

APPENDIX

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University

All rights reserved

Appendix A

All plants used in this research

- 1. Scientific name** : *Anethum graveolens* Linn. ^(1,2)
- Thai name** : Puk-Chee-Lao
- Family** : Apiaceae
- Botanical note** : The slender, spindle-shaped root is slightly branched, the stem erect, hollow, finely furrowed and branched in the upper part. The alternate leaves are divided three or four times into thin pinnate sections, and are petiolate in the lower part of the stem. The stems and branches terminate in compound umbels up to 15cm in diameter with 30-50 rays which have neither covering nor capsules. The yellowish-brown or reddish fruits develop into ovoid flattened achenes and are compressed together in pairs.
- Compounds** : (*S*)- α -phellandrene, (3*r*,4*S*,8*S*)-3,9-epoxy-1-menthene, myristicin, methyl-2-methylbutanoate, (*R*)-limonene
- Pharmacological activity:** The carminative volatile oil improves the appetite and aids digestion. Chewing the seeds is helpful in cases of halitosis. *Anethum* stimulates milk flow in lactating mothers, and is often given to cattle for this reason. An infusion of the flowering plant is recommended for urinary complaints and or coughs while soaking the hands in a decoction of the seeds is said to strengthen the nails.

2. Scientific name : *Apium graveolens* Linn. ^(3,4)

Common name : Celery

Thai name : Khun-Chai

Family : Apiaceae

Botanical note : strong-smelling, slender, erect biennial herb, up to 60 cm high. It has shiny pinnate leaves and large toothed leaflets, the upper trifoliate and unsalted. The flowers are white, in four to twelve simple umbels in a compound umbel which is often opposite a leaf; there are no upper or lower bracts. The petals are small and entire with a small inflected point, and the fruits are very small and slightly compressed laterally.

Compounds : α -pinene, β -pinene, myrcene, limonene, γ -terpinene, cymene, β -elemene, β -caryophyllene, α -humulene, β -humulene, β -selinene

Pharmacological activity: mild diuretic, urinary antiseptic, urinary calculi, calming effect, relief of flatulence, griping pains, reduce visceral spasm, uterus readjust, encourages, antispasmodic, sedative, diuretic, kidneys, elimination of water, hypoglycemic, diabetes, anti-platelet activity *in vitro*. The volatile oil; antifungal activity, against many bacteria including *Staphylococcus aureus*, *Staphylococcus albus*, *Shigella dysenteriae*, *Salmonella typhi*, *Streptococcus faecalis*, *Streptococcus pyogenes* and *Pseudomonas solanacearum*. No activity was observed against *Escherichia coli* or *Pseudomonas aeruginosa*.

- 3. Scientific name** : *Centella asiatica* Urban ^(5,6)
- Common name** : Asiatic Pennywort, Tiger Grass
- Thai name** : Bua-Bok
- Family** : Apiaceae
- Botanical note** : Perennial creeping herb. Leaf simple, alternate, kidney-shaped, 2-5 cm in diameter. Inflouescence in axillary umbel; flowers small, violet. Fruit dry, dehiscent.
- Compounds** : asiaticoside, madecassic acid, madecassosid, asiatic acid
- Pharmacological activity:** Fresh leaf: treatment of sore throat, thirst, antipyretic, diuretic, antidiarrheal; externally used for burns and wounds. Active constituents are madecassic acid, asiaticoside, which are astringent and stimulate tissue repairment at affected area, lower the probability of keloid formation, inhibits growth of *Staphylococcus spp.*, possess antifungal activity against tinea versicolor and reduce inflammation. Commercialized product as anti-inflammatory cream for surgical wounds.
- 4. Scientific name** : *Coleus amboinicus* Lour.,
Plectranthus amboinicus (Lour.) Spreng
- Common name** : Country Borage, Indian Borage, Oregano
- Thai name** : Neum-Hoo-Suea
- Family** : Lamiaceae
- Botanical note** : Perennial herb, quadrilateral or circular trunk, leaves; sharp, circular, easy to break, 1.5-9.5 cm wide, 1.5-11.5 cm long, the nib of a leaf wide 0.75-7.5 cm, aroma. Flower white or purple, 14-39 cm long.
- Compounds** : caryophyllene, p-cymene, limonene, terpinen-4-ol, thymol, carvacrol, camphor, ascorbic acid, vitamin B1, fatty acid.
- Pharmacological activity:** anti-fungal, anti-bacterea, anti-yeast, killed insect, remedy deaf, stop bleeding.

5. Scientific name : *Coriandrum sativum* L. ^(7,8,9)

Thai name : Puk-Chee

Family : Apiaceae

Botanical note : It is an annual, with erect stems, 1 to 3 feet high, slender and branched. The lowest leaves are stalked and pinnate, the leaflets roundish or oval, slightly lobed. The segments of the uppermost leaves are linear and more divided. The flowers are in shortly-stalked umbels, five to ten rays, pale mauve, almost white, delicately pretty. The seed clusters are very symmetrical and the seeds fall as soon as ripe. The plant is bright green, shining, glabrous and intensely foetid.

Compounds : linalool, α -pinene, β -pinene, limonene, anethole, camphor, oleic, petroselinic, linolenic fatty acids, flavonoid glycosides, chlorogenic, caffeic acids, tannins, sugar proteins

Pharmacological activity: Stimulant, aromatic and carminative. The powdered fruit, fluid extract and oil are chiefly used medicinally as flavouring to disguise the taste of active purgatives and correct their griping tendencies. It is an ingredient of the following compound preparations of the Pharmacopoeia: confection, syrup and tincture of senna, and tincture and syrup of Rhubarb, and enters also into compounds with angelica gentian, jalap, quassia and lavender. As a corrigent to senna, it is considered superior to other aromatics

6. Scientific name : *Emilia sonchifolia* DC. ^(10,11,12)

Thai name : Hang-Pla-Chon

Family : Asteraceae

Botanical note : Annual growing to 0.6 m. It is hardy to zone 9 and is frost tender. The flowers are hermaphrodite (has both male and female organs) and are pollinated by insects. The plant is self-fertile. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils and requires well-drained soil. The plant

prefers acid, neutral and basic (alkaline) soils. It cannot grow in the shade. It requires moist soil.

Compounds : simiral, β -sitosterol, stigmasterol, palmitic acid, honey acid

Pharmacological activity: depurative, diaphoretic, diuretic, expectorant, febrifuge, odontalgic, and ophthalmic. A tea made from the leaves is used in the treatment of dysentery. The juice of the leaves is used in treating eye inflammations, night blindness, cuts and wounds and sore ears. The plant is astringent, depurative, diuretic, expectorant, febrifuge and soporific. It is used in the treatment of infantile tympanitis and bowel complaints. The juice of the root is used in the treatment of diarrhea. The flower heads are chewed and kept in the mouth for about 10 minutes to protect teeth from decay.

7. Scientific name : *Eryngium foetidum* Linn. ^(13,14)

Common name : Fitweed, Stink Weed

Thai name : Phak-Chee-Pha-Rang

Family : Apiaceae

Botanical note : Aromatic herb with fusiform root. Flowering stems much branched. Leaves rosette, lanceolate-spathulate, sessile, margin dentate, 3-20 by 1-3 cm. Inflorescence bracteates, \pm spiny, heads cylindrical, 5-10 mm. Flowers sessile, yellowish green. Fruits glabrous, densely warty. In partial shaded, moist and fertile localities, nursery beds. Reproduced by seeds. Native of tropical America. In Thailand: throughout the country, 0-1,400 m. blooming period: October-April. Young plants are eaten raw or cooked.

Compounds : α -muurolene, β -gurjunene, δ -cadinene, valencene, 2,4,5-trimethylbenzaldehyde, (e)-2-dodecenal, carotol, 3-dodecenal, γ -terpinene

- 8. Scientific name** : *Eupatorium odoratum* Linn. ^(15,16)
- Thai name** : Sap-Suea
- Family** : Asteraceae
- Botanical note** : Erect, branched, annual herb, up to 1.5 m high; younger parts densely pubescent. Leaves simple, opposite decussate, ovate, sparsely pubescent on both sides, 2-6.5 cm wide, 5.5-11.5 cm long. Inflorescence in terminal corymbose panicle; flower white with lilac hue. Fruit achene, linear, flat, sparsely puberulous.
- Compounds** : flavone, salvigenin, triterpenoids, lupeol, β -amyirin
- Pharmacological activity:** Fresh leaf: tropically apply to stop bleeding; contains 4, 5, 6, 7-tetramethoxyflavone and calcium, which decrease blood clotting time. Whole plant: insecticide. Root ; an ingredient in antimalarial formula. Chloroform and acetone extracts of stem and leaf inhibit the growth of *Staphylococcus aureus* and *Bacillus subtilis*, which cause pus formation
- 9. Scientific name** : *Houttuynia cordata* Thunb. ^(5,17, 18)
- Thai name** : Phlu-Khao
- Family** : Saururaceae
- Botanical note** : Erect perennial herb with deep-lying rhizome, 15-30 cm high. Leaf simple, alternate, cordate, 4-6 cm wide, 6-10 cm long; petioles long covered by leaf-sheath. In florescence in terminal spike, 4 involucrel whitish bracts; flowers small, without petals and pedicels. Fruit obovoid capsule.
- Compounds** : In Japan; decanoyl acetaldehyde, capric acid, capric aldehyde, lauric aldehyde, myrcene, methyl-n-nonylketone. In China; decanoyl acetaldehyde, bornyl acetate, D-limonene, myrcene, camphene, α -pinene, carylphyllene, 2-undecanone, dodecanaldehyde, potassium chloride compounds, potassium sulphate compounds, cordarine.

Pharmacological activity: Leaf: treatment of venereal diseases; externally used for skin diseases. Whole plant: diuretic, genitor-urinary antiseptic.

- 10. Scientific name** : *Mentha cordifolia* Opiz. ^(4,5)
- Common name** : Kitchen Mint
- Thai name** : Sa-Ra-Nae
- Family** : Lamiaceae
- Botanical note** : Aromatic creeping herb, stems quadrangular, 10-30 cm high, brownish purple, much-branched. Leaves simple, opposite, broadly elliptic, 1.5-2.5 cm wide, 2-3 cm long, many sunken nerves on the upper surface, serrate-dentate, aromatic. In florescence in axillary cyme; flowers small, violet. Fruit ellipsoid nutlets.
- Compounds** : menthol, menthone, α -pinene, β -pinene, limonene, cineol, ethylamylalcohol, menthyl acetate, piperitone, menthofuran
- Pharmacological activity:** Fresh whole plant: carminative, antifatulent; apply to temple to relieve headache; treatment of faintness, sprains.
- 11. Scientific name** : *Ocimum basilicum* Linn. ^(5,19)
- Common name** : Sweet Basil, Common Basil
- Thai name** : Ho-Ra-Pha
- Family** : Lamiaceae
- Botanical note** : Erect perennial, herb, 30-90 cm high, aromatic; stems quadrangular; young shoots greenish purple. Leaves simple, opposite, ovate or elliptic, 2-3 cm wide, 4-6 cm long, serrate. Inflorescence in terminal verticillaster; flower white, bilabiate; lower lip reddish purple blotches; bracts purplish green. Fruit four nutlets.
- Compounds** : cineol, D-linalool, methyl chavicol, eugenol, geraniol, p-cymene, α -terpineol, sesquiterpene hydrocarbons from leaves; ocimene, α -pinene, 1,8-cineole, eucalyptol, linalool, geraniol,

limonene, eugenol, methyl chavicol, eugenol methyl ether, methyl cinnamate, 3-hexen-1-ol, estragol.

Pharmacological activity: Leaf: carminative; contains volatile oil which stimulates movement of small intestine, antifatulence, diaphoretic, expectorant, digestant, flavoring agent. Fruit: laxative; soak in water, wait until completely swollen, take two or three times daily.

- 12. Scientific name** : *Ocimum canum* Sims. ^(5,20)
Common name : Hairy basil
Thai name : Maeng-Lak
Family : Lamiaceae
Botanical note : Habit, leaf, flower and fruit similar to *O. basilicum*, different odor, paler green leaf and green bract.
Compounds : Essential oil: borneol, bornrol acetate, α -cadinene, β -cadinene, β -cadinol, camphene, chavicol methyl ether, camphor, caryophyllene oxide, β -caryophyllene, 1-8-cineol, cinnamic acid methyl ester, ρ -cymene, β -elemene, elemol, estragole, eugenol, eugenol methyl ether, geraniol, geraniol acetate, limonene, linalool, linalool acetate, α -pinene, tricyclene.

Pharmacological activity: Fruit: bulk laxative, stool softener, increases the amount of stool and frequency of purging. Leaf: carminative.

- 13. Scientific name** : *Ocimum sanctum* Linn. ⁽⁵⁾
Common name : Holy Basil, Sacred Basil
Thai name : Ka-Phrao
Family : Lamiaceae
Botanical note : *O. sanctum* consists of three varieties, red, white and hybrid. General characteristics similar to *O. basilicum*, different in

odor, pubescent branch, paler green leaf in white variety, purplish green leaf in red variety, purplish pink florets.

Compounds : In leaves; apigenin, ocimol, linalool, essential oil, chavibetal, citric acid, fumaric acid, luteolin, tartaric acid, ursolic acid, malic acid, methyl chavicol, linalool

Pharmacological activity: Leaf or whole plant: carminative, antidiarrheal, antiemetic. Eugenol from leaf stimulates bile secretion, increases fat digestion, antifatulent. Leaves and twigs both fresh and dried-antiflatulence; treatment of stomachache, nausea and vomiting. Roots-antipyretic; treatment of gonorrhoea. Seeds- contain mucilage as a lubricant.

14. Scientific name : *Piper sarmentosum* Roxb. ^(5, 21)

Thai name : Cha-Phlu

Family : Piperaceae

Botanical note : Stoloniferous small shrub, 30-80 cm high. Leaf simple, alternate, cordate, 5-10 cm wide, 7-15 cm long. Inflorescence in axillary spike, tubular, unisexual. Fruit obovoid berry.

Compounds : neolignans, sarmentosine. Amide 8 types; pellitorine, guineensine, brachystamide-B, sarmentine, brachyamide-B, 1-piperetyl pyrrolidine, 3',4',5'-trimethoxycinna, moyl pyrrolidine, sarmentosine, Lignans 2 types ; (+)-asarinin, sesamin. Other compounds; (3,4-methylenedioxyphenyl)-1E-tetradecene, methyl piperate, mixture of β -sitosterol and stigmasterol

Pharmacological activity: Whole plant: expectorant, antiflatulence. Leaf: carminative, expectorant. Whole plant extract stimulates intestinal movement and relaxes skeletal muscles.

15. Scientific name : *Polygonum odoratum* Lour. ⁽²²⁾

Vietnamese Coriander, Persicaria odorata

Common name : Smartweed, Laksa plant, Vietnamese mint

- Thai name** : Puk-Preaw, Puk-Pai
- Family** : Polygonaceae
- Botanical note** : The Vietnamese coriander is a perennial plant that grows best in tropical and subtropical zones in warm and damp conditions. It can not live above the 32° parallel or in places with too much water. In advantageous conditions, it can grow up to 15 to 30 cm. In the winter or when the temperature is too high, it can wither. The top of its leaf is dark green, with chestnut-colored spots while the leaf's bottom is burgundy red. Its stem has sections. In Vietnam it can be cultivated or grown in the wild.
- Compounds** : In the essential oil, long-chain aldehydes were found, e.g., decanal (28%) and dodecanal (44%), furthermore decanol (11%). Sesquiterpenes (α -humulene, β -caryophyllene) account for about 15% of the essential oil.
- Pharmacological activity:** bitter and spicy taste, nontoxic, and can detoxify food. They claim that it can be used to treat swellings, acne, indigestion, flatulence, and stomach aches. It is used to repress sexual urges. *Polygonum odoratum* Lour. **activity:** has the ability to reduce fertility, while live bean sprouts have the opposite effect. Many Buddhist monks grow it in their private gardens and eat it frequently.

16. Scientific name : *Polyscias fruticosa* Harms. ⁽²³⁾

Nothopanax fruticosus (L.) Miq.

Panax fruticosus L.

Common name : Ming aralia, Black aralia

Thai name : Leb-Krut

Family : Araliaceae

Botanical note : Large, erect shrubs, up to 3 m or taller, with few, rapidly ascending branches; and normally compound pinnate, ovoid to orbicular to deeply toothed or laciniate, green, bright yellow to variously variegated leaves, depending on the variety.

Compounds : leaves and roots revealed eight new oleanolic acid saponins named polysciosides A to H and three known saponins.

Pharmacological activity: Chemicals found in plant shown to be effective for the ailment medicated.

17. Scientific name : *Spilanthes acmella* Murr. ^(5,20)

Common name : Para Cress, Tooth-ache plant

Thai name : Puk-Khrat-Hua-Waen

Family : Asteraceae

Botanical note : Creeping herb, 30-40 cm high; stems cylindric, succulent, purplish red. Leaves simple, opposite, triangle-shaped, 3-4 cm wide, 3-6 cm long, serrate. Inflorescence in axillary, obconical head; flowers light yellow. Fruit ovate achene.

Compounds : Root; olean-12-ene-3-O- β -D-galactopyranosyl (1 \rightarrow 4)-O- α -L-rhamnopyranoside. Whole plant; α -amyrin, α -amyrin acetate, β -amyrin, β -amyrin acetate, daucosterol, lauric and linoleic acids, myricyl alcohol, myristic, oleic and palmetic acids, spilanthol, stigmasterol.

Pharmacological activity: Whole plant: antipyretic; apply locally for toothache; crush and mix with alcohol or vinegar, squeeze, gargles the solution to relieve pharyngitis and inflammation of salivary glands. Leaf, peduncle and inflorescence: contain local anesthetic compounds, spilanthol. Clinical trials revealed that alcoholic extract of whole plant showed earlier onset but shorter duration of anesthetic action compared to lidocaine.

18. Scientific name : *Vernonia cinerea* (L.) Less. ⁽²⁴⁾

Blumea chinensis (L.) DC., *Conyza chinensis* L.,

Conyza cinerea L., *Cyanthillium cinereum* (L.) H. Rob,

Serratula cinerea (L.) Roxb., *Vernonia arguta* Baker,

Vernonia betonicaefolia Baker, *Vernonia exilis* Miq.,

Vernonia vialis DC.

Common name : ironweed, little ironweed, small ironweed, vernonia
Thai name : Ya-La-Oong, Ya-Dok-Khao, Mor-Noi (young doctor),
 Ya-sam-Wan (three-days-grass)

Family : Asteraceae

Botanical note : Erect branching annual herb to 60 cm tall, with ovate acute, or variably shaped leaves, mostly 1-5 (rarely to 7-8) cm long, 1.5-2 (-3) cm wide, the upper ones narrower, smaller and sessile, the lower ones with petioles 1-3 cm long, surfaces minutely puberulent, base decurrent; heads 20-25-flowered, more or less corymbose, shortly pedunculate, 6-7 mm long; bracts of involucre acute-acuminate, 4-5 mm long, in 4 series, puberulent; corollas violet or pinkish-violet, about 4 mm long, exserted; achenes appressed-pubescent, 1.5-2 mm long; inner hairs of pappus 4-5 mm long, outer ones very short.

Compounds : sesquiterpene lactones, flavonoids, steroids, polysaccharides, terpenoids

Pharmacological activity: Thai Prescription: Neurasthenia, Aversion of food: approx. 15 gm. of fresh whole herb boiled with 1.5 liter of water for approx. 5 minutes. Drink as tea, one glass a time, 5-6 times a day, for 3 days. Chinese Prescription: Dose: 15 – 30 gm. (dried-fresh), Colds and fever, cough: *Vernonia cinerea*, *Vitex negundo*, *Sapindus mukorossi* roots, *Ficus retusa* leaves, 15 gm. each. Boil in water. Neurasthenia: *Vernonia cinerea*, *Siegesbeckia orientalis*, 15 gm. each; *Oxalis repens*, *Marsilea quadrifolia*, 12 gm. each; *Alpinia oxphylla* 6 gm. Boil in water. Hypertension: *Vernonia cinerea*, *Oxalis repens*, *Siegesbeckia orientalis*, 15 gm. each. Boil in water.

19. Scientific name : *Cinnamomum bejolghota* Sweet. ^(4,5)

Common name : Cinnamom

Thai name : Op-Choei

Family : Lauraceae

Botanical note : Tree, 4-10 m high, bark and leaf fragrant. Leaves simple, opposite, elliptic or elliptic-oblong, 3-6 cm wide, 7-13 cm long, coriaceous, 3-nerved. Influrescence in terminal and axillary panicle; flowers small, light yellow. Fruit berry.

Compounds : In leaves; pinene, phellandrene, dipentene, linalool, benzylaldehyde, borneol, terpineol, safrol, caryophyllene, eugenol, cinnamic alcohol, cinnamic aldehyde, geraniol

Pharmacological activity: Stem bark; cardiac tonic, antifatulent, relieves fatigue; ingredient in Ya-Hom and Ya-Nut.

20. Scientific name : *Knema lenta* Warb.

Knema angustifolia (Roxb.) Warb.

Knema cinerea var *andamanica* auct. Non (Warb.) J.Sinclair

Knema erratica Warb.

Myristica corticosa auct. Non (Lour.) Hook.f. & Thomson

Common name : Dareugruba

Thai name : Lueat khwai

Family : Myristicaceae

Botanical note : Large trees to small trees. Bark green, red, brown, black; not peeling in strips. Slash red, brown. Vertical stripes in the inner bark or wood absent. Odor absent. Exudate present; red; flowing, or spotty; not blackening on exposure hairs present, or absent; star-like. Stinging hairs absent. Mature twing indumentums absent. Leaves two-ranked; not scale-like; simple.

Reference:

1. Haughton C. (2007). *Anethum graveolens* (L.) [Online]. Available: <http://www.purplesage.org.uk/profiles/dill.htm> [2007, January 10].
2. Imre B., Alina S., and Werner G. (1992). Sensory study on the character-impact flavour compounds of dill herb *Anethum graveolens* L.). *Food Chemistry*, 43(5), 337-343.
3. (2007). *Apium graveolens* (L.) [Online]. Available: <http://www.purplesage.org.uk/profiles/celery.htm> [2007, January 10].
4. Yoshiro M. (1919). Analysis of essential oils by gas chromatography and Mass Spectrometry. New York: Halsted Press.
5. Saralamp P., Chuakul W., Temsirirkkul R., and Clayton T. (1996). Medicinal Plants in Thailand Volume 1. Bangkok: Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University.
6. Vogel H.G., De Souza N.J., D' Sa A. (1990). Effect of terpenoids isolated from *Centella asiatica* on granuloma tissue. Hoechst A.-G., Frankfurt/Main. *Fed Rep Ger. Acta Ther*, 16(4), 285-98.
7. Grieve M. (1995-2007). Coriander [Online]. Available: <http://botanical.com/botanical/mgmh/c/corian99.html> [2007, January 10].
8. Blumenthal M., Goldberg A., and Brinckmann J. (2000). Herbal Medicine Expanded commission Monographs. United States of America: Integrative Medicine, Communications.
9. Grieve M. (1995-2007). Coriander [Online]. Available: <http://botanical.com/botanical/mgmh/c/corian99.html> [2007, January 10].
10. Morris R. (1996-2003). Plants for a future [Online]. Available: <http://www.pfaf.org/database/plants.php?Emilia+sonchifolia> [2007, January 10].
11. Gao J.J., Cheng D.L., and Liu X.P. (1993). Chemical constituents of *Emilia sonchifolia* L. (DC.). *PubMed*, 18(2), 102-127.
12. Plants For A Future. (1996-2003). *Emilia sonchifolia* - (L.)DC. [Online]. Available: <http://www.pfaf.org/database/plants.php?Emilia+sonchifolia> [2007, January 10].
13. Sefidkon F., Dabiri M., and Alamshahi A. (2004). Chemical composition of the essential oil of *Eryngium billardieri* F. delaroché from Iran. *Journal of essential Oil Research*, 16(1), 42-43.

14. Cardozo E., Rubio M., Rojas L.B., Usubillaga A. (2004). "Composition of the essential oil from the leaves of *Eryngium foetidum* L. from the Venezuelan Andes." *Journal of essential oil research*. 16(1), 42-43.
15. Chuakul W., Saralamp P., Pacnil W., Temsirirkkul R., and Clayton T. (1997). *Medicinal Plants in Thailand. Volume 2*. Bangkok: Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University.
16. Sunil K.T., Durga S.B., and Bani T. (1974). Flavonoid and terpenoid constituents of *Eupatorium odoratum*. *Phytochemistry*, 13(1), 284-285.
17. Cheng-Jian X., Yi-Zeng L. and Foo-Tim C. (2005). Identification of essential components of *Houttuynia cordata* by gas chromatography/mass spectrometry and the integrated chemometric approach. *Talanta*, 68(1), 108-115.
18. Thengburanatham W. (1988). *Dictionary of Thai Medicinal Plants*. Bangkok: Odeonstore Press.
19. Henry H., BPharm M.B.E., and Ffst F.P.S. (1973). Herbs and Spices-A Bibliography. Part 11, 4. Basil. *The Flavour Industry*, 4, 65.
20. Fornsworth N.R., and Banyapraphatsara N. (1994). *Thai medicinal plants: recommended for primacy health care system*. Bangkok: Medicinal Plant Information Center, Faculty of Pharmacy, Mahidol University.
21. Thitima R., Puttan S., Kanchanawadee S., Chanika W., Phongpan R., Phaopong W., and Apichart S. (2004). Chemical constituents and bioactivity of *Piper sarmentosum*. *Journal of Ethnopharmacology*, 93(2-3), 173-176.
22. Ning-hon L. (1991). *Chinese Medicinal Herbs of Hong Kong Vol. 1*. Hong Kong: Chinese Medical Research Institute.
23. Clarke W.C. and Thaman R.R. (1993). *Agroforestry in the Pacific Islands: Systems for Sustainability*. Japan: United Nations University Press.
24. Stone B.C. (1970). The flora of Guam. *Micronesica*, 6, 1-659.

Appendix B

Cytotoxicity test

1. Trypan Blue Dye technique

Chemicals / cell

1. RPMI media 1640 with 10% Newborn Calf Serum (NCS): media
2. 70% alcohol
3. 0.4% Trypan blue dye
4. P388 cells (Mouse Lymphocytic Leukemia cells)

Subculture of suspension cell culture

Cleaning media with 70% alcohol around cover bottle for prevent contaminate. Pipette 5 ml RPMI 1640 medium with 10% NCS into new cell culture bottle. Pipette 1 drop P388 cells into flask that have media in flask. Soft knock for cell to float up media and put flask in a horizontal line. Incubator at 37 °C.

Cell count suspension cell

Pipette 0.2 ml Trypan blue dye into test tube, used new pipette suck up-down many times for spread cells. Pipette 0.2 ml P388 cells into the same test tube, suck up-down many times for spread cells and blend dye, wait the die cell to adjoin dye (Trypan blue dye) for 5 min. Cleaning hemacytometer and cover slide. Pipette P388 cell dropping into hemacytometer, counting cancer cells that not stain in 5 sq. Calculating dilution number for find cell beginning that wanted, counting every day in the same time by viable cell count method of day 1 to day 7, record results.

Preparation of solution

1. Cell culture

$$\begin{aligned} \text{Cell count} &= 37 \text{ cells/5sq} \\ \text{Cell concentration} &= (37/5) \times 2 \times 10^4 \text{ cells/ml} \\ &= 14.8 \times 10^4 \text{ cells/ml} \\ \text{Want cell concentration begin} &= 5 \times 10^4 \text{ cells/ml} \\ \text{Therefore, dilution number} &= 14.8 \times 10^4 / 5 \times 10^4 \text{ cells/ml} \\ &= 2.96 \end{aligned}$$

Because of in the real test can not pipette solution in decimal system, so disregard the amount remaining to 3-fold for easily test.

Thus, dilution method = 3 ml cell stock + 7 ml Medium

Therefore, real cell concentration begin

$$\begin{aligned} \text{2.96-fold have cells} &= 5 \times 10^4 \text{ cells/ml} \\ \text{If 3-fold have cells} &= 14.8 \times 10^4 / 3 \text{ cells/ml} \\ &= 4.93 \times 10^4 \text{ cells/ml} \end{aligned}$$

2. Sample stock 100 µg/ml, 10 ml

Volatile oil 0.001 g + DMSO 0.25 ml + RPMI with NCS 9.75 ml

3. 2.5 % DMSO in RPMI, 10 ml

Pipette DMSO 0.25 ml + RPMI 9.75 ml

Then prepared 3 solution, pipette solution in following into flask (Table B.1)

Table B.1 Ratio of adding solution into flask

Flask	Medium (ml)	2.5 % DMSO in RPMI (ml)	Sample 100 µg/ml (ml)	Cell (ml)	Final Conc. (µg/ml)
1	3	1	-	1	Control
2	3	1	-	1	Control
3	3	-	1	1	20
4	3	-	1	1	20
5	3	0.5	0.5	1	10
6	3	0.5	0.5	1	10

Calculation ED₅₀ (Effect Dose 50)

ED₅₀ is minimum concentration values of drug that can destroy cancer cells to die half. Can find ED₅₀ from % growth of day4 of culture cancer cells by cells in flask control is 100% and prepare with flask that have drug. The calculation in following:

$$\% \text{ growth} = \frac{\text{Average amount cells of this concentration}}{\text{Average amount cells of control}} \times 100$$

Therefore, in the research valuable %growth of each concentration in following:

$$\begin{aligned} \% \text{ growth at concentration } 10 \mu\text{g/ml} &= (100 \times 4.96 \times 10^6) / 6.34 \times 10^6 \\ &= 78.33\% \\ \% \text{ growth at concentration } 20 \mu\text{g/ml} &= (100 \times 2.15 \times 10^6) / 6.34 \times 10^6 \\ &= 33.91\% \end{aligned}$$

Table B.2 % growth value of cancer cells in day 4

Sample concentration (μg/ml)	average amount cells	% growth
Control	6.34 x 10 ⁶	100
10	4.96 x 10 ⁶	78.33
20	2.15 x 10 ⁶	33.91

Bring % growth values plot graph with concentration of sample for find ED₅₀ values, replace y = 50 in an algebraic equation $y = -3.3045X + 103.79$

$$\text{Will get } 50 = -3.3045X + 103.79$$

$$X = (50 - 103.79) / (-3.3045)$$

$$= 16.28 \mu\text{g/ml}$$

$$\text{ED}_{50} = 16.28 \mu\text{g/ml}$$

Preparation media: RPMI medium 1640 with 10% Newborn Calf Serum (NCS)

RPMI powder 20.9 g, Sterile water for injection 2 L, HEPES 3.00 g, Sodium hydrogen carbonate (NaHCO₃) 2.00 g. Adjust pH with NaHCO₃ 7.5 % (pH~7.0-7.2), add sodium penicillin G 0.4 ml, add streptomycin 1.0 ml. Membrane filtration

electron N₂ (pass through Millipore filter), dispense into bottles and sterility test (all bottles in 37°C incubate 24 h.)

Preparation of 0.4% Trypan blue dye

Trypan blue	0.40 g
Sodium chloride (NaCl)	0.81 g
Potassium phosphate monobasic	0.06 g
Methyl-p-hydroxybenzoate	0.05 g
Distilled water	~95.0 ml

Boiling this solution for boil and waiting for cool. Adjust pH = 7.2 – 7.3 with 10 N sodium hydroxide. Adjust volume to 100 ml. This solution was stable in room temperature.

2. Protein Determination technique

Chemicals / cell

1. EMEM (Eagle's Minimum Essential Medium): media
2. 0.25% Trypsin solution
3. HBSS
4. Human Mammary Carcinoma (MCF-7)
5. Human Carcinoma of the Floor of Mouth (KB)

Subculture of monolayer cell culture

Bring MCF-7 cell or KB cell that culture for 4 day was suck Tissue culture medium. Wash cell with HBSS 5 ml, digest cells to single cell with 0.25% Trypsin in HBS 5 ml for 2-3 min, drain 0.25% Trypsin solution. Add 10% EMEM 3 ml, used pipette suck up-down many times to spread cells. Bring suspension dilute with media (1 ml suspension cell: 4 ml media). Incubate at 37°C.

Colorimetric assay

Measure by spectrophotometer at 660 nm

Preparation media: EMEM, 2 L

EMEM powder 19.02 g, sterile water for injection 2 L, HEPES 2 g, NaHCO₃ 2 g. Add sodium penicillin G 0.4 ml, add streptomycin 1.0 ml and adjust pH with NaHCO₃ 7.5 % (pH~7.0-7.2). Filtrate with Membrane filtration electron N₂, dispense into bottles. Sterility test (All bottles store in 37°C incubator for 2 days and observe turbidity if contaminated)

Preparation of 0.25% Trypsin in HEPES-Buffered Saline (HBS)**HEPES-Buffered Saline (HBS)**

NaCl	8.00 g
KCl	0.40 g
Na ₂ HPO ₄	0.10 g
Dextrose	1.00 g
HEPES	2.38 g
Distilled water	1,000 ml

Solute Trypsin 2.5 g in HEPES-Buffered Saline 1,000 ml and stirred with magnetic stirrer, adjust pH~7.0-7.2. Filtrate pass millipore filter, dispense into bottles, check contaminate, and store in 37°C.

Preparation of HBSS (Hank's Balance Salt Solution)**Solution A**

Sodium chloride (NaCl)	8.00 g
Calcium chloride dihydrate (CaCl ₂ ·2H ₂ O)	1.90 g
Potassium chloride (KCl)	4.00 g
Magnesium sulfate heptahydrate (MgSO ₄ ·7H ₂ O)	2.00 g
Distilled water	<500 ml

Solution B

Disodium hydrogen phosphate dodecahydrate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$)	1.52 g
Potassium dihydrogen phosphate (KH_2PO_4)	0.60 g
Glucose	10.00 g
1% phenol red	16.00 g
Distilled water	<500 ml

Slowly pouring for the solution B into the solution A and stirring with magnetic stirrer. Then dilute with distilled water fully in 1,000 ml. filtrate with Millipore filter.

Appendix C

Standard curve of antioxidant activity

Standard curve of Trolox for ABTS assay:

Prepare Trolox stock solution 500 μM by weigh Trolox 0.0125 g, solute with ethanol and adjust to 100.0 ml in volumetric flask. The Trolox stock solution was diluted in ethanol to give several concentrations in Table C.1.

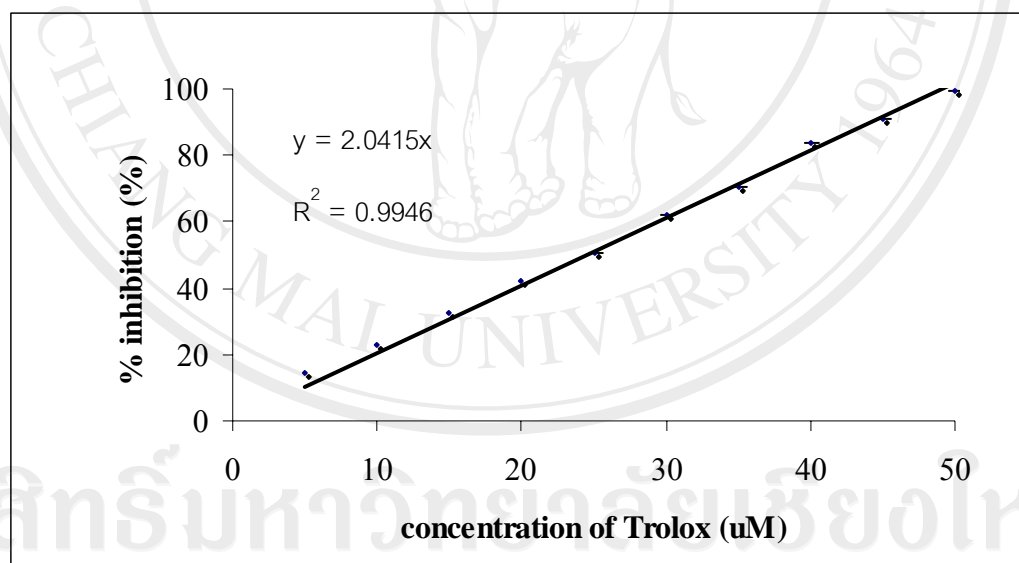
The percentage inhibition of absorbance at 740 nm is calculated and plotted as concentration of standard Trolox solution is illustrated in Table C.2. The representative regression coefficient (r^2) was 0.9946 and the linear regression equation was $y = 2.0415x$. (Figure C.1)

Table C.1 Preparing of series Trolox stock solution

Concentration of Trolox (μM)	Volume of Trolox stock solution (μl)	Volume of ethanol (μl)
500	1000	0
450	900	100
400	800	200
350	700	300
300	600	400
250	500	500
200	400	600
150	300	700
100	200	800
50	100	900

Table C.2 Absorbance values and %inhibition of standard Trolox

Concentration (μM)	Absorbance at 5 min					%inhibition mean
	control	1	2	3	$\bar{X} \pm \text{SD}$	
50	0.980	0.007	0.006	0.007	0.007 ± 0.06	99.32
45		0.086	0.088	0.086	0.087 ± 0.12	91.16
40		0.160	0.159	0.157	0.159 ± 0.16	83.81
35		0.290	0.285	0.285	0.287 ± 0.29	70.75
30		0.376	0.370	0.374	0.373 ± 0.31	61.90
25		0.483	0.481	0.482	0.482 ± 0.10	50.82
20		0.571	0.564	0.569	0.568 ± 0.37	42.04
15		0.664	0.662	0.666	0.664 ± 0.20	32.24
10		0.757	0.753	0.757	0.756 ± 0.24	22.89
5		0.839	0.838	0.839	0.839 ± 0.06	14.42

**Figure C.1** Standard curves of Trolox

Standard curve of ferrous sulfate (FeSO₄·7H₂O) for FRAP assay:

Prepare FeSO₄·7H₂O stock solution 1000 μM by weigh FeSO₄·7H₂O 0.0140 g, solute with water and adjust to 100.0 ml in volumetric flask. The FeSO₄·7H₂O stock solution was diluted in water to give several concentrations in Table C.3.

Table C.3 Preparing of series FeSO₄·7H₂O stock solution

Concentration of FeSO ₄ ·7H ₂ O (μM)	Volume of FeSO ₄ ·7H ₂ O stock solution (μl)	Volume of water (μl)
1000	1000	0
800	800	200
600	600	400
400	400	600
200	200	800
100	100	900

Table C.4 Absorbance value of FeSO₄·7H₂O

Concentration (μM)	Absorbance at 5 min			
	1	2	3	$\bar{X} \pm SD$
100	1.579	1.564	1.574	1.572 ± 0.008
80	1.395	1.393	1.391	1.393 ± 0.002
60	0.990	0.995	0.992	0.992 ± 0.003
40	0.585	0.581	0.578	0.581 ± 0.004
20	0.240	0.237	0.239	0.239 ± 0.002
10	0.107	0.106	0.108	0.107 ± 0.001

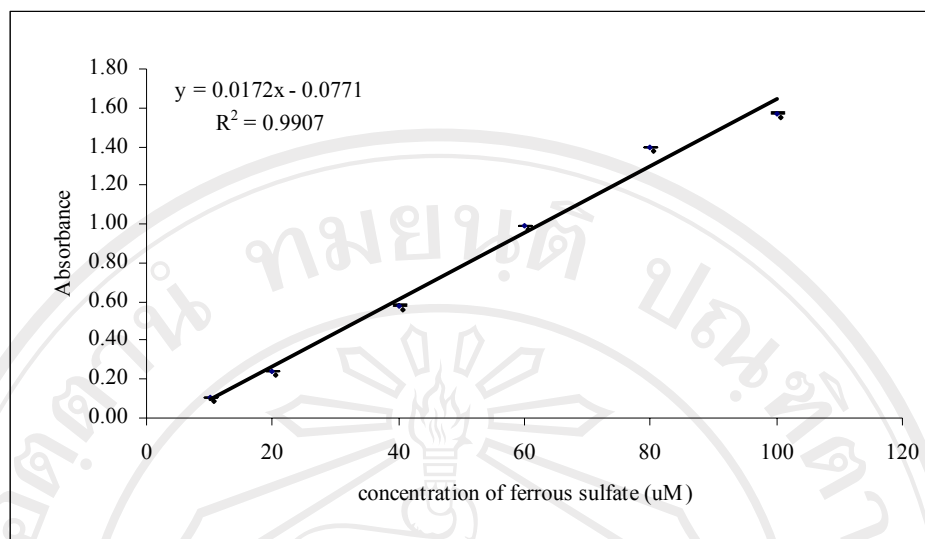


Figure C.2 Relations between concentration of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and absorbance

Standard curve of gallic acid for Folin-Ciocalteu assay:

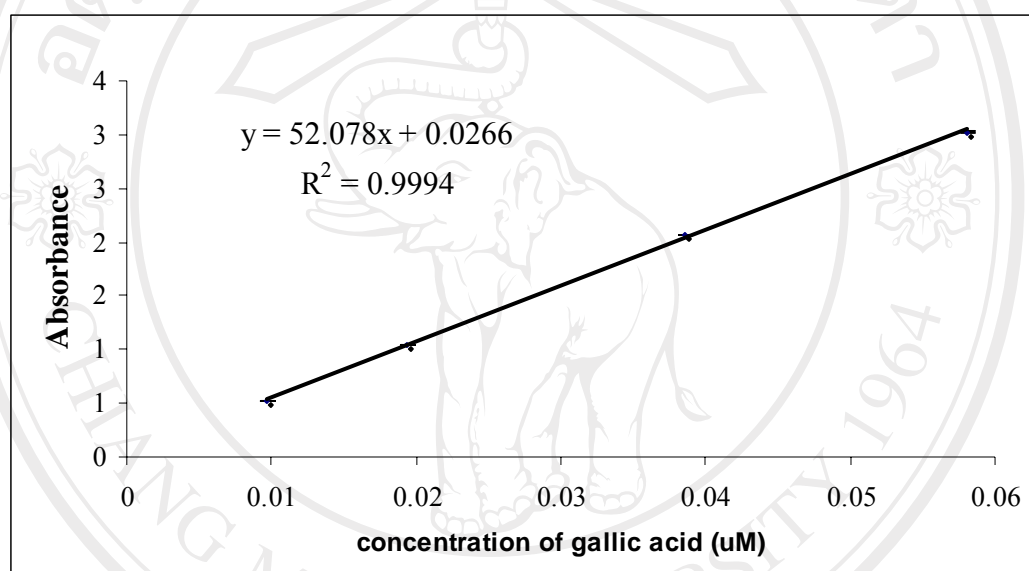
Prepare gallic acid stock solution $0.580 \mu\text{M}$ by weigh gallic acid 0.0097 g , solute with ethanol and adjust to 100.0 ml in volumetric flask. The gallic acid stock solution was diluted in ethanol to give several concentrations in Table C.5.

Table C.5 Preparing of series gallic acid stock solution

Concentration of gallic acid (μM)	Volume of gallic acid stock solution (μl)	Volume of ethanol (μl)
0.580	1000	0
0.386	665	335
0.193	333	667
0.097	167	833

Table C.6 Absorbance values of gallic acid

Concentration (μM)	Absorbance at 5 min			
	1	2	3	$\bar{X} \pm \text{SD}$
0.058	3.026	3.023	3.025	3.025 ± 0.002
0.039	2.073	2.074	2.073	2.073 ± 0.001
0.019	1.035	1.034	1.035	1.035 ± 0.001
0.010	0.513	0.515	0.516	0.515 ± 0.002

**Figure C.3** Relation between concentration of gallic acid and absorbance

Appendix D

Sample for calculation antioxidant activity

ABTS assay:

From Sample = 8.2 mg/ml
Abs sample = 0.205 in 200 μ l
Abs control = 0.950 in 200 μ l

$$\begin{aligned}\% \text{ Inhibition} &= \left[\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right] \times 100 \\ &= \frac{0.950 - 0.205}{0.950} \times 100 \\ &= 78.42 \%\end{aligned}$$

Find % inhibition/mg of sample

$$\begin{aligned}\% \text{ inhibition/mg} &= \frac{\% \text{ Inhibition}}{(20 \times 8.2 / 1000)} \\ &= \frac{78.42 \%}{(20 \times 8.2 / 1000)} \\ &= 478.17 \%\end{aligned}$$

From $y = 2.0415 x$

replace $y = \% \text{ inhibition/mg} = 478.17 \%$

Thus, $478.17 = 2.0415 x$

$$x = \frac{478.17}{2.0415}$$

TEAC = 234.22 μ M/ mg of sample

$$\text{TEAC} = \frac{234.22}{1000}$$

= 0.234 mM/mg of sample

FRAP assay:

$$\begin{aligned} \text{From Sample} &= 8.6 \text{ mg/ml} \\ \text{Abs sample} &= 0.810 \text{ in } 200 \mu\text{l} \end{aligned}$$

Find Abs/mg of sample

$$\begin{aligned} \text{Abs/mg} &= \frac{\text{Abs}}{(20 \times 8.6 / 1000)} \\ &= \frac{0.810}{(20 \times 8.6 / 1000)} \\ &= 4.709 \end{aligned}$$

From $y = 0.0172x - 0.0771$ Replace $y = \text{Abs sample/mg} = 4.709$

$$\begin{aligned} \text{Thus, } 4.709 &= 0.0172x - 0.0771 \\ x &= \frac{4.709 + 0.0771}{0.0172} \end{aligned}$$

$$\text{EC1} = 287.26 \mu\text{M/ mg of sample}$$

Lipid peroxidation:

$$\text{From Sample} = 10.3 \text{ mg/ml}$$

$$\text{Abs sample at 0 min} = 0.700$$

$$\text{Abs control at 0 min} = 0.661$$

$$\text{Abs sample at 150 min} = 0.608$$

$$\text{Abs control at 150 min} = 0.097$$

Find % Antioxidant Activity (% AA) of sample

$$\% \text{ AA} = \left\{ 1 - \frac{(\text{Abs}_{t0} - \text{Abs}_{t150}) \text{ of sample}}{(\text{Abs}_{t0} - \text{Abs}_{t150}) \text{ of control}} \right\} \times 100$$

$$= \left\{ 1 - \frac{(0.700 - 0.608)}{(0.661 - 0.097)} \right\} \times 100$$

$$= 83.58 \%$$

Amount of phenolic compounds:

$$\text{From Sample} = 9.8 \text{ mg/ml}$$

$$\text{Abs sample at 2 hour} = 0.456 \text{ in } 200 \mu\text{l}$$

Find Abs/mg of sample

$$\begin{aligned} \text{Abs/mg} &= \frac{\text{Abs}}{(20 \times 9.8 / 1000)} \\ &= \frac{0.456}{(20 \times 9.8 / 1000)} \\ &= 2.33 \end{aligned}$$

$$\text{From } y = 52.078x + 0.0266$$

$$\text{Replace } y = \text{Abs sample/mg} = 2.33$$

$$\begin{aligned} \text{Thus, } 2.33 &= 52.078x + 0.0266 \\ x &= \frac{2.33 - 0.0266}{52.078} \\ &= 0.044 \end{aligned}$$

$$\begin{aligned} \text{EC1} &= 0.044 \mu\text{g/ mg of sample} \\ &= 44.23 \text{ mg/ mg of sample} \end{aligned}$$

CURRICULUM VITAE

Name Miss. Rungthip Kawaree

Date of Birth 28 April 1980

Place of Birth Lamphun

Instituted attended Suanboonyopatum Lamphun School, Lamphun, 1998,
Certificated of Matayom VI.

Maejo University, Chiang Mai, 2001, BSC. (Chemistry)

Publication: International Journal

Rungthip Kawaree, Weerachai Phutdhawong, Porntipa Picha, Jarunya Ngamkham and Sombat Chowwanapoonpohn. 2006. "Chemical compounds, anticancer and antioxidant activities of volatile oil from *Piper samentosum* Roxb., *Polyscias fruticosa* Harms. and *Polygonum odoratum* Lour." KMITL International Conference on Science and Applied Science 2006, 8-10 March, 2006. Bangkok, Thailand.

Rungthip Kawaree, Weerachai Phutdhawong, Siriporn Okonogi and Sombat Chowwanapoonpohn. 2007. "Chemical Composition and Antioxidant Evaluation of Volatile oils from Thai Medicinal Plants." The international workshop on medicinal and aromatic plants, January 15-18, 2007, Lotus Pang Suan Kaew Hotel, Chiang Mai, Thailand. Poster presentation in Isolation and Characterization. Page 96.