



A Pilot Study of Chemical Composition and Major Compounds of Conservation Plant: A Case Study of Ma-Kwan from Doi Phu Kha, Nan Province

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ABSTRACT

Introduction: The plant genetic conservation project under the royal initiative of Her Royal Highness Princess Maha Chakri Sirindhorn in response to the royal initiative by Mae Fah Luang University Botanical Garden has been a continuous survey and study on the utilization of indigenous plants. The results found that 391 types of Lua people and residents of Doi Phu Kha, Nan Province, categorized as food plants, medicines, housing, equipment and utensils. plants used in ceremonies and found an important spice is Ma-kwan (*Zanthoxylum limonella*).

Objective: The aim of this pilot to study the chemical composition of conservation plant and create a database of chemical composition of Ma-kwan that found in Doi Phu Kha, Nan Province.

Methods: The fresh Ma-kwan were dried, pericarps and seeds were separated and coarsely grinded then the essential oils were collected by water distillation method. The essential oils from pericarp and seed's part were analyzed for their chemical composition using Gas Chromatography-Mass Spectrometry (GC-MS).

Results: 35 compounds were found in essential oil; Piperitenol and p-Cymene were the most major compounds from pericarp and seed respectively. Moreover, essential oils obtained from the pericarp and seed have 16 identical compounds.

Conclusion: From the results of this study, the quantity and chemical composition of the essential oils from the fresh fruits will be recorded for comparison with fresh or dried fruits from different sources. They also know the major compounds found in the essential oils of fresh Ma-kwan found in Doi Phu Kha, Nan Province.

Keywords: Ma-kwan; *Zanthoxylum limonella*; Chemical composition; Essential oils

Introduction

Thailand has abundant natural resources and high biodiversity, especially in the northern region or the area known as the former Lanna Kingdom but after high economic growth and rapid population growth, there have been developments in various fields that have resulted in a change in the old way of life of the people in the area. However, in the northern area of Thailand, it is also the habitat of various ethnic groups that have the wisdom to use resources in the area. Plants used as a medicine as food and others, resulting in these plants being widely used. But, the utilization of some plants in the local area has not been scientifically studied to support it. The study of the chemical composition and major compounds of local plants is therefore an important study in the conservation of local knowledge

in terms of plant utilization and can also be disseminated to interested people or related groups to develop applications in parallel with current knowledge. Because of the plant genetic conservation project due to the royal initiative Her Royal Highness Princess Maha Chakri Sirindhorn Her Royal Highness Princess Maha Chakri Sirindhorn In response to the royal initiative by Mae Fah Luang University. There has been a continuous survey and study on the utilization of indigenous plants. The results of the study found that a study of the indigenous botany of Lua people and residents of Doi Phu Kha, Nan Province found a total of 391 types of plants, categorized as food plants, medicines, housing, equipment and utensils. plants used in ceremonies and found the important spice is Ma-kwan (*Zanthoxylum limonella*), which is widely used in cooking in the

northern region of Thailand. It is commonly used as a spice for flavoring food. It has a spicy flavor, while the bark and fruit can be used as a carminative. and as a sedative or pain reliever in India, coconut oil is commonly used as an anti-inflammatory in the name of “Mullilam oil”, therefore, Ma-kwan samples were collected from Doi Phu Kha, Nan province, to extract volatile oil and analyze its chemical composition. The aim of this pilot to study the chemical composition of conservation plant and create a database of chemical composition of Ma-kwan that found in Doi Phu Kha, Nan Province. Ma-kwan has a scientific name that *Zanthoxylum limonella* is in the family Rutaceae is a perennial plant that the northern people used as fruit and dried seeds for cooking; including used to make herbal medicine. Because, the dried fruit has a strong smell and has a spicy flavor helps to deodorize the fishy smell and enhance the flavor of food, it is also popular to use the dried fruit to extract essential oils for massage, used as a cosmetic ingredient or use anti-mosquito spray [1]. Botanical characteristics of Ma-kwan, it is a medium to large deciduous tree, 12-20 m tall, bark white, with conical spikes at the end, straight or slightly curved, growing along the trunk, branches and petioles 10-28 leaflets, may or may not have leaflets at the ends. Petiole short 0.5-1.0 cm, leaf width 4-5 cm, length 10-14 cm, oval, ovate or oblong, base ovate, margin smooth or wavy. The tip of the leaf is slender and pointed. The flowers are panicle-like panicles at the apex or axillary leaves. Inflorescences are 10-21 cm long, peduncles are long, small, greenish-white flowers are clustered at the end of the bouquet. The pistil and the male flower are on different plants, 4 secondary petals, 4 petals arranged alternately with 4 stamens, 1 pistil above the stamens, inside there is 1 cavity. Fruit round shape, light green fruit, 0.5-0.7 cm in diameter. The taste is very spicy when the skin is brown and crack open to see the black seeds into it. Flowering - fruiting during March – April [2]. Ma-kwan is found in the north of Thailand, naturally found in dry evergreen forests or evergreen forest in addition, it was also found that farmers prefer to plant interspersed with wild garden plants, which is planted together with other crops to increase their income. Sources of quality Ma-kwan such as Pha Chang Noi Sub-district, Pong District, Phayao Province, Doi Hang Subdistrict, Mueang District, Chiang Rai Province, Pa Pae Sub-District, Mae Taeng District, Chiang Mai Province, Doi Phu Kha, Pua District, Nan Province [2]. Ma-kwan can grow in areas with an altitude of 800 - 1,000 meters above sea level in cool climates and high humidity. It thrives in outdoor conditions, does not require much water likes soil that drains well, so it should be planted along the hillside or steep areas. Generally, Ma-kwan is used by using mature seeds and it is the fresh seeds that come out of the new husks, not dry. Ma-kwan will begin to produce at the age of 3 - 5 years. There are heavy varieties and light varieties, light varieties will begin to bloom in July and harvested in

October - November, the heavy varieties will begin to bloom in August and harvested in November-December, 3 - 5 years of age has yield approximately 1 - 5 kg dry per plant, 6 - 10 years old has yield 10 - 15 kg dry per plant, 11 - 15 years old has yield 30 - 35 kg dry per plant and 21 - 25 years old yields up to 50 kg dry per plant [1]. Because Ma-kwan is a spice plant used in local food. Most of the Ma-kwan market is in the northern area. The price of Ma-kwan that farmers can sell in the range of 50 - 70 baht per kg dry. In addition to farmers selling their products, then it is also processed into products of soup, stew, chili paste, etc. The parts used in cooking are young leaves and fruits, leaves and young leaves can be eaten as fresh vegetables. The fruit is a popular spice “Larb chili paste” seasoning has a spicy flavor that helps to improve the taste of food. Ma-kwan has folk wisdom that it is used as a side dish for meat-rich dishes because it can digest meat. In the south, it is popular to mix mackerel in spices such as pumpkin curry, eel curry, etc., which gives the curry a hot and spicy flavor and smells good. Ma-kwan has used as drug properties in traditional medicine; the roots and wood are used as intestinal carminatives, reduces dizzy, reduces blood pressure and it is a menstrual blood pill for women but not for pregnant women. Seeds can extract essential oils for treat dizziness, nourish blood, nourish the heart, increase blood circulation. Using in Chinese medicine textbooks to cure stomachache, flatulence, indigestion, vomiting, diarrhea, Ma-kwan seed extract, it has an anti-inflammatory effect caused by the poisoning of Formalin and Carrageenin and can suspend inflammation on the skin when applied with oil extracted from the Ma-kwan has the effect of suicide intestinal parasites [3]. From the study of chemotaxonomy of plants found that extracts from plants in the family Rutaceae have anti-inflammatory effects, especially citrus plants (Citrus) [4]. The main chemical composition of the skin is monoterpene, containing α -terpinene, α -terpineol, terpinen-4-ol, terpineol, α -pinene, sabinene and limonene, which has the highest concentration as seen in the effects of anti-inflammatory [5] by mechanism can inhibit the production of nitric oxide, prostaglandin E2 and TNF- α [6]. In addition, studies on the bioactivity of limonene showed that at a concentration of 50 μ g/ml suppressed the expression of limonene. The cyclooxygenase-2 (COX-2) gene was not significantly different from the 20 μ M concentration of celecoxib by the induction of cyclooxygenase-2 (COX-2) genes. Lipopolysaccharides (LPS) in RAW 264.7 macrophages [7] and 0.04% concentrations inhibited prostaglandin E2, equivalent to that of 20 μ M dexamethasone inhibited pro-inflammatory cytokines (TNF- α , IL-1 β , and IL-6) compared to levels of TNF- α , IL-1 β and IL-6 production in RAW 264.7 macrophages by induction of LPS and suppressing iNOS Protein and COX-2 Protein expression [8]. In vitro study found that extracts from the Ma-kwan fruit which is obtained by water

distillation has an inhibitory effect Cyclooxygenase-1 (70.4%), cyclooxygenase-2 (88.9%) and 5-lipoxygenase (7.57%) reported in recent studies have shown the bioavailability of extract to inhibit the neurotransmitter mediator of inflammation [9].

Methodology

Raw materials and preparation

The Ma-kwan fruits used in this study came from Doi Phu Kha in Nan province, harvested in October 2021. The fresh fruits were dried by the solar drying for 6 hours. Total weight of raw materials fruits in this study, when dried, was 30.88 kg, divided into pericarps weight 17.78 kg, seeds weight 13.10 kg. The pericarps and dried seeds were then separated and coarsely ground with a grinder for 20 seconds/kg, their weight and appearance were recorded.

Distillation of essential oils

The essential oil from the pericarps and seeds were extracted by water distillation using 150 liters of water and distilled for 6 hours in Clevenger apparatus at 60 Celsius degrees. Recorded the yellow oil content and appearance.

Chemical composition analysis

The essential oils obtained from the pericarps and dried seeds were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) with column TG-5 slim. Helium gas was carrier with flow rate 1.2 ml/min. The initial temperature of column oven was programed 120°C and then heated to 280°C with a rate of 8°C/min and kept constant at 280°C for 15 minutes. The mass spectrum was recorded. Chemical compositions were analyzed by Science and Technology Service Center, Faculty of Science, Chiang Mai University (STSC-CMU).

Results

The total weight of raw materials in this study, was 30.88 kg, divided into dried pericarps weight 17.78 kg and dried seeds weight 13.10 kg. Essential oil was extracted from the pericarps of 143 g (0.8%yield) and 66 g (0.5%yield) of the seeds.

35 compounds were found in essential oil, essential oils obtained from the pericarps and seeds have 16 identical compounds and 11 compounds from both parts, the chemical composition could not be identified. Piperitenol (30.6%yield), 3-Cyclohexene-1-methanol, alpha, alpha, 4-trimethyl-, acetate, (1R) (14.8%yield), p-Cymene (6.8%yield), Linalool (6.7%yield) and Limonene (4.8%yield) were major compounds from the pericarps (Figure 1) and p-Cymene (20%yield), Bicyclohexane, 4-methylene-1-(1-methylethyl), 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R) (16%yield), Limonene (6.5%yield) and Linalool (4.6%yield) were major compounds from

the seeds (Figure 2).

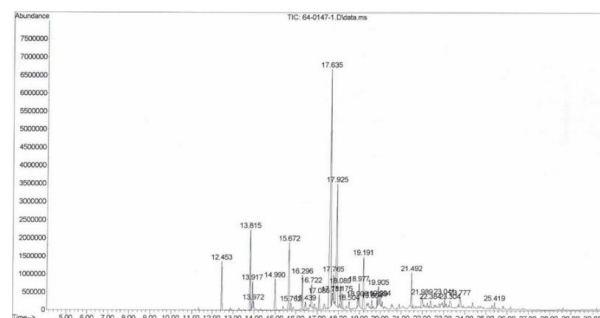


Figure 1 GC chromatogram of the pericarps

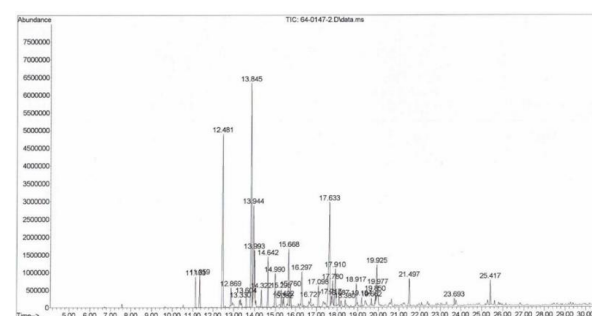


Figure 2 GC chromatogram of the seeds

Discussion

The chemical composition of this study was found to be similar in number of compounds to the study of Chemical compositions and pharmacological effects of essential oil from the fruit of *Zanthoxylum limonella*, evaluation of the oil composition was achieved by GC/MS analysis; Limonene, terpineol and sabinene were found to be the major components [10]. At the time of this study, the chemical composition of the compounds was found, but with different quantities. Because, this study found that Piperitenol, 3-Cyclohexene-1-methanol, alpha, alpha, 4-trimethyl-, acetate, (1R) and p-Cymene were major compounds from the pericarps and p-Cymene, Bicyclohexane, 4-methylene-1-(1-methylethyl), 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R) and Limonene were major compounds from the seeds. Also consistent with the study of characterization of the essential oil and fatty oil from Ma-kwan fruit (*Zanthoxylum rhetsa*; *Zanthoxylum limonella*), the 24 compounds were identified in the essential oil, including monoterpenes, oxygenated monoterpenes, sesquiterpenes, hydrocarbon and ketones group; the major compounds from essential oil of Ma-kwan fruit were α -phellandrene, terpineol, sabinene and α -pinene [5]. From the above comparative studies, it can be concluded that the chemical composition of the essential oils of the Ma-kwan fruit is mainly Terpenes, but the difference may be due to the type of raw material, source of raw material and different in drying methods affect to result in different

chemical compositions and quantities as shown in this study above.

Conclusion

From the results of this study, the quantity and chemical composition of the essential oils from the fresh fruits will be recorded for comparison with fresh or dried fruits from different sources. They also know the major compounds found in the essential oils of fresh Ma-kwan found in Doi Phu Kha, Nan Province.

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