องค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพของน้ำมันหอมระเหยใบคำแสด

Chemical Composition and Biological Activity of *Bixa orellana* L. Leaf Essential Oil

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บทคัดย่อ

สกัดน้ำมันหอมระเหยจากใบคำแสด (*Bixa orellana* L.) โดยวิธีกลั่นด้วยน้ำแล้วนำมาวิเคราะห์องค์ประกอบของน้ำมันหอมระเหยโดยใช้เทคนิค GC-MS และทดสอบฤทธิ์ต้านเชื้อแบคทีเรีย ได้เปอร์เซ็นต์การสกัดของน้ำมันหอมระเหย 1.04 การวิเคราะห์ GC-MS แสดงให้เห็นว่ามีสารพฤกษเคมี เช่น เทอร์พีนอยด์ (terpenoids) เซสควิเทอร์ปีน (sesquiterpenes) และสารประกอบอะโรมาติก การทดสอบฤทธิ์ต้านเชื้อแบคทีเรียของน้ำมันหอมระเหยพบว่ามีฤทธิ์ต้านเชื้อ *Escherichia coli*, *Staphylococcus aureus* และ *Staphylococcus epidermidis* เส้นผ่าศูนย์กลางเฉลี่ยของโซนการยับยั้งเชื้อ *E. coli*, *S. aureus* และ *S. epidermidis* เท่ากับ 12.0±0.2 11.0±0.1 และ 12.5±0.3 ตามลำดับ สรุปได้ว่าน้ำมันหอมระเหยของใบคำแสด มีฤทธิ์ทางชีวภาพต่อเชื้อ *E. coli*, *S. aureus* และ *S. epidermidis* โดยมีสารประกอบทางชีวภาพบางชนิด เช่น 1H-cycloprop[e]azulene หรือ (-)-alpha-gurjunene, camphor, D-germacrene และ guaiacol

ABSTRACT

The leaves of Thai Annatto Tree (*Bixa orellana* L.) were subjected to water distillation followed by analysis of the essential oil constituents using GC-MS technique as well as antibacterial activity test. The percent of extraction of the essential oil was found to be 1.04. GC-MS analysis showed the presence of phytochemicals such as terpenoids, sesquiterpenes and aromatic compounds. The antibacterial test of the essential oil showed the activity against *Escherichia coli*, *Staphylococcus* aureus and *Staphylococcus epidermidis*. The average diameter of inhibition zone of *E. coli*, *S.* aureus and *S. epidermidis* were 12.0±0.2, 11.0±0.1 and 12.5±0.3 respectively. In conclusion, the essential oil of Annatto leaf showed biological activity against *E. coli*, *S.* aureus and *S. epidermidis* with the presence of some biological active compounds such as 1H-Cycloprop[e]azulene or (-)-alpha-Gurjunene, camphor, D-germacrene and guaiacol.

คำสำคัญ: น้ำมันหอมระเหย การกลั่นด้วยน้ำ ใบคำแสด ฤทธิ์ต้านเชื้อแบคทีเรีย

Keywords: Essential oils, Water distillation, *Bixa orellana* L., Annatto leaf, Antibacterial activity.

INTRODUCTIONS

Natural products are chemical compounds which produced by living organisms. In plants, some chemical compounds show bioactivity and the plant extracts are widely used as pharmaceuticals, dye preparations, cosmetics, food, agricultural products and medicals. Phytochemicals are naturally occurring chemical compounds in plants (Atideshmukh et al., 2011; Shilpi et al., 2006 and Venugopalan et al., 2013). There may be as many as 4,000 different compounds in plants with potential for biological activity. Essential oil or volatile oil contains organic compounds of plants normally have fragrance and are volatile at room temperature. The essential oil can be found in all part of aromatic plants such as roots, leaves, flowers and seeds. The essential oil found in plants range from 0.01% to 10% and contains more than 100 chemicals. Essential oil is generally extracted by distillation; usually steam distillation. Other techniques which are also used include expression, solvent extraction, enfleurage and super-critical carbon dioxide extraction. Recently, the essential oil is widely used for bactericidal, virucidal, fungicidal, antiparasitical, insecticidal, medicinal and cosmetic application, especially in pharmaceutical, sanitary, cosmetic, agricultural and food industry because the essential oil contain variety bioactive compounds such as terpenes and terpenoids, phenol-derived aromatic components and aliphatic components (Bakkali et al., 2008).

"Bixa orellana L." is normally called Annatto tree or Comsaed in Thai. Annatto tree is a shrub or small tree originating from the tropical region of the Americas, North, Central and South Americas originally used to make red color paint and lipstick. Annatto tree riches in carotenoide, beta-carotene, essential oil, saturated and unsaturated fatty acids, flavonoids and vitamin C. Islam et al. and Tamil et al. found that the phytocamicals of Annatto extract were carotenoids, apocarotenoids, terpenes, terpenoids, steroids, alkaloids and aliphatic compounds. They are main compounds found in all parts of this plant. The extract of Annatto tree exhibit anti-inflammatory, antioxidant, antibacterial, antifungal and anticancer (Islam et al., 2011 and Tamil et al., 2015). Bhatnagar et al. found that leaf of Annatto tree extracted by methanol showed significant inhibition against all the tested bacteria and fungus while seed extract was less efficacious in most of the tested (Bhatnagar et al.,2015). Vilar et al. reported the presence of chemical constituents in different part of Annatto without suggestion of the biological activities of any compounds (Vilar et al., 2014). Trobi, et al. also studied the antimicrobial activity of Annatto extract. They found that the zones of inhibition against susceptible bacteria were 15-17 mm while those obtained in assays with chloramphenicol and phenol positive controls were 12-18 mm and 10-28 mm respectively. In this work, the biological active compounds in the extracted were not identified (Trobi, et al., 1996).

An interesting to development of the medicinal plants for the bioactive of essential oil. The objectives of this research are to obtain the essential oil of Annatto leaves by water distillation and to analyse the chemical composition and test for antibecterial activities of those essential oil.

METHODS

1. Annatto tree Essential Oil Extraction

The fresh leaves of Thai Annatto tree were collected from Chiang Mai Thailand. The leaves were dried and pulverized followed by using 200 g of the powder for water distillation in 1.2 L of deionized water for 6 h. The essential oil was separated and

water was removed using sodium sulfate anhydrous (Na_2SO_4).

2. Analysis of Chemical Composition by Gas Chromatography-Mass Spectrometry (GC-MS)

The essential oil of Thai Annatto tree leaves was analyzed for the chemical composition using GC-MS. The investigation of essential oil was analysed using Agilent 6890 interfaced to a 5973 mass selective detector, operating in IE mode at 70 eV and equipped with a splitless injector at 250 ºC. The capillary column HP-5MS (30 m x 0.25 mm x 0.25 µm film thickness) was used and helium was used as carrier gas (1 mL/min). The oven temperature was initially maintained at 50 ºC for 3 min and then programmed to 260 ºC at 7 ºC min^{-1}, with split ratio of 10:1. The analysis for chemical composition was done by comparing the mass spectra to the compounds compiled in the data library.

3. Antimicrobial Assay

Antimicrobial assay was tested using disc diffusion (Kirby-Bauer) method. *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis* were grown in nutrient broth (NB) at room temperature (32±2 °C) for 8 h and maintained by culturing process. The amount of bacterial to be tested was 106-108 CFU/mL, which is equivalent to 0.5 Mc Farland by opacity tube method. The essential oil and 0.24 mg/mL tetracycline HCl control were loaded on 6 mm sterile individual discs at room temperature (32 ± 2 °C) for 24 h. Inhibition zones formed around the discs were measured with transparent ruler.

RESULTS AND DISCUSSION

1. Annatto tree Essential Oil Extraction

The essential oil of dry Thai Annatto tree leaves were obtained with the percent of extraction of 1.04.

2. Analysis of Chemical Composition by Gas Chromatography-Mass Spectrometry (GC-MS)

Identification of compounds of Annatto tree leaves essential oil was based on GC-MS spectroscopic data, corresponding to peaks and molecular mass of compounds. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST). The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The GC-MS analysis of the Annatto leaves revealed the presence of fourteen compounds (Figure 1). The related identified chemicals are listed in table 1.

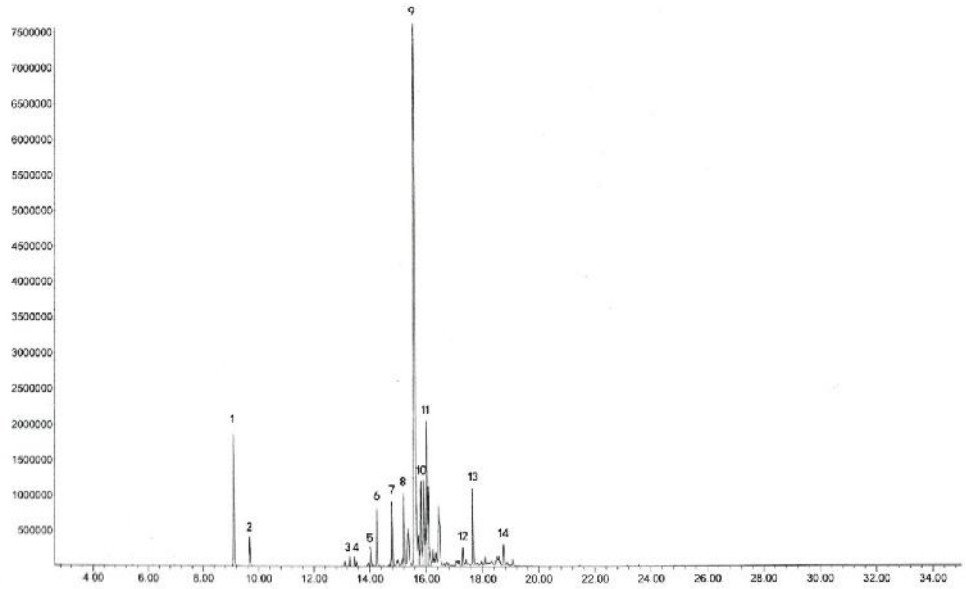
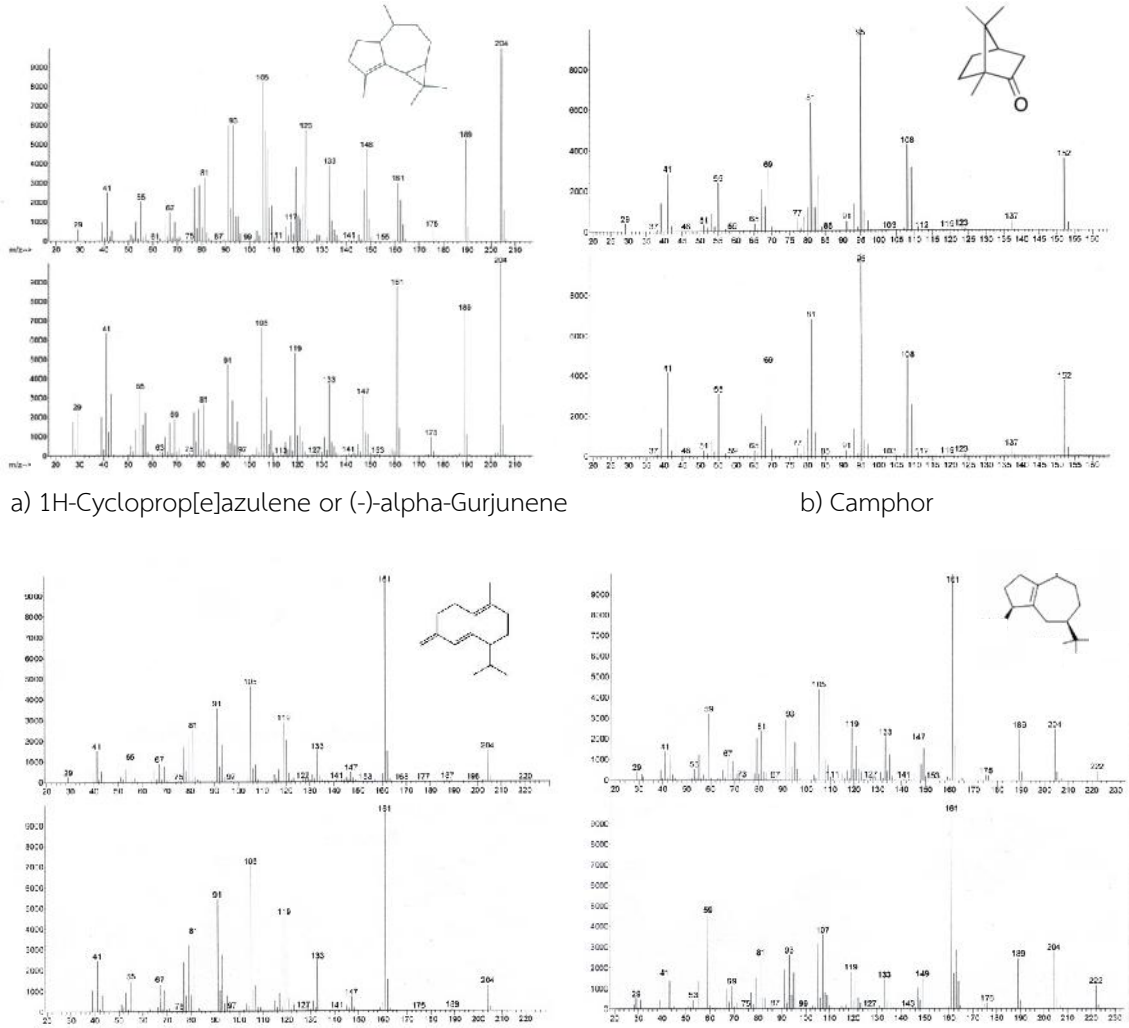


Figure 1 The GC-MS chromatogram of the essential oil of Annatto tree leaves.

Table 1 The chemical compositions of essential oil of Annatto Tree leaves.

Peak no.	Retention time (min)	Chemicals	Relative % area
1	9.1	Camphor	6.5
2	9.7	L-Borneol	1.4
3	13.2	δ-Elemene	0.5
4	13.6	α-Guaiene	0.4
5	14.0	α-Copaene	0.9
6	14.2	β-Elemene	2.8
7	14.8	*Trans*-Caryophyllene	3.3
8	15.2	β-Selinene	3.8
9	15.6	1H-Cycloprop[e]azulene or (-)-alpha-Gurjunene	50.0
10	15.8	D-Germacrene	5.4
11	15.9	2-Isopropenyl-4a 8-dimethyl-1 2 3 4 4a 5 6 7-octahydronaphthalene	9.5
12	17.3	1H-Cycloprop(e)azulene-7-ol	1.0
13	17.6	Guaiacol	3.9
14	18.7	2-Napthalenemethanol	1.3

Some chemicals identified in the essential oil of Thai Annatto tree leaves were analyzed by GC-MS. It was found that the oil contains many phytochemicals such as terpenoids, sesquiterpe-noids and aromatic compounds. The main compositions are 1H-cycloprop[e]azulene or (-)-alpha-gurjunene (50.0 %), camphor (6.5 %), D-germacrene (5.4 %) and guaiacal (3.9 %). The mass spectra of the dominant compositions in the essential oil of Thai Annatto tree leaves compared with the spectrum of reference components stored in the NIST library are presented in Figure 2.



a) 1H-Cycloprop[e]azulene or (-)-alpha-Gurjunene b) Camphor

c) D-Germacrene d) Guaiacol

Figure 2 The mass spectra of the main identified compositions in the essential oil of Annatto tree leaves.

3. Antimicrobial Assay

The essential oil of Thai Annatto tree leaves exhibited dose-dependent antimicrobial activity against the tested pathogens; *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis* as shown in Table 2 compared to the tetracycline HCl control at the concentration of the 0.24 mg/mL.

Table 2 The bioactivity of essential oil of Annatto Tree leaves extracts.

Bacteria	Diameter of inhibition zone (mm)	
	Essential oil of Annatto tree	Tetracycline (0.24 mg/mL)
Escherichia coli	12.0±0.2	21.3±0.2
Staphylococcus aureus	11.0±0.1	19.3±0.2
Staphylococcus epidermidis	12.5±0.3	29.0±0.3

Form the result, the essential oil of Thai Annatto tree leaves was moderate effective against bacteria compared to tetracycline at the concentration of the 0.24 mg/mL. Due to the composition of essential oil is the presence of phenols, aldehydes and alcohols which is the cytotoxic (Bruni et al., 2003 and Sacchetti et al., 2005). This cytotoxic property of the essential oil is a great importance in the applications in human or animal.

Among the identified compounds from Thai Annatto leaves extract, the dominant peaks are 1H-cycloprop[e]azulene or (-)-alpha-Gurjunene, camphor, D-germacrene and guaiacol are possibly belived to be biological active compounds. Camphor is an aromatic, volatile, terpene ketone derived from the wood of *Cinnamomum camphora* (Silva et al., 2016). The compounds were revealed to possesses a range of useful biological activity being an antiviral, antimicrobial, antitussive and analgesic agents Sokolova et al., 2003). D-Germacrene also possesses antibacterial property. It was stated that D-germacrene and caryophyllene; found in the species *Origanum vulgare*; have significant antibacterial and antifungal activies (Sahin et al., 2004). Guaiacol is also one of the compounds having antimicrobial activity (Cooper et al., 2004).

CONCLUSION

The essential oil of Annatto tree leaves possess a number of phytochemical constituents and it showed antibacterial activity. The possible biological active components belived to be 1H-cycloprop[e] azulene, (-)-alpha-Gurjunene camphor, D-germacrene or guaiacol.

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