CHAPTER I

INTRODUCTION

1. Review of the Rutaceae Family

The Rutaceae is a shrub, or tree, or sometimes herb, sometimes scrambling or scandent, sometimes armed, with aromatic volatile oils contained in glands visible at surface of at least leaves, young branchlets, inflorescences, flower parts, fruit, or cotyledons in seed. Stipules absent (or stipular excrescences rarely present). Leaves alternate, opposite (or whorled), simple (petiole neither apically swollen nor articulate with leaf blade), 1-foliolate (in individual specimens at least some 1-foliolate leaves with petiole apically swollen and/or articulate with leaf blade), or variously compound. Flowers bisexual or unisexual, usually 3–5-merous, actinomorphic or rarely zygomorphic, hypogynous (or rarely perigynous). Perianth in 2 series, with clearly differentiated calyx and corolla or sometimes in 2 irregular series or 1 series, with ± undifferentiated tepals. Sepals distinct or connate to their full length. Petals distinct (or rarely coherent or connate for part of their length). Stamens usually as many as or 2 × as many as petals or sometimes more numerous; filaments distinct or sometimes coherent or connate for at least part of their length; anthers introrse or sometimes latrorse, longitudinally dehiscent. Disk (rarely lacking) within androecium, nectariferous, flattened, annular, cup-shaped, pulvinate, or sometimes columnar, bell-shaped, conic, or hourglass-shaped. Gynoecium of 1–5 distinct 1-loculed carpels or 2 to many partially to completely connate carpels; placentation axile (very rarely becoming parietal); ovules 1 to many per locule. Fruit of 2–5 follicles (drupes or samaras) or a single follicle, capsule, or berry (or samara). Seeds with relatively large embryo; endosperm present and fleshy or lacking (1-2).

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The Rutaceae is a moderately-sized plant family with about 1700 species in 158 genera. There are trees and herbs also outside the tropics. Thailand has about 28 genera with 70 species. All plants in this family have oil glands with a nice aromatic to fetid smell. The family is most known for the fruits in genus Citrus: orange, mandarins, lime, citron and many more. Some area these fruits are only cultivated for the volatile oil in the peel of the fruit (3).

2. Historical Background of *Citrus hystrix* DC.

Citrus hystrix DC. is commonly known in Thai as “Ma-kruut”. *C. hystrix* is a small tree about 2-8 m high. Branchlets have spines. Leaves ark red when young; petiole winged, apex rounded to truncate; leaf blade ovate, 5–8 × 2.5–4.5 cm, 1–2.5 cm longer (rarely same length) and 0.5–1 cm wider than winged petiole, tertiary veins conspicuous, margin apically conspicuously and sparsely crenate, apex narrowly obtuse. Inflorescences with (1 or) 3–5 flowers; peduncle 1–5 mm. Flower buds globose. Calyx lobes 4 or 5, broadly triangular, ca. 4 × 6 mm. Petals white but pinkish red outside, 7–10 mm. Stamens ca. 30; filaments distinct. Styles short, thick. Fruit hesperidium, ellipsoid to subglobose, 5–7 × 3–5 cm, yellow or yellowish green, slightly coarse or smooth, oil dots numerous and prominent, apex rounded; pericarp thick; sarcocarp in 11–13 segments, very acidic and slightly bitter. Fruit-pulp is yellowish green, sour and slightly bitter. Seeds numerous 1.5–1.8 × 1–1.2 cm, ridged; embryo solitary; cotyledons milky white. The figure of *C. hystrix* are shown in Figure 1.1

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Figure 1.1 *Citrus hystrix* DC.

This plant, *C. hystrix* has been recognized as medicinal plant whose parts have been used as components in traditional medicine of various purposes;

Leaves contain essential oil and used as a condiment.

Fruit is a source of flavour and shampooing agent. Salted prickled fruit is taken orally for improving the quality of menstrual blood and as hematinic.

Peel of fruit can be a tonic, carminative and relieves abdominal discomfort.

Root is used as a detoxicant. It can be used for treatment of stomachache; expectorant.

Fresh juice is used for antiscurvy, expectorant; shampoo hair as antidandruff (1-2, 4-7).

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3. Historical Background of *Feronia limonia* Swing.

In Thailand, *Feronia limonia* Swing. is commonly known as "Ma-khwit". The tree is about 6-10 m high. The leaves are pinnate, imparipinnate alternate; leaflets 5 or 7, less often 3, 6 or 9, oblong-obovate, 0.5-1 cm wide, 1.5-4.5 cm long, pellucid-dotted only among the margin. Inflorescene in terminal or axillary panicle,

polygamomonoecious (unisexual flowers and bisexual flowers); flowers are greenish yellow, tinged with red. The fruit is a berry 5-9 cm diameter, and may be sweet or sour. It has a very hard rind which can be difficult to crack open, and contains sticky brown pulp and small white seeds.

Figure 1.2 *Feronia limonia* Swing.

This plant, *F. limonia* has been recognized as medicinal plant whose parts have been used as components in traditional medicine of various purposes;

The leaves are aromatic, astringent, carminative, constipating, antiemetic, expectorant and cardiotonic. They are useful in gastropathy, anorexia, diarrhea, vomiting, cough, bronchitis, hiccough, cardiac debility and antidiaraheal. Leaves can treatment of bloody vaginal discharge; timing of menstruation; crush and topically apply to treat trauma, abscesses and some skin diseases. Leaves extract can inhibit growth of *Vibrio cholerae in vitro*.

The unripe fruits are sour, aromatic, astringent, constipating and alexipharmic, and are useful in diarrhea, pruritus and pharyngodynia. The ripe fruits

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are sweet, sour, astringent, bitter, refrigerant, aromatic, anodyne, constipating, aphrodisiac, antiscorbutic, alexipharmic, cardiotonic, diuretic, vulnerary, expectorant, stomachic and antiemetic. They are useful in diarrhea, dysentery, vomiting, hyperdipsia, hiccough, pharygodynia, stomatitis, gingivitis, tumours, cough, asthma, consumption, ophthlmia, otalgia, cephalalgia, leucorrhoea, wounds and ulcers, anorexia, dyspepsia, cardiac, debility, strangury.

The gum is demulcent and constipating and is useful in diarrhea, dysentery, gastropathy, haemorrhoids and diabetes (1-2, 7-9).

4. Historical Background of *Aegle marmelos* Corr.

In Thailand, “Ma-tum” is Thai name of the plant *Aegle marmelos* Corr. This tree is 10-15 m high. Tops of branchlets short-hairy; spines 2.5 to 5 cm; petioles 1.5 to 5.5 cm; leaflets with a rounded or cuneate base, obtusely acuminate, shallowly crenate-dentate, 4 to 13.5 cm by 1.75 to 7.5 cm; central leaflet largest, its stalk 2 to 3 cm; stalks of lateral leaflet 0.33 to 0.5 cm. Leaf digitatety trifoliolate, alternate; leaflets elliptic or lanceolate-ovate, 2-7 cm wide, 4-3cm long. Inflorescence3 to 6 cm; pedicels short-hairy, articulate below the middle, 4 to 5 mm long; calyx pubescent, 2 to 3 mm high; segments acute; petals rounded or obtuse, thick, green on the outside, greenish white on the inner side, 10 to 17 mm long; filaments greenish white, glabrous, 5 to 8 mm long; style below the stigma 2.5 to 3.5 mm long; stigma green, 2 to 3 mm long; flowers greenish white on inner side, green on outer side; leaf and flower aromatic. Fruit fleshy, globose or ellipsoid, pericarp, pericarp very hard, pulp yellow or orange colored, 5 to 12.5 cm diameter; cell of young fruits filled with very viscid juice.

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Figure 1.3 *Aegle marmelos* Corr.

This plant, *A. marmelos* has been recognized as medicinal plant whose parts have been used as components in traditional medicine of various purposes;

Flesh fruit is eatable or processed into drinks or flavoring.

Young fruits can be sliced, dried and roasted to yellowish, then boiled in water for drinking

Mature fruits can be crystallized as dessert.

Ripe pulp is used as a digestive aid, stomachic and a laxative.

Unripe pulp is used to treat diarrhea and dysentery.

Fruit pulp is sometimes used as a detergent and adhesive.

Leaves are poisonous and flavor (1-2, 4, 7).

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5. Historical Background of *Citrus aurantifolia* Swing.

Citrus aurantifolia Swing. is commonly known in Thai as “Ma-nao”. *C. aurantifolia* is a small tree. Branchlets numerous and irregular have short stout spines.

Leaves slightly stiff with a short, conspicuous petiole; leaf blade broadly ovate to elliptic, 5–8 × 2–4 cm, base rounded, margin crenulate, apex obtuse and sometimes mucronate. Inflorescences have about 7 flowers or rarely flowers solitary. Calyx cup-shaped have lobes 4 or 5. Petals (4 or) 5, white, 1–1.2 cm, rather thick. Stamens 20–25. Ovary globose has style short, as long as stigma; stigma large. Fruit has greenish yellow, globose, ellipsoid, or obovoid, usually 4–5 cm in diameters, smooth, with prominent oil glands, apex with a papilla; pericarp thin; sarcocarp with 9–12 segments, very acidic. Seeds have few, ovoid; seed coat smooth; cotyledons milky white.

Figure 1.4 *Citrus aurantifolia* Swing.

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This plant, *C. aurantifolia* has been recognized as medicinal plant whose parts have been used as components in traditional medicine of various purposes;

Fresh fruit juice can be used as expectorant, cough remedy, antiscurvy due to high vitamin C content and used as solvent for other cough formulae. Dried preserved fruit can also be used.

Fruit can be used for treatment flatulence, stomach pain, vitamin A deficiency, ulcers, cough and insect bites.

Seeds are used for treatment of chronic gastro-intestinal ailment of children between the ages of 5 and 13 characterized by marked malnutrition, usually

associated with intestinal parasitism, respiratory distress and internal abscesses; antipyretic and expectorant.

Roots can be treated to exanthematous fever, recurrent fever and abscesses, antipyretic, analgesic and antiinflammation.

Leaves have been used to treatment for hepatomegaly due to injuries or chronic diseases, improves the quality of menstrual blood.

Flowers can be used for treatment of flatulence, stomach pain, nausea, vomiting and cough; as an expectorant (1-2, 4, 7, 10-11).

In Thailand, *C. hystrix, F. limonia*, *A. marmelos* and *C. aurantifolia* are Thai medicinal plants, great valuable heritage have been used for health and curing illness for a long time. Previous reports revealed that the extract from stem and bark of *C. hystrix* showed the antibacterial activity on *B. subtilis*, *B. cereus*, *S. epidermidis*, *S. aureus* and *P. acnes* (12). The methanolic extracts from the stem, sac, seed, peel, leaves, and callus of *C. hystrix* showed antibacterial, antiamebic, anti-tumor, hypotensive activity. The volatile oil from the callus of *C. hystrix* inhibited *S. aureus* (13). The extracts from stem bark and fruits of *F. limonia* showed anti-microbial and anti-tumor (14-16). The extracts from *A. marmelos* are able to inhibit the *in vitro* proliferation of human tumor cell lines, including the leukemic K562, T-lymphoid Jurkat, Blymphoid Raji, erythroleukemic HEL, melanoma Colo38, and breast cancer MCF7 and MDAMB-231 cell lines (17). The alcoholic and aqueous extracts of the leaves had similar effects as digoxin in amplitude and contractions of the frog heart and methanolic extracts of roots inhibited the beating rate by approximately 50% of cultured mouse myocardial cells (18). Alcoholic extracts of the roots and fruits showed hypoglycaemic activity (19). The roots were astringent, bitter and febrifuge. They were used to treat in diarrhoea, dysentery, dyspepsia, stomachalgia (20), cardiopalmus, seminal weakness, vomiting, intermittent fever and swellings. The leaves of *A. marmelos* were used as laxative, febrifuge and expectorant, also in ophthalmia, deafness, inflammations, catarrah, diabetes, asthmatic and antifungal complaints (21). The alcoholic extract of its roots were hypoglycemic (22), the ripe fruits extract showed antiviral (23), essential oils exhibited antifungal (24) and seeds oil showed antibacterial activities (25). Fruits of *C. aurantifolia* volatile oil had potential benefits in colon cancer prevention (26). Some experiments provided

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evidences of anticancer, antidepressant, anti-inflammatory and antibacterial properties of various Citrus juices and fragrance (27-30). Flavonoids, limonoids, and ascorbic acid were groups of citrus micronutrients, which are responsible for the anti-inflammatory and antitumor activities of citrus juice (31-34). According to these, the biological activities from fruit, seed, stem bark, root and bark of *C. hystrix, F. limonia*, *A. marmelos* and *C. aurantifolia* have been reported, but the antioxidant, anticancer, antibacterial and antifungal activities together with chemical constituent of *C. hystrix, F. limonia*, *A. marmelos* and *C. aurantifolia* leaves have a few reported. Therefore, this present study is aimed to assess the antioxidant, anticancer, antibacterial and antifungal activities and chemical constituents of *C. hystrix, F. limonia*, *A. marmelos* and *C. aurantifolia* leaves.

6. Research Aim

6.1 To study the antioxidant activity and biological activities of *C. hystrix, F. limonia*, *A. marmelos* and *C. aurantifolia* leaves.

6.2 To search for chemical constituents of essential oil and crude extracts from leaves of *C. hystrix, F. limonia*, *A. marmelos* and *C. aurantifolia*.

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