neutrophils (37).

6. Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) (19)

Physicochemical property

Molecular formular: $C_{15}H_{10}O_6$

Molecular weight: 286.23

Melting point: 276-278 °C

Solubility: water

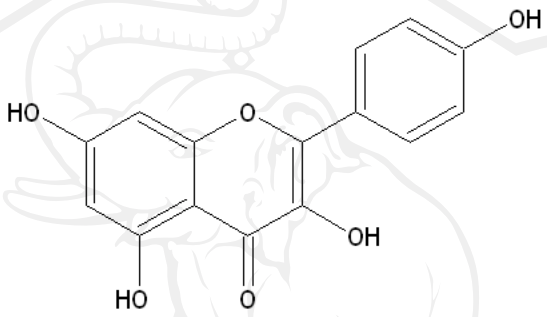


Figure 2.9 Kaempferol structure (24)

Anti-inflammatory activity of kaempferol has been reported as following:

In 2010, Mahat and colleagues studied anti-inflammatory activity on cyclooxygenase pathway of kaempferol via inhibition of nitric oxide synthesis using carrageenan induced rat air pouch model. The doses at 50 and 100 mg/kg inhibited nitric oxide synthesis about 40.12% and 59.74%, respectively. It also inhibited prostaglandin E2 synthesis about 64.23% and 78.55% (36).

7. Ursolic acid (7)

Ursolic acid is triterpenoids found in several herbal medicines. Ursolic acid was found in *T. catappa* Linn. leaves about 0.0086% of dry extract.

Physicochemical property

Molecular forlular: $C_{30}H_{48}O_3$

Molecular weight: 456.70

Solubility: ethanol, chloroform

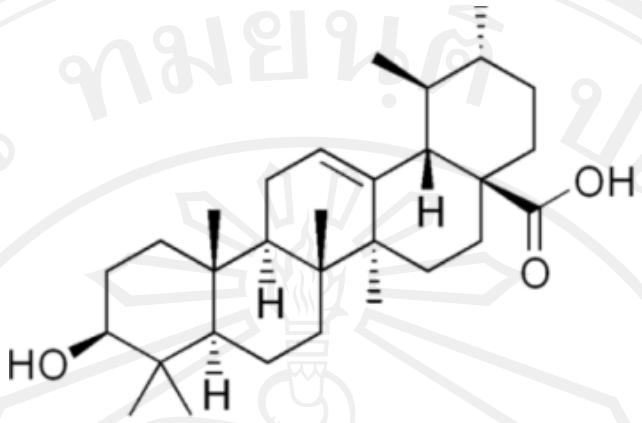


Figure 2.10 Ursolic acid structure (24)

Anti-inflammatory activity of ursolic acid has been reported as following:

In 2000, Subbaramaiah and colleagues found that ursolic acid inhibited COX-2 enzyme gene expression (38).

In 2008, Kang and colleagues studied on anti-arthritis of ursolic acid using zymosan-induced acute inflammation model in mice and complete Freund's adjuvant (CFA)-induced arthritis model in rats by oral rout. It was found that ursolic acid can inhibit arthritis generation. The most effective dose was 50 mg/kg. The mechanisms were inhibition of prostaglandin E2 (PGE2) synthesis, edema reduction and pain relieve (39).

Table 2.1 Phytochemicals with anti-inflammatory activity found in *T. catappa* Linn. leaves (33, 34, 40-43)

Phytochemicals group	Phytochemical/MW*	Anti-inflammatory mechanism	Dose / IC_{50}	References
Phenolic acid	Gallic acid	- Inhibit histamine release and pro-inflammatory cytokine synthesis from mast cell - Inhibit COX-2 - Inhibit COX-1	IC_{50}= 4.4 nM IC_{50}= 3.5 nM	(33, 34, 43)
Tannins	Ellagic acid/302.19	- Inhibit COX-2 - Inhibit NO	IC_{50} = 1.1 M IC_{50} = 660 nM	(43)
Tannins	Punicalagin	- Inhibit COX-2 protein expression - Inhibit NO	- IC_{50} = 69.8 mM	(26)
Tannins	Corilagin	- Inhibit COX-2 - Inhibit NO	IC_{50} = 0.92 µM IC_{50} = 69 µM	(43)
Tannins	Chebulagic acid	- Inhibit COX-2 - Regulation via CD4+, CD25+T-cells	IC_{50} = 0.92 µM	(43)
Flavonoids	Kaempferol	- Anti-inflamatory activity - Inhibit COX-2 - Inhibit iNOS	20 – 200 mg/kg IC_{50} = 20 M IC_{50} = 13.9 µM	(43)
Flavonoids	Quercetin	- Inhibit tumor necrosis factor alpha (TNF-α) gene expression, pro-inflammatory cytokine and monocyte chemotactic protein-1 (MCP-1) on mononuclear cells - Regulate mRNA transcription of COX-2 and inhibit PGE2 and macrophage-inflammatory protein-2 (MIP-2) synthesis		(35, 40)

Table 2.1 Phytochemicals with anti-inflammatory activity found in *T. catappa* Linn. leaves (Cont.) (33, 34, 40-43)

Phytochemicals group	**Phytochemical/MW***	**Anti-inflammatory mechanism**	**Dose / IC_{50}**	**References**
		1. Inhibit cyclooxygenase 2. Inhibit lipoxygenase 3. Anti-inflammatory activity 4. Inhibit NO	IC_{50} = 16 M IC_{50} = 3.5 M 150 mg/kg IC_{50} = 18.5μM	(35, 40)
Flavonoids	Isovitexin/432.38	- Inhibit COX-2	IC_{50} = 80.0 μM	(43)
Flavonoids	Apigenin/270.24	- Inhibit COX-2 - Anti-inflammatory activity	IC_{65} = 1 mM = indomethacin	(43)
Flavonoids	Vitexin/432.38	- Inhibit COX-2	IC_{50} = 9.1 μM	(43)
Flavonoids	Rutin/610.52	- Inhibit lipoxygenase - Anti-inflammatory activity - Suppress oxidative stress in rheumatoid arthritis leukocytes	IC_{50}= 2.5 mM 20 mg/kg	(43, 44)
Triterpenoids	Ursolic acid	- inhibit lipoxygenase - Inhibit COX-2 - Inhibit NO	IC_{50} = 0.18 mM IC_{50} = 130 μM IC_{50} = 34.60 μg	(43)

Note: * Molecular weight (g/mole) refers to pubchem compound

Apart from the research studies, there are patents of *T. catappa* Linn. leaf extract as cosmetic products.

In 2002, Pauly got the patent title "Cosmetic, dermatological and pharmaceutical use of an extract of *T. catappa* (45) (United States Patent 6217876)". The patent claimed the cosmetic containing *T. catappa* Linn. leaf extract used for skin and nail. The product possessed anti-inflammatory activity, soothing, astringent, firming and repairing property. The anti-inflammatory activity of the extract was obtained by thermal extraction using solvent as following: water, hydroalcoholic solvents, ketones, halogenated hydrocarbons, esters, polyols and cosolvent. The effective concentration of the extract was 0.10-3% by weight.

In 2002, Renimel and colleagues got the patent title "Use of an extract of the plant *T. catappa* in the cosmetic and pharmaceutical fields, especially the dermatological field (46) (United States Patent 6413519)". The patent claimed the usage of the extract for sensitive skin and dermatitis as topical formulation. The mechanism was inhibition of phospholipase A2 and lipoxygenase enzymes at 0.02-1% by weight.

The topical preparation was emphazised to cosmetic field commercial market such as body firming gel® by Body shape, kangzen-charming nano whitening roll on passion®, charming passion perfume body lotion® by Kangzen-kenko, milky cleansing lotion® and eye repair® by Bio-Vera Lab.

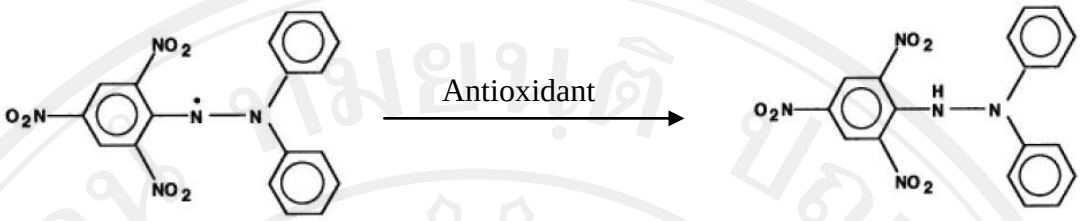
2.3 Antioxidant activity determination and anti-inflammatory activity test

The inflammatory response relates to immune system and radicals generated during inflammation and radicals also role in inflammation controling. Therefore antioxidant activity and anti-inflammatory activity should be simultaneously studied.

2.3.1 Antioxidant activity determination (47)

(1) Scavenging effect on DPPH

This method used for antioxidant activity screening test. The reaction can be followed by measuring absorbance at 517-520 nm.



1, 1- diphenyl-2-picrylhydrazyl radicals (Purple)

1, 1- diphenyl-2-picrylhydrazine (Yellow)

(2) Hydroxyl radical scavenging activity

Hydroxyl radical is very highly active radical role in DNA, protein destruction. Therefore hydroxyl radicals is one factor can lead to several diseases. The reaction can be followed using Fenton reaction (Figure 2.3).

(3) FRAP (Ferric reducing antioxidant power) assay

This method can directly determine reducing capacity of the sample and be followed absorbance increased at 595 nm.

$$Fe^{3+} + TPTZ \longrightarrow Fe^{2+} - TPTZ$$

(4) Superoxide scavenging activity

The method is a study of superoxide ($O_2^{\cdot-}$) scavenging capacity by following the reduction of NBT. The reaction is that $O_2^{\cdot-}$ reduces NBT and reduced NBT is blue color. When there is an antioxidant in the reaction, $O_2^{\cdot-}$ will be scavenged and reduced NBT will be less occurred. The absorbance of blue color is 560 nm.

(5) Nitric oxide scavenging activity

Nitric oxide (NO) generated in the body can activate COX-2 enzyme in inflammatory response. Therfore inhibition of NO generation is one mechanism of anti-inflammatory activity. The study can be followed using Griess reaction.

(6) ABTS assay (2,2'-Azino-*bis* (3-ethylbenzothiazoline-6-sulfonic acid) cation radicals scavenging assay)

This method is to study on $ABTS^{\cdot+}$ (blue-green color) scavenging activity. The reaction can be followed at absorbance 734 nm.

2.3.2 *In vitro* anti-inflammatory activity study

(1) Cyclooxygenase inhibition activity

Cyclooxygenase (COX) enzyme is responsible for the formation of prostaglandins, thromboxane and prostacyclins from arachidonic acid. Especially cyclooxygenase-2 (COX-2) roles in inflammatory mediators formation of acute inflammation and relates to cytokines. Inhibition of COX enzyme is one mechanism of anti-inflammatory drugs.

(2) Pro-inflammatory / Cytokine inhibition test

This is the test of inhibition on inflammatory mediators (TNF-α, interleukins and prostaglandin E2) via macrophage activation by lipopolysaccharide (LPS) from negative bacterial cell wall. Then activated macrophage releases the mediators. The released mediators can be measured.

2.3.3 *In vivo* anti-inflammatory activity study

(1) Ethyl phenylpropiolate (EPP)-induced ear edema in rats (48-50)

Ethyl phenylpropiolate (EPP)-induced ear edema in rats is the acute inflammation study model for evaluation of sample in reducing edema caused by increased vascular permeability via topical route.

2.4 Topical preparations (51)

There are 3 types of topical preparation.

1. Solid (powders, patchs, sticks)
2. Semi-solid (ointments, creams, pastes, gels, foams)
3. Liquid (emulsions, solutions, suspensions)

Topical preparations for joints and muscle are usauly semi-solid form due to adhere on skin surface and also can be massaged and rubbed. The preparation for joints and muscle are ointments, creams, gels, emulgels and sprays.

Selection topical preparations should be considered on the ease of application and the ability of product to penetrate into the dermis layer. For treatment arthritis and muscle, the preparation should penetrate into dermis layer and usually be cream or gel.

Table 2.2 Product types in marget for topical application

Product type	Application
Liquid preparations	wound cleansing, skin rash
Gels	topical steroid, NSAIDs
Powders	skin protection, weeping wound
Ointments	emollient, skin allergic condition, antimicrobial, pain
Creams	skin allergic condition, topical steroids, NSAIDs, antimicrobial
Pastes	skin protection, weeping wound
Aerosols	skin allergic condition, topical steroids, NSAIDs, antimicrobial

2.4.1 Gels

Gel is a semi-solid dosage form consists of small inorganic molecules dispersed in liquid or large organic molecules in liquid. There are 2 types of gels: two-phase gels and single-phase gels. In pharmaceutical sciences and cosmetics, single-phase gels is more use due to semi-solid property, transparency, easily apply and drug is released promptly without depending on solubility when compared to creams and ointments.

Gelling agent can be divided into 2 types. There are hydrophilic gels and lipophilic gels which gel base consists of colloidal silica in liquid paraffin and fatty oil. Almost of pharmaceutical preparations are hydrophilic gels.

Hydrophilic gel consists of gelling agent in water, glycerol or propylene glycol. Gelling agent are included non-charged cellulose derivative (methylcellulose (MC) and hydroxypropyl methyl cellulose (HPMC) are form gel in water) and charged cellulose derivative (sodium carboxymethylcellulose (SCMC) can dissolve without thermal.

1. Carbomer is polymeric acid disperse but not dissolve in water. This gel is stable at lower basic pH.
2. Sodium alginate is dissolved and stable at pH 4.5-10

Each gelling agent can be prepared at various concentrations such as 2-5% of tragacanth, 2-10% of sodium alginate, 2-15% of gelatin, 3-5% of methylcellulose 450, 2-5% of sodium carboxymethylcellulose, 15-50% of poloxamer and 0.3-5% of carbomer.

2.4.2 Gel preparation

Principle of gel preparation is to gradually disperse gelling agent into dispersion medium to prevent mass gel formation. Then gel is mixed with other ingredients.

Ingredients in gel preparation:

1. Gelling agents (poloxamer, starch, cellulose derivatives, carbomer)
2. Dispersion medium (water)
3. Preservative (0.2% paraben, 0.2% benzoic acid)
4. Humectant as a peeling effect
5. Skin enhancer (ethanol, propylene glycol, fatty alcohol)

2.5 Releasing / permeation study (52-54)

Topical preparations can penetrate into skin via 3 routes that are:

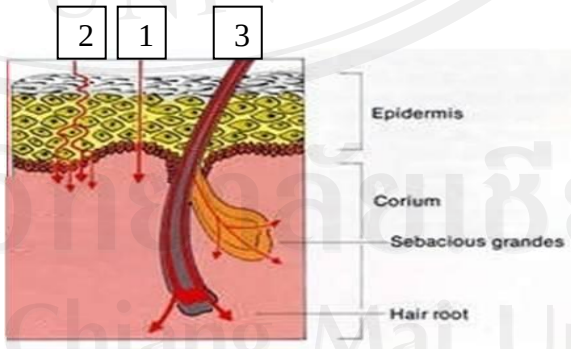


Figure 2.11 Routes of skin absorption (55)

1: Transcellular route; 2: intercellular route; 3: transappendageal route as shown in the Figure 2.11. The pathway of skin penetration is as ordered:

1. Drug releasing from base adhere on skin surface
2. Drug penetrating into stratum corneum
3. Drug diffusion to viable epidermis
4. Drug diffusion to dermis
5. Drug absorption and action

Topical preparation for joints and muscle is targeted on dermis absorption to reduce pain and inflammation. Therefore releasing test of the preparations should be evaluated to confirm whether the preparations can release active compounds on target area of inflammation. The study can be evaluated as followed:

In vitro skin permeation study

The principle is to study on diffusion of drug from diffusion cell at donor part through membrane mimic the skin and release into receiver fluid that mimic viable epidermis. The released drug is then quantitatively measured.