รายงานวิจัยฉบับสมบูรณ์

โครงการ การศึกษาฤทธิ์ต้านเชื้อแบคทีเรียและความเป็นพิษแบบ *in vitro* ของเนื้อไม้ชะเอมไทย

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กรกฎาคม 2557

สัญญาเลขที่ MRG5580107

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สนับสนุนโดยสำนักงานคณะกรรมการการอุดมศึกษา สำนักงานกองทุนสนับสนุนการวิจัย และมหาวิทยาลัยสงขลานครินทร์

(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกอ. และสกว. ไม่จำเป็นต้องเห็นด้วยเสมอไป)

ACKNOWLEDGMENTS

This work was supported by TRF-CHE Research Grant for New Scholar (MRG5580107) funded by the Thailand Research fund (TRF) and the Commission on Higher Education (CHE).

The authors would like to thank BIOTEC laboratories, NSTDA, Thailand for the evaluation of cytotoxic activity of the plant extracts and pure compounds.

The authors also acknowledge the Scientific Equipment Center, Prince of Songkla University for performing part of spectroscopic experiment.

Executive Summary

Traditional knowledge of herbal medicines plays an important role in the search for novel chemotherapeutic agents. Recently, due to the adverse effects of synthetic drugs, there has been a rapid growth of interest from the western population in herbal remedies which are safe and effective. Traditional and herbal remedies offer a valuable alternative treatment in developing countries where traditional therapies are considered cheap and readily available. The studies of medicinal plants have been receiving attention in various areas of health research; much information has been documented as regards their various biological effects. The usage of herbal medicine for primary health care needs by people in local communities due to limited availability and affordability of pharmaceutical products is still occupying a prominent position. Therefore, the awareness of local communities should be enhanced by incorporating the traditional knowledge with scientific findings in order to promote cautious use of herbal medicine. *Albizia myriophylla* Benth is a tree, widely distributed in southeastern Asian countries. In Thailand, it is commonly called "Cha-am Thai". This plant species is used in Thai traditional medicine as antitussive (root), expectorant (fruit and root), demulcent (root), and tonic (wood). *A. myriophylla* is among the most important medicinal plant which is used alone or in combination with other medicinal plants in various Thai herbal formulas, especially those for remedy of oral diseases including dental caries and aphthous ulcer. In our previous work, the herbal formula comprising four different medicinal plants including *Albizia myriophylla* Benth. (Leguminosae), *Alpinia galanga* (L.) Willd. (Zingiberaceae), *Avicennia marina* (Forssk.) Vierh. (Acanthaceae), and *Ocimum sanctum* L. (Lamiaceae) was the most active against *Streptococcus mutans*, responsible for dental caries, with MIC of 250 μg/ml as compared with the other formulas tested. Subsequent MIC determination of these medicinal plants revealed that the wood extract of *A. myriophylla* exhibited the strongest antibacterial activity against *S. mutans* ATCC 25175 with MIC value of 3.9 μg/mL. This result suggests the presence of phytochemical components with good antibacterial potency in this plant extract. Up to now, only one phytochemical investigation from its wood extract has been reported while no biological activity data, especially anticariogenic activity, is currently available. This work was carried out in order to investigate the chemical compositions from the wood of *A. myriophylla* as well as the cytotoxic and antibacterial activities of its isolated compounds. Phytochemical investigation of *A. myriophylla* wood has led to the isolation of five flavonoids 3,4,7,3′-tetrahydroxyflavan (**1**), 7,3',4'-trihydroxyflavanone (**2**), 8-methoxy-7,3′,4′-trihydroxyflavone (**3**), 7,8,3′,4′-

tetrahydroxyflavone (**4**), lupinifolin (**5**), a triterpenoid lupeol (**6**) as well as two set of mixtures belonging to the class of sterols including a mixture of β-sitosterone (**7**) and stigmasta-5,22-dien-3-one (**8**) and a mixture of β-sitosterol (**9**) and stigmasterol (**10**) were isolated from the wood of this plant species. The structures of all these compounds were determined by extensive spectroscopic studies, including comparisons of their UV, IR, MS, and NMR data with those previously reported. Some of the isolated compounds, particularly those belonging to flavonoid group were evaluated for their antibacterial activity against *S. mutans* ATCC 25175, *Bacillus cereus* ATCC 11778, and *Staphylococcus aureus* ATCC 29213 using broth microdilution method as well as cytotoxicity against oral cavity cancer (KB) cell line using resazurin microplate assay. All the tested compounds, except for compound **1** and **2**, exhibited antibacterial activity against *S. mutans* ATCC 25175 with minimum inhibitory concentration (MIC) ranging from 0.98–250 µg/ml. Among the compounds tested, lupinifolin (**5**) showed the most potent anti-*S mutans* activity with MIC of 0.98 µg/ml comparable with the reference standard chlorhexidine with MIC of 0.5 µg/ml. Compound **5** also displayed marked antibacterial activity against *B. cereus* ATCC 11778 and *S. aureus* ATCC 29213 with the same MIC of 15.6 µg/ml. Compounds **3** and **4** showed moderate activity against the three tested bacterial strains with MIC values ranging from 62.5—250 µg/ml, whereas compounds **1** and **2** exhibited no antibacterial activity against the tested pathogens at the highest concentration tested of 250 µg/ml. Regarding the cytotoxicity, lupinifolin (**5**) was found to have potent anticancer activity against KB cell with IC_{50} of 4.9 µg/ml, whereas the other tested compounds at the highest concentration of 50 µg/ml did not exert cytotoxic effect against cancer cell tested. These results could be used as scientific evidences for the traditional use of *A. myriophylla* as a remedy for dental caries. Our study also reported the bioactive ingredients of *A. myriophylla*, which support its ethnomedical claims as well. Lupinifolin may have a potential to be a natural anticariogenic agent. Further research is necessary to establish the antibacterial mechanisms of action of this compound against *S. mutans* or other cariogenic bacterial strains. The study on the quality control of *A. myriophylla* extract is important for further development of this plant species as herbal oral care product as well.

We described herein the isolation and structure identification of chemical substances from the wood of *A. myriophylla* as well as the cytotoxic and antibacterial activities of the semi-purified fractions and the isolated compounds. The results of this study could be used as the scientific evidences for the traditional uses of *A. myriophylla*. Besides, our finding may contribute to the increase of knowledge of the chemotaxonomy and biological activity in *Albizia* species. In addition, if such very promising bioactive molecules are isolated, they will be used for the development of novel therapeutic agents in the future.

บทคัดย่อ

Albizia myriophylla Benth ได้ถูกใช้มาเป็นเวลานานโดยหมอพื้นบ้านไทย ในการเป็นพืชสมุนไพรองค์ประกอบที่สำคัญในตำรับยาสมุนไพรไทยสำหรับการรักษาอาการปวดฟันที่เกิดจากโรคฟันผุ ในการศึกษานี้ สารในกลุ่มฟลาวานอยด์ 5 ชนิด ได้แก่ 3,4,7,3′-tetrahydroxyflavan (**1**), 7,3',4'-trihydroxyflavanone (**2**), 8-methoxy-7,3′,4′-trihydroxyflavone (**3**), 7,8,3′,4′-tetrahydroxyflavone (**4**) และ lupinifolin (**5**) สารในกลุ่มไตรเทอร์พีนอยด์ 1 ชนิด คือ lupeol (**6**) ตลอดจนสารผสม 2 ชนิด ในกลุ่มสเตอรอยด์ คือ สารผสมของ β-sitosterone (**7**) และ stigmasta-5,22-dien-3-one (**8**) และ สารผสมของ β-sitosterol (**9**) และ stigmasterol (**10**) ได้ถูกแยกจากเนื้อไม้ของพืชชนิดนี้ การพิสูจน์เอกลักษณ์ของสารเหล่านี้ได้ใช้เทคนิคทางด้านสเปกโทรสโกปี รวมถึงการเปรียบเทียบข้อมูลของ UV, IR, MS และ NMR กับข้อมูลที่เคยมีรายงานก่อนหน้านี้ บางส่วนของสารที่แยกได้ โดยเฉพาะอย่างยิ่งสารที่อยู่ในกลุ่ม flavonoids ได้ถูกนำมาศึกษาฤทธิ์ต้านเชื้อแบคทีเรีย ได้แก่ *Streptococcus mutans* ATCC 25175, *Bacillus cereus* ATCC 11778 และ *Staphylococcus aureus* ATCC 29213 โดยใช้วิธี broth microdilution ตลอดจนศึกษาฤทธิ์เป็นพิษต่อเซลล์มะเร็งช่องปาก (KB cell) โดยใช้วิธี resazurin microplate assay สารทั้งหมดที่ทดสอบยกเว้นสารที่ **1** และ **2** แสดงฤทธิ์ต้านเชื้อ *S. mutans* ATCC 25175 โดยมีความเข้มข้นต่ำสุดที่สามารถยับยั้งการเจริญของเชื้อ (MIC) ในช่วง 1-250 µg/ml ในบรรดาสารทดสอบ lupinifolin (**5**) มีฤทธิ์ต้าน *S-mutans* ATCC 25175 ที่มีศักยภาพมากที่สุด โดยมีค่า MIC 0.98 µg/ml เมื่อเปรียบเทียบกับสารมาตรฐาน chlorhexidine ซึ่งมีค่า MIC เท่ากับ 0.5 µg/ml สาร **5** ยังแสดงฤทธิ์ที่ดีในการต้านเชื้อแบคทีเรีย *B. cereus* ATCC 11778 และ *S. aureus* ATCC 29213 โดยมีค่า MIC เท่ากับ 15.6 µg/ml สารประกอบที่ **3** และ **4** แสดงฤทธิ์ระดับปานกลางต่อเชื้อแบคทีเรียสามชนิดที่ใช้ทดสอบ ด้วยค่า MIC ในช่วง 62.5-250 µg/ml ในขณะที่สาร **1** และ **2** ที่ความเข้มข้นสูงสุดในการทดสอบเท่ากับ 250 µg/ml ไม่แสดงฤทธิ์ต้านเชื้อแบคทีเรียที่ใช้ทดสอบ จากการศึกษาฤทธิ์ความเป็นพิษ พบว่าสาร lupinifolin (**5**) ออกฤทธิ์ต้านเซลล์มะเร็ง KB โดยมีค่า IC_{50} เท่ากับ 4.9 µg/ml ในขณะที่สารทดสอบอื่น ๆ ที่ความเข้มข้นสูงสุด 50 µg/ml ไม่มีฤทธิ์ยับยั้งเซลล์มะเร็งที่ใช้ทดสอบ การค้นพบนี้แสดงให้เห็นถึงสารองค์ประกอบที่ออกฤทธิ์ทางชีวภาพของ *A. myriophylla* และยังสนับสนุนข้อมูลการใช้ทางการแพทย์พื้นบ้านของพืชชนิดนี้อีกด้วย การศึกษานี้ได้รายงานการแยกสาร **1—5** จากพืชในสกุล *Albizia* เป็นครั้งแรก สารประกอบ **6—8** ได้ถูกแยกจากเนื้อไม้ของ *A. myriophylla* ในการศึกษานี้เป็นครั้งแรก สาร lupinifolin (**5**) อาจมีศักยภาพที่จะเป็นสารต้านฟันผุจากธรรมชาติ การวิจัยเกี่ยวกับกลไกของสารชนิดนี้ในการต้านเชื้อแบคทีเรีย *S. mutans* หรือเชื้อแบคทีเรียสายพันธุ์อื่น ๆ ที่ก่อโรคฟันผุ เป็นสิ่งจำเป็นที่ต้องดำเนินการต่อไป

คำหลัก: ชะเอมไทย ฤทธิ์เป็นพิษต่อเซลล์ ฟันผุ พืชสมุนไพร เชื้อ *Streptococcus mutans*

ABSTRACT

Albizia myriophylla Benth has been used for long by Thai traditional healers as an important ingredient herb in Thai herbal formulas for treating toothache caused by dental caries. In this study, five flavonoids 3,4,7,3′-tetrahydroxyflavan (**1**), 7,3',4'-trihydroxyflavanone (**2**), 8-methoxy-7,3′,4′-trihydroxyflavone (**3**), 7,8,3′,4′-tetrahydroxyflavone (**4**), lupinifolin (**5**), a triterpenoid lupeol (**6**) as well as two set of mixtures belonging to the class of sterols including a mixture of β-sitosterone (**7**) and stigmasta-5,22-dien-3-one (**8**) and a mixture of β-sitosterol (**9**) and stigmasterol (**10**) were isolated from the wood of this plant species. The structures of all these compounds were determined by extensive spectroscopic studies, including comparisons of their UV, IR, MS, and NMR data with those previously reported. Some of the isolated compounds, particularly those belonging to flavonoid group were evaluated for their antibacterial activity against *Streptococcus mutans* ATCC 25175, *Bacillus cereus* ATCC 11778, and *Staphylococcus aureus* ATCC 29213 using broth microdilution method as well as cytotoxicity against oral cavity cancer (KB) cell line using resazurin microplate assay. All the tested compounds, except for compounds **1** and **2**, exhibited antibacterial activity against *S. mutans* ATCC 25175 with minimum inhibitory concentration (MIC) ranging from 1–250 µg/ml. Among the compounds tested, lupinifolin (**5**) showed the most potent anti-*S mutans* activity with MIC of 0.98 µg/ml comparable with the reference standard chlorhexidine with MIC of 0.5 µg/ml. Compound **5** also displayed marked antibacterial activity against *B. cereus* ATCC 11778 and *S. aureus* ATCC 29213 with the same MIC of 15.6 µg/ml. Compounds **3** and **4** showed moderate activity against the three tested bacterial strains with MIC values ranging from 62.5—250 µg/ml, whereas compounds **1** and **2** exhibited no antibacterial activity against the tested pathogens at the highest concentration tested of 250 µg/ml. Regarding the cytotoxicity, lupinifolin (**5**) was found to have potent anticancer activity against KB cell with IC_{50} of 4.9 µg/ml, whereas the other tested compounds at the highest concentration of 50 µg/ml did not exert cytotoxic effect against cancer cell tested. These findings demonstrate the bioactive ingredients of *A. myriophylla* and thus support its ethnomedical claims as well. Compounds **1—5** were reported herein from the genus *Albizia* for the first time. Compounds **6—8** were firstly isolated in this study from the wood of *A. myriophylla*. Lupinifolin (**5**) may have a potential to be a natural anticariogenic agent. Further research is necessary to establish the antibacterial mechanisms of action of this compound against *S. mutans* or other cariogenic bacterial strains.

Keywords: *Albizia myriophylla*, Cytotoxicity, Dental caries, Medicinal plant, *Streptococcus mutans*

CONTENTS

1. Introduction

Plant-derived natural products, particularly those used in folk medicine play an important role as a source of medically valuable products in the prevention and treatment of human diseases (Bown, 1995; Newman, 2003). Bioactive secondary metabolites produced by plants have proven to possess a wide range of therapeutic effects for human diseases (Newman, 2003; Ferrazzano *et al*., 2011). Recently, the scientific interest in these metabolites has increased with the search of new therapeutic agents from plant source, due to the fact that currently used drugs are relatively toxic or, to a certain extent, ineffective by the spreading of resistance (Khaomek *et al*., 2008; Ekuadzi *et al*., 2014). Herbal remedies offer a valuable alternative treatment in developing countries where traditional therapies are considered cheap and readily available (WHO, 2002). The usage of herbal medicine for primary health care needs by people in local communities due to the limited availability and high prices of most pharmaceutical products is still occupying a prominent position (Bodeker & Burford, 2007). Some products from herbal plants have been used successfully as medicinal agents against a variety of disorders and had advantages over synthetic drugs due to their minimal side effects (Venkatesh *et al*., 2010). Plants can be rationally selected for phytochemical and biological studies based on their uses in traditional medicine (Joycharat *et al*., 2012; Neamsuvan *et al*., 2012; Ekuadzi *et al*., 2014). In Thailand, medicinal plants are increasingly used by the traditional healers and herbalists for the treatment of various ailments (Ifesan *et al*., 2009; Limsuwan *et al*., 2009; 2011; Joycharat *et al*., 2012; Neamsuvan *et al*., 2012). Thus, further scientific studies are necessary to confirm the effectiveness of the plants used in traditional medicine. Toxicity tests are also important, even if the plants have already been used in traditional medicine.

The genus *Albizia* (also *Albizzia*) belongs to Fabaceae/Leguminosae family (Mimosoideae subfamily). Most *Albizia* species are deciduous woody trees and shrubs. They are generally identified by their bipinnately compound leaves. Inflorescence is usually racemose, globose or pedunculate heads. Their small flowers are sessile or pedicellate, bisexual. Calyx is gamosepalous, dentate or shortly lobed. Corolla is gamopetalous, funnel shaped, petals are connate beyond the middle. Stamens are generally indefinite; filaments elongated, white, rose or rarely purple, anthers small, eglandular. Ovary is shortly stipitate or sessile; style filiform, stigma capitate or minute. Pod is broadly linear, thin, compressed, dehiscent or indehiscent. Seeds are ovate or orbicular, compressed, funicle filiform (Singh, 2004).

The genus *Albizia* consists of approximately 150 species. Many members of this genus are endemic to Indian subcontinent (Singh, 2004; Wang *et al*., 2006). Some are widely distributed in Asia, Africa, Australia,

and tropical and subtropical America (Zheng *et al*., 2004; Kim *et al*., 2007). In Thailand, seven species can be found as follows; *A. chinesis* (Kang Luang), *A. lebbeck* (Preug), *A. lebbeckoides* (Kang), *A. lucidior* (Pan Thae), *A. myriophylla* (Cha-am Thai; Cha-am Pa), *A. odoratissima* (Kang Khi Mot), and *A. procera* (Thing Thon) (Smitinand, 2001).

***Albizia myriophylla* Benth.**

Family: Leguminosae-Mimosoideae

Common name: Littleleaf sensitive-briar, Sensitive briar

Botanical description:

Plant habit Large liana with prickles at stem and branch

Leaves Compound, bipinnate; pinnae 12-18 pairs; leaflet oblong

Flower Inflorescence in axillary head; flowers white, aromatic

Fruit Pod, yellow to brown, swollen at seed position

Seed Ovate or orbicular

Treditional uses: - Single plant parts : **Root** sweet tasting, antitussive, demulcent; **Stem & Wood** sweet tasting, relieves sore throat, tonic; **Fruit** expectorant (MRD, 1998).

- Combination with other medicinal plants in Thai herbal formulas: For the treatment of oral diseases and diabetes (Neamsuvan *et al*., 2012).

Phytochemicals: **Bark** phenolic acids, lignan glycosides; **Stem** triterpene saponins**; Wood** alkaloids (Ito *et al*., 1994; Yoshikawa *et al*., 2002; Asano *et al*., 2005; Panmei *et al*., 2007)

Pharmacological activities: Antioxidant, anticandidal, and antibacterial activities (Cholticha *et al*., 2006; Rukayadi *et al*., 2008; Steinrut *et al*., 2011)

Ethnopharmacological data of *Albizia* spp.

Albizia species are highly valued multipurpose tree legume. They are socially significant for producing high quality timber. Their wood can be used for building and furniture-making. Some members of this genus are used as the valuable resources of gum and resin. The young leaves are edible (Daniei *et al*., 2002; Zheng *et al*., 2004). The seeds are a source of oil (Wang *et al*., 2006) and as a food for livestock and wildlife (Cholticha *et al*., 2006; Nehdi, 2011). Some *Albizia* species were a plant of choice for silviculture and secondary plantation because of their thick foliage and quick growing characteristics. Some species such as *A. lebbeck* and *A. procera* have shown great potential in soil redevelopment process during early phase of mine spoil restoration in tropical dry forests (Singh *et al*., 2004). Many members of this genus are important in ayurvedic medicine (Kokwaro, 1976). *Albizia lebbeck* is used in folk remedies for abdominal tumors, boils, cough, eye ailments, flu, and lung ailments. It is also reported to be astringent, pectoral, rejuvenant, and tonic (Hartwell, 1969; Balandrin *et al*., 1993). The powdered seed is used in scrofulous swellings and its oil for leprosy. In India, the flowers are employed for spermatorrhea. Its leaves are used for the treatment of diarrhoea, dysentery, and pruritis (Sudharameshwar & Radhika, 2007). In some African contries, the sweet-smelling gum or resin of *A. lebbeck* is used in cosmetics. An infusion from its bark and roots is used to treat skin diseases for instance scabies as well as inflamed eyes and bronchitis (Babu *et al*., 2009). In China, the barks of *A. julibrissin* have been recommended as a sedative and anti-inflammatory drug for treating swelling and pain of the lungs, skin ulcers, and wounds bruises, abscesses, boils, haemorrhoids, and fractures (Higuchi *et al*., 1992; Ikeda *et al*., 1997; Pharmacopoeia, 2005). In Asian countries, the bark of *A. julibrissin* is prescribed to treat insomnia, diuresis, sthenia, and confusion (Zhu, 1998). Its flowers have been commonly used to treat anxiety, depression, and insomnia (Kang *et al*., 2000; 2007). Similarly, the seeds of *A. julibrissin*, *A. lebbeck*, *A. procera*, and *A. amara* are regarded as astringent and used in the treatment of piles, diarrhea, and gonorrhea (Anonymous, 1989). In the Vietnamese system of traditional medicine, the stems of *A. myriophylla* are used to substitute for licorice due to their sweet taste (Yoshikawa *et al*., 2002). In Thai traditional medicine, the root of this plant species is used as antitussive and demulcent. The fruit and root are also employed as expectorant. Its wood is recommended as tonic (MRD, 1998). In Ethiopia, *A. gummifera* is documented as anthelmintics in livestock and human (Eguale *et al*., 2011). In Uganda, the decoction of fresh fruit of *A. coriaria* is drunk for the treatment of cough (Namukobe *et al*., 2011). In Kenya, *A. anthelmintica* is used for the remedy of helminthiasis, malaria, stomachache, and emetic (Muthee *et al*., 2011). The stem bark of *A. ersicolor* is used for the treatment of venereal diseases, coughs,

joint pains, tapeworms, and fever while that of *A. schimperana* is used for malaria, general discomforts, and as a pain reliever (Rukunga & Waterman, 2001).

Chemical constituents of *Albizia* spp.

According to previous phytochemical studies, a number of compounds have been isolated from plant species of the genus *Albizia*. They can be classified as saponins, lignan glycosides, alkaloids, iminosugars, and flavonoids. The distribution of these compounds in *Albizia* spp. and the chemical structures are summarized in **Table 1**.

Biological activities of plants in the genus *Albizia*

Many experimental evidences have revealed that several species of the genus *Albizia* were shown to possess high cytotoxicity against many different cancer cell lines (Ikeda *et al*., 1997; Liang *et al*., 2005; Zou *et al*., 2000; 2005**;** Melek *et al*., 2007; Roy *et al*., 2008; Liu *et al*., 2010; Zhang *et al*., 2011). Furthermore, biological screenings worldwide showed that many plant species of this genus have demonstrated antimalarial (Desjardins *et al*., 1979; Ofulla *et al*., 1995; Freiburghaus *et al*., 1996; Ovenden *et al*., 2002; Rukunga *et al*., 2007; Elizabeth *et al*., 2009), immunomodulatory (Barua *et al*., 2000), antioxidant (Jung *et al*., 2004), antimicrobial (Colin *et al*., 1997; Geyid *et al*., 2005; Duraipandiyan *et al*., 2006: Runyoro *et al*., 2006; Lam *et al*., 2011), anthelmintic (Eguale et al., 2011), and anti-inflammatory (Tripathi *et al*., 1979; Besra *et al*., 2002; Venkatesh *et al*., 2010; Yadav *et al*., 2010) properties. The *Albizia* genus is known to be a rich source of bioactive saponins (Krief *et al*., 2005; Zheng *et al*., 2006). These compounds were shown to possess high cytotoxicity against various cancer cell lines. Apart from saponins, alkaloids found in some plant species of this genus also possessed significant antimalarial activity (Geoffrey *et al*., 1996). In addition, phenolic compounds, especially those belonging to flavonoids isolated from some *Albizia* spp. showed various biological activities such as antioxidant, antiparasitic, and anti-obesity activities. Summary of biological activities of *Albizia* plants is shown in **Table 2**. The chemical structures of bioactive compounds are shown in **Figure 1**.

The present work was carried out in order to isolate and characterize the bioactive ingredients from the wood of *A. myriophylla*. *In vitro* antibacterial activity and cytotoxicity of the semi-purified fractions and the isolated compounds from the wood of *A. myriophylla* were established. The results of this study could be used as the scientific evidences for the traditional uses of *A. myriophylla*. Furturemore, our finding may contribute to the increase of knowledge of the chemotaxonomy and biological activity in *Albizia* species. In addition, some bioactive molecules isolated in this work may be used for the development of novel therapeutic agents in the future.

Table 1. Chemical constituents of plants in the genus *Albizia*.

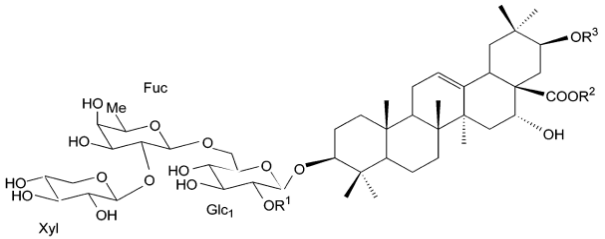
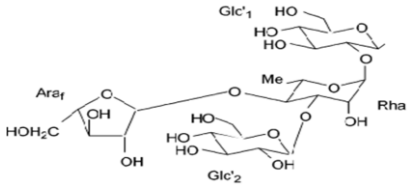
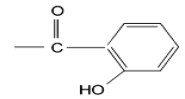
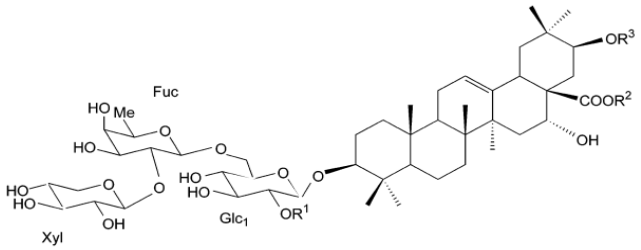
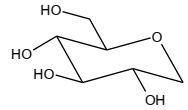
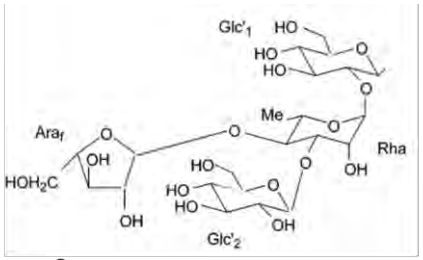
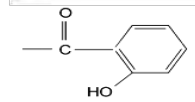
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
1. Triterpene saponins  (1) Adianthifolioside A	R_1=$HNCOCH_3$  	*A. adianthifolioside* (root)	Haddad *et al*., 2003
 (2) Adianthifolioside B	R_1=  R_2=  R_3= 	*A. adianthifolioside* (root)	Haddad *et al*., 2003

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

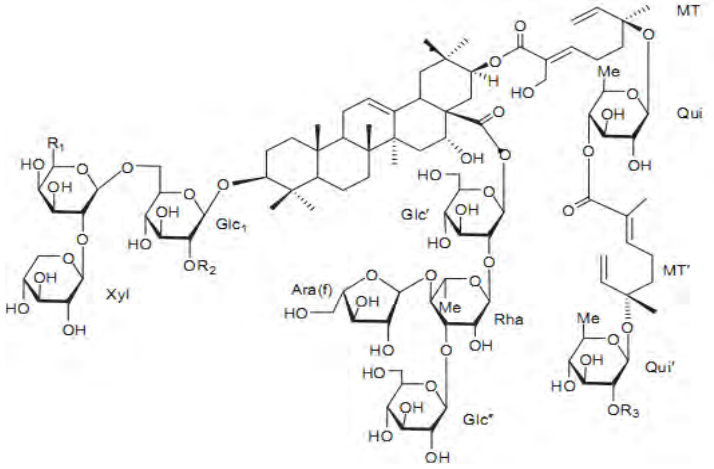
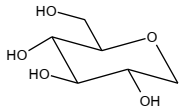
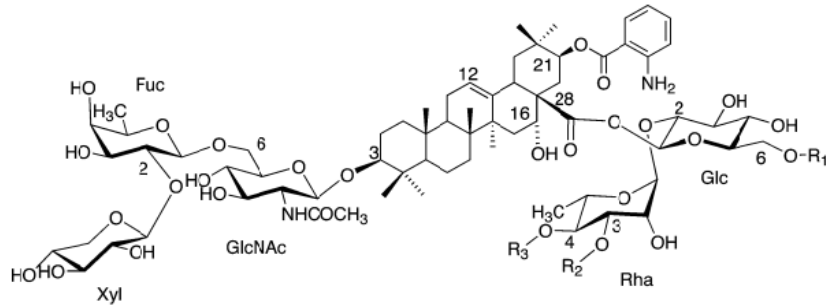
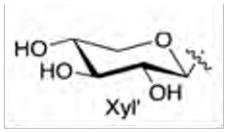
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (3) Albizoside D	R_1=CH_3 R_2=  R_3=H	*A. chinnensis* (stem bark)	Liu *et al*., 2010
 (4) Grandibracteriosides A	R_1=R_2=H R_3= 	*A. grandibracteata* (stem bark)	Krief *et al*., 2005

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

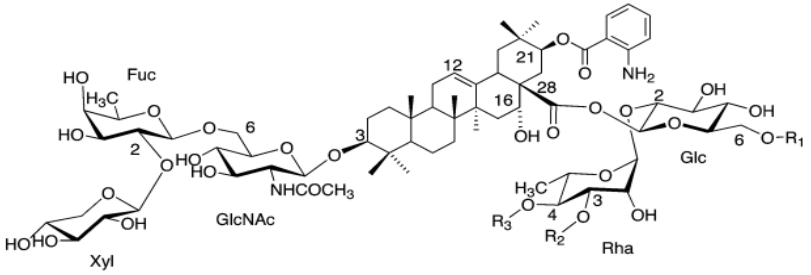
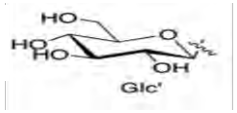
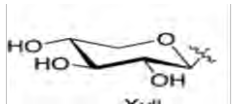
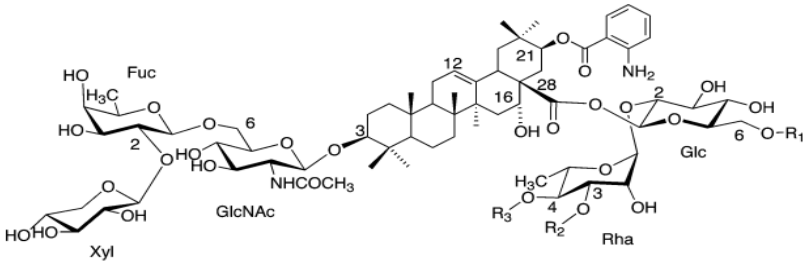
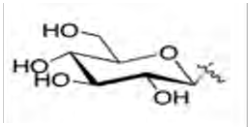
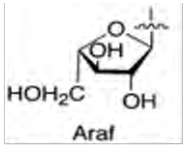
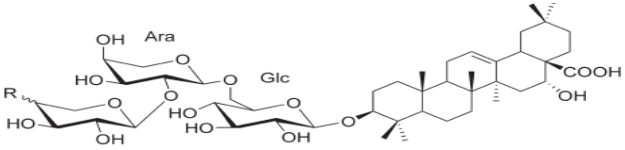
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (5) Grandibracteriosides B	R_1=  R_2=H R_3= 	*A. grandibracteata* (leaves, flower)	Krief *et al*., 2005
 (6) Grandibracteriosides C	R_1=H R_2=  R_3= 	*A. grandibracteata* (leaves, flower)	Krief *et al*., 2005
 (7) 3-O-[β-D-xylopyranosyl(1-->2)-α-L-arabinopyranosyl(1-->6)]-β-D-gluco-pyranosyl oleanolic acid	R=β-OH	*A. inundata* (stem bark)	Zhang *et al*., 2011

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

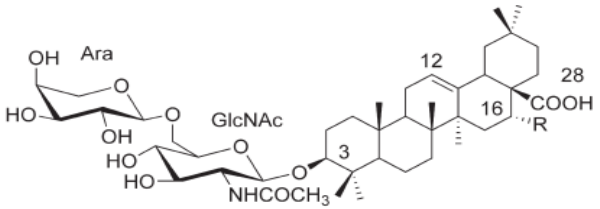
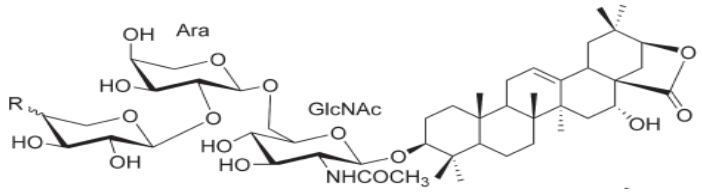
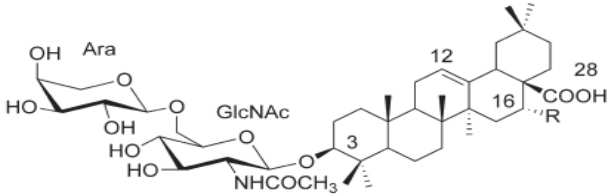
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (8) 3-O-[α-L-arabinopyranosyl(1-->6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl oleanolic acid.	R=H	*A. inundata* (stem bark)	Zhang *et al*., 2011
 (9) 3-O-[α-L-arabinopyranosyl (1-->2)-α-L-arabinopyranosyl (1->6)]-2-acetamido-2- deoxy-D-glucopyranosyl acacic acid lactone	R=β-OH	*A. inundata* (stem bark)	Zhang *et al*., 2011
 (10) 3-O-[α-L-arabinopyranosyl (1-->6)]-2-acetamid-O-2-deoxy-β-D-glucopyranosyl echinocystic acid	R=OH	*A. inundata* (stem bark)	Zhang *et al*., 2011

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

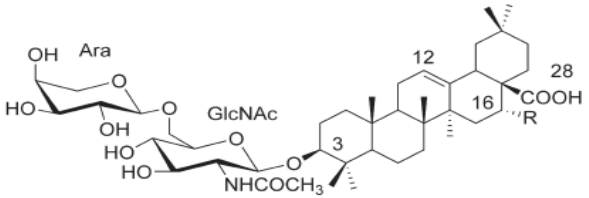
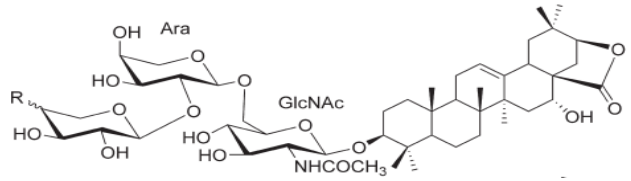
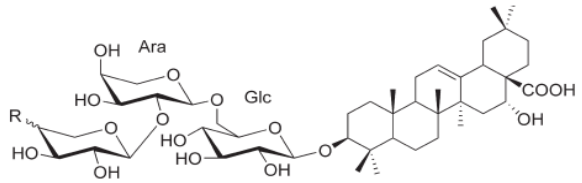
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (11) 3-O-[α-L-arabinopyranosyl(1-->6)]-2-acetamid O-2-deoxy-β-D-glucopyranosyl echinocystic acid	R=OH	*A. inundata* (stem bark)	Zhang *et al*., 2011
 (12) 3-O-[β-D-xylopyranosyl (1-->2)-α-L-arabinopyranosyl (1-->6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl acacic acid lactone	R=α-OH	*A. inundata* (stem bark)	Zhang *et al*., 2011
 (13) 3-O-[α-L-arabinopyranosyl (1-->2)-α-L-arabinopyranosyl (1->6)]-β-D-glucopyranosyl oleanolic acid	R=α-OH	*A. inundata* (stem bark)	Zhang *et al*., 2011

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

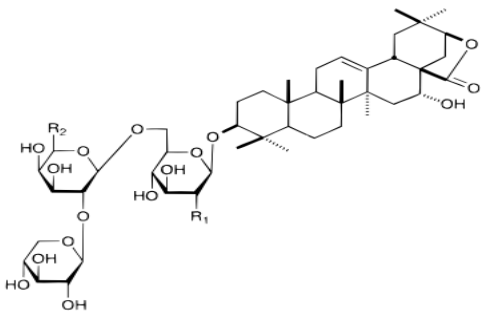
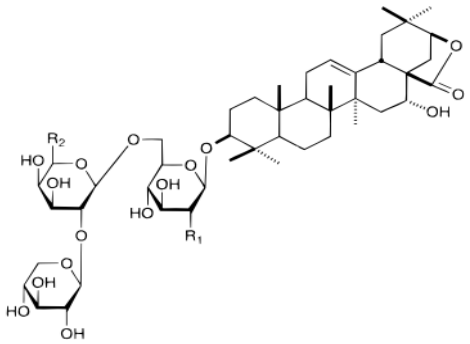
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (14) Prosagenin 3	R_1=OH R_2=H	*A. julibrissin* (stem bark) *A. adiabthifolia* (root)	Ikeda *et al*., 1997, Haddad *et al*., 2003
 (15) Prosagenin 5	R_1=OH R_2=CH_3	*A. julibrissin* (stem bark) *A. adiabthifolia* (root)	Ikeda *et al*., 1997, Haddad *et al*., 2003

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

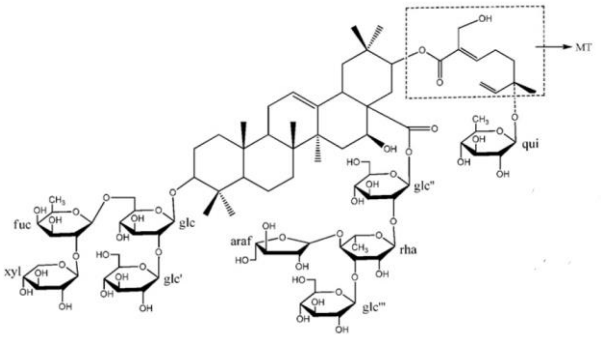
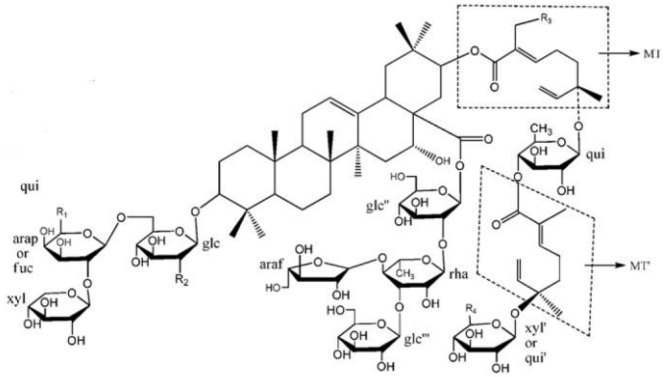
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (16) Julibroside J_{32}		*A. julibrossin* (stem bark)	Zheng *et al*., 2010
 (17) Julibroside J_{35}	R_1=R_3=R_4=H, R_2=OH	*A. julibrossin* (stem bark)	Zheng *et al*., 2010

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

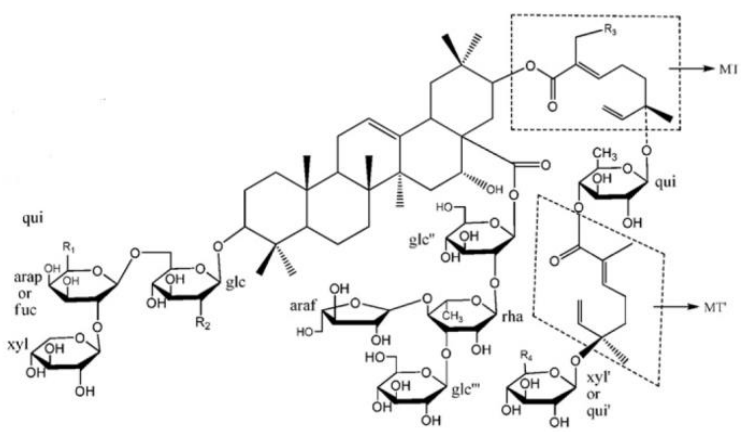
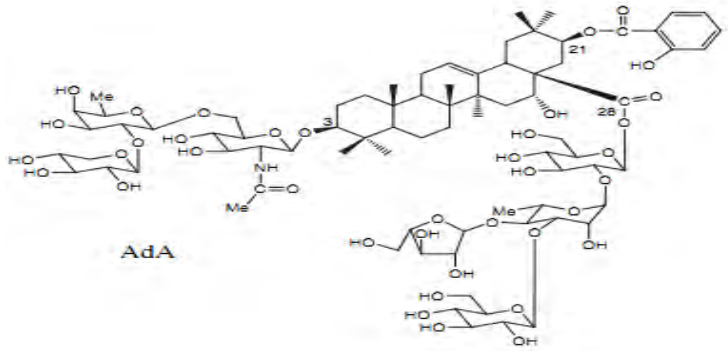
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (18) JulibrosideJ_{36}	R_1=R_4=CH_3 R_2=O-β-D-glcp R_3=OH	*A. julibrossin* (stem bark)	Zheng *et al*., 2010
 (19) Adianthifolia A		*A. adianthifolia* (root)	Haddad *et al*., 2004

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

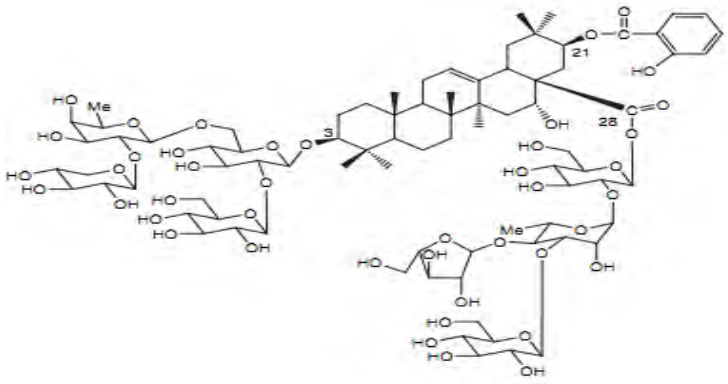
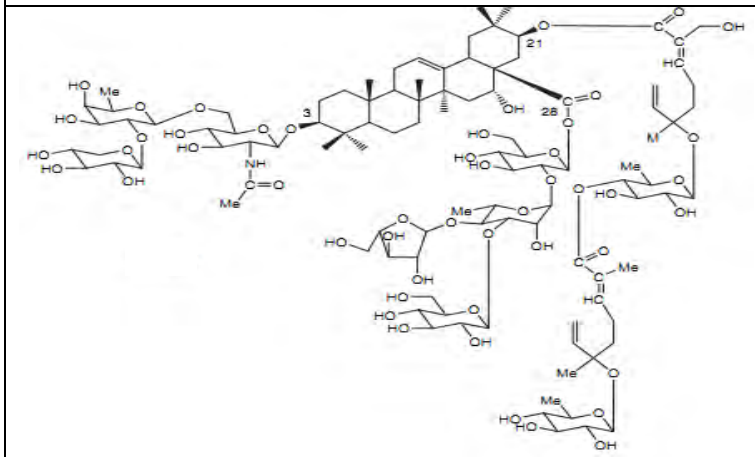
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (20) Adianthifolia B		*A. adianthifolia* (root)	Haddad *et al*., 2004
 (21) Adianthifolia D		*A. adianthifolia* (root)	Haddad *et al*., 2004

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

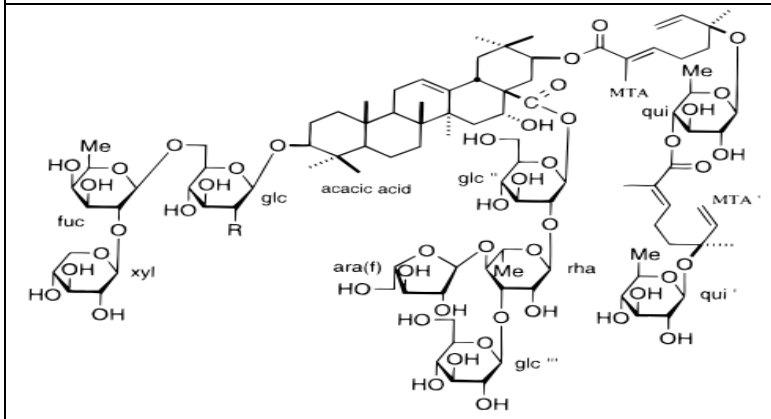
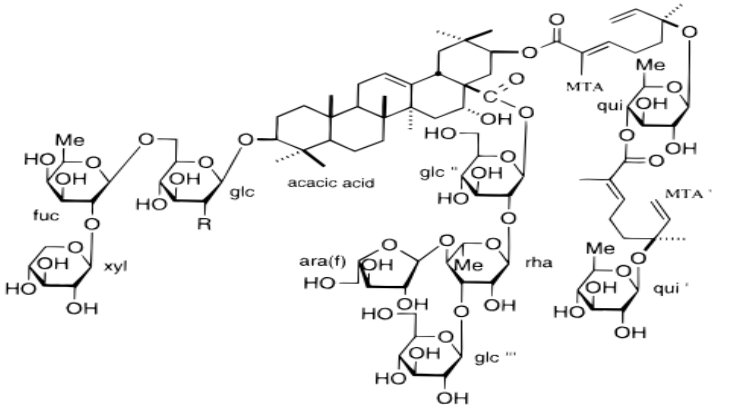
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (22) Julibroside J_1	R=O-glc'	*A. julibrissin* (stem bark)	Ikeda *et al*., 1997, Zou *et al*., 2000
 (23) Julibroside J_2	R=OH	*A. julibrissin* (stem bark)	Ikeda *et al*., 1997

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

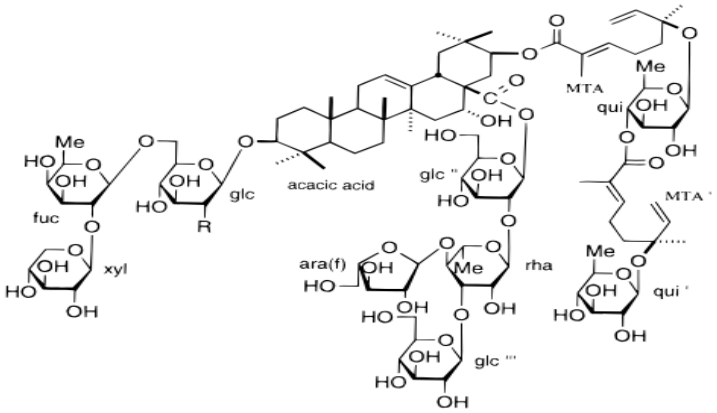
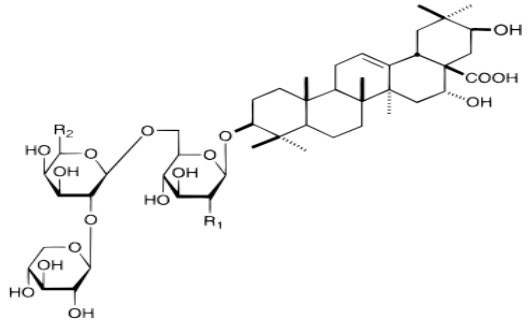
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (24) Julibroside J_3	R=$HNCOCH_3$	*A. julibrissin* (stem bark)	Ikeda *et al*., 1997
 (25) Prosapogenin 1	R_1=OH R_2=CH_3	*A. julibrissin* (stem bark)	Ikeda *et al*., 1997
(26) Prosapogenin 2	R_1=$HNCOCH_3$, R_2=H	*A. julibrissin* (stem bark)	Ikeda *et al*., 1997

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

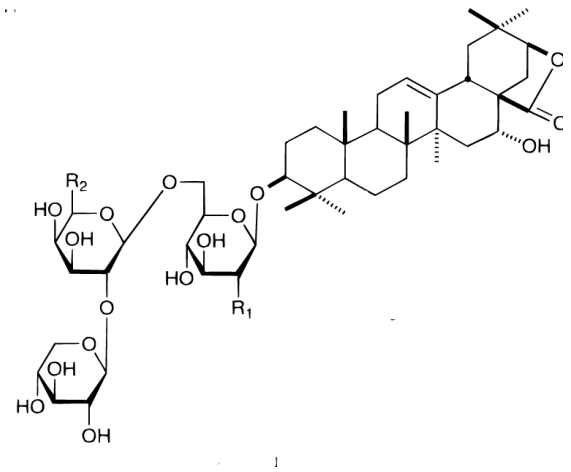
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (27) Prosapogenin 3	R_1=OH R_2=H	*A. julibrissin* (stem bark)	Ikeda et al., 1997
(28) Prosapogenin 4	R_1=$NHCOCH_3$ R_2=H	*A. julibrissin* (stem bark)	Ikeda et al., 1997
(29) Prosapogenin 5	R_1=O-glc R_2=CH_3	*A. julibrissin* (stem bark)	Ikeda et al., 1997
(30) Prosapogenin 6	R_1=OH R_2=CH_3	*A. julibrissin* (stem bark)	Ikeda et al., 1997
(31) Prosapogenin 7	R_1=$NHCOCH_3$ R_2=CH_3	*A. julibrissin* (stem bark)	Ikeda et al., 1997

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

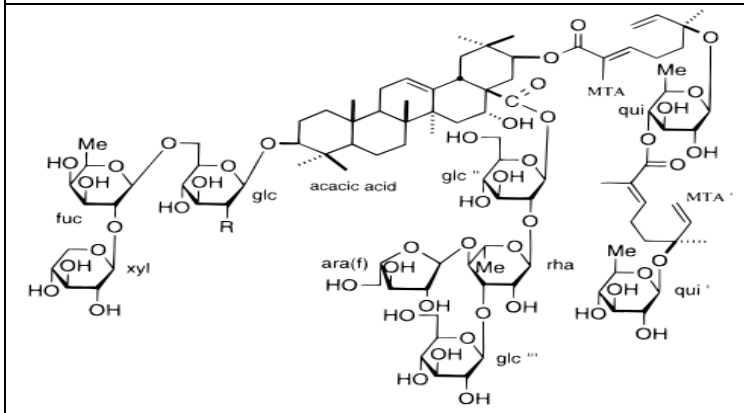
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (32) Prosapogenin 8	R_1=glc' R_2=OH R_3=H	*A. julibrissin* (stem bark)	Ikeda *et al*., 1997, Yoshakawa *et al*., 1998, Hua *et al*., 2009
(33) Prosapogenin 9	R_1=H R_2=OH R_3=H	*A. julibrissin* (stem bark) *A. procera* (seed)	Ikeda *et al*., 1997, Yoshakawa *et al*., 1998, Zheng *et al*., 2010
(34) Prosapogenin 10	R_1=H,R_2=OH, R_3=OH	*A. julibrissin* (stem bark)	Ikeda *et al*., 1997
(35) Prosapogenin 11	R_1=R_2=R_3=H	*A. julibrissin* (stem bark)	Ikeda *et al*., 1997
(36) Prosapogenin 12	R_1=glc, R_2=OH, R_3=H	*A. julibrissin* (stem bark)	Ikeda *et al*., 1997

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

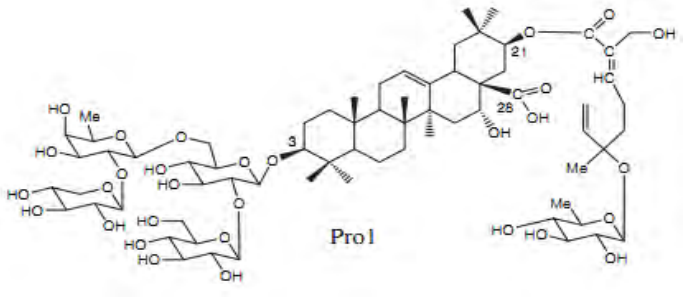
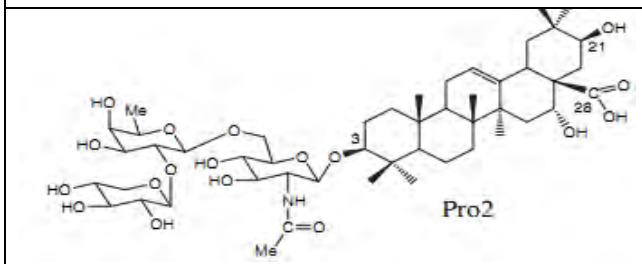
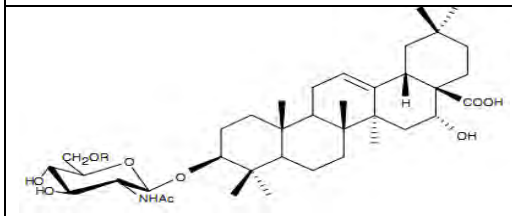
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (37) Prosapogenin Prol 1		*A. adianthifolia* (root)	Haddad *et al*., 2004
 (38) Prosapogenin Prol 2		*A. adianthifolia* (root)	Haddad *et al*., 2004
 (39) 3-O-[β-D-xylopyranosyl-(1-->2)-α–L-arabinopyranosyl-(1-->6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid	R=β-D-xylp-(1-->2)-α-L-arap	*A. procera* (bark)	Melek *et al*., 2007

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

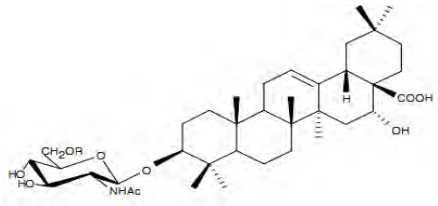
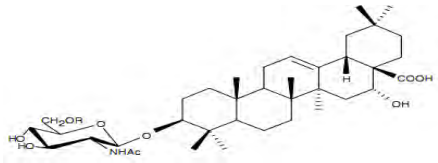
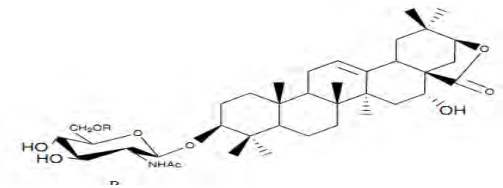
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (40) 3-O-[α-L-arabinopyranosyl-(1-->2)-β-D-fucopyranosyl-(1-->6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid	R=α-D-aylp-(1-->2)-α-L-fucp	*A. procera* (bark)	Melek *et al*., 2007
 (41) 3-O-[α-L-arabinopyranosyl-(1-->2)-α-L-arabinopyranosyl-(1-->6)]-2-acetamido-2-deoxy-β-D-glucopyranosyl echinocystic acid	R=α-D-xylp-(1-->2)-α-L-arap	*A. procera* (bark)	Melek *et al*., 2007
 (42) 3-O-[β -D-xylopyranosyl-(1-->2)- α -L-ara binopyranosyl-(1-->6)]-2-acetamido-2-deoxy - β-D-glucopyranosyl] acacic acid lactone	R=β-D-xylp-(1-->2)-α-L-arap	*A. procera* (bark)	Melek *et al*., 2007

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

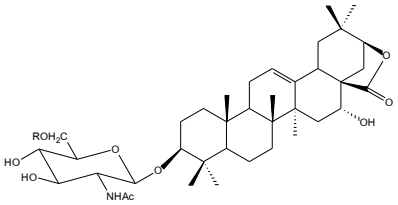
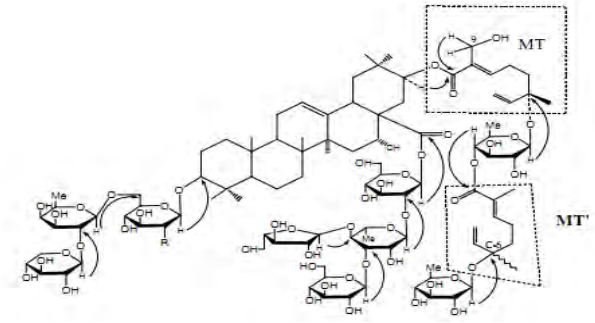
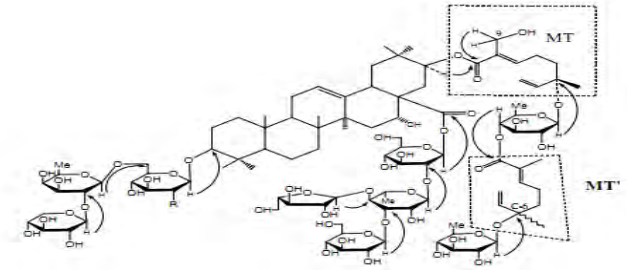
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (43) Juibroside J_8	R_1=CH_3, R_2=R_3=H	*A. chinnensis* (stem bark) *A. julibrissin* (stem bark)	Zou *et al*., 2005, Liu *et al*., 2010
 (44) Julibroside J_5	R=OH	*A. julibrissin* (stem bark)	Zou *et al*., 2005
 (45) Julibroside J_{12}	R=$NHCOCH_3$	*A. julibrissin* (stem bark)	Zou *et al*., 2005

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

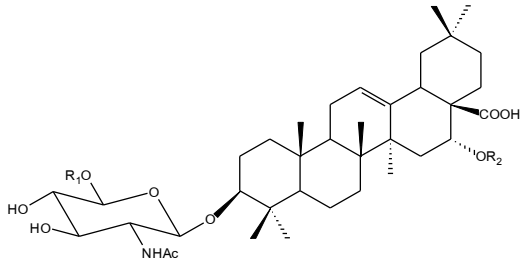
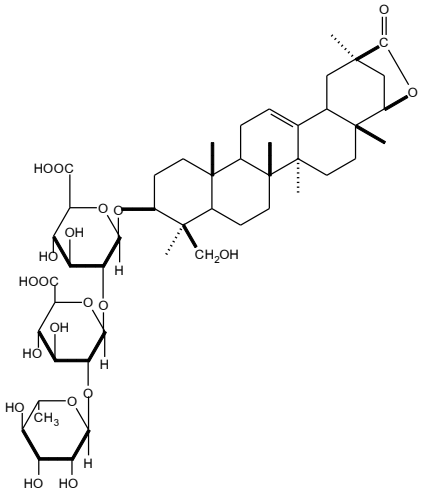
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (46) Echinocystic acid 3,16-O-bisglycosides	R_1=β-D-xylp-(1-->2)-β-D-galp R_2=β-D-xylp	*A. procera* (bark)	Miyase *et al*., 2010
 (47) Albiziasaponin A		*A. myriophylla* (stem)	Yoshikawa *et al*., 2002

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

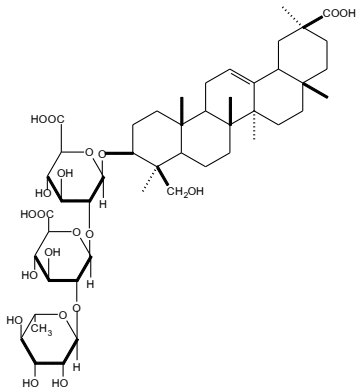
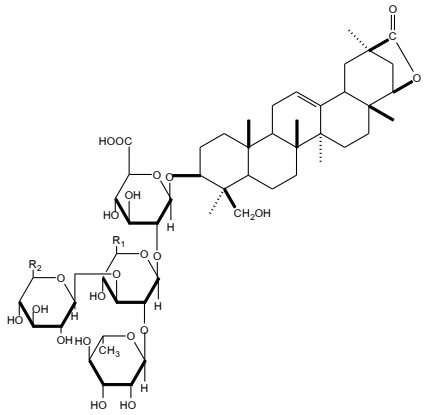
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (48) Albiziasaponin B		*A. myriophylla* (stem)	Yoshikawa *et al*., 2002
 (49) Albiziasaponin C	R_1=R_2=CH_2OH	*A. myriophylla* (stem)	Yoshikawa *et al*., 2002

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

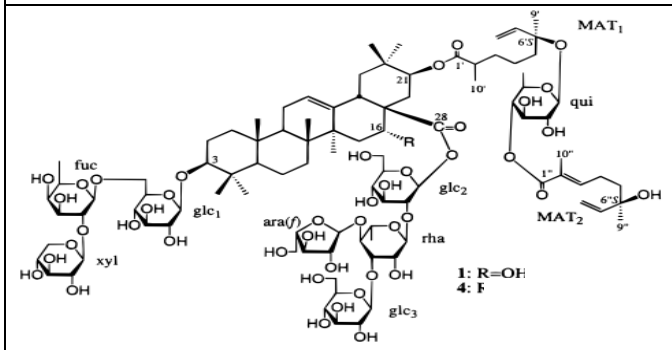
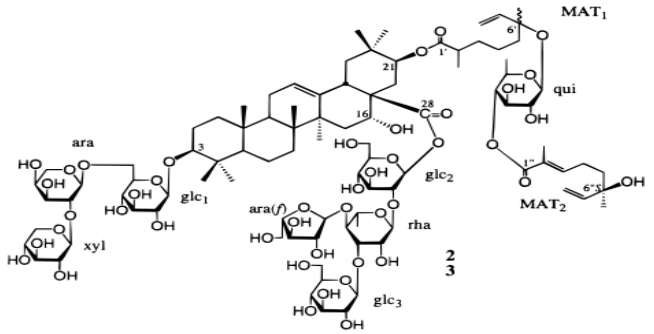
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
(50) Albiziasaponin D	R_1=CH_2OH, R_2=H	*A. myriophylla* (stem)	Yoshikawa *et al*., 2002
(51) Albiziasaponin E	R_1=COOH, R_2=H	*A. myriophylla* (stem)	Yoshikawa *et al*., 2002
 (52) Proceraoside A	R=OH	*A. procera* (seed)	Yoshikawa *et al*., 1998
(53) Proceraoside D	R=H	*A. procera* (seed)	Yoshikawa *et al*., 1998
 (54) Proceraoside C		*A. procera* (seed)	Yoshikawa *et al*., 1998

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

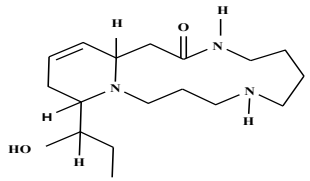
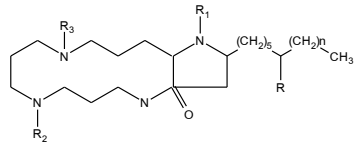
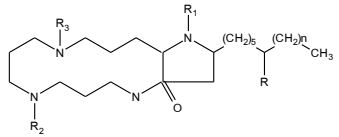
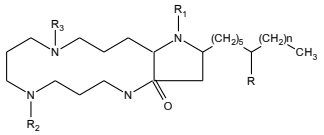
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
2. Alkaloids  (55) Palustrine		*A. myriophylla* (stem bark)	Phavanantha *et al*., 1990
 (56) Budmunchiamine G	R= R_1=H R_2=R_3=CH_3 n=6	*A. gummifera* (stem bark)	Rukunga & Waterman, 1996
 (57) Budmunchiamine K	R=H R_1=R_2=R_3=CH_3 n=8	*A. gummifera* (stem bark)	Rukunga & Waterman, 1996, Rukunga *et al*., 2007
 (58) 6'-Hydroxybudmunchiamin K	R=OH R_1=R_2=R_3=CH_3 n=8	*A. gummifera* (stem bark)	Rukunga & Waterman, 1996

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

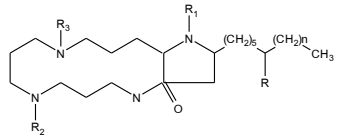
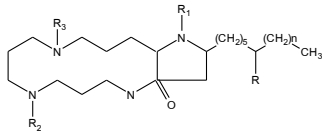
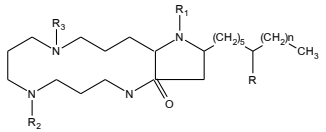
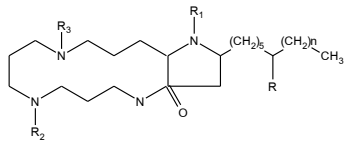
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (59) Budmunchiamine A	R=H R_1=R_2=R_3=CH_3 n=4	*A. schiperana* (stem bark)	Rukunga & Waterman, 2001
 (60) 6'-Hydroxybudmunchiamin C	R=OH R_1=R_2=R_3=CH_3 n=6	*A. schiperana* (stem bark) *A. gummifera* (root)	Rukunga & Waterman, 2001, Rukunga *et al*., 2007
 (61) 5-Normethylbuchiamine K	R=R_1=H R_2=R_3=CH_3 n=8	*A. schiperana* (stem bark) *A. gummifera* (root)	Rukunga & Waterman, 2001, Rukunga *et al*., 2007
 (62) 6'-Hydroxy-5-normethylbuchiamine K	R=OH R_1=H R_2=R_3=CH_3 n=8	*A. schiperana* (stem bark) *A. gummifera* (root)	Rukunga & Waterman, 2001, Rukunga *et al*., 2007

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

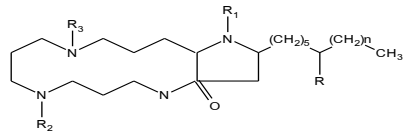
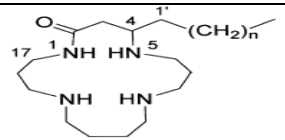
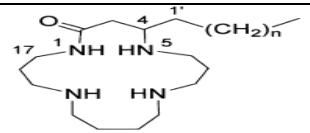
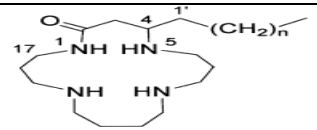
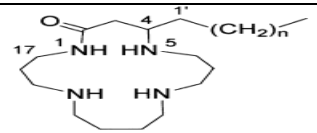
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (63) 9-Normethylbuchiamine K	$R=R_1= CH_3$ n=8	*A. gummifera* (stem bark)	Rukunga & Waterman, 2001
 (64) Budmunchiamine L_1	n=14	*A. adinocephala* (bark, leaf)	Ovenden *et al*., 2002
 (65) Budmunchiamine L_2	n=12	*A. adinocephala* (bark, leaf)	Ovenden *et al*., 2002
 (66) Budmunchiamine L_4	n=11	*A. adinocephala* (bark, leaf)	Ovenden *et al*., 2002
 (67) Budmunchiamine L_5	n=13	*A. adinocephala* (bark, leaf)	Ovenden *et al*., 2002

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

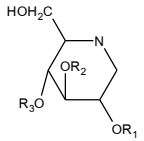
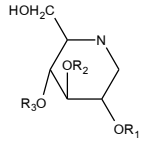
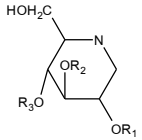
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
3. Iminosugars  (68) 1-Deoxymannojirimycin (DMJ)	R_1=R_2=R_3=H	*A. myriophylla* (bark)	Asano *et al*., 2005
 (69) 4-O- β-D- Glucopyranosyl-DMJ	R_1=R_2=H R_3=β-D-glucopyranose	*A. myriophylla* (bark)	Asano *et al*., 2005
 (70) 2-O-β-Glucopyranosyl-DMJ	R_1=β-D-glucopyranose R_2=R_3=H	*A. myriophylla* (bark)	Asano *et al*., 2005

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

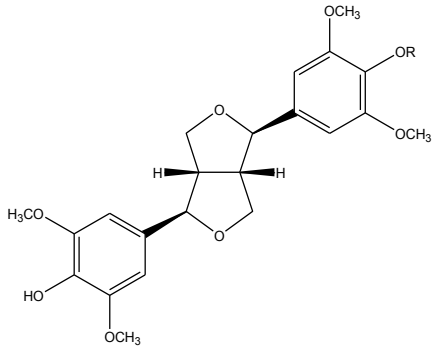
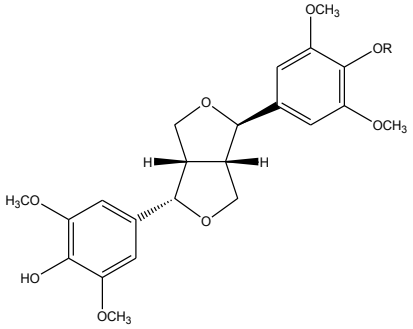
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
4. Lignan glycosides  (71) 6-epi-syringaresinol-4-O-β-D-apiofuranosyl-(1-->2)- β-D-glucopyranoside (Albizzioside A)	R = -Glc^{2}-Api	*A. myriophylla* (bark)	Ito *et al*., 1994
 (72) Albizzioside B	R=Glc^{2}-Api	*A. myriophylla* (bark)	Ito *et al*., 1994

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

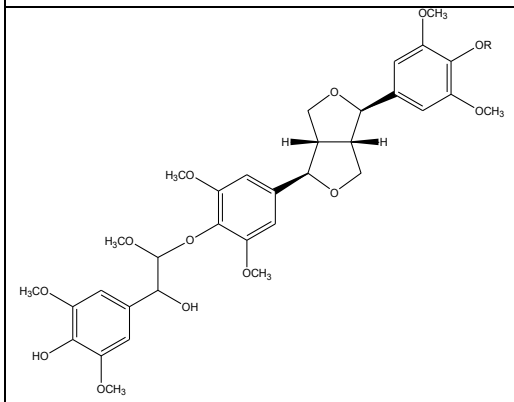
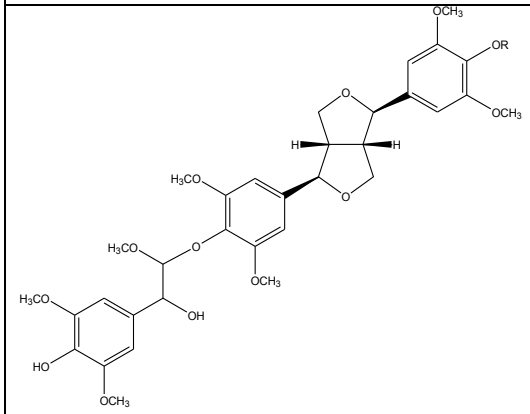
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
 (73) Albizzioside C	R=Glc	*A. myriophylla* (bark)	Ito *et al*., 1994
 (74) Syringaresino1-4-O-β-D-apiofuranosyl(1⟶2)-β-D-glucopyranoside	R = Glc	*A. myriophylla* (bark)	Ito *et al*., 1994

Table 1. Chemical constituents of plants in the genus *Albizia* (*cont*).

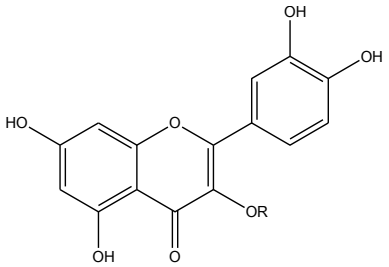
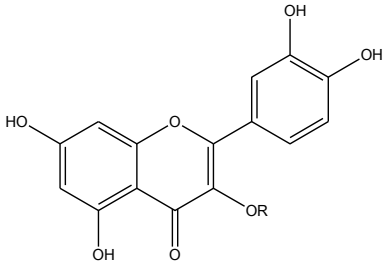
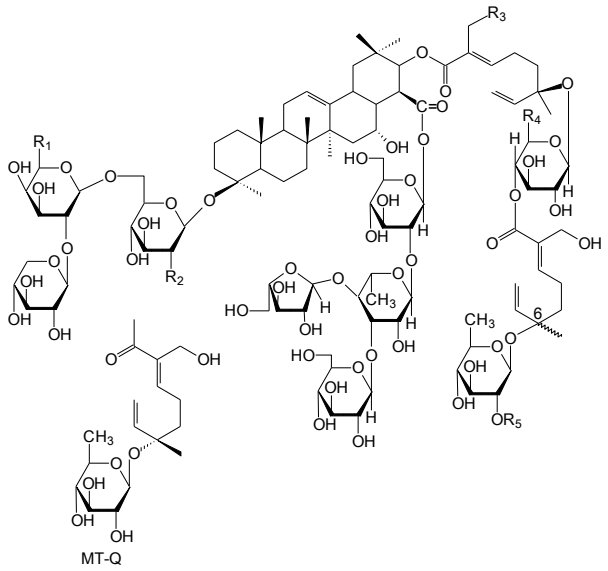
Chemical group/Structure	Substituted group (R-group)	Plant (Parts)	References
5. Flavonoids  (75) Quercetrin	R=α-L-rhamnopyranose	*A. julibrissin* (flower)	Kang *et al*., 2000, Lau *et al*., 2007
 (76) Isoquercetrin	R=β–D-glucopyranose	*A. julibrissin* (flower)	Kang *et al*., 2000, Lau *et al*., 2007

Table 2. Pharmacological activities of *Albizia* spp.

Pharmacological activities	*Albizia* spp.	Active compounds	References
Cytotoxicity against cancer cell lines	*A. adianthifolia*	**15**-**17**	Haddad *et al*., 2004
	A. chinensis	**6**-**10**	Liu *et al*., 2009; Liu *et al*., 2010
	A. coriaria	**22**-**23**	Note *et al*., 2009
	A. grandibracteata	**18**-**20**	Krief *et al*., 2005
	A. inundata	**24**-**27**	Zhang *et al*., 2011
	A. julibrissin	**1**-**5**, **11**-**13**	Moon *et al*., 1985; Liang *et al*., 2005; Zou *et al*., 2005; Zheng *et al*., 2006; Hua *et al*., 2009; Hua *et al*., 2011
	A. lebback	-	Lam and Ng, 2011
	A. procera	**21**	Melek *et al*., 2007
	A. subdimidiata	**14**	Abdel-Kader *et al*., 2001
Antioxidant activity	*A. anthelmintica*	**57**-**59**	Mohamed *et al*., 2013
	A. chinensis	**33**-**38**	Chaudhary *et al*., 2011
	A. julibrissin	**28**-**31**, **33**-**35**, **39**	Jung *et al*., 2004a; Jung *et al*., 2004b; Lau *et al*., 2007; Vaughn *et al*., 2007; Liu *et al*., 2010
	A. lebbeck	-	D'Souza *et al*., 2004; Resmi *et al*., 2006
	A. myriophylla	-	Steinrut *et al*., 2011
Anti-inflamatory activity	*A. chinensis*	-	Perumal *et al*., 2010
	A. julibrissin	-	Qiao *et al*., 2007
	A. lebbeck	-	Pratibha *et al*., 2004; Babu *et al*., 2009; Saha and Ahmed, 2009; Yadav *et al*., 2010
Antimicrobial activity	*A. amara*	**40**-**42**	Mar *et al*., 1991
	A. anthelmintica	-	Runyoro *et al*., 2006
	A. gummifera	**43**-**45**	Merz *et al*., 1986; Rukunga and Waterman, 1996

Table 2. Pharmacological activities of *Albizia* spp. (*cont*.)

Pharmacological activities	*Albizia* spp.	Active compounds	References
Antimicrobial activity	*A. gummifera*	**43-45**	Unasho *et al*., 2009; Mbosso *et al*., 2010; Mmushi *et al*., 2010
	A. julibrissin	-	Yadav *et al*., 2010
	A. lebback	-	Sudharameshwari and Radhika, 2006
	A. myriophylla	**60-62**	Amornchat *et al*., 2006; Rukayadi *et al*., 2008; Joycharat *et al*., 2012
	A. schimperiana	**45-47**	Samoylenko *et al*., 2009
Antiparasitic activity	*A. adenocephara*	**49-50**	Ovenden *et al*., 2002
	A. anthelmintica	-	Grade *et al*., 2008
	A. coriaria	-	Kigondu *et al*., 2009
	A. lebbek	-	Gathuma *et al*., 2004; Eguale *et al*., 2011
	A. gummifera	**51-52**	Muregi *et al*., 2007; Rukunga *et al*., 2007
	A. schimperiana	-	Nibret and Wink, 2011
	A. zygia	**29**	Kigondu *et al*., 2009 ; Abdalla and Laatsch, 2011
CNS depressant	*A. inopinata*	-	Assis *et al*., 2001
Anti-anxiety	*A. julibrissin*	-	Jung *et al*., 2005
Anti-depressant	*A. julibrissin*	-	Kim *et al*., 2007
Anti-diabetic	*A. odoratissima*	-	Kumar *et al*., 2011
Anti-obesity	*A. julibrissin*	**53-56**	Yahagi *et al*., 2012
Wound healing	*A. lebbek*	-	Joshi *et al*., 2013



	R_1	R_2	R_3	R_4	R_5	C-6
Julibroside III (**1**)	CH_3	$NHCOCH_3$	H	CH_3	H	S
Julibroside J_{28} (**2**)	CH_3	$NHCOCH_3$	H	CH_3	H	R
Julibroside J_8 (**3**)	CH_3	OH	OCH_3	CH_3	H	S
Julibroside J_{13} (**4**)	CH_3	$NHCOCH_3$	OCH_3	CH_3	H	S
Julibroside J_{12} (**5**)	CH_3	$NHCOCH_3$	OCH_3	CH_3	H	R
Albiziaoside A (**6**)	CH_3	OH	OH	CH_3	MT-Q	-
Albiziaoside B (**7**)	CH_3	O-glc	OH	CH_3	MT-Q	-
Albiziaoside C (**8**)	H	O-glc	OH	CH_3	H	-
Albiziaoside D (**9**)	CH_3	O-glc	OH	CH_3	H	-
Albiziaoside E (**10**)	H	OH	OH	CH_3	MT-Q	-

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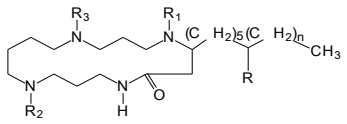
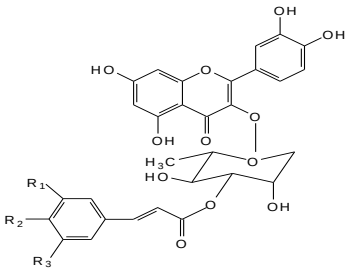
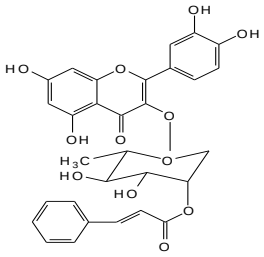
Figure 1. Chemical structures of bioactive compounds from *Albizia* species



	R	R_1	R_2	R_3	n
Budmunchiamine A (**40**)	H	CH_3	CH_3	CH_3	4
Budmunchiamine B (**41**)	H	CH_3	CH_3	CH_3	2
Budmunchiamine C (**42**)	H	CH_3	CH_3	CH_3	6
Budmunchiamine G (**43**)	H	H	CH_3	CH_3	6
Budmunchiamine K (**44**)	H	CH_3	CH_3	CH_3	8
6′-Hydroxybudmunchiamine K (**45**)	OH	CH_3	CH_3	CH_3	8
6′-Hydroxybudmunchiamine C (**46**)	OH	CH_3	CH_3	CH_3	6
5-Normethylbudmunchiamine K (**47**)	H	H	CH_3	CH_3	8
5,14-Dimethylbudmunchiamine L1 (**48**)	H	CH_3	CH_3	H	12
Budmunchiamine L4 (**49**)	H	H	H	H	6
Budmunchiamine L5 (**50**)	H	H	H	H	8
6′-Hydroxy-5-normethylbudmunchiamine K (**51**)	OH	H	CH_3	CH_3	8
9-Normethylbudmunchiamine K (**52**)	H	CH_3	CH_3	H	8

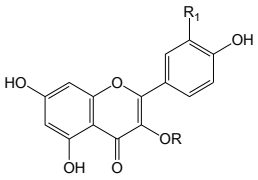


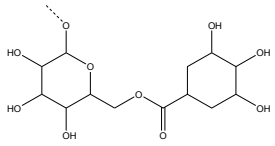
	R_1	R_2	R_3
3″-(*E*)-*p*-Coumaroylquercitrin (**53**)	H	OH	H
3″-(*E*)-Feruloylquercitrin (**54**)	OH	OH	H
3″-(*E*)-Cinnamoylquercitrin (**55**)	H	H	H



2″-(*E*)-Cinnamoylquercitrin (**56**)

Figure 1. Chemical structures of bioactive compounds from *Albizia* species (*cont*.)



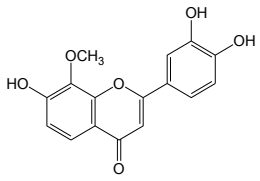


A

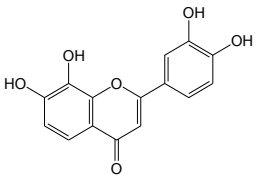
Quercetin-3-O-(6β-O-galloyl-β-D-glucopyranoside) (**57**): R = A, R_1 = OH

Kaempferol-3-O-β-D-glucopyranoside (**58**) :R = Glu, R_1 = H

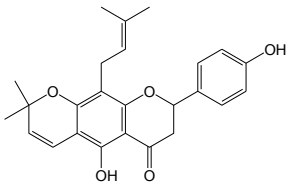
Kaempferol-3-O-(6β-O-galloyl-β-D-glucopyranoside (**59**) :R = A, R_1 = H



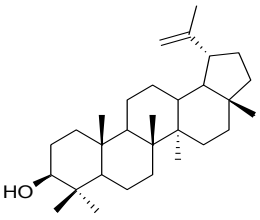
8-Methoxy-7,3′,4′-trihydroxyflavone (**60**)



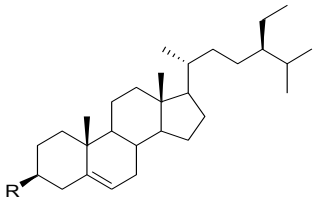
7,8,3′,4′-Tetrahydroxyflavone (**61**)



Lupinifolin (**62**)

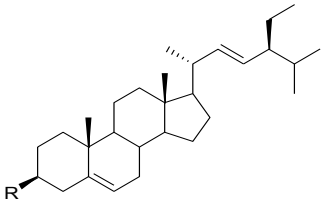


Lupeol (**63**)



β-Sitosterone (**64**) : R = =O,

β- Sitosterol (**65**) : R = OH



Stigmasta-5,22-dien-3-one (**66**) : R = =O

Stigmasterol (**67**) : R = OH

Figure 1. Chemical structures of bioactive compounds from *Albizia* species (*cont*.)

2. Experimental

2.1 General experimental procedures

The following apparatus was used: Mps, Electrothermal 9100; Optical rotation, Jasco P-1020 polarimeter; IR, EQUINOX 55, Bruker FT-IR; 1D and 2D-NMR, FT-NMR Bruker Advance 400 MHz, FT-NMR Varian Inova 500 MHz. Column chromatography (CC) was performed using silica gel (Merck, 70-230 mesh) and Sephadex LH 20 ((Sigma, 20-100 μm). Thin-layer chromatography (TLC) was carried out on precoated sheets of silica gel 60 F254. Compounds were monitored by TLC sprayed with an anisaldehyde–sulfuric acid solution. Bacterial culture media, brain heart infusion (BHI) agar and tryptic soy broth (TSB) were purchased from Difco (Detroit, MI). Dimethyl sulfoxide (DMSO) and penicillin G were purchased from Merck (Darmstadt, Germany). Chlorhexidine was purchased from Fluka BioChemika (Buchs, Switzerland). The microtiter plate was purchased from Corning Life Sciences (Californis, USA). All solvents for column chromatography were of laboratory reagent grade and were purchased from commercial sources.

2.2 Plant material

The wood of *Albizia myriophylla* Benth was collected from Phatthalung province of the southern region of Thailand in June 2011. Botanical identification was performed by Assit. Prof. Dr. Oratai Neamsuvan, an ethnobotanist at the Faculty of Traditional Thai Medicine, Prince of Songkla University, where the voucher specimen (NJ0611) was deposited.

2.3 Isolation and purification

The wood of *A. myrophylla* (2.7 kg) was dried, ground, and exhaustively extracted with ethanol (EtOH) at room temperature three times, filtered, and concentrated. The EtOH extract (98.89 g, 3.66%) was resuspended in a mixture of methanol and water and then extracted with hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and *n*-butanol (BuOH), successively. Each filtrate was pooled and evaporated till dry under reduced pressure at 40 °C. The yield of hexane fraction (11.79 g, 0.44%), CH_2Cl_2 fraction (14.13 g, 0.52%), EtOAc fraction (12.11 g, 0.45%), and BuOH fraction (16.73 g, 0.62%) was obtained.

The hexane fraction (6.9 g) was subjected to CC using silica gel as adsorbent and eluted with acetone-CH_2Cl_2 gradient, followed by washing with MeOH. The fractions were then combined and numbered (IH-VIIIH) on the basis of their TLC profiles. Fraction IIH (728 mg) precipitated as white residues during the concentration process was further recrystallized from acetone to get a mixture (54.5 mg) of β-

sitosterone (**7**) and stigmasta-5,22-dien-3-one (**8**). Fraction IIIH (481 mg) was subjected to silica gel CC and eluted with acetone-CH_2Cl_2 gradient. Column fractions eluted with 20% and 25% acetone in CH_2Cl_2 afforded lupeol (**6**, 20.2 mg) and a mixture (9.1 mg) of β-sitosterol (**9**) and stigmasterol (**10**), respectively. Fraction VIIH (494 mg) was purified on silica gel column with 35% acetone in hexane as an eluent to yield lupinifolin (**5**, 40.3 mg).

The dichloromethane fraction (12.36 g) was applied to silica gel CC using gradient elution with acetone-CH_2Cl_2, and finally washed down with MeOH. The fractions were then combined and numbered (ID-VID) according to their TLC patterns. Fraction IID (1.84 g) was further subjected to silica gel CC using gradient elution with acetone-Hexane, and finally washed down with MeOH. The fractions were then combined according to their TLC patterns to give five subfractions (I-V). Subfraction III (750 mg) was further purified on silica gel CC using $CHCl_3$-MeOH (90:10) as the eluent, followed by three successive Sephadex LH-20 columns eluted with MeOH to yield 3,4,7,3′-tetrahydroxyflavan (**1**, 5.7 mg). Fraction IIID (1.46 g) was further applied to silica gel CC using gradient elution with acetone-CH_2Cl_2, and finally washed down with MeOH. The fractions were then combined according to their TLC chromatograms to yield six subfractions (I-VI). Subfraction IV (127 mg) was purified on silica gel CC eluted with acetone-CH_2Cl_2 (40:60), followed by repeated gel filtration chromatography, using three successive Sephadex LH 20 columns eluted with MeOH to yield 7,3',4'-trihydroxyflavanone (syn. butin) (**2**, 11.7 mg). Subfraction V (46.6 mg) was purified on silica gel CC eluted with CH_2Cl_2-EtOAc (40:60), followed by repeated gel filtration chromatography, using three successive Sephadex LH 20 columns eluted with MeOH to yield 8-methoxy-7,3′,4′-trihydroxyflavone (**3**, 9.9 mg) and 7,8,3′,4′-tetrahydroxyflavone (**4**, 5.9 mg). The structures of all isolated compounds are shown in **Figure 2**.

The EtOAc (10 g) and *n*-BuOH (15 g) fractions were also isolated and purifield separately by chromatographic tecniques using both siliga gel and Sephadex LH 20 columns. Various solvent systems were employed as the eluents depending on materials but no compound could be isolated from both of the EtOAc and *n*-BuOH fractions in this study.

2.4 Compound identification

Physical properties of some purified compounds such as melting points and optical rotations were determined. The structures of the isolated compounds were determined by extensive NMR techniques mainly one-dimensional and two-dimensional nuclear magnetic resonance spectroscopy (1D

and 2D NMR; ^{1}H, ^{13}C, DEPT, HMQC, HMBC, COSY), ultraviolet spectroscopy (UV), infrared spectroscopy (IR), electron-impact mass spectroscopy (EIMS), and comparison with the literature values. TLC co-spotting with an authentic sample was also used to support the identity of some compounds.

3,4,7,3′-Tetrahydroxyflavan (**1**): Brown amorphous solid; UV (MeOH, nm) λmax: 313, 208. IR (Neat, cm^{-1}) νmax: 3497, 3398, 3319, 1621, 1461, 1382, 1155, 1119, 829, 781. ^{1}H and ^{13}C NMR, HMBC data (DMSO-d_6, 400 MHz): see **Table 3**. HRESIMS: $[M+Na]^+$ m/z 297.2593 (calcd. for $C_{15}H_{14}O_5$: 297.2597).

7,3',4'-Trihydroxyflavanone (**2**): Yellow solid; UV (MeOH, nm) λmax: 311, 277; EIMS m/z $[M]^+$; 272.8; ^{1}H and ^{13}C NMR data (MeOH-d_4, 500 MHz): see **Table 4**.

8-Methoxy-7,3′,4′-trihydroxyflavone (**3**): Yellow solid; 270–271 °C; UV (MeOH, nm) λmax: 221, 250, 349; IR (Neat, cm^{-1}) νmax: 3458, 3399, 3318, 3138, 2561, 2464, 2343, 1599, 1581, 1506, 1440, 1386, 1250, 1207, 1178, 1107, 1024; EIMS m/z 300.7 $[M]^+$; ^{1}H and ^{13}C NMR data (DMSO-d_6, 400 MHz): see **Table 5**.

7,8,3′,4′-Tetrahydroxyflavone (**4**): Yellow solid; UV (MeOH, nm) λmax: 209; IR (Neat, cm^{-1}) νmax: 3435, 2920, 2852, 1626, 1608, 1499, 1270, 1121, 1020; EIMS m/z 286.9 $[M]^+$; ^{1}H and ^{13}C NMR data (DMSO-d_6, 400 MHz): see **Table 6**.

Lupinifolin (**5**): Yellow needles; 119–120 °C; -9.7 (c 0.1, MeOH); UV (MeOH, nm) λmax: 313; IR (Neat, cm^{-1}) νmax: 3422, 2973, 2915, 1643, 1619, 1520, 1452, 1380, 1239, 1196, 1123; EIMS m/z 406.7 $[M]^+$; ^{1}H and ^{13}C NMR data ($CDCl_3$, 400 MHz): see **Table 7**.

2.5 Antibacterial assay

Bacterial strains and culture conditions

Three bacterial strains including *Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 11778, and *Streptococcus mutans* ATCC 25175 were obtained from Department of Microbiology, Faculty of Science, Prince of Songkla University, Thailand. The bacterial cultures were stored in brain heart infusion (BHI) broth (Difco, France) with 20% glycerol at -80 °C until use. All bacterial strains were cultured on BHI agar and incubated separately with 5% CO_2 at 37 °C for 24 h for *S. mutans* ATCC 25175 or at 37 °C for 24 h for *S. aureus* ATCC 29213 and *B. cereus* ATCC 11778.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts and compounds isolated from *A. myriophylla* were determined using a modified broth microdilution method (CLSI, 2009). Briefly, the test agents including fractions and compounds were separately dissolved in 10% DMSO (Merck, Germany) and two-fold dilutions were prepared. Suspension of bacterial cultures in BHI broth was made from the overnight broth culture. The bacterial suspension (180 µL) was mixed with the diluted test agents (20 µL) in 96 wells microtiter plate (Corning Life Sciences, USA). The final bacterial concentration was approximately 5×10^5 CFU/mL. The final concentration of the tested extracts was ranging from 0.49-1000 µg/mL and that for the tested compounds was 0.5-250 µg/mL. Chlorhexidine and penicillin G were included as the positive controls. DMSO was used as a negative control. The lowest concentration of the tested agents required to completely inhibit bacterial cell growth after incubation at 37 °C for 24 h was recorded as MIC. Each assay was performed in triplicate.

Minimum bactericidal concentration (MBC)

After MICs were recorded, bactericidal properties of compound isolates were evaluated by MBC test. In short, an aliquot (100 µl) from the broth with no growth was pipetted and dropped onto agar plate and incubated with at 37 °C for 48 h. The lowest concentration of the tested agents required to completely preventing bacterial growth was reported as MBC. Each assay was performed in triplicate.

2.6 Cytotoxicity assay

Cytotoxic activity of the extracts and isolated compounds against KB cells was carried out using the method described by Brien *et al*. (2000). Briefly, cells at a logarithmic growth phase were harvested and diluted to $2.2\text{x}10^4$ cells/ml for KB and $3.3\text{x}10^4$ cells/ml for MCF-7 and NCI-H187, in fresh medium. Successively, 5 µl of test sample prepared in 5% DMSO, 45 µl of cell suspension wre added to 384-well (the final DMSO content in each well is 0.5%). Plates were incubated at 37°C in 5% CO_2 incubator (3 days for KB and MCF-7 and 5 days for NCI-H187). After the incubation period, 12.5 µl of 62.5 µg/ml resazurin solution were added to each well of the plate. The plates were then incubated at 37°C for 4 h. Fluorescence signal was measured using SpectraMax M5 multi-detection microplate reader at the excitation and emission wavelengths of 530 nm and 590 nm, respectively. Percent inhibition of cell growth was calculated by the following equation, where FU_T and FU_C are the mean fluorescent unit from treated and untreated conditions, respectively:

$$\% \text{ Inhibition} = [1- (FU_T / FU_C)]*100$$

Dose response curves were plotted from 6 concentrations of 2-fold serially diluted test samples and the sample concentration that inhibited cell growth by 50% (IC_{50}) can be derived using the SOFTMax Pro software. Ellipticine and doxorubicin were used as positive controls whereas 0.5% DMSO was used as a negative control.

Cytotoxicity against the non-tumor cell line of test samples was performed using green fluorescent protein (GFP) method (Hunt *et al.*, 1999). Briefly, the GFP-expressing vero cell line was generated in-house by stably transfecting the african green monkey kidney cell line (vero, ATCC CCL-81) with pEGFP-N1 plasmid (Clontech). The cell line was maintained in minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 1.5 g/L sodium bicarbonate and 0.8 mg/ml geneticin, at 37°C in a humidified incubator with 5% CO_2. The assay was carried out by adding 45 µl of cell suspension at $3.3x10^4$ cells/ml to each well of 384-well plates containing 5 µL of test samples previously diluted in 0.5% DMSO, and then incubating for 4 days in 37°C incubator with 5% CO_2. Fluorescence signals were measured by using SpectraMax M5 microplate reader in the bottom-up reading mode with excitation and emission wavelengths of 485 and 535 nm, respectively. Fluorescence signal at day 4 was subtracted with background fluorescence at day 0. The percentage of cytotoxicity was calculated by the following equation, where FU_T and FU_C represent the fluorescence units of cells treated with test sample and untreated cells, respectively:

$$\% \text{ cytotoxicity} = [1-(FU_T / FU_C)] \times 100$$

IC_{50} values were derived from dose-response curves, using 6 concentrations of 2-fold serially diluted samples, by the SOFTMax Pro software. Ellipticine and 0.5% DMSO were used as a positive and a negative controls, respectively.

3. Results and Discussion

3.1 Chemical constituents from the wood of Albizia myriophylla

Chromatographic separation of the hexane fraction of the ethanol extract from the wood of *A. myriophylla* yielded a prenylated flavanone lupinifolin (**5**), a triterpenoid lupeol (**6**) and four common sterols namely β-sitosterone (**7**), stigmasta-5,22-dien-3-one (**8**), β-sitosterol (**9**), and stigmasterol (**10**). The dichloromethane fraction of *A. myriophylla* was separated by extensive column chromatography to get four flavonoids, 3,4,7,3′-tetrahydroxyflavan (**1**), 7,3',4'-trihydroxyflavanone (**2**), 8-methoxy-7,3′,4′-trihydroxyflavone (**3**) and 7,8,3′,4′-tetrahydroxyflavone (**4**). The structures of these compounds (**Figure 2**) were identified on the basis of spectroscopic data (1D, 2D NMR and MS) as well as comparison with

literature values (Porter and Foo, 1982; Reynolds *et al*., 1986; Mahidol *et al*., 1997; Tian *et al*., 2004; Chokchasiri *et al*., 2009; Herath *et al*., 2009; Yoon *et al*., 2011; Wu *et al*., 2012).

3,4,7,3′-Tetrahydroxyflavan (**1**) was obtained as a brown amorphous solid and had a molecular formula $C_{15}H_{14}O_5$, as deduced from HRESIMS at *m/z* 297.2593 $[M + Na]^+$ (calcd. *m/z* 297.2597). Its IR spectrum showed absorption bands at 3423, 3398, and 3319 cm^{-1}, suggesting the presence of hydroxy function. The ^1H NMR spectrum (**Table 3**) of **1** showed one typical trisubstituted benzene system at δ_H 7.28 (1H, dd, *J* = 8.5,1.0 Hz, H-5), 6.28 (1H, d, *J* = 2.5 Hz, H-6), and 6.25 (1H, d, *J* = 2.5 Hz, H-8), and four additional aromatic protons at δ_H 7.02 (1H, d, *J* = 2.0 Hz, H-2′), 6.42 (1H, m, H-4′), 6.41 (1H, m, H-5′), and 7.12 (1H, d, *J* = 8.5 Hz, H-6′). The ^{13}C NMR (**Table 3**) together with DEPT and HMQC data of **1**, exhibited 12 aromatic carbons of the two aromatic rings and three oxymethine carbon signals at δ_C 78.11 (d, C-2), 66.69 (d, C-3), and 68.66 (d, C-4). The assignment of the four hydroxy groups to the flavan nucleus of **1** was accomplished by a detailed analysis of HMBC spectral data (**Table 3**). These findings revealed that compound **1** was a flavan-3,4-diol derivative with the OH-substitution on positions C7 in ring A and C3′ in ring B of the flavonoid. The 2,3-*cis* configuration of **1** was determined according to a broad singlet at δ_H 4.89 for H-2 and its corresponding oxymethine carbon signal at δ_C 78.11 of C-2 (Wu *et al*., 2012). The low value of $J_{3,4}$ 3.0 Hz indicated the 3,4-*cis* configuration of **1** (Porter and Foo, 1982). Thus, the structure of compound **1** was proposed to be 3,4,7,3′-tetrahydroxyflavan (**1**). The isolation and characterization of this flavan-3,4-diol from the wood of *A. myriophylla* in this study supported the previous literature of the general observation of the members of flavan-3,4-diols to be the wood components of plants (Stafford and Lester, 1985). Previous studies have demonstrated that flavan-3,4-diols were involved as intermediate substances in flavan-3-ol biosynthesis catalyzed by reductase reaction (Drewes and Roux, 1965).

7,3',4'-Trihydroxyflavanone (**2**) or butin has been characterized for the first time as aglycone unit of the glycoside butrin isolated by Lal and Dutt (1937) from the flowers of *Butea frondosa*. Flavones and flavonols commonly constitute the vast majority of the flavan-based flavonoids. Contrastly, flavanones, which lack the C ring 2-3 double bond, are less abundant than those two and have received less attention. Flavanone **2** has been previously reported to have a number of biological activities including estrogenic, postcoital anti-conceptive, antimycobacterial, anti-lipoxygenase and anti-tyrosinase activities (Malik *et al*., 2004; Lee *et al*., 2006; Chokchasiri *et al*., 2009). This compound has been proved to be an effective component of some medicinal plants in reducing tyrosinase activity without significant cytotoxic

to human epidermal melanocytes. There has been documented that this compound was among the components of whitening formulation (Munekyo et al., 1994). This trihydroxyflavone **2** was previously isolated from many plant species of Fabaceae family such as *Butea monosperma, Cassia nomame, Erythrina sigmoidea, Indigofera hetrantha* and *Spatholobus suberectus* (Kitanaka *et al*., 1992; Malik *et al*., 2004; Lee *et al*., 2006; Chokchasiri *et al*., 2009; Ali *et al*., 2011). This study reported the isolation of this compound from *Albizia* species for the first time. Its NMR spectral data are summarized in **Table 4**.

8-Methoxy-7,3′,4′-trihydroxyflavone (**3**), yellow solid; 270-271 °C, is a flavone derivative previously reported as microbial metabolite of 7,8-dihydroxyflavone transformed by *Mucor ramannianus* (Herath *et al*., 2009). This work reported the isolation of this compound from *Albizia* species for the first time. Its NMR spectral data are summarized in **Table 5**.

7,8,3′,4′-Tetrahydroxyflavone (**4**), yellow solid, has the ^{1}H NMR spectral data closely related to those of compound **3**, but differed in the absence of signal at δ_H 3.74 of methoxy proton in spectrum of compound **4**. This observation suggested this compound to be a flavone derivative of **3**. This tetrahydroxyflavone **4** was isolated from the heart wood of *Acacia confula* (Wu *et al*., 2008; Tung *et al*., 2010). As far as we know this is also the first report of the flavone **4** in *Albizia* spp. Its interesting properties such as xanthine oxidase and telomerase inhibitory activities were previously reported (Tung *et al*., 2010; Menichincheri *et al*., 2004). Its NMR spectral data are summarized in **Table 6**.

Lupinifolin (**5**), yellow needles; 119-120 °C; -9.7 (*c* 0.1, MeOH), is a prenylated flavonoid first described from the root of *Tephrosia lupinifolia* Burch (DC) (Lin, *et al*., 1991). Its ^{1}H and ^{13}C NMR spectral data (**Table 7**) macthed well with those of a flavanone already reported (Mahidol *et al*., 1997). This compound was previously demonstrated to have interesting therapeutic properties such as anti-inflammatory (Ganapaty *et al*., 2006), antimalarial (Khaomek *et al*., 2008), antioxidant, antibacterial, and cytotoxic (Khaomek *et al*., 2008) activities.

Lupeol (**6**), white needles, is a lupine-type triterpenoid previously isolated from various members of *Albizia* (Rukunga and Waterman, 2001; Hussain *et al*., 2008; Baek *et al*., 2010). It is among the most common plant triterpenoid previously demonstrated to have interesting therapeutic properties such as antimalarial (Alves *et al*., 1997), anti-inflammatory (Geetha and Varalakshmi, 2001), anticancer (Moriarity *et al*., 1998; Aratanechemuge *et al*., 2004), anti-angiogenic (You *et al*., 2003), and antimicrobial (Ajaiyeoba *et al*., 2003) activities. Its ^{1}H and ^{13}C spectral data are summarized in **Table 8**.

A mixture of β-sitosterone (**7**) and stigmasta-5,22-dien-3-one (**8**), colorless needles, has the ^{13}C spectral data (**Table 9**) closely similar to its stigmastane-3-ol derivatives previously described (Wright *et al*., 1978). These steroid-type compounds have been described previously from the stem of *Annona cherimola* (Chen *et al*., 1997) and the bark of *Hernandia nymphaefolia* (Chen *et al*., 2000). Sitosterone (**8)** has been shown previously to exhibit antioxidant activity (Mazid *et al*., 2011).

A mixture of β-sitosterol (**9**) and stigmasterol (**10**), colorless needle crystals, is a 3-hydroxy derivative of compound 1. It has the ^{1}H resonances similar to those of its keto derivative 1, except for an extra signal of carbinolic methine proton resonated at δ_H 3.58. Further TLC-co spotting with an authentic sample agreed well with the proposed structures. This mixture is the most common plant sterol previously demonstrated to have interesting therapeutic properties such as antihyperglycemic (Invorra *et al*., 1988), anti-inflammatory, and antipyretic (Gupta *et al*., 1980) activities.

The *Albizia* genus is known to be a rice source of bioactive saponins (Krief *et al*., 2005; Zheng *et al*., 2006). Some members of this chemical group were shown to possess high cytotoxicity against many different cancer cell lines. In addition, compounds belonging to the classes of alkaloids and flavonoids were among the bioactive components commonly described from the members of this genus. To our knowledge, few phytochemical studies have been previously reported for *A. myriophylla*. Various chemical classes including phenolic acids (bark), triterpene saponins (stem), lignan glycosides (bark), and iminosugars (wood) (Ito *et al*., 1994; Yoshikawa *et al*., 2002; Asano *et al*., 2005; Panmei *et al*., 2007) were characterised previously from this plant species. Up to now, only one phytochemical investigation from the wood extract of this species has been reported (Asano *et al*., 2005). In this case, five iminosugars including DMJ, DMDP, 3-*O*-β-D-glucopyranosyl-DMDP, DIDG, and 4-*O*-β-D-glucopyranosyl-DMJ were characterized from the ethanol extract of this plant part. The present findings showed for the first time of the flavonoids **1-5**, a triterpenoid **6,** and the steroids **7-10** isolated from this plant species.

3.2 Antibacterial activity and cytotoxicity of semi-purified fractions and the isolated compounds from the wood of A. myriophylla

Antibacterial and cytotoxic activities of various extracts including those of EtOH, Hexane, CH_2Cl_2, EtOAc, and *n*-BuOH as well as the isolated compounds **1—8** from *A. myriophylla* were presented in Tables 10 and 11. Anti-*Streptococcus mutans* activity of various extracts and pure compounds from *A. myriophylla* was evaluated using broth microdilution method (**Table 10**). As can be seen in table 10, the Hexane fraction was the most active with its respective MIC and MBC of 250 and 500μg/ml, followed by

those of CH_2Cl_2 and BuOH both of which showed the same activity with the same MIC and MBC of 1000 μg/ml. The EtOAc fraction showed no activity at the highest concentration tested. All tested compounds, except for compounds **1** and **2**, were effective against *S. mutans* ATCC 25175 with MIC and MBC values ranging from 1-250 and 2-250 μg/ml, respectively. However, MIC and MBC values of penicillin G and chlorhexidine were 0.0015 and 0.0015 μg/ml and 0.5 and 1 μg/ml, respectively. The compounds belong to triterpenoid and steroid classes exhibited almost same anti-*S. mutans* activity as those of 8-methoxy-7,3′,4′-trihydroxyflavone (**3**) and 7,8,3′,4′-tetrahydroxyflavone (**4**) with MIC and MBC values ranging from 62.5-125 and 125-250 μg/ml, respectively. Among the compounds isolated from *A. myriophylla*, lupinifolin (**5**) displayed the highest activity with MIC and MBC of 0.98 and 1.96 μg/ml, respectively. Compound **5** also displayed pronounced antibacterial activity against *B. cereus* ATCC 11778 and *S. aureus* ATCC 29213 with the same MIC and MBC values of 15.6 and 31.2 μg/ml, respectively. Compounds **3** and **4** showed moderate activity against the three tested bacterial strains with MIC values ranging from 62.5—250 μg/ml, whereas compounds **1** and **2** exhibited no antibacterial activity against the all the tested pathogens at the highest concentration tested of 250 μg/ml (**Table 10**). Besides antibacterial activity, the isolated compounds **1**–**7** were evaluated for their cytotoxicity against KB cells using resazurin microplate assay. The prenylated flavonoid **5** demonstrated significant *in vitro* cytotoxic activity against KB cells with IC_{50} of 4.95 μg/ml while the remainder exhibited no activity against KB cells at the highest concentrations tested (**Table 11**).

In previous study, the EtOH extract of this plant species purchased from herb shop in southern province of Thailand exhibited pronounced anti-*S. mutans* activity with MIC of 3.9 μg/ml (Joycharat *et al*., 2012). In contrase of previous observation, the EtOH extract of *A. myriophylla* collected from sounthern province of Thailand in this study showed much lower activity against *S. mutans* ATCC 25175 with MIC of 500 μg/ml. The differences in phytochemical composition of *A. myriophylla* collected from sampling sites differing in environmental conditions may result in the variation in the efficacy of its folk remedies for oral diseases. The significant difference in the antibacterial result of the ETOH extracts of two diferent collections of this plant species could be explained by many factors which may affect the variation of its bioactive components including genetic and environment (Wahyuni *et al*., 2013). The variation of plant secondary metabolites under differing geographical conditions has been described previously (Zhao *et al*., 2012). This work reported antibacterial activity of compounds **1**-**8** against the three bacterial strains of medically importance including *S. mutans* ATCC 25175, *B. cereus* ATCC 11778, and *S. aureus* ATCC 29213

for the first time. Three out of five flavonoids tested including the two flavones **3** and **4** and one prenylated flavanone **5** displayed antibacterial activity against the three pathogens tested, whereas the falvan 3,4-diol **1** and the flavanone **2** exibited no activity. Previous study has shown that 7-hydroxyflavanone **2** exhibited no *in vitro* antibacterial activity, therefore our finding supported that from previous literature (Mikell *et al*., 2012). Antibacterial activity against various kind of pathogenic bacteria has been widely described for the members of prenylated flavonoids (Khaomek *et al*., 2008; Sutthivaiyakit *et al*., 2009). In this study, the higher antibacterial activity of lupinifolin (**5**) compared with those of the other isolated flavonoids **1**-**4** could be partly associated with the more lipophilic property of its structure. In addition, the members of prenylated flavonoids also demonstrated other interesting pharmacological activities, especially anticancer activity. Lupinifolin (**5)** were previously reported to be active against P-388 leukemia cell (Mahidol *et al*., 1997; 2002). The cytotoxic activity of **5** against KB cells in this study was in accordance with that previously described (Sutthivaiyakit *et al*., 2009). The relationships between the flavonoid structures and their antibacterial or cytotoxic activities could not be justified herein from the existing results. However, the bioassay results indicated that the more lipophilicity of compound **5** compared with the weak or inactive flavonoids **1**-**4** may play an importance role in the *in vitro* antibacterial property as well as cytotoxic activity.

The antibacterial activity of *A. myriophylla* against *S. mutans* has been shown in previous studies. In particular, the mouthwash of *A. myriophylla* significantly reduce mutans streptococci counts in saliva of schoolchildren (Cholticha *et al*., 2006). In the present study, we found that *A. myriophylla* components including three flavonoids (**3-5**), a triterpenoid **6**, and four steroids (**8-10**) have an antibacterial effect on *S. mutans* that associated with dental plaque formation and caries development. Among the three antibacterial flavonoids, lupinifolin (**5**), a prenylated flavonoid bearing hydrophobic moiety of an isoprenyl and a dimethylchromene groups attached to the flavanone skeleton, exhibited the strongest activity against *S. mutans* compared with those of two more polar flavones, 8-methoxy-7,3′,4′-trihydroxyflavone (**3**) and 7,8,3′,4′-tetrahydroxyflavone (**4**). Antibacterial or antiplaque activities against *S. mutans* of several compounds in flavonoids class such as apigenin (Koo *et al*., 2003), kurarinone (Chen *et al*., 2005), guaijaverin (Prabu *et al*., 2006), and patulitrin (Rhama and Madhavan, 2011) have been reported previously. Furthermore, antibacterial activity against the diverse range of pathogenic bacteria is widely found in prenylated flavonoids (Nomura, 1988). It has been postulated that their hydrophobic properties are especially suitable for protection against microorganisms because they may easily penetrate through the bacterial cell membrane (Chi *et al*., 2001). Flavonoids that have crossed the bacterial cell membrane

could be possibly active against bacterial enzymes and proteins (Cushnie & Lamb, 2011). Thus, the strong anti-*S. mutans* activity of lupinifolin (**5**) could be partly due to its lipophilic characteristic. Antibacterial mechanisms of action of various flavonoids including cytoplasmic membrane damage, inhibition of nucleic acid, cell wall, and cell membrane synthesis as well as inhibition of energy metabolism have been proposed previously (Cushnie & Lamb, 2011). In addition, previous investigations have reported the antibacterial activity of triterpenoids and steroids containing plants against *S. mutans* (Song *et al*., 2006*a*; Zheng *et al*., 2010). However, our study is the first report on the MIC and MBC values of the triterpene lupeol (**6**) and other four common sterols, β-sitosterone (**7**), stigmasta-5,22-dien-3-one (**8**), β-sitosterol (**9**), and stigmasterol (**10**), against cariogenic *S. mutans*.

Several antiseptics and natural products such as chlorhexidine, sanguinarine, essential oils, tea extract, and propolis have been reported for their antibacterial activity against cariogenic bacteria. Some of these agents are incorporated into various commercial oral care products including toothpaste, mouthwash, chewing gum, and dental floss. However, there are some undesirable side effects following their use. Chlorhexidine, a gold standard antiseptic for an antiplaque, produces brown staining on the teeth and other oral surfaces which is very difficult to remove. Moreover, it increases the calculus formation and has an unpleasant taste (Eley, 1999). Our results demonstrated that anti-*S. mutans* activities of lupinifolin (**5**) were almost comparable to chlorhexidine. Sanguinarine, a benzophenanthridine alkaloid from the rhizome of *Sanguinaria canadensis*, has been used in many oral care products. This alkaloid displayed broad spectrum and strong antibacterial activity against many oral pathogenic bacteria (Dzink & Socransky, 1985; Eley, 1999), however, it was reported to be associated with oral leukoplakia (Mascarenhas *et al*., 2001; Mascarenhas *et al*., 2002). The MIC value of sanguinarine against *S. mutans* was 1-32 μg/ml (Dzink & Socransky, 1985; Park *et al*., 2003; Hwang *et al*., 2004; Chung *et al*., 2006). A number of essential oils from many plant species have been studied for their anti-*S. mutans* activity. The MIC ranges of essential oils, their fractions, and isolated compounds such as carvacal, eucalyptol, menthol, thymol, isoeugenol, and methyl salicylate were in range of 15.6-1,000 μg/ml (Hwang *et al*., 2004; Chung *et al*., 2006; Galvao *et al*., 2012). Green tea extracts, one of the most popular agents combined with oral hygiene products, have been demonstrated for anti-*S. mutans* activity with MIC ranging from 125-800 μg/ml (Park *et al*., 2003; Hwang *et al*., 2004; Cho *et al*., 2010; Awadalla *et al*., 2011; Tahir & Moeen, 2011; Subramaniam *et al*., 2012). Several active compounds, mainly polyphenols, such as gallocatechin, epigallocatechin, and epigallocatechin gallate have been isolated (Sakanaka *et al*., 1989; Otake *et al*., 1991) with MIC values against *S. mutans* ranging from 250-1,000 μg/ml (Sakanaka *et al*.,

1989). Propolis, a resinous bee product, has been investigated for anti-*S. mutans* activity both *in vitro* (Park *et al*., 1998*a*, Park *et al*., 1998*b*; Ugur & Arslan, 2004; Castro *et al*., 2009; Kouidhi *et al.*, 2010; Liberio *et al.*, 2011; Elbaz & Elsayad, 2012) and *in vivo* (Duailibe *et al*., 2007). The MIC ranges of propolis against *S. mutans* were 17.5-100 μg/ml (Duarte *et al.*, 2003; Kim *et al.*, 2011). The compounds present in propolis such as sesquiterpene (*tt*-farnesol), flavone (baicalein), flavanones (pinocembrin, sakuranetin, isosakuranetin), and dihydroflavonol (pinobanksin-3-acetate) have been reported for their anti-*S. mutans* activity with MIC of 125-≥500 μg/ml (Koo *et al.*, 2002). In comparison to the compounds reported above, the anti-*S. mutans* activity of lupinifolin (**5**) from the this study possesses better than or at least equivalent antibacterial activity. In addition, lupinifolin (**5**) did not produce any toxic signs or deaths via the oral route in mice at administered doses of 5 g/kg and 60 mg/kg in acute and subacute toxicity studies, respectively (Chivapat *et al*., 2009). Therefore, oral use of lupinifolin (**5**) for various medicinal purposes could be considered as safe.

Recently, researchers have recognized that there are many phytochemicals affecting the healing properties of herbal plants. The inconsistency of the active constituents affects the variation in the chemical quality of medicinal plant materials and may result in the reproducible therapeutic effect of their herbal products. The variation of bioactive phytochemicals in plants is common and often associated with geographical parameter. Among the compounds studied, lupinifolin (**5**) which has the highest antibacterial and anticancer activities is most relevant with the bioactivities of this plant. As the batch-to-batch consistency of herbal products could be affected by many factors for instance genetic and ecological factors, the quality control has become an important role to support the clinical applications (Shi et al., 2014). Herbal products contain normally a complex mixture of phytochemicals therefore selection of appropriate marker compounds that are relevant to the biological activities is currently an important approach for their quality control (Shi et al., 2014). Similarly, in order to facilitate efficient standardization procedure for the future development of *A. myriophylla* extract as herbal product, it is essential to define chemical markers responsible for its therapeutic activity. Further research on correlations between lupinifolin content and biological activities among geographically distinct plant populations would allow obtaining information relating to the quality control of *A. myriophylla* wood.

Table 3. The NMR spectral data of 3,4,7,3′-tetrahydroxyflavan (**1**) (in DMSO-d_6, 100 MHz)

Position	Compound **1**		
	^{1}H (mult., J in Hz)	^{13}C (mult.)	HMBC
2	4.89 (br s)	78.11 (d)	C3, C1′
3	4.86 (m)	66.69 (d)	C-2, C-4
4	4.56 (d, 3.0)	68.66 (d)	C-3, C4a
4a		115.72 (s)	
5	7.28 (dd, 8.5,1.0)	128.41 (d)	C-4a, C-6
6	6.28 (d, 2.5)	107.83 (d)	C-5, C-7
7		157.10 (s)	
8	6.25 (d, 2.5)	101.52 (d)	C7, C9
8a		154.90 (s)	
1'		115.24 (s)	
2'	7.02 (d, 2.0)	114.79 (d)	C-2, C-4'
3'		144.57 (s)	
4'	6.41 (m)	114.21 (d)	C-3', C-5'
5'	7.12 (d, 8.5)	130.26 (d)	C-4', C-6'
6'	6.42 (m)	118.47 (d)	C-1', C-5'

Table 4. The NMR spectral data of 7,3',4'-trihydroxylflavanone (**2**) (in MeOH-d_4, 125 MHz)

Position	Compound **2**	
	^{1}H (mult., J in Hz)	^{13}C (mult)
2	5.23 (dd, 12.9, 2.9)	81.08 (d)
3	H-3eq 2.67 (dd, 16.9, 2.9)	45.03 (t)
	H-3ax 2.93 (dd, 16.9, 12.9)	
4		193.54 (s)
4a		165.54 (s)
5	7.70 (d, 8.7)	129.84 (d)
6	6.44 (dd, 8.7, 2.1)	111.74 (d)
7		166.41 (s)
8	6.31 (d, 2.1)	103.81 (d)
8a		114.96 (s)
1'		132.03 (s)
2'	6.87 (d, 1.6)	114.68 (d)
3'		146.87 (s)
4'		146.09 (s)
5'	6.78 (d, 8.1)	116.23 (d)
6'	6.73 (dd, 8.1, 1.6)	119.22 (d)

Table 5. The NMR spectral data of 8-methoxy-7,3',4' trihydroxylflavone (**3**) (in DMSO-d_6, 125 MHz)

Position	Compound **3**		
	^{1}H (mult., J in Hz)	^{13}C (mult)	HMBC
2		157.89 (s)	
3	6.87 (s)	103.13 (d)	C-6', C-4a
4		176.51 (s)	
4a		117.75 (s)	
5	7.88 (d, 8.48)	127.82 (d)	C-4, C-7, C-8a
6	6.91 (d, 8.4)	116.1 (d)	C-5, C-4a, C-7
7		164.6 (s)	
8		141.21 (s)	
8a		158.64 (s)	
1'		123.33 (s)	
2'	7.52 (d, 2.0)	116.57 (d)	C-2, C-3', C-6'
3'		146.45 (s)	
4'		149.74 (s)	
5'	6.89 (d, 8.2)	116.41 (d)	C-3', C-1'
6'	7.42 (dd, 8.2, 2.0)	122.28 (d)	
8-OCH_3		60.34 (s)	C-8

Table 6. The NMR spectral data of 7,8,3',4'-tetrahydroxylflavone (**4**) (in DMSO-d_6, 125 MHz)

Position	Compound **4**		
	^{1}H (mult., J in Hz)	^{13}C (mult)	HMBC
2		146.25 (s)	
3	6.87 (s)	102.99 (d)	C-2, C-6'
4		174.43 (s)	
4a		115.49 (s)	
5	7.91 (d, 9.32)	127.51 (d)	C-4, C-7, C-8a
6	6.91 (d, 9.32)	116.25 (d)	C-5, C-4a, C-7
7		164.26 (s)	
8		138.58 (s)	
8a		158.53 (s)	
1'		124.39 (s)	
2'	7.67 (d, 2.0)	116.02 (d)	C-2, C-6'
3'		147.55 (s)	
4'		148.66 (s)	
5'	6.89 (d, 8.5)	115.97 (d)	C-3', C-1'
6'	7.53 (dd, 8.5, 2.0)	121.65 (d)	C-2, C-2', C-4'

Table 7. The NMR spectral data of lupinifolin (**5**) (in $CDCl_3$, 125 MHz)

Position	Compound **5**		
	^{1}H (mult., J in Hz)	^{13}C (mult)	HMBC
2	5.32 (dd, 3.1, 12.8)	78.4 (d)	C-3, C-4, C-1', C-6'
3ax	3.03 (dd, 12.8, 17.1)	43.1 (t)	C-4, C-8a
3eq	2.78 (dd, 3.2, 17.1)	43.1 (t)	-
4	-	196.5 (s)	-
4a	-	102.6 (s)	-
5	-	156.5 (s)	-
6	-	102.7 (s)	-
7	-	159.8 (s)	-
8	-	108.6 (s)	-
8a	-	159.3 (s)	-
1'	-	130.9 (s)	-
2'	7.29 (d, 8.2)	127.6 (d)	C-2, C-3'
3'	6.85 (d, 8.2)	115.6 (d)	C-1', C-4'
4'	-	155.8 (s)	-
5'	6.85 (d, 8.2)	115.6 (d)	C-1'
6'	7.29 (d, 8.2)	127.6 (d)	C-4', C-5', C-2
2''	-	78.1 (s)	-
3''	5.49 (d, 10.1)	125.9 (d)	-
4''	6.62 (d, 10.1)	115.4 (d)	C-5, C-6, C-7
5''	1.42 (s)	28.3 (q)	-
6''	1.41 (s)	28.2 (q)	C-2'', C-3''
1'''	3.19 (d, 7.6)	17.8 (t)	C-8, C-8a
2'''	5.13 (tp,7.6,1.5)	122.4 (d)	C-8, C-1'''
3'''	-	131.1 (s)	-
4'''	1.62 (s)	25.8 (q)	C-3''', C-5'''
5'''	1.62 (s)	25.8 (q)	C-2''', C-3''', C-4'''
HO-5	12.22 (s)	-	C-4, C-5
4'''	1.62 (s)	25.8 (q)	C-3''', C-5'''
5'''	1.62 (s)	25.8 (q)	C-2''', C-3''', C-4'''

Table 8. The ^{1}H and ^{13}C NMR spectral data of lupeol (**6**) (in $CDCl_3$, 125 MHz)

Position	Compound **3**	
	^{1}H (mult., J in Hz)	^{13}C (mult)
1		38.7 (t)
2		27.4 (t)
3	3.18 (dd, 10.8, 5.4)	78.9 (d)
4		38.8 (t)
5		55.2 (d)
6		18.2 (s)
7		34.2 (t)
8		40.7 (s)
9		50.4 (d)
10		37.1 (s)
11		20.8 (t)
12		25.0 (t)
13		37.9 (s)
14		42.8 (s)
15		27.4 (t)
16		35.5 (t)
17		42.9 (s)
18		48.2 (d)
19		47.9 (d)
20		150.9 (s)
21		29.8 (t)
22		39.9 (t)
23	0.94 (s)	27.9 (q)
24	0.73 (s)	15.3 (q)
25	0.80 (s)	16.1 (q)
26	1.02 (s)	15.9 (q)
27	0.92 (s)	14.5 (q)
28	0.76 (s)	17.9 (q)
29	4.66 /4.54 (d, 2.5 each m)	109.3 (t)
30	1.65 (s)	19.2 (q)

Table 9. The ^{13}C NMR spectral data of β-sitosterone (**1**) and stigmasta-5,22-dien-3-one (**2**) (in $CDCl_3$, 125 MHz)

Position	Compound **1**	Compound **2**
	^{13}C (mult)	^{13}C (mult)
1	38.1 (t)	38.1 (t)
2	30.3 (t)	30.0 (t)
3	211.9 (s)	211.9 (s)
4	42.8 (t)	42.8 (t)
5	139.4 (s)	139.4 (s)
6	116.9 (d)	119 .9 (d)
7	33.8 (t)	31.85 (t)
8	31.8 (d)	30.08 (d)
9	48.8 (d)	48.8 (d)
10	36.5 (s)	36.5 (s)
11	21.0 (t)	21.0 (t)
12	39.4 (t)	39.4 (t)
13	43.2 (s)	43.2 (s)
14	56.03 (d)	56.03 (d)
15	25.3 (t)	25.3 (t)
16	28.4 (t)	29.1 (t)
17	55.8 (d)	55.8 (d)
18	11.9 (q)	12.0 (q)
19	19.8 (q)	19.8 (q)
20	36.5 (d)	40.8 (d)
21	18.8 (q)	21.3 (q)
22	33.8 (t)	138.0 (t)
23	26.2 (d)	129.5 (t)
24	45.8 (d)	51.2 (d)
25	29.1 (d)	34.3 (d)
26	19.8 (q)	21.6 (q)
27	18.9 (q)	18.9 (q)
28	22.9 (d)	26.1 (t)
29	11.9 (q)	12.4 (q)

Table 10. Antibacterial activity of extracts and isolated compounds from two different collections of *Albizia myriophylla* Benth.

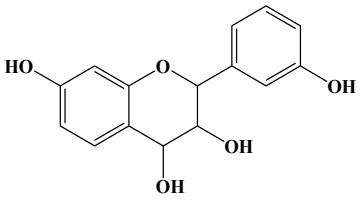
Extracts/ Compounds	Antibacterial activity (µg/ml)					
	S. mutans ATCC 25175		*S. aureus* ATCC 29213		*B. cereus* ATCC 11778	
	MIC	MBC	MIC	MBC	MIC	MBC
EtOH	500	1000	ND	ND	ND	ND
Hexane	250	500	ND	ND	ND	ND
CH_2Cl_2	1000	1000	ND	ND	ND	ND
EtOAc	>1000	>1000	ND	ND	ND	ND
n-BuOH	1000	1000	ND	ND	ND	ND
1	>250	ND	>250	ND	>250	ND
2	>250	ND	>250	ND	>250	ND
3	125	250	250	>250	250	>250
4	62.5	125	250	>250	250	>250
5	0.98	1.96	15.6	31.2	15.6	31.2
6	125	250	ND	ND	ND	ND
Mixture of **7** and **8**	125	250	ND	ND	ND	ND
Chlorhexidine	0.5	1	ND	ND	ND	ND
Penicillin G	0.0015	0.0015	0.05	0.05	0.5	0.5

ND = Not Determined

Table 11. Anticancer activity of isolated compounds from *Albizia myriophylla* Benth.

Extracts/Compounds	Cytotoxicity			
	KB cell		Vero cell	
	% Inhibition (50 µg/ml)	IC50 (µg/ml)	% Cell growth (50 µg/ml)	IC50 (µg/ml)
EtOH	< 50%	ND	> 50%	ND
Hexane	< 50%	ND	> 50%	ND
CH_2Cl_2	< 50%	ND	> 50%	ND
EtOAc	< 50%	ND	< 50%	ND
n-BuOH	< 50%	ND	> 50%	ND
1	< 50%	ND	> 50%	ND
2	< 50%	ND	> 50%	ND
3	< 50%	ND	> 50%	ND
4	99.72	12.55	> 50%	ND
5	95.18	4.95	< 50%	1.99
6	< 50%	ND	> 50%	ND
Mixture of **7** and **8**	< 50%	ND	> 50%	ND
Ellipticine	ND	1.96	ND	0.38
Doxorubicin	ND	0.916	ND	ND

ND = Not Determined



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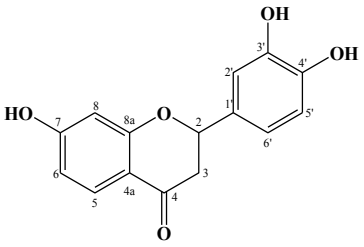

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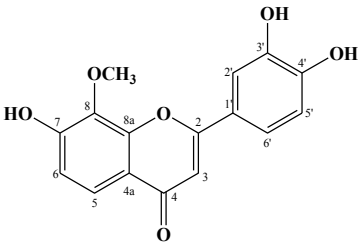

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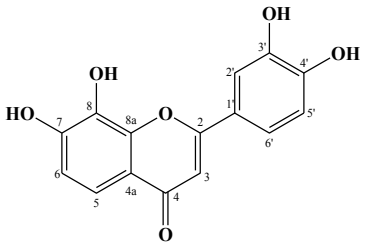

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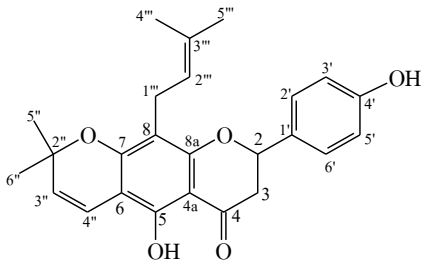

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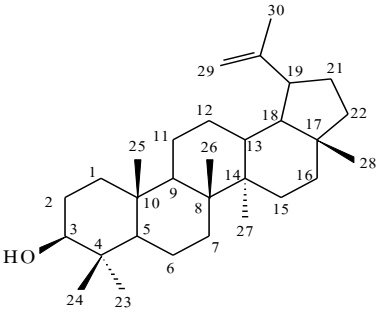
Figure 2 Chemical structures of compounds isolated from *A. myriophylla* wood



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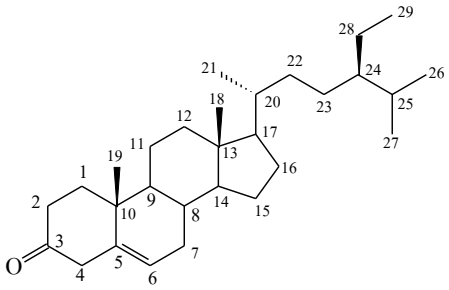

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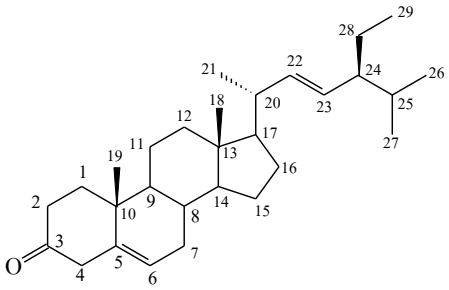

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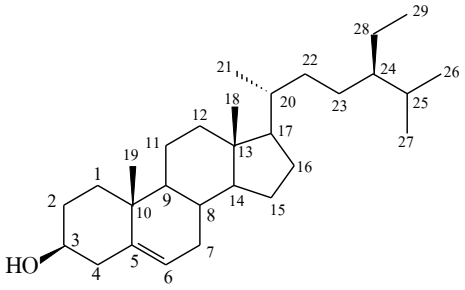

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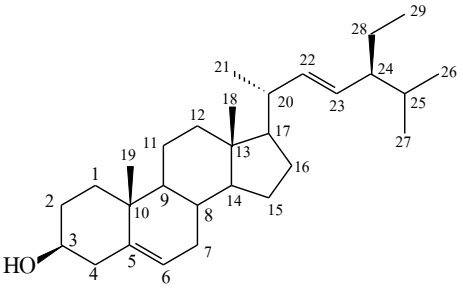

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Figure 2 Chemical structures of compounds isolated from *A. myriophylla* wood *(cont.)*

4. Conclusion

In our previous study, we reported the interesting *in vitro* anti-*S. mutans* activity of the ethanolic wood extract of *A. myriophylla*. This gave rise to identify the compounds responsible for its antibacterial activity. The aim of our study was, on the one hand, to evaluate the *in vitro* antibacterial activity and the cytotoxicity of *A. myriophylla* wood used in the treatment of dental caries, with the objective of promoting the development of improved traditional medicines and, on the other hand, to identify the active ingredients in this plant species. The chemical investigation of the wood of *A. myriophylla* resulted in the isolation of a rare flavan-3,4-diol **1** together with nine known compounds (**2—10**). Compounds **2—5** were isolated herein from the genus *Albizia* for the first time. All the isolated compounds were evaluated for their cytotoxic activity against KB cells whereas those belonging to flavonoids were also tested for their antibacterial activity against three pathogens of medically importance. Among the tested compound, lupinifolin (**5)** was found to have the hightest anticancer and antibacterial activities. The awareness of local communities should be enhanced by incorporating the traditional knowledge with scientific findings in order to promote cautious use of herbal medicine. This study reveals the preliminary scientific evidences for the traditional uses of *A. myriophylla* for dental caries. Our study also reported the significant result of the bacteriostatic property of *A. myriophylla* and its components against *S. mutans*, responsible for dental caries. Among the isolated compounds, lupinifolin (**5**) was found to be most active against *S. mutans*, and thus, it might contribute to the anticariogenic property of *A. myriophylla*. This result reveals the correlation between scientific evidence and the ethnomedical use of this plant against dental caries. Although lupinifolin (**5**) has been reported in several plants of Leguminosae including the root and aerial part of *Tephrosia lupinifolia* (Smalberger et al., 1974), the root of *Derris laxiflora* (Lin et al., 1991), the stem of *D. reticulata* (Mahidol et al, 1997), and the root of *Euchresta formosana* (Matsuura et al., 1995), there is no record in the genus *Albizia*. This study revealed for the first time the role of lupinifolin (**5)** as the main anti-compound in *A. myriophylla.* To best of our knowledge, this is the first report of lupinifolin (**5**) isolated from *A. myriophylla* wood and the antibacterial activity of this compound against cariogenic *S. mutans*. Considering the strong anti-*S. mutans* activity of lupinifolin (**5**), this flavonoid may have a potential for further development as natural anti-cariogenic agent. Further research is necessary to establish the antibacterial mechanisms of action of this compound against *S. mutans* or other cariogenic bacterial strains.

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Research Outputs

Publication

1) **Joycharat N**, Thammavong S, Limsuwan S, Homlaead S, Voravuthikunchai SP, Yingyongnarongkul BE, Dej-Adisai S, Subhadhirasakul S. 2013. Antibacterial substances from *Albizia myriophylla* wood against cariogenic *Streptococcus mutans*. *Arch Pharm Res*. 36(6): 723-730.

Proceeding

1) **Joycharat N**, Limsuwan S, Thammavong S, Yingyongnarongkul B, Voravuthikunchai SP. Chemical constituents and biological properties of *Albizia myriophylla* wood. The International Journal of Arts and Sciences (IJAS) Conference. Paris, France, 31 March - 3 April 2014.

Submitted manuscript

1) **Joycharat N**, Thammavong S, Yingyongnarongkul B, Limsuwan S, Voravuthikunchai SP. 2014. A new flavan-3,4-diol and bioactive constituents from the wood of *Albizia myriophylla* Benth. *Pharm Biol*. (MS: NPHB 2014-1159).

APPENDICES

Arch. Pharm. Res. (2013) 36:723–730
DOI 10.1007/s12272-013-0085-7

Archives of Pharmacal Research
www.springer.com/12272

RESEARCH ARTICLE

Antibacterial substances from *Albizia myriophylla* wood against cariogenic *Streptococcus mutans*

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Received: 31 August 2012 / Accepted: 2 February 2013 / Published online: 12 March 2013

Abstract *Albizia myriophylla* has been used for long by Thai traditional healers as an important ingredient herb in Thai herbal formulas for caries. In this study, three flavonoids lupinifolin (**6**), 8-methoxy-7,3′,4′-trihydroxyflavone (**7**), and 7,8,3′,4′-tetrahydroxyflavone (**8**), a triterpenoid lupeol (**3**) as well as four sterols β-sitosterone (**1**), stigmasta-5,22-dien-3-one (**2**), β-sitosterol (**4**), and stigmasterol (**5**) were isolated from *A. myriophylla* wood. The antibacterial activity of these compounds against *Streptococcus mutans* ATCC 25175 was performed using broth microdilution method. All compounds exhibited antibacterial activity against *S. mutans* with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) ranging from 1–256 and 2–256 μg/ml, respectively. Among the isolated compounds, lupinifolin (**6**) was found to be the most potent with MIC and MBC of 1 and 2 μg/ml, respectively. Lupinifolin (**6**) also showed a strong activity against ten clinical isolates of *S. mutans* with MIC and MBC ranging from 0.25–2 and 0.5–8 μg/ml, respectively. These results reported the bioactive ingredients of *A. myriophylla* which support its ethnomedical claims as well. Lupinifolin (**6**) may have a potential to be a natural anticariogenic agent.

Keywords *Albizia myriophylla* · Leguminosae · Lupinifolin · Flavonoid · *Streptococcus mutans* · Medicinal plant

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Introduction

Natural products of plant origin, especially those used in traditional medicine are shown to be a potential source of novel therapeutic agents for many diseases (Newman et al. 2003). Plant-derived compounds have been reported for a wide range of biological activities including preventing oral disease, especially dental caries (Katsura et al. 2001; Koo et al. 2003; Koo et al. 2010; Zheng et al. 2010). Plants can be rationally selected for biological studies based on their relevant ethnomedical uses. Antibacterial properties of plant compounds have been drawn increased attention (Katsura et al. 2001; Koo et al. 2003; Zheng et al. 2010; Limsuwan et al. 2011). Many scientific studies have reported the bioactive ingredients and medical properties of various medicinal plants in order to confirm their traditional uses (Song et al. 2006a, 2006b; Zheng et al. 2010). However, there are many herbal plants still required to be scientifically validated for ethnomedical claims.

The genus *Albizia*, belonging to Fabaceae (Leguminosae), consists of approximately 150 species distributed in Asia, Africa, Australia as well as tropical and subtropical

America. Several members of this genus have been widely used in Asian and African Traditional medicine for the treatment of cough, diarrhea, insomnia, lung diseases, malaria, and parasitic infection (Watt and Breyer-Brandwijk 1962; Bown 1995; Tang and Eisenbrand 2011). Many experimental evidences of this genus have exhibited a broad pharmacological effects, including anticancer (Liang et al. 2005; Zhang et al. 2011), antimalarial (Rukunga et al. 2007), immunomodulatory (Barua et al. 2000), antioxidant (Jung et al. 2004), antimicrobial (Lam and Ng 2011), anthelmintic (Egualea et al. 2011), and anti-inflammatory (Venkatesh et al. 2010) activities.

Albizia myriophylla, locally known as "Cha-em Thai", commonly used in the Thai traditional medicine as antitussive (root), tonic (wood), digestant (flower), menstrual stimulant (leaves), expectorant (wood and root), and demulcent (wood and root) (Medical Registration Division 1998). In addition, *A. myriophylla* is an important herb among Thai herbal formulas used against dental caries by Thai traditional healers in southern Thailand. Our previous work revealed that *A. myriophylla* possessed best activity against cariogenic *Streptococcus mutans* compared with other herbal components among Thai herbal formulas for dental carries. In this case, the wood ethanol extract of this plant species exhibited pronounced antibacterial activity against *S. mutans* with MIC of 3.9 μg/ml (Joycharat et al. 2012). A number of compounds classified as phenolic acids, iminosugars, triterpene saponins, lignan glycosides, and alkaloids (Ito et al. 1994; Yoshikawa et al. 2002; Asano et al. 2005; Panmei et al. 2007) were previously described from this species. During our literature review, we have found only one phytochemical investigation from its wood however, biological activities, particularly anticariogenic activity, of its wood components have not been investigated. As a part of our continued interest in anticariogenic property of *A. myriophylla* wood, the aim of the present study was to isolate and characterize the bioactive ingredients from the wood of *A. myriophylla*. *In vitro* antibacterial activity of the compounds isolated from *A. myriophylla* wood against *S. mutans* ATCC 25175 and ten clinical isolates was established.

Materials and methods

General

The following instruments were used: Mps, Electrothermal 9100; Optical rotation, Jasco P-1020 polarimeter; UV, Genesys 10 Series; IR, EQUINOX 55, Bruker FTIR; 1D- and 2D-NMR, FTNMR Bruker Advance 400 MHz; EI-MS, MAT 95 XL mass spectrometer (Thermo Finigan). Column chromatography (CC) was performed using silica gel (70–230 mesh, Merck) and Sephadex LH-20 (20–100 μm, Sigma). Thin-layer chromatography (TLC) was carried out on precoated sheets of silica gel 60 F254 (20 × 20 cm, 0.25 mm, Merck). Compounds were monitored by TLC sprayed with an anisaldehyde-sulfuric acid solution. All solvents for CC were of laboratory reagent grade and were purchased from commercial sources.

Plant materials

The wood of *Albizia myriophylla* was collected from the southern region of Thailand in June 2011. Botanical identification was performed by Dr. Oratai Neamsuvan, an ethnobotanist at the Faculty of Traditional Thai Medicine, Prince of Songkla University, where the voucher specimen (NJ0611) was deposited.

Extraction, fractionation, and isolation

The wood of *A. myrophylla* (2.7 kg) was dried, ground, and exhaustively extracted with ethanol (EtOH) at room temperature three times, filtered, and concentrated. The EtOH extract (98.89 g, 3.66 %) was resuspended in a mixture of methanol and water and then extracted with hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and *n*-butanol (BuOH), successively. Each filtrate was pooled and evaporated till dry under reduced pressure at 40 °C. The yield of hexane fraction (11.79 g, 0.44 %), CH_2Cl_2 fraction (14.13 g, 0.52 %), EtOAc fraction (12.11 g, 0.45 %), and BuOH fraction (16.73 g, 0.62 %) was obtained.

The hexane fraction (6.9 g) was subjected to CC using silica gel as adsorbent and eluted with acetone–CH_2Cl_2 gradient, followed by washing with MeOH. The fractions were then combined and numbered (IH–VIIIH) on the basis of their TLC profiles. Fraction IIH (728 mg) precipitated as white residues during the concentration process was further recrystallized from acetone to get a mixture (54.5 mg) of β-sitosterone (**1**) and stigmasta-5,22-dien-3-one (**2**). Fraction IIIH (481 mg) was subjected to silica gel CC and eluted with acetone–CH_2Cl_2 gradient. Column fractions eluted with 20 and 25 % acetone in CH_2Cl_2 afforded lupeol (**3**, 20.2 mg) and a mixture (9.1 mg) of β-sitosterol (**4**) and stigmasterol (**5**), respectively. Fraction VIIH (494 mg) was purified on silica gel column with 35 % acetone in hexane as an eluent to yield lupinifolin (**6**, 40.3 mg).

The dichloromethane fraction (12.36 g) was applied to silica gel CC using gradient elution with acetone–CH_2Cl_2, and finally washed down with MeOH. The fractions were then combined and numbered (ID–VID) according to their TLC patterns. Fraction IID (1.46 g) was purified on repeated silica gel chromatography (CC acetone–CH_2Cl_2; 40:60 and CC CH_2Cl_2–EtOAc; 40:60), followed by repeated gel filtration chromatography, using three successive

Sephadex LH-20 columns eluted with MeOH to yield 8-methoxy-7,3′,4′-trihydroxyflavone (**7**, 9.9 mg) and 7,8,3′,4′-tetrahydroxyflavone (**8**, 5.9 mg). The structures of all isolated compounds are shown in Fig. 1.

Lupinifolin (**6**)

Yellow needles; 119–120 °C; −9.7 (*c* 0.1, MeOH); UV (MeOH, nm) λ_{max}: 313; IR (Neat, cm^{-1}) ν_{max}: 3422, 2973, 2915, 1643, 1619, 1520, 1452, 1380, 1239, 1196, 1123; EIMS *m*/*z* 406.7 $[M]^+$; ^1H NMR ($CDCl_3$): δ 5.32 (1H, dd, $J = 12.8$, 3.1 Hz, H-2), 3.03 (1H, dd, $J = 17.1$, 12.8 Hz, H3α), 2.78 (1H, dd, $J = 17.1$, 3.1 Hz, H3β), 7.29 (2H, d, $J = 8.2$ Hz, H2′/H6′), 6.85 (2H, d, $J = 8.2$ Hz, H3′/H5′), 5.49 (1H, d, $J = 10.1$ Hz, H3″), 6.62 (1H, d, $J = 10.1$ Hz, H4″), 1.42 (1H, s, H5″), 1.41 (1H, s, H6″), 3.19 (2H, d, $J = 7.6$ Hz, H1‴), 5.13 (1H, t, $J = 7.6$, 1.5 Hz, H2‴), 1.62 (6H, s, H-4‴/H-5‴), 12.22 (1H, s, 5-OH); ^{13}C NMR ($CDCl_3$): δ 78.48 (C-2), 43.18 (C-3), 196.50 (C-4), 102.62 (C-4a), 156.53 (C-5), 102.79 (C-6), 159.89 (C-7), 108.61 (C-8), 159.33 (C-8a), 130.98 (C-1′), 127.69 (C-2′,6′), 115.60 (C-3′,5′), 155.83 (C-4′), 78.12 (C-2″), 125.98 (C-3″), 115.48 (C-4″), 28.39 (C-5″), 28.29 (C-6″), 17.80 (C-1‴), 122.43 (C-2‴), 131.10 (C-3‴), 25.80 (C-4‴,5‴).

8-Methoxy-7,3′,4′-trihydroxyflavone (**7**)

Yellow solid; 270–271 °C; UV (MeOH, nm) λ_{max}: 221, 250, 349; IR (Neat, cm^{-1}) ν_{max}: 3458, 3399, 3318, 3138, 2561, 2464, 2343, 1599, 1581, 1506, 1440, 1386, 1250, 1207, 1178, 1107, 1024; EIMS *m*/*z* 300.7 $[M]^+$; ^1H NMR (DMSO-d_6): δ 6.87 (1H, s, H-3), 7.88 (1H, d, $J = 8.48$ Hz, H-5), 6.91 (1H, d, $J = 8.48$ Hz, H-6), 7.52 (1H, d, $J = 2.0$ Hz, H-2′), 6.89 (1H, d, $J = 8.2$ Hz, H-5′), 7.42 (1H, dd, $J = 8.2$, 2.0 Hz, H-6′), 3.74 (3H, s, 8-OC<u>H</u>$_3$); ^{13}C NMR (DMSO-d_6): δ 157.89 (C-2), 103.13 (C-3), 176.51 (C-4), 117.75 (C-4a), 127.82 (C-5), 164.61 (C-6), 116.14 (C-7), 141.21 (C-8), 158.64 (C-8a), 123.33 (C-1′),

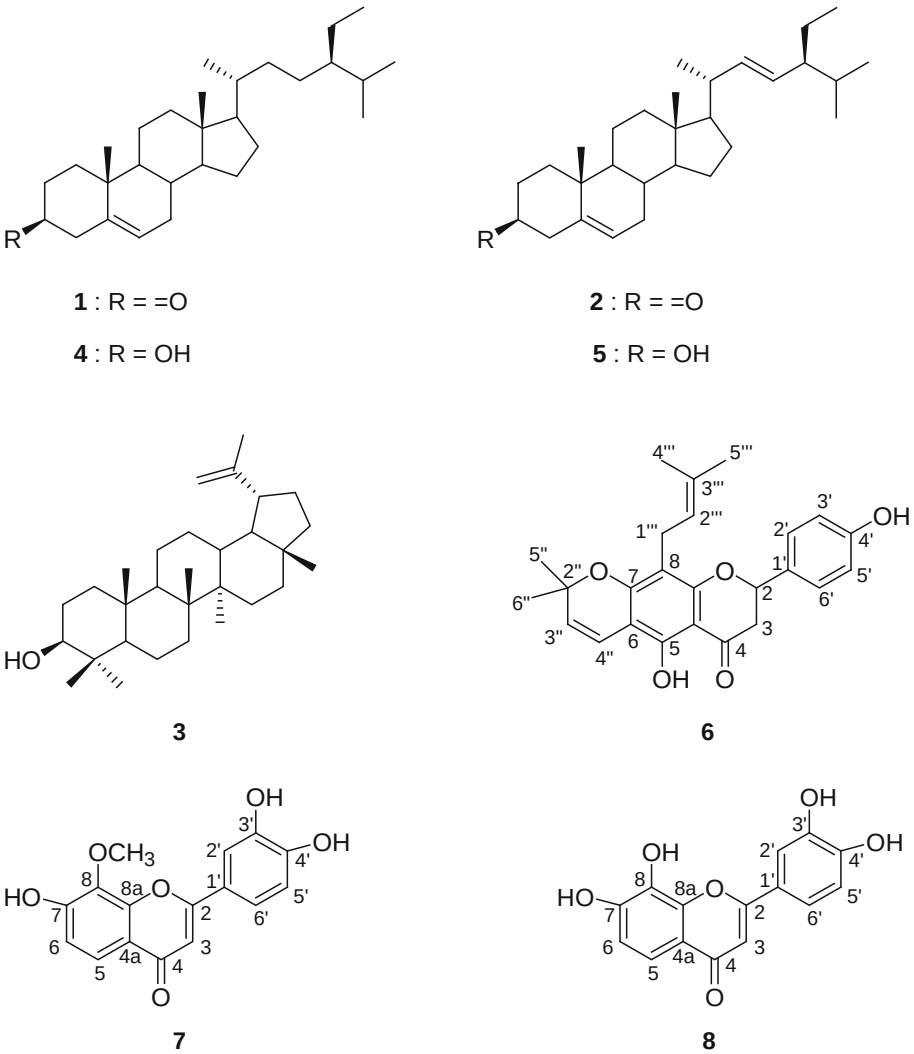

Fig. 1 Chemical structures of compounds isolated from *A. myriophylla* wood

116.57(C-2′), 146.45 (C-3′), 149.74 (C-4′), 116.41 (C-5′), 122.28 (C-6′), 60.34 ($O\underline{C}H_3$).

7,8,3′,4′-Tetrahydroxyflavone (**8**)

Yellow solid; UV (MeOH, nm) λ_{max}: 209; IR (Neat, cm^{-1}) ν_{max}: 3435, 2920, 2852, 1626, 1608, 1499, 1270, 1121, 1020; EIMS *m/z* 286.9 $[M]^+$; ^{1}H-NMR (DMSO-d_6): δ 6.87 (1H, s, H-3), 7.91 (1H, d, $J = 9.32$ Hz, H-5), 6.91 (1H, d, $J = 9.32$ Hz, H-6), 7.67 (1H, d, $J = 2.0$ Hz, H-2′), 6.89 (1H, d, $J = 8.5$ Hz, H-5′), 7.53 (1H, dd, $J = 8.5$, 2.0 Hz, H-6′); ^{13}C NMR (DMSO-d_6): δ 146.25 (C-2), 102.99 (C-3), 174.43 (C-4), 115.49 (C-4a), 127.51 (C-5), 164.25 (C-6), 116.26 (C-7), 138.58 (C-8), 158.53 (C-8a), 124.39 (C-1′), 116.02 (C-2′), 147.55 (C-3′), 148.68 (C-4′), 115.97 (C-5′), 121.65 (C-6′).

Bacterial strains and culture conditions

Ten clinical isolates of *S. mutans* (NPRC801–NPRC810) from carious lesions of patient's teeth were obtained from Department of Microbiology, Faculty of Science, Prince of Songkla University, Thailand. *Streptococcus mutans* ATCC 25175 was used as a reference strain. The bacterial cultures were stored in brain heart infusion (BHI) broth (Difco, France) containing 20 % glycerol at −80 °C until use. All isolates were cultured on BHI agar and incubated with 5 % CO_2 at 37 °C for 24 h.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

A modified broth microdilution method according to Clinical and Laboratory Standards Institute Guidelines (CLSI, 2009) was used to determine MIC and MBC of compounds isolated from *A. myriophylla*. The compounds were dissolved in 10 % dimethyl sulfoxide (DMSO, Merck, Germany) and two-fold dilutions were made. Suspension of *S. mutans* in BHI broth was prepared from the overnight broth culture. The bacterial suspension (180 µl) was mixed with the diluted test agents (20 µl) in 96 wells flat bottom microtiter plate (Corning Life Sciences, USA). The final bacterial cell concentration was approximately 5×10^5 cfu/ml. The final concentration of the test agents was ranging from 0.5-1024 µg/ml. Chlorhexidine (Fluka BioChemika, Switzerland) and 1 % DMSO were used as positive and negative controls, respectively. The microtiter plates were incubated with 5 % CO_2 at 37 °C for 24 h. The MIC was recorded as the lowest concentration that completely suppressed the visible growth. An aliquot (20 µl) from the broth with no growth was dropped onto BHI agar and incubated with 5 % CO_2 at 37 °C for 48 h. The MBC was defined as the lowest concentration of the test agents completely preventing bacterial growth. All tests were performed in triplicate independent experiments.

Results

Chromatographic separation of the hexane fraction of the ethanol extract of *A. myriophylla* wood yielded a flavanone lupinifolin (**6**), a triterpenoid lupeol (**3**) and four common sterols namely β-sitosterone (**1**), stigmasta-5,22-dien-3-one (**2**), β-sitosterol (**4**), and stigmasterol (**5**). The dichloromethane fraction of *A. myriophylla* was separated by extensive CC to get two flavones, 8-methoxy-7,3′,4′-trihydroxyflavone (**7**) and 7,8,3′,4′-tetrahydroxyflavone (**8**). The structures of these compounds were identified on the basis of their spectroscopic data as well as comparison with previously reported data (Reynolds et al. 1986; Mahidol et al. 1997; Herath et al. 2009; Yoon et al. 2011). All the isolated compounds were evaluated for their antibacterial activity against *S. mutans* using broth microdilution method.

Antibacterial activities of *A. myriophylla* components against *S. mutans* ATCC 25175 were presented as MIC and MBC values (Table 1). All tested compounds were effective against *S. mutans* ATCC 25175 with MIC and MBC values ranging from 1-256 and 2-256 µg/ml, respectively. However, MIC and MBC values of penicillin G and chlorhexidine were 0.0156 and 0.0156 µg/ml and 0.5 and 1 µg/ml, respectively. The compounds belong to triterpenoid and steroid classes exhibited almost same anti-*S. mutans* activity as those of 8-methoxy-7,3′,4′-trihydroxyflavone (**7**) and 7,8,3′,4′-tetrahydroxyflavone (**8**) with MIC and MBC values ranging from 64-128 and 128-256 µg/ml, respectively. Among the compounds isolated from *A. myriophylla*, lupinifolin (**6**) displayed the highest activity with MIC and MBC of 1 and 2 µg/ml, respectively.

On the basis of antibacterial activity of lupinifolin (**6**) against *S. mutans* ATCC 25175, it was selected to further

Table 1 The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Albizia myriophylla* components against *Streptococcus mutans* ATCC 25175

Chemical classes	Compounds	MIC (µg/ml)	MBC (µg/ml)
Flavonoids	**6**	1	2
	7	128	256
	8	64	128
Triterpenoid	**3**	128	256
Steroids	Mixture of **1** and **2**	128	256
	Mixture of **4** and **5**	128	256
Antimicrobial agents	Penicillin G	0.0156	0.0156
	Chlorhexidine	0.5	1

determine the antibacterial activities against clinical isolates of *S. mutans*. The MIC and MBC values of this compound against ten clinical isolates of *S. mutans* are shown in Table 2. Lupinifolin (**6**) exhibited anti-*S. mutans* activities with the MIC and MBC ranging from 0.25-2 and 0.5-8 μg/ml, respectively. The MIC and MBC values of chlorhexidine were 0.25-0.5 and 0.5-2 μg/ml, respectively. However, all test strains were susceptible to penicillin. The susceptibility breakpoint concentrations were in accordance with the interpretive standards for *Streptococcus* spp. viridans group recommended by the Clinical and Laboratory Standards Institute (CLSI 2009). Strains were classified for penicillin susceptibility as follows: susceptible, MIC ≤ 0.12 μg/ml; intermediately resistant, MIC 0.25–2 μg/ml and resistant, MIC ≥ 4 μg/ml.

Discussion

Dental caries are among the most common oral diseases throughout the world (Bowen 2002). It is hypothesized that bacterial infection is an important cause of dental caries (Tanzer 1995). The mutans streptococci, especially *S. mutans*, are mostly responsible for the induction of tooth decay (Loesche 1986). The antibacterial activity of *A. myriophylla* against *S. mutans* has been shown in previous studies. In particular, the ethanol extract of this species exhibited pronounced anti-*S. mutans* activity with MIC of 3.9 μg/ml (Joycharat et al. 2012). In clinical study, the mouthwash of *A. myriophylla* significantly reduce mutans streptococci counts in saliva of schoolchildren (Cholticha et al. 2006). In this study, we found that *A. myriophylla* components including three flavonoids (**6**–**8**), a triterpenoid **3**, and four steroids (**1**, **2**, **4**, **5**) have an antibacterial effect on *S. mutans* that associated with dental plaque formation and caries development. As almost all isolated compounds, β-sitosterone (**1**), stigmasta-5,22-dien-3-one (**2**), lupeol (**3**), β-sitosterol (**4**), stigmasterol (**5**), 8-methoxy-7,3′,4′-trihydroxyflavone (**7**), and 7,8,3′,4′-tetrahydroxyflavone (**8**), exhibited similar MICs against the organisms tested, structure–activity relationships do not seem to play a role here. However, among the three antibacterial flavonoids, lupinifolin (**6**), a prenylated flavonoid bearing hydrophobic moiety of an isoprenyl and a dimethylchromene groups attached to the flavanone skeleton, exhibited the strongest activity against *S. mutans* compared with those of two more polar flavones, 8-methoxy-7,3′,4′-trihydroxyflavone (**7**) and 7,8,3′,4′-tetrahydroxyflavone (**8**). Antibacterial or antiplaque activities against *S. mutans* of several compounds in flavonoid class such as apigenin (Koo et al. 2003), kurarinone (Chen et al. 2005), guaijaverin (Prabu et al. 2006), and patulitrin (Rhama and Madhavan 2011) have been reported previously. Furthermore, antibacterial activity against the diverse range of pathogenic bacteria is widely found in prenylated flavonoids (Nomura 1988). It has been postulated that their hydrophobic properties are especially suitable for protection against microorganisms because they may easily penetrate through the bacterial cell membrane (Chi et al. 2001). Flavonoids that have crossed the bacterial cell membrane could be possibly active against bacterial enzymes and proteins (Cushnie and Lamb 2011). Thus, the strong anti-*S. mutans* activity of lupinifolin (**6**) could be partly due to its lipophilic characteristic. Antibacterial mechanisms of action of various flavonoids including cytoplasmic membrane damage, inhibition of nucleic acid, cell wall, and cell membrane synthesis as well as inhibition of energy metabolism have been proposed previously (Cushnie and Lamb 2005). In addition, previous investigations have reported the antibacterial activity of triterpenoids and steroids containing plants against *S. mutans*

Table 2 The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of lupinifolin from *Albizia myriophylla* against ten clinical isolates of *Streptococcus mutans* compared with penicillin G and chlorhexidine

Streptococcus mutans	Lupinifolin (**6**)		Penicillin G		Chlorhexidine	
	MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)
NPRC 801	0.5	0.5	0.0156	0.0156	0.5	0.5
NPRC 802	0.5	0.5	0.0156	0.0156	0.5	0.5
NPRC 803	0.25	0.5	0.0156	0.0312	0.5	0.5
NPRC 804	1	1	0.0156	0.0156	0.5	1
NPRC 805	2	4	0.0156	0.0156	0.25	0.5
NPRC 806	0.5	1	0.0156	0.0156	0.5	1
NPRC 807	1	8	0.0156	0.0156	0.5	1
NPRC 808	2	8	0.0156	0.0312	0.5	2
NPRC 809	1	1	0.0156	0.0156	0.5	0.5
NPRC 810	1	1	0.0156	0.0312	0.5	1
ATCC 25175	1	2	0.0156	0.0156	0.5	1

(Song et al. 2006a; Zheng et al. 2010). However, our study is the first report on the MIC and MBC values of the triterpene lupeol (**3**) and other four common sterols, β-sitosterone (**1**), stigmasta-5,22-dien-3-one (**2**), β-sitosterol (**4**), and stigmasterol (**5**), against cariogenic *S. mutans*.

Several antiseptics and natural products such as chlorhexidine, sanguinarine, essential oils, tea extract, and propolis have been reported for their antibacterial activity against cariogenic bacteria. Some of these agents are incorporated into various commercial oral care products including toothpaste, mouthwash, chewing gum, and dental floss. However, there are some undesirable side effects following their use. Chlorhexidine, a gold standard antiseptic for an antiplaque, produces brown staining on the teeth and other oral surfaces which is very difficult to remove. Moreover, it increases the calculus formation and has an unpleasant taste (Eley 1999). Our results demonstrated that anti-*S. mutans* activities of lupinifolin (**6**) were almost comparable to chlorhexidine. Sanguinarine, a benzophenanthridine alkaloid from the rhizome of *Sanguinaria canadensis*, has been used in many oral care products. This alkaloid displayed broad spectrum and strong antibacterial activity against many oral pathogenic bacteria (Dzink and Socransky 1985; Eley 1999), however, it was reported to be associated with oral leukoplakia (Mascarenhas et al. 2001; Mascarenhas et al. 2002). The MIC value of sanguinarine against *S. mutans* was 1–32 μg/ml (Dzink and Socransky 1985; Park et al. 2003; Hwang et al. 2004; Chung et al. 2006). A number of essential oils from many plant species have been studied for their anti-*S. mutans* activity. The MIC ranges of essential oils, their fractions, and isolated compounds such as carvacal, eucalyptol, menthol, thymol, isoeugenol, and methyl salicylate were in range of 15.6–1,000 μg/ml (Hwang et al. 2004; Chung et al. 2006; Galvao et al. 2012). Green tea extracts, one of the most popular agents combined with oral hygiene products, have been demonstrated for anti-*S. mutans* activity with MIC ranging from 125–800 μg/ml (Park et al. 2003; Hwang et al. 2004; Cho et al. 2010; Awadalla et al. 2011; Tahir and Moeen 2011; Subramaniam et al. 2012). Several active compounds, mainly polyphenols, such as gallocatechin, epigallocatechin, and epigallocatechin gallate have been isolated (Sakanaka et al. 1989; Otake et al. 1991) with MIC values against *S. mutans* ranging from 250–1,000 μg/ml (Sakanaka et al. 1989). Propolis, a resinous bee product, has been investigated for anti-*S. mutans* activity both in vitro (Park et al. 1998a, 1998b; Ugur and Arslan, 2004; Castro et al. 2009; Liberio et al. 2009; Kouidhi et al. 2010; Liberio et al. 2011; Elbaz and Elsayad 2012) and in vivo (Duailibe et al. 2007). The MIC ranges of propolis against *S. mutans* were 17.5–100 μg/ml (Duarte et al. 2003; Kim et al. 2011). The compounds present in propolis such as sesquiterpene (*tt*-farnesol), flavone (baicalein), flavanones (pinocembrin, sakuranetin, isosakuranetin), and dihydroflavonol (pinobanksin-3-acetate) have been reported for their anti-*S. mutans* activity with MIC of 125–≥500 μg/ml (Koo et al. 2002). In comparison to the compounds reported above, the anti-*S. mutans* activity of lupinifolin (**6**) from the present study possesses better than or at least equivalent antibacterial activity. In addition, lupinifolin (**6**) did not produce any toxic signs or deaths via the oral route in mice at administered doses of 5 g/ kg and 60 mg/kg in acute and subacute toxicity studies, respectively (Chivapat et al. 2009). Therefore, oral use of lupinifolin (**6**) for various medicinal purposes could be considered as safe.

We have reported the antibacterial activity of *A. myriophylla* components against *S. mutans* ATCC 25175 and clinical isolates. Among the isolated compounds, lupinifolin (**6**) was found to be most active against *S. mutans*, and thus, it might contribute to the anticariogenic property of *A. myriophylla*. This result reveals the correlation between scientific evidence and the ethnomedical use of this plant against dental caries. Although lupinifolin (**6**) has been reported in several plants of Leguminosae including the root and aerial part of *Tephrosia lupinifolia* (Smalberger et al. 1974), the root of *Derris laxiflora* (Lin et al. 1991), the stem of *D. reticulata* (Mahidol et al. 1997), and the root of *Euchresta formosana* (Matsuura et al. 1995), there is no record in the genus *Albizia*. To best of our knowledge, this is the first report of lupinifolin (**6**) isolated from *A. myriophylla* wood and the antibacterial activity of this compound against cariogenic *S. mutans*. Considering the strong anti-*S. mutans* activity of lupinifolin (**6**), this flavonoid compound may have potential for further development as natural anti-cariogenic agents. Further research is necessary to establish the antibacterial mechanisms of action of this compound against *S. mutans* or other cariogenic bacterial strains.

Acknowledgments This paper was supported by research funds from Prince of Songkla University, the Thailand Research Fund, and the Office of the Higher Education Commission (MRG5580107 and TTM5406815).

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Chemical constituents and biological properties of *Albizia myriophylla* wood

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ABSTRACT

A new flavan-3,4-diol, 3,4,7,3′-tetrahydroxyflavan (**1**), together with eight known compounds including 1,2,3-benzenetriol (**2**), butin (**3**), 2″,3″-dihydroxylupinifolin (**4**), lupinifolin (**5**), 8-methoxy-7,3′,4′-trihydroxyflavone (**6**), 7,8,3′,4′-tetrahydroxyflavone (**7**), lupeol (**8**), and stigmasta-5,22-dien-3-one (**9**), and a mixture of β-sitosterol and stigmasterol were isolated from the wood of *Albizia myriophylla* Benth. Their structures were established by extensive spectroscopic analysis and by comparison of their NMR spectroscopic data with those reported in the literature. Compounds **4**–**9** exhibited antibacterial activity against *Streptococcus mutans* ATCC 25175 with MIC values ranging from 1.56–250 µg/ml. Flavanone **5** showed the best anti-*S. mutans* activity with MIC of 1.56 µg/ml, followed by its dihydroxy derivative **4** with MIC of 62.5 µg/ml. Both **4** and **5** also displayed marked antibacterial activity against *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 25923 with MIC values ranging from 12.5–250 µg/ml. In addition, compound **5** demonstrated significant cytotoxic activity against human epidermoid carcinoma (KB) cells with IC_{50} of 4.95 µg/ml.

Keywords: *Albizia myriophylla*, antibacterial activity, cytotoxicity, flavan-3,4-diol

A new flavan-3,4-diol and bioactive constituents from the wood of *Albizia myriophylla* Benth

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Abstract

Context: *Albizia myriophylla* Benth is a medicinal plant widely used in folk medicine in Thailand and other Asian countries for the remedy of many ailments.
Objective: To investigate the chemical compositions, antibacterial activity and cytotoxicity of *A. myriophylla* wood.
Materials and methods: The structure identification was established using spectroscopic methods. *In vitro* antibacterial activity against *Streptococcus mutans*, *Staphylococcus aureus*, and *Bacillus cereus* was performed using broth microdilution assay. The cytotoxicity against human oral epidermoid carcinoma (KB) cells was carried out using resazurin microplate assay. The lupinifolin content in *A. myriophylla* extracts was quantified by HPLC.
Results: A new flavan-3,4-diol, 3,4,7,3′-tetrahydroxyflavan (**1**), together with eight known compounds (**2**–**9**) were isolated from the wood of *A. myriophylla*. Compounds **4**–**9** exhibited anti-*S. mutans* activity, of which lupinifolin (**5)** was the most potent with MIC of 0.98 μg/mL, followed by its dihydroxy derivative **4** with MIC of 62.5 μg/mL. Compounds **4** and **5** also displayed marked antibacterial activity against *B. cereus* and *S. aureus* and showed strong cytotoxic activity against KB cells. The lupinifolin contents in ethanol extracts from two different collections of this plant originating from central and southern Thailand were 93.85 and 0.04 mg/g, respectively.
Conclusion: Compound **1** was isolated and characterized herein for the first time. This is the first report of compounds **2**–**4** from *Albizia* spp. Compounds **4** and **5** showed potent antibacterial and cytotoxic activities compared to other isolates. The anti-*S. mutans* activity of *A. myriophylla* extracts seems to have a relationship with the lupinifolin content.

Keywords: *Albizia myriophylla*, antibacterial activity, cytotoxicity, dental caries, flavan-3,4-diol, lupinifolin

Introduction

Plant-derived natural products, particularly those used in folk medicine play an important role as a source of medically valuable products in the prevention and treatment of many ailments (Newman, 2003). Bioactive secondary metabolites produced by plants have proven to possess a wide range of therapeutic effects for human diseases (Ferrazzano et al., 2011). Recently, the scientific interest in these metabolites has increased with the search of new therapeutic agents from plant source, due to the fact that currently used drugs are relatively toxic or, to a certain extent, ineffective by the spreading of resistance (Khaomek et al., 2008; Joycharat et al., 2013). In Thailand, medicinal plants are increasingly used by the traditional healers and herbalists for the treatment of various ailments (Joycharat et al., 2012; Neamsuvan et al., 2012). Plants can be rationally selected for phytochemical and biological studies based on their uses in traditional medicine (Ekuadzi et al., 2014). Some products from herbal plants have been used successfully as medicinal agents against a variety of disorders and had advantages over synthetic drugs due to their minimal side effects (Venkatesh et al., 2010).

Albizia species are greatly valued multipurpose tree legume. Many members of this genus are important in traditional medicines in some Asian and African countries (Kokwaro, 1976). *Albizia myriophylla* Benth (Thai local name "Cha Em Thai") belonging to the family Leguminosae is a medicinal plant, widely distributed in southeastern Asian countries (Yoshikawa et al., 2002). There have been reported in the Thai and the Vietnamese systems of traditional medicines that the stems of this plant

have been used to substitute for licorice due to their sweetness (Yoshikawa et al., 2002). *A. myriophylla* is among the most important medicinal plant which is used alone or in combination with other medicinal plants in various Thai herbal formulas, especially those for remedy of oral diseases including dental caries and aphthous ulcer (Neamsuvan et al., 2012). Many experimental evidences of *A. myriophylla* have exhibited a broad pharmacological effects, including antioxidant, anticandidal, and antibacterial activities (Steinrut et al., 2011; Joycharat et al., 2013). Regarding the biological activity of the isolated secondary metabolites from *A. myriophylla*, only one report is currently available. In our previous work, the flavanone lupinifolin isolated from the wood extract of that originating from southern Thailand exhibited very strong antibacterial activity against *Streptococcus mutans* ATCC 25175 and its clinical isolates, with MIC values ranging from 0.5–4 μg/mL (Joycharat et al., 2013). Preliminary antibacterial activity test of the ethanol extracts of *A. myriophylla* from two different areas in Thailand showed that the respective MIC values of those from central and southern parts of Thailand against *S. mutans* ATCC 25175 were 4 and 500 μg/mL. This interesting observation prompted our continued interest in bioactive substances from *A. myriophylla* wood, the aim of this study was to isolate and characterize the bioactive ingredients from the wood of two different collections of *A. myriophylla* originating from central and southern provinces of Thailand. Selected pharmacological assays based on its relevant folk remedies including *in vitro* antibacterial activity against three pathogenic bacterial strains, *S. mutans* ATCC 25175, *Staphylococcus aureus* ATCC 29213, and *Bacillus cereus* ATCC 11778, as well as cytotoxicity against human oral epidermoid carcinoma (KB) cells of the isolated compounds were established. The content of lupinifolin in ethanol extracts from two different collections

of *A. myriophylla* originating from central and southern Thailand was quantified using HPLC method and the anti-cariogenic activity against *S. mutans* of extracts from both collections of this plant was evaluated using broth microdilution assay.

Materials and methods

General

The following instruments were used: UV, Genesys 10 Series; IR, EQUINOX 55, Bruker FTIR; 1D- and 2D-NMR, FTNMR Bruker Advance 400 MHz; ESIMS, MAT 95 XL mass spectrometer (Thermofinigan), and HRESIMS, Bruker Daltonics microTOF; HPLC, Agilent Technologies. Column chromatography (CC) was performed using silica gel (70–230 mesh, Merck) and Sephadex LH-20 (20–100 μm, Sigma). Thin-layer chromatography (TLC) was performed on precoated sheets of silica gel 60 F254 (20x20 cm, 0.25 mm, Merck). Fractions and compounds were monitored by TLC sprayed with an anisaldehyde-sulfuric acid solution. Bacterial culture media, brain heart infusion (BHI) agar and tryptic soy broth (TSB) were purchased from Difco (Detroit, MI). Dimethyl sulfoxide (DMSO) was purchased from Merck (Darmstadt, Germany). The microtiter plate was purchased from Corning Life Sciences (Californis, USA). All solvents for CC were of laboratory reagent grade and were purchased from commercial sources.

Plant materials

The wood of two different collections of *Albizia myriophylla* Benth originating from southern and central regions of Thailand was collected during June 2011 to October 2012. Botanical identification was performed by Dr. Oratai Neamsuvan of the Faculty

of Traditional Thai Medicine, Prince of Songkla University where the voucher specimens (NJ0611 and NJ1012) were deposited.

Extraction and isolation

The details relating to the extraction method of *A. myriophylla* wood collected from southern region of Thailand were previously described (Joycharat et al., 2013). The wood of *A. myriophylla* originating from central region of Thailand (2.9 kg) was dried, ground, and exhaustively macerated with ethanol (EtOH) at room temperature three times, filtered, and concentrated to give a residue of EtOH extract (213 g, 7.34%). The EtOH extract was resuspended in a mixture of methanol and water and then partitioned with hexane, dichloromethane (CH_2Cl_2), and *n*-butanol (*n*-BuOH), successively. The yield of hexane extract (41.12 g, 1.42%), CH_2Cl_2 extract (32.17 g, 1.11%), and *n*-BuOH extract (34.53 g, 1.19%) was obtained.

For the isolation of compounds **1** and **3**, the CH_2Cl_2 extract (12.36 g) of *A. myriophylla* from southern Thailand was applied to silica gel CC using gradient elution with acetone-CH_2Cl_2, and finally washed down with MeOH. The fractions were then combined and numbered (ID-VID) according to their TLC patterns. Fraction IID (1.84 g) was further subjected to silica gel CC using gradient elution with acetone-Hexane, and finally washed down with MeOH. The fractions were then combined according to their TLC patterns to give five subfractions I-V. Subfraction III (750 mg) was further purified on silica gel CC using MeOH-$CHCl_3$ (10:90) as the eluent, followed by three successive Sephadex LH-20 columns eluted with MeOH to yield 3,4,7,3′-tetrahydroxyflavan (**1**, 5.7 mg). Fraction IIID (1.46 g) was further applied to silica gel CC using gradient elution with acetone-CH_2Cl_2, and finally washed down with MeOH. The fractions were then combined according to their TLC chromatograms to yield six

subfractions I-VI. Subfraction IV (127 mg) was purified on silica gel CC eluted with acetone-CH_2Cl_2 (40:60), followed by repeated gel filtration chromatography, using three successive Sephadex LH-20 columns eluted with MeOH to yield butin (**3**, 11.7 mg).

For the isolation of compounds **2** and **4**, the CH_2Cl_2 extract (20 g) of *A. myriophylla* from central Thailand was applied to silica gel CC using gradient elution with acetone-CH_2Cl_2, and finally washed down with MeOH. The fractions were then combined and numbered (ID-XID) according to their TLC profiles. Fraction VID (0.35 g) was further separated on repeated silica gel CC ((i) CC acetone-CH_2Cl_2 gradient and (ii) CC acetone-$CHCl_3$ gradient), followed by Sephadex LH-20 column eluted with MeOH to yield 2″,3″-dihydroxylupinifolin (**4**, 21.3 mg). Fraction VIID (0.29 g) was further purified over repeated silica gel CC ((i) CC acetone-CH_2Cl_2 gradient and (ii) CC $CHCl_3$-MeOH gradient) to afford 1,2,3-benzenetriol (**2**, 5.5 mg).

Additional quantities of lupinifolin (**5**, 98.6 mg), 8-methoxy-7,3′,4′-trihydroxyflavone (**6**, 4.3 mg), 7,8,3′,4′-tetrahydroxyflavone (**7**, 3.7 mg), lupeol (**8**, 14.8 mg), and stigmasta-5,22-dien-3-one (**9**, 13.4 mg) as well as a mixture of *β*-sitosterol and stigmasterol were obtained from the hexane or CH_2Cl_2 extracts of *A. myriophylla* from central Thailand according to the isolation method mentioned previously (Joycharat et al., 2013). The structures of all the isolated compounds are shown in Figure 1.

3,4,7,3′-tetrahydroxyflavan (**1**): brown amorphous solid; UV λ_{max} (MeOH) nm (log ε): 313, 208. IR ν_{max} (KBr) cm^{-1}: 3497, 3398, 3319, 1621, 1461, 1382, 1155, 1119, 829, 781. ^1H and ^{13}C NMR, HMBC data (DMSO-d_6): see Table 1. HRESIMS: $[M+Na]^+$ *m/z* 297.2593 (calcd. for $C_{15}H_{14}O_5$: 297.2597).

Antibacterial assay

Three bacterial strains including *Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 11778, and *Streptococcus mutans* ATCC 25175 were obtained from Department of Microbiology, Faculty of Science, Prince of Songkla University, Thailand. The bacterial cultures were stored in brain heart infusion (BHI) broth (Difco, France) with 20% glycerol at -80 °C until use. All bacterial strains were cultured on BHI agar and incubated separately with 5% CO_2 at 37 °C for 24 h for *S. mutans* ATCC 25175 or at 37 °C for 24 h for *S. aureus* ATCC 29213 and *B. cereus* ATCC 11778.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts and compounds isolated from *A. myriophylla* were determined using a modified broth microdilution method (CLSI, 2009). Briefly, the extracts and compounds were dissolved in 10% DMSO (Merck, Germany) and two-fold dilutions were prepared. Suspension of bacterial cultures in BHI broth was made from the overnight broth culture. The bacterial suspension (180 μL) was mixed with the diluted test agents (20 μL) in 96 wells microtiter plate (Corning Life Sciences, USA). The final bacterial concentration was approximately 5×10^5 CFU/mL. The final concentration of the tested extracts was ranging from 0.49-1000 μg/mL and that for the tested compounds was 0.49-250 μg/mL. Chlorhexidine and penicillin G were included as the positive controls. DMSO was used as a negative control. The lowest concentration of the tested agents required to completely inhibit bacterial cell growth after incubation at 37 °C for 24 h was recorded as MIC value. After MICs were recorded, an aliquot (100 μL) from the broth with no growth was pipetted and dropped onto agar plate and incubated with at 37 °C for 48 h. The lowest concentration of the tested agents required to completely preventing bacterial growth was reported as MBC value. Each assay was performed in triplicate.

Cytotoxicity assay

Cytotoxic activity of the isolated compounds against KB cells was carried out using the method described by Brien *et al*. (2000). In short, cells at a logarithmic growth phase were harvested and diluted to 2.2 x 10^4 cells/mL in growth medium. Successively, 5 µL of the tested compounds diluted in 5% DMSO and 45 µL of cell suspension were added into each well of 384-well plates. Plates were incubated at 37 °C in 5% CO_2 incubator for 3 days. After incubation period, 12.5 µL of 62.5 µg/mL resazurin solution was added into each well and the plates were incubated for a further 4 h. The absorbance was measured on a SpectraMax M5 multi-detection microplate reader (Molecular Devices, USA) at the excitation and emission wavelengths of 530 nm and 590 nm. The reference compounds were ellipticine and doxorubicin. Water and 0.5% DMSO were used as negative controls. The activity is expressed as 50% inhibitory concentration (IC_{50}). The IC_{50} values of more than 20 µg/mL are considered inactive.

Quantification of lupinifolin content by high-performance liquid chromatography (HPLC)

Standard stock solution of lupinifolin (100 mg/L) was prepared in methanol (HPLC grade, Merck). The extract solutions were prepared separately by dissolving 300 mg of individual extracts in 1 mL of methanol (HPLC grade, Merck) and vigorously shaken. Lupinifolin content in the ethanol extracts of *A. myriophylla* was analyzed by HPLC–UV technique. The HPLC system (Agilent Technologies, Germany) consists of binary pump, auto injector, column thermostat, and variable wavelength detector. The stationary phase was a Zorbax Eclipse XDB-C8 column (4.6 x 250 mm, 5 µm) connected to a cartridge guard column (Agilent Technologies). The mobile phase was an isocratic elution system consisting of methanol (HPLC grade, Merck) and 15%

glacial acetic acid (AR grade, Lab scan) in DI water (90:20, v/v%). The mobile phase was filtered through a 0.45μm nylon membrane (Whatman, Sigma) and degassed by sonicating prior to use. The flow rate of mobile phase was kept at 1.2 mL/min. throughout the analysis and the detector wavelength was set at 254 nm. The injection volume of each sample was 5 μL. The column temperature was maintained at 25 °C. The chromatograms were processed using Chemstation software (Agilent Technologies). The peak of lupinifolin in the samples was characterized by direct comparison of its retention time with that of standard solution. The linearity of the analysis method was evaluated by regression analysis. Standard stock solution of lupinifolin was diluted with methanol (HPLC grade, Merck) and six concentrations ranging from 1–50 mg/L were prepared. The calibration curve was obtained by plotting the value of peak area against each concentration of standard solution. The regression equation was given as $y = ax + b$, where y and x correspond to peak area and concentration of standard, respectively.

Results

A new flavan-3,4-diol, 3,4,7,3′-tetrahydroxyflavan (**1**), and a flavanone butin (**3**) were isolated from the CH_2Cl_2 extract of *Albizia myriophylla* Benth collected from southern Thailand. A phenolic compound, 1,2,3-benzenetriol (**2**), and a prenylated flavonoid, 2″,3″-dihydroxylupinifolin (**4**), were isolated from this plant species originating from central Thailand. Furthermore, five other known compounds including lupinifolin (**5**), 8-methoxy-7,3′,4′-trihydroxyflavone (**6**), 7,8,3′,4′-tetrahydroxyflavone (**7**), lupeol (**8**),

and stigmasta-5,22-dien-3-one (**9**) as well as a mixture of *β*-sitosterol and stigmasterol were obtained from both collections of *A. myriophylla* in this study as well.

3,4,7,3′-tetrahydroxyflavan (**1**) was obtained as a brown amorphous solid and had a molecular formula $C_{15}H_{14}O_5$, as deduced from HRESIMS at *m*/*z* 297.2593 $[M + Na]^+$ (calcd. *m*/*z* 297.2597). Its IR spectrum showed absorption bands at 3423, 3398, and 3319 cm^{-1}, suggesting the presence of hydroxy function. The ^1H NMR spectrum (Table 1) of **1** showed one typical trisubstituted benzene system at δ_H 7.28 (1H, dd, *J* = 8.5,1.0 Hz, H-5), 6.28 (1H, d, *J* = 2.5 Hz, H-6), and 6.25 (1H, d, *J* = 2.5 Hz, H-8), and four additional aromatic protons at δ_H 7.02 (1H, d, *J* = 2.0 Hz, H-2′), 6.42 (1H, m, H-4′), 6.41 (1H, m, H-5′), and 7.12 (1H, d, *J* = 8.5 Hz, H-6′). The ^{13}C NMR spectrum (Table 1) of **1**, exhibited 12 aromatic carbons of the two aromatic rings and three oxymethine carbon signals at δ_C 78.11 (d, C-2), 66.69 (d, C-3), and 68.66 (d, C-4). The assignment of the four hydroxy groups to the flavan nucleus of **1** was accomplished by a detailed analysis of HMBC spectral data (Table 1). These findings revealed that compound **1** was a flavan-3,4-diol derivative with the OH-substitution on positions C7 in ring A and C3′ in ring B of the flavonoid. The 2,3-*cis* configuration of **1** was determined according to a broad singlet at δ_H 4.89 for H-2 and its corresponding oxymethine carbon signal at δ_C 78.11 of C-2 (Wu et al., 2012). The low value of $J_{3,4}$ 3.0 Hz indicated the 3,4-*cis* configuration of **1** (Porter and Foo, 1982). Thus, the structure of compound **1** was proposed to be 3,4,7,3′-tetrahydroxyflavan (**1**).

Antibacterial and anticancer activities of various extracts including those of EtOH, Hexane, CH_2Cl_2, and *n*-BuOH as well as the isolated compounds **1**–**9** from *A. myriophylla* were presented in Tables 2 and 3. In our continuing study on the anti-*Streptococcus mutans* activity of *A. myriophylla*, the MIC values of all tested extracts

from both collections of *A. myriophylla* against *S. mutans* ATCC 25175 were ranging from 2−1000 μg/mL, of which the CH_2Cl_2 extract from central collection of this plant was found to be the most active with the same MIC and MBC of 2 μg/mL. Considering some phenolic compounds, those belonging to flavonoids have been described to have moderate or strong antibacterial activity (Cushnie et al., 2005). Thus, antibacterial activities against three pathogens including *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 29213, and *S. mutans* ATCC 25175 were carried out with compounds **1**−**7** using broth microdilution method. The results showed that flavanone **5** exhibited the best anti-*S. mutans* activity with MIC of 0.98 μg/mL, followed by its dihydroxy derivative **4** with MIC of 62.5 μg/mL. Both **4** and **5** also displayed pronounced antibacterial activity against *B. cereus* ATCC 11778 and *S. aureus* ATCC 29213 with MIC and MBC values ranging from 15.63−125 and 31.25−250 μg/mL, respectively. Compounds **6** and **7** showed moderate activity against the three tested bacterial strains with MIC values ranging from 62.5−250 μg/mL, whereas compounds **1**−**3** exhibited no antibacterial activity against the tested pathogens at the highest concentration tested of 250 μg/mL (Table 2). Besides antibacterial activity, the isolated compounds **1**−**9** were evaluated for their cytotoxicity against KB cells using resazurin microplate assay. Both prenylated flavonoids **4** and **5** demonstrated significant cytotoxic activity against KB cells, of which **5** was found to be more active with IC_{50} of 4.95 μg/mL. The other tested compounds were inactive against KB cells at the highest concentrations tested (Table 3).

The quantity of lupinifolin (**5**) present in the ethanol extracts of two different collections of *A. myriophylla* was determined using HPLC method. Both samples were assessed in triplicate to guarantee the reproducibility of the quantitative result. As can

be seen from Table 4, the lupinifolin contents in ethanol extracts of *A. myriophylla* originating from central and southern Thailand were 93.85 and 0.04 mg/g (crude EtOH extract), respectively. It was interested that the ethanolic wood extract of *A. myriophylla* from central collection, that contained a high amount of lupinifolin, showed anti-cariogenic activity with MIC value against *S. mutans* much better than that from southern collection.

Discussion

The isolation and characterization of a new flavan-3,4-diol from the wood of *Albizia myriophylla* Benth in this study supported the previous literature of the general observation of the members of flavan-3,4-diols to be the wood components of plants (Stafford and Lester, 1985). Previous studies have demonstrated that flavan-3,4-diols were involved as intermediate substances in flavan-3-ol biosynthesis catalyzed by reductase reaction (Drewes and Roux, 1965). The known compounds were successively identified as 1,2,3-benzenetriol (**2**), butin (**3**), 2″,3″-dihydroxylupinifolin (**4**), lupinifolin (**5**), 8-methoxy-7,3′,4′-trihydroxyflavone (**6**), 7,8,3′,4′-tetrahydroxyflavone (**7**), lupeol (**8**), stigmasta-5,22-dien-3-one (**9**), and a mixture of β-sitosterol and stigmasterol by analysis of their NMR spectra and by comparison with the data reported in literature (Porter and Foo, 1982; Reynolds et al., 1986; Mahidol et al., 1997; 2002; Herath et al., 2009). Compounds **2**–**4** were firstly isolated herein from the genus *Albizia*.

The differences in phytochemical composition of *A. myriophylla* collected from sampling sites differing in environmental conditions may result in the variation in the efficacy of its folk remedies for oral diseases. It is interesting to note that all the

extracts of *A. myriophylla* from central Thailand demonstrated stronger activity against *Streptococcus mutans* ATCC 25175 as compared to those from southern Thailand. In previous study, the EtOH extract of this plant species purchased from herb shop in southern province of Thailand exhibited pronounced anti-*S. mutans* activity with MIC of 3.9 μg/mL (Joycharat et al., 2012). Similarly, the EtOH extract of *A. myriophylla* from central Thailand in this study showed very good activity against *S. mutans* ATCC 25175 with MIC of 4 μg/mL. The significant differences in the antibacterial results of the extracts of this plant species collected from two geographically distinct regions could be explained by many factors which may affect the variation of their bioactive components including genetic and environment (Wahyuni et al., 2013). The variation of plant secondary metabolites under differing geographical conditions has been described previously (Zhao et al., 2012). Anti-*S. mutans* activity of several compounds isolated in this work, especially lupinifolin (**5**), has been reported previously (Joycharat et al., 2013). However, antibacterial activity of **5** against some other bacterial strains of medically importance including *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 29213 has not been reported so far. A diverse range of antibacterial mechanisms of action of various flavonoids including inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function , and inhibition of energy metabolism have been well documented (Cushnie and Lamb, 2005). Antibacterial activity against various kind of pathogenic bacteria has been widely described for the members of prenylated flavonoids (Khaomek et al., 2008; Sutthivaiyakit et al., 2009). Previous research has revealed that combining the flavonoid nucleus with a lipophilicity of the prenyl group may result in the great potential for biological activity (Smejkal, 2014). In this study, the higher antibacterial activity of lupinifolin (**5**) compared with its

closely related dihydroxy derivative **4** or other weak and inactive compounds tested could be partly associated with the more lipophilic property of its structure. The strongly cytotoxic activity of the members of flavonoids possesing an A-ring prenyl substitution against several cancer cell lines has been reported previously (Groweiss et al. 2000). Moreover, the decrease in cytotoxic activity of prenylated flavonoids that have an oxidative change in the linear prenyl group has been observed and reported (Groweiss et al. 2000) . The presence of an A-ring prenyl side chain at C-8 and the oxidative modification of the prenly residue in compound **5** may play an importance role in its cytotoxicity. Both **4** and **5** were previously reported to be active against P-388 leukemia cell (Mahidol et al., 1997; 2002). For compound **5**, it was also observed to possess strong to moderate activity against KB, BC, and NCI-H187 cancer cell lines (Innok et al., 2009). The cytotoxic activity of **5** against KB cells in this study was in accordance with that previously described (Sutthivaiyakit et al., 2009). We reported, for the first time, the cytotoxicity of compound **4** against KB cells, with IC_{50} of 12.55 μg/mL.

Recently, researchers have recognized that there are many phytochemicals affecting the healing properties of herbal plants. The inconsistency of the active constituents affects the variation in the chemical quality of medicinal plant materials and may result in the reproducible therapeutic effect of their herbal products. The variation of bioactive phytochemicals in plants is common and often associated with geographical parameter. In this work, the variation of anti-*S. mutans* property of *A. myriophylla* extracts seems to be affected by sampling sites differing in geographical origins. Chemically both collections of *A. myriophylla* originating from central and southern parts of Thailand showed uniformity in presence of compounds **5**–**9** in the wood.

However, among the compounds studied, lupinifolin (**5**) which has the highest antibacterial and anticancer activities is most relevant with the bioactivities of this plant. As the batch-to-batch consistency of herbal products could be affected by many factors for instance genetic and ecological factors, the quality control has become an important role to support the clinical applications (Shi et al., 2014). Herbal products contain normally a complex mixture of phytochemicals therefore selection of appropriate marker compounds that are relevant to the biological activities is currently an important approach for their quality control (Shi et al., 2014). Similarly, in order to facilitate efficient standardization procedure for the future development of *A. myriophylla* extract as herbal product, it is essential to define chemical markers responsible for its therapeutic activity. Our finding revealed that the quantity of lupinifolin in ethanolic wood extracts of *A. myriophylla* from two different geographical origins had a relationship with the anti-*S.mutans* activity in dose dependent manner. Thus, lupinifolin may be an effective marker compound that is representative of the bioactivities of *A. myriophylla*.

Conclusion

The chemical investigation of the wood of *Albizia myriophylla* Benth resulted in the isolation of a new flavan-3,4-diol **1** together with eight known compounds (**2**–**9**). Compounds **2**–**4** were isolated herein from the genus *Albizia* for the first time. All the isolated compounds were evaluated for their anticancer activity against KB cells whereas those belonging to phenolic group were also tested for their antibacterial activity against some pathogens of medically importance. Compounds **4** and **5** showed

significantly anticancer and antibacterial activities. The variation of anti-cariogenic activity against *Streptococcus mutans* of *A. myriophylla* extracts seems to be affected by its sampling locations that were different in geographical origins. The HPLC analysis of lupinifolin (**5**) content in the ethanol extracts of two different collections of *A. myriophylla* revealed the relationship between the amount of **5** and the anti-*S.mutans* activity in dose dependent manner. Lupinifolin may be a marker compound responsible for the bioactivities of *A. myriophylla*. Further research on correlations between lupinifolin content and biological activities among geographically distinct plant populations would allow obtaining information relating to the quality control of *A. myriophylla* wood.

Acknowledgments

This work was supported by research grant for General Researcher, The Annual Income Budget of Prince of Songkla University (TTM570374S) and by TRF-CHE Research Grant for New Scholar (MRG5580107) funded by the Thailand Research fund (TRF) and the Commission on Higher Education (CHE).

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Table 1. The NMR spectral data of 3,4,7,3′-tetrahydroxyflavan (**1**) (in DMSO-d_6, 100 MHz)

Position	Compound **1**		
	^{1}H (mult., J in Hz)	^{13}C (mult.)	HMBC
2	4.89 (br s)	78.11 (d)	C3, C1′
3	4.86 (m)	66.69 (d)	C-2, C-4
4	4.56 (d, 3.0)	68.66 (d)	C-3, C4a
4a		115.72 (s)	
5	7.28 (dd, 8.5,1.0)	128.41 (d)	C-4a, C-6
6	6.28 (d, 2.5)	107.83 (d)	C-5, C-7
7		157.10 (s)	
8	6.25 (d, 2.5)	101.52 (d)	C7, C9
8a		154.90 (s)	
1'		115.24 (s)	
2'	7.02 (d, 2.0)	114.79 (d)	C-2, C-4'
3'		144.57 (s)	
4'	6.41 (m)	114.21 (d)	C-3', C-5'
5'	7.12 (d, 8.5)	130.26 (d)	C-4', C-6'
6'	6.42 (m)	118.47 (d)	C-1', C-5'

Table 2. Antibacterial activity of extracts and the isolated compounds from two different collections of *A. myriophylla*

Extracts/ Compounds	Antibacterial activity (μg/mL)					
	S. mutans ATCC 25175		*S. aureus* ATCC 29213		*B. cereus* ATCC 11778	
	MIC	MBC	MIC	MBC	MIC	MBC
Central Thailand						
EtOH	4	4	ND	ND	ND	ND
Hexane	16	32	ND	ND	ND	ND
CH_2Cl_2	2	2	ND	ND	ND	ND
n-BuOH	4	16	ND	ND	ND	ND
2	>250	ND	>250	ND	>250	ND
4	62.5	>250	125	>250	62.5	62.5
5	0.98	1.96	15.63	31.25	15.63	31.25
Southern Thailand						
EtOH	500	1000	ND	ND	ND	ND
Hexane	250	500	ND	ND	ND	ND
CH_2Cl_2	1000	1000	ND	ND	ND	ND
n-BuOH	1000	1000	ND	ND	ND	ND
1	>250	ND	>250	ND	>250	ND
3	>250	ND	>250	ND	>250	ND
6	125	250	250	>250	250	>250
7	62.5	125	250	>250	250	>250
8	125	250	ND	ND	ND	ND
9	125	250	ND	ND	ND	ND
Chlorhexidine	0.5	1	ND	ND	ND	ND
Penicillin G	0.0015	0.0015	0.05	0.05	0.5	0.5

ND = Not Determined

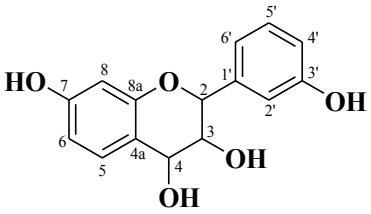
Table 3. Anticancer activity of the isolated compounds from *A. myriophylla*

Compounds	Cytotoxicity (KB cell)	
	% Inhibition (50 μg/mL)	IC50 (μg/mL)
1	< 50%	ND
2	< 50%	ND
3	< 50%	ND
4	99.72	12.55
5	95.18	4.95
6	< 50%	ND
7	< 50%	ND
8	< 50%	ND
9	< 50%	ND
Ellipticine	ND	1.00
Doxorubicin	ND	0.916

ND = Not Determined

Table 4. Lupinifolin content and anti-cariogenic activity against *S. mutans* ATCC 25175 of two different collections of *A. myriophylla*

Sources of EtOH extracts	Lupinifolin (mg/g)	MIC (μg/mL)
Central collection	93.85 ± 0.07	4
Southern collection	0.04 ± 0.0006	500



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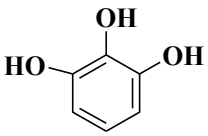

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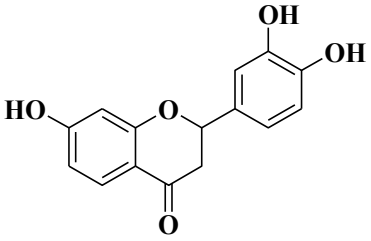

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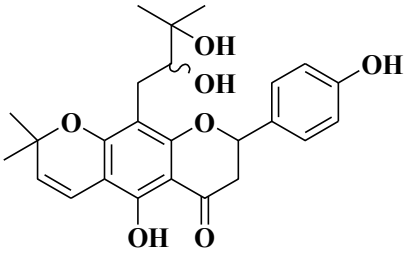

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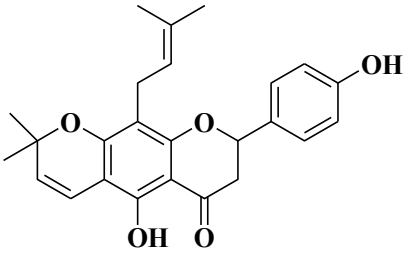

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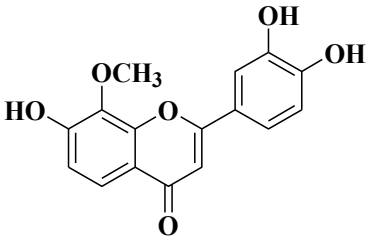

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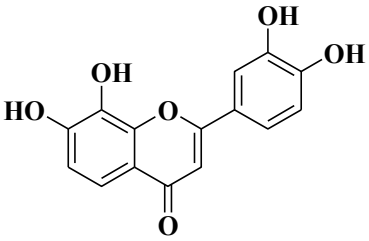

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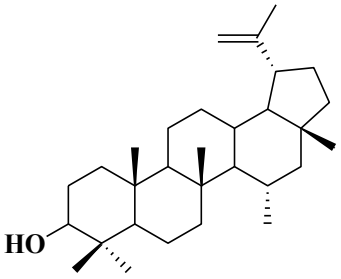

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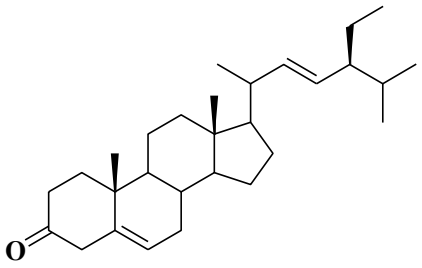

9

Figure 1. Chemical structures of isolated compounds from *Albizia myriophylla* Benth.