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RESEARCH ARTICLE

Anti-*Streptococcus mutans* efficacy of Thai herbal formula used as a remedy for dental caries

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Abstract

Context: Traditional knowledge of herbal remedies plays an important role in the search for more effective alternative treatment of a variety of disorders. The ethnobotanical surveys in southern Thailand have revealed that 35 Thai herbal formulas have been used by Thai traditional healers against dental caries. However, the scientific evaluation to confirm their rational uses is scarce.

Objective: To test *in vitro* anti-*Streptococcus mutans* activity of Thai herbal formulas used against dental caries (THF-DC).

Materials and methods: Ethanol extracts of Thai herbal formulas were evaluated for antibacterial activity against *S. mutans*. Agar disc diffusion was employed as a preliminary screening assay, followed by broth microdilution assay to assess minimum inhibitory concentration (MIC). Furthermore, medicinal plants contained in the most active THF-DC were investigated for their phytochemicals.

Results: Eleven THF-DC extracts exhibited clear inhibition zones of 7.0–22.5 mm against *S. mutans*. Subsequent determination of their MIC revealed that the formula containing *Albizia myriophylla* Benth. (Leguminosae), *Alpinia galanga* (L.) Willd. (Zingiberaceae), *Avicennia marina* (Forssk.) Vierh. (Acanthaceae), and *Ocimum sanctum* L. (Lamiaceae) was the most active, with MIC at 250 µg/mL. Among these medicinal plants, *A. myriophylla* gave the strongest activity with MIC at 3.9 µg/mL, followed by *A. marina* with MIC at 62.5 µg/mL. Various classes of bioactive phytochemicals including tannins, flavonoids, alkaloids, and terpenoids were found in these extracts.

Conclusion: Anti-*S. mutans* activity of THF-DC extracts was established. Further investigations may be required for the isolation and chemical characterization of the active ingredients in *A. myriophylla*.

Keywords: *Albizia myriophylla*, medicinal plants, *Streptococcus mutans*, traditional medicine

Introduction

Traditional knowledge of herbal medicines plays an important role in the search for novel chemotherapeutic agents. The uses of medicinal plants for primary health care have steadily increased worldwide in recent years. Traditional and herbal remedies offer a valuable alternative treatment in developing countries where traditional therapies are considered cheap and readily available (WHO, 2002). 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 (Dubey et al., 2004). The studies of single medicinal plants have been receiving attention in various areas of health research; much information has been documented as regards their various biological effects (Ifesan et al., 2009; Limsuwan et al., 2009). However, the scientific evaluation to ensure the rational use of herbal formulas containing two or more different single medicinal plants in traditional medicine is scarce. Thai traditional medicine is one of the most valuable

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heritages of Thai culture. The usage of Thai herbal formulas 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 (Bodeker & Burford, 2007). The ethnobotanical surveys in the southern part of Thailand have revealed that 35 Thai herbal formulas have been used by Thai traditional healers for treating toothache caused by dental caries (Prakob et al., 2004). These formulas are currently not marketed products and no scientific data is available for their traditional uses.

Recent research has focused on the potential of natural products in the prevention of oral disease, particularly plaque-related diseases, such as dental caries (Limsong et al., 2004; Xiao et al., 2007; Furiga et al., 2008; Sharma et al., 2009). Dental caries is an infectious disease caused by interactions of multiple factors including diet constituents, cariogenic bacteria, and the composition of the tooth enamel (Samaranayake et al., 2002). Streptococci especially, *Streptococcus mutans*, have been implicated as a principal etiological organism of dental caries (Rawashdeh et al., 2008). The ability of *S. mutans* to colonize the tooth surface and to initiate plaque formation by synthesis of water-insoluble glucan from sucrose using the cooperative actions of glucosyltransferase is believed to be associated with the cariogenicity of this pathogen (Samaranayake et al., 2002). Consequently, the prevention of caries is aimed mainly at the eradication of *S. mutans*, the primary microbial cause of tooth decay in humans.

The present work was carried out in order to examine the anticariogenic activity against *S. mutans* of the crude extracts from 35 Thai herbal formulas used against dental caries (THF-DC) by Thai traditional healers in southern region of Thailand. Additionally, the active plant extracts in some Thai herbal formulas were investigated for their phytochemical components.

Materials and methods

Plant materials

The majority of selected plant species (66 genera/75 species in 39 family; see Table 1) and their corresponding parts used (see Table 1) e.g. leaves (L), stem bark (SB), flower (Fl), bulb (Bl), rhizome (Rh), root (R), fruit (F), seed (S), Gall (G), and whole plant (Wp) were collected from different area of southern region of Thailand during summer months from March to May, 2010. Some plant materials were purchased from an herb shop in Songkhla, Thailand. Botanical identification was performed by Dr. Oratai Neamsuvan, an ethnobotanist at the Faculty of Traditional Thai Medicine, Prince of Songkla University, where the voucher specimens (S. Pratumwan 001–075; for some species see Table 2) are deposited.

Preparation of extracts

Each single plant material was rinsed thoroughly and cleaned from foreign matter with tap water, oven-dried at 60°C, grounded with an electric-grinder, weighed, and stored in a dry place at room temperature (25–30°C). For every 200 g of dry THF-DC, each dried, powdered single plant contained in the corresponding THF-DC (see Table 1) was combined together at a same ratio. Each THF-DC was separately macerated with 95% ethanol for 7 days at room temperature. The ethanol extracts were filtered and evaporated to dryness under reduced pressure at 45°C in vacuum rotary evaporator. The ethanol extracts of single plants contained in the active THF-DC were prepared similarly to those of THF-DC.

Bacterial strains and culture conditions

The cariogenic bacterial strains tested in this study were *S. mutans* ATCC 25175 and GS-5. The culture was grown in tryptic soy broth (TSB) (Difco, Sparks, MD) at 37°C for 24 h and incubated in CO_2 incubator. Glycerol stock of the bacteria was kept at –80°C.

Antibacterial assay

Agar disc diffusion method

The extracts were dissolved in dimethyl sulfoxide (DMSO) to give a concentration of 250 mg/mL and then 10 µL of the extracts were loaded onto sterile filter paper disc (diameter 6 mm) (CLSI, 2009a). Three to five colonies of overnight culture on Brain Heart Infusion (BHI) agar were transferred to TSB and incubated for 24 h. The bacterial suspension was adjusted to McFarland standard No. 0.5 and spread over Petri dishes containing BHI agar. The discs were placed on the cultured plates and incubated in a 5% CO_2 at 37°C for 24 h. DMSO and the standard drug recommended for Gram-positive bacteria, penicillin G (10 U/disc), were used as controls. All experiments were performed in duplicate. The diameters of the inhibition zones (mm) were measured and the mean was recorded.

Determination of minimum inhibitory concentration

The THF-DC extract that demonstrated inhibition zones against both *S. mutans* ATCC 25175 and GS-5 was subjected to minimum inhibitory concentration (MIC) evaluation using broth microdilution method (CLSI, 2009b). Serial 2-fold dilutions of the extracts were diluted in 1% DMSO to the concentrations at 0.49–1000 µg/mL. They were mixed with BHI broth in the ratio of 1:8 in 96-well sterile microtiter plates. Twenty microlitre of an overnight culture, containing approximately 10^5 cfu/mL, were applied to BHI broth supplemented with the THF-DC extracts. The microtiter plates were incubated in a 5% CO_2 at 37°C for 24 h. The MIC evaluation of plant extracts was performed in the same manner as for that of THF-DC extract. The controls comprised pure growth medium and inoculated growth medium without tested extract. MICs were observed in triplicate as the lowest concentration of THF-DC extracts or plant extracts that produced a complete suppression of colony visible growth.

Table 1. Antibacterial activity against two strains of *Streptococcus mutans* of 35 Thai herbal formulas used against dental caries by disc diffusion and broth microdilution methods.

THF-DC No.	Herbal components	Inhibition zone (mm) GS-5	Inhibition zone (mm) ATCC 25175	MIC (μg/mL)
1	*Allium sativum* (Bl), *Alpinia galanga* (Rh), *Avicennia marina* (L)	≤6.0	≤6.0	nd*
2	*Acmella oleracea* (Wp), *Avicennia marina* (L)	≤6.0	≤6.0	nd
3	*Avicennia marina* (L), *Uncaria gambir* (L)	≤6.0	≤ 6.0	nd
4	*Albizia myriophylla* (SB), *Alpinia galangal* (Rh), *Avicennia marina* (L), *Ocimum sanctum* (L)	7.0	8.0	250
5	*Avicennia marina* (L), *Borussus flabellifer* (L), *Corypha lecomtei* (L), *Pouzolzia pentandra* (L)	≤6.0	7.0	nd
6	*Avicennia marina* (L), *Azadirachta indica* (SB), *Mimusops elengi* (F), *Syzygium aromaticum* (F), *Tamarindus indica* (SB)	≤6.0	8.0	nd
7	*Tamarindus indica* (SB), *Phyllanthus emblica* (SB), *Pithecellobium dulce* (SB)	≤6.0	≤6.0	nd
8	*Avicennia marina* (L), *Combretum quadrangulare* (SB), *Pouzolzia pentandra* (L), *Tinospora crispa* (Wp)	≤6.0	≤6.0	nd
9	*Avicennia marina* (L), *Mimusops elengi* (F), *Pouzolzia pentandra* (L)	≤6.0	≤6.0	nd
10	*Hopea odorata* (SB), *Ficus religiosa* (W)	≤6.0	≤6.0	nd
11	*Barringtonia acutangula* (F), *Quercus infectoria* (G), *Uncaria gambir* (L)	≤6.0	7.5	nd
12	*Capparis micracantha* (SB), *Clerodendrum petasites* (SB), *Harrisonia perforata* (SB), *Tiliacora triandra* (SB)	≤6.0	≤6.0	nd
13	*Avicennia marina* (L), *Curcuma longa* (Rh)	7.0	≤6.0	nd
14	*Avicennia marina* (L), *Holarrhena pubescens* (L)	≤6.0	≤6.0	nd
15	*Avicennia marina* (L), *Ocimum sanctum* (L), *Sapium indicum* (L)	10.0	7.0	>1000
16	*Avicennia marina* (L), *Pouzolzia pentandra* (L), *Pterocarpus indicus* (SB)	8.0	8.5	1000
17	*Pouzolzia pentandra* (L), *Syzygium cumini* (L)	9.5	≤6.0	nd
18	*Avicennia marina* (L), *Psidium guajava* (L), *Senna alata* (L)	≤6.0	≤6.0	nd
19	*Avicennia marina* (L), *Euphorbia neriifolia* (L), *Senna alata* (L)	8.0	≤6.0	nd
20	*Euphorbia neriifolia* (L), *Terminalia bellerica* (F), *Terminalia chebula* (F)	9.0	≤6.0	nd
21	*Avicennia marina* (L), *Annona sguamosa* (L), *Carica papyya* (R)	9.5	≤6.0	nd
22	*Avicennia marina* (L), *Cocos nucifera* (R)	≤6.0	≤6.0	nd
23	*Coccinia grandis* (L), *Rhinacanthus nasutus* (L)	≤6.0	≤6.0	nd
24	*Angelica dahurica* (R), *Abroma augusta* (S), *Aquilaria crassna* (SB), *Euphorbia antiquorum* (SB), *Lawsonia inermis* (SB), *Syzygium aromaticum* (Fl), *Oroxylum indicum* (SB), *Piper nigrum* (F), *Piper retrofractum* (Fl), *Terminalia triptera* (SB), *Zingiber cassumunar* (Rh), *Zingiber officinale* (Rh)	8.0	7.5	1000
25	*Abroma augusta* (S), *Amomum krevanh* (L), *Anethum graveolens* (S), *Angelica dahurica* (R), *Angelica sinensis* (R), *Artemisia indica* (Rh), *Artemisia vulgaris* (Rh), *Asclepias curassavica* (S), *Atractylodes lancea* (R), *Cladogynos orientalis* (SB), *Eurycoma longifolia* (L), *Foeniculum vulgare* (S), *Hyptis suaveolens* (L), *Ligusticum chuanxiong* (R), *Lawsonia inermis* (S), *Myristica fragrans* (F), *Raphistemma pulchellum* (SB), *Ocimum basilicum* (L), *Phyllanthus emblica* (SB), *Piper nigrum* (F), *Piper retrofractum* (Fl), *Syzygium aromaticum* (Fl), *Terminalia chebula* (F), *Zingiber officinale* (Rh)	7.0	≤6.0	nd
26	*Areca catechu* (F), *Avicennia marina* (L), *Phyllanthus emblica* (SB), *Streblus asper* (SB)	≤6.0	≤6.0	nd
27	*Areca catechu* (F), *Nicotiana tabacum* (L), *Piper betle* (L)	≤6.0	≤6.0	nd
28	*Avicennia marina* (L), *Peperomia pellucida* (L), *Phyllanthus emblica* (SB), *Streblus asper* (SB), *Syzygium aromaticum* (Fl)	20.5	22.5	1000
29	*Acmella oleracea* (Wp), *Avicennia marina* (L), *Piper betle* (L), *Streblus asper* (SB), *Syzygium aromaticum* (Fl)	18.5	19.5	1000
30	*Avicennia marina* (L), *Areca catechu* (F), *Phyllanthus emblica* (SB), *Zingiber officinale* (Rh)	19.0	16.5	1000
31	*Abroma augusta* (S), *Avicennia marina* (L), *Anethum graveolens* (S), *Asclepias curassavica* (S), *Foeniculum vulgare* (S), *Lawsonia inermis* (S), *Pouzolzia pentandra* (L), *Streblus asper* (SB), *Syzygium aromaticum* (Fl)	8.0	10.5	>1000
32	*Avicennia marina* (L), *Amomum krevanh* (L), *Areca catechu* (F), *Flacourtia indica* (SB), *Pouzolzia pentandra* (L), *Syzygium aromaticum* (Fl)	≤6.0	≤6.0	nd
33	*Avicennia marina* (L), *Acmella oleracea* (Wp), *Murraya paniculata* (L)	15.0	16.5	1000
34	*Acmella oleracea* (Wp), *Avicennia marina* (L), *Plectranthus amboinicus* (L), *Pouzolzia pentandra* (L)	16.5	18.0	500
35	*Acmella oleracea* (Wp), *Areca catechu* (F), *Avicennia marina* (L), *Streblus asper* (SB)	14.0	17.0	1000
Pen G		52	42	nd

*Not done.
MIC, minimum inhibitory concentration.

Table 2. Ethnopharmacological, phytochemical, and minimum inhibitory concentration data of active medicinal plants in Thai herbal formulas used against dental caries.

Scientific name	Voucher number (Local name)	Part used	Thai traditional uses	Phytochemical	MIC (μg/mL)
Acmella oleracea (L.) R.K. Jansen (Compositae)	S. Pratumwan 003 (Pukkad huawan)	Whole plant	As diuretic, laxative, against fever, headache, toothache	Tannins, flavonoids, anthraquinones	1000
Albizia myriophylla Benth. (Leguminosae)	S. Pratumwan 006 (Cha-em thai)	wood	Antitussive, expectorant, demulcent, tonic	Tannins, flavonoids, alkaloids, terpenoids	3.9
Alpinia galanga (L.) Willd. (Zingiberaceae)	S. Pratumwan 001 (Kha)	Rhizome	Carminative, against skin disease	Tannins, flavonoids, anthraquinones, alkaloids, terpenoids	500
Avicennia marina (Forssk.) Vierh. (Acanthaceae)	S. Pratumwan 068 (Samae thale)	Leaves	Blood and circulation, diuretic, against menstrual disorders	Tannins, flavonoids, anthraquinones, alkaloids, terpenoids	62.5
Ocimum sanctum L. (Lamiaceae)	S. Pratumwan 005 (Kapao dang)	Whole plant	Carminative, antidiarrhoea, antiemetic	Tannins, flavonoids, anthraquinones, alkaloids	1000
Plectranthus amboinicus (Lour.) Spreng. (Lamiaceae)	S. Pratumwan 063 (Neam huseu)	Leaves	Otitis acuta	Tannins, flavonoids, anthraquinones	1000
Pouzolzia pentandra (Roxb.) Benn. (Urticaceae)	S. Pratumwan 009 (Kob-chanang-dang)	Leaves	Diuretic, against menstrual disorders, skin inflammation, periodentitis, cancer	Tannins, flavonoids, anthraquinones, alkaloids, terpenoids	1000

MIC, minimum inhibitory concentration.

Preliminary phytochemical components analysis

The chemical components of the active extracts were preliminarily determined for the presence of terpenoids, alkaloids, tannins, flavonoids, and anthraquinones as previously described (Houghton & Raman, 1998).

Test for terpenoids

Liebermann-Burchard test was used for the presence of terpenoids. Each extract was dissolved in dry chloroform in a dry test tube. Several drops of acetic anhydride were added, followed by two drops of concentrated sulfuric acid and then mixed carefully. Dark pink or red coloration of the solution indicated the presence of terpenoids.

Test for alkaloids

Dragendorff's reagent was used for the presence of alkaloids. A drop of ethanol extract was spotted on a small piece of thin layer chromatography precoated plate (TLC plate) and the plate was sprayed with modified Dragendorff's reagent. Orange coloration of the spot indicated the presence of alkaloids.

Test for tannins

Ferric chloride reagent was used for the presence of tannins. Each extract was dissolved in 70% ethanol in a dry test tube. Two drops of 10% alcoholic ferric chloride solution were added. An indication of a positive test was dark blue or greenish grey coloration of the solution.

Test for flavonoids

Shinoda test was used for the presence of flavonoids. In 2–3 mL of ethanol extract, a piece of magnesium ribbon and 0.5 mL of concentrated hydrochloric acid were added. Orange to margenta bubble solution indicated the presence of flavonoids in the extract.

Test for anthraquinones

The Brontrager's test was performed for the presence of anthraquinones. In 10 mL of KOH-soluble extract, 1 mL of 3% hydrogen peroxide solution was added and boiled for 10 min. After cooling, the mixture was acidified with acetic acid and filtered. The filtrate was extracted with 10 mL of chloroform. In the chloroform layer, 5 mL of ammonium hydroxide was added. A positive reaction for the presence of anthraquinones was evidenced by the formation of a red color in the alkaline layer.

Results

The ethanol extracts of all THF-DC were screened for their antibacterial activity against *S. mutans* ATCC 25175 and GS-5 by disc diffusion method. The objective of this screening test was to exclude the THF-DC extracts that showed no activity (zone of inhibition ≤6.0 mm) against either *S. mutans* ATCC 25175 or GS-5. It was apparent from the measured values of diameters of inhibition zones (Table 1) that 11 THF-DC extracts were effective against both strains of *S. mutans* with inhibition zones ranged from 7.0 to 22.5 mm. These 11 THF-DC extracts were further determined for their MIC values against *S. mutans* ATCC 25175 using broth microdilution test. As can be seen from Table 1, the most active formula was THF-DC4 with MIC value of 250 μg/mL, followed by THF-DC34 with MIC value of 500 μg/mL. The other active formulas were THF-DC16, THF-DC24, THF-DC28, THF-DC29, THF-DC30, THF-DC33, and THF-DC35 with the same MIC value of 1000 μg/mL. Both of the tested formulas including THF-DC15 and THF-DC31 showed no activity at the tested concentration. Subsequent MIC evaluation of seven medicinal plants contained in two of the most active formulas (THF-DC4 and THF-DC34) against *S. mutans* ATCC 25175 has revealed that *Albizia myriophylla* Benth. (Leguminosae) showed the strongest activity with MIC value of 3.9 μg/mL, followed by *Avicennia marina* (Forssk.) Vierh. (Acanthaceae) with MIC value of 62.5 μg/mL (Table 2). Percentage yield of ethanol extract was 2.42 for the former while that of the latter was 1.52. The other five medicinal plant species including *Acmella oleracea* (L.) R.K. Jansen (Compositae), *Alpinia galanga* (L.) Willd. (Zingiberaceae), *Ocimum sanctum* L. (Lamiaceae), *Plectranthus amboinicus* (Lour.) Spreng. (Lamiaceae), and *Pouzolzia pentandra* (Roxb.) Benn. (Urticaceae) were moderately active against the tested bacterial strain, with MIC values ranged from 500 to 1000 μg/mL. Phytochemical screening revealed the presence of tannins and flavonoids in all the tested active medicinal plant extracts (Table 2). Alkaloids and terpenoids were also present in the ethanol extract of the most active plant, *A. myriophylla*. All chemical classes including alkaloids, flavonoids, tannins, terpenoids, and anthraquinones were observed in that of *A. marina*, the second most active medicinal plant.

Discussion

Dental caries is among the most common and costly oral infectious diseases worldwide (Moynihan & Petersen, 2004). It has been well established that *S. mutans* is the major etiological agent in dental caries (Samaranayake et al., 2002). This ethnopharmacological investigation of the Thai herbal formulas used against dental caries in Thailand allowed the identification of seven medicinal plants with potential anticariogenic property. The medicinal plants contained in the effective formulas were active against the tested organism with MIC ranged from 3.9 to 1000 μg/mL. These results suggest the presence of phytochemical components with good antibacterial potency in the plant extracts. This work demonstrates the correlation between scientific observations and the traditional recommendations of the tested Thai herbal formulas against dental caries. Furthermore, our study also indicated a medicinal plant species, *A. myriophylla*, which may contain promising antibacterial compounds. Among the single medicinal plant extracts tested, the wood extract of this species showed the best activity against *S. mutans* ATCC 25175 with MIC value of 3.9 μg/mL. The present study was an initial trial of the plants that used only one reference strain. Further studies should be carried out in order to determine the antimicrobial activity of the plants against other reference strains and against clinical isolates. Many active plant species have probably minimal side effects as these plants are wildly used as dietary intake as well as in the Thai folk medicine. Toxicity tests are also important, even if these plants have already been used in traditional medicine. Previous toxicity studies were carried out on *A. galanga* (Qureshi et al., 1992), *A. marina* (Ali & Bashir, 1998), *O. sanctum* (Mondal et al., 2009), and *P. amboinicus* (Pillai et al., 2011). In these cases, their *in vivo* toxicity properties have been investigated in acute and subchronic toxicity tests using oral or intraperitoneal routes of administration. Least adverse reactions have been found for these plants while there is no reported toxicity study for the plants including *A. myriophylla*, *A. oleracea*, and *P. pentandra*. However, for *A. myriophylla*, its closely related species, *Albizia lebbeckoides* was shown previously to have no toxicity by oral or subcutaneous routes (Makkhasmit et al., 1971).

A. myriophylla is a tree, widely distributed in southeastern Asian countries. In Thailand, it is commonly called "Cha-em Thai". This plant species is used in Thai traditional medicine (Table 2) as antitussive (root), expectorant (fruit and root), demulcent (root), and tonic (wood) (MRD, 1998). The species exhibited pronounced antibacterial activity against *S. mutans* with MIC value of 3.9 μg/mL. Our present finding is in accordance with previous clinical study, in which a mouthwash of *A. myriophylla* was shown to significantly reduce mutans streptococci counts in saliva of schoolchildren (Cholticha et al., 2006). Previous phytochemical studies have revealed the presence of 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). Some of these chemical groups such as phenolics and alkaloids have been established to be the major phytochemical classes of antibacterial metabolites (Samy & Gopalakrishnakone, 2008).

A. marina is a small evergreen tree planted in many coasts, creeks, and islands throughout Thailand. The wood of which is used in Thai traditional medicine as diuretic, for blood and circulation conditions, as well as against menstrual disorders (MRD, 1998). In the United Arab Emirates, leaves are traditionally used for toothache (El-Ghonemy, 1993). The effectiveness of using the leaves of *A. marina* for toothache is probably related to the bacteriostatic property of this species against *S. mutans* (MIC 62.5 μg/mL). Hydrocarbons, sterols, triterpene alcohols, iridoid glycosides, and significant amount of carbohydrate, lipids, and proteins were identified previously in the leaves of this species (Sharaf et al., 2000).

A. galanga, a perennial herb found commonly in Thailand, possesses several therapeutic properties. The rhizome of this species is used topically for the treatment of skin disease caused by bacterial and fungal infection (MRD, 1998). Additionally, this plant part is also used for its carminative properties (MRD, 1998). Previous studies have demonstrated that various extracts from the rhizome of this species exhibited a broad spectrum of antibacterial activity against Gram-positive bacteria (Burt, 2004; Sunilson et al., 2009). A diverse group of antibacterial agents had been isolated from this species comprising of terpenoids, flavonoids, and coumarin derivatives (Verma et al., 2011). The potential of anti-*S. mutans* activity was supposed to be partly due to a bioactive phenolic component of *A. galanga*, 1′-acetoxychavicol acetate, which was described previously (Vuddhakul et al., 2007).

A. oleracea is largely grown for ornamental and medicinal purposes. “Pukkad Huawan” is a Thai local name often used for this plant species. The species is used for the remedy of several diseases (Table 2) such as toothache (leaves, flowers, roots), headache (leaves and roots), and fever (whole plant). Roots of this species are also used as diuretic and laxative (MRD, 1998). The applications of this species for antitoothache formulation and in mouthwashes have been described previously (Prachayasittikul et al., 2009). A number of bioactive constituents had been isolated from the species such as phenolics, coumarins, and triterpenoids (Prachayasittikul et al., 2009).

O. sanctum is a medicinal plant found commonly in various areas of Thailand. Every part of the “Kapao dang” (Thai local name) can be used in herbal remedies to treat a variety of conditions (MRD, 1998). Leaves are used in Thai traditional medicine for the treatment of digestive disorders. Root decoction is used as an antidiarrheal medicine. The whole plant is also recommended as carminative (MRD, 1998). The plant species has been reported previously to possess antibacterial activity against *Staphylococcus aureus* (Rahman et al., 2010). This species is known as a rich source of essential oils which were shown previously to be effective against both Gram-positive and Gram-negative bacteria. Besides oil, alkaloids, glycosides, saponins, and tannins have also been reported from this plant species (Mondal et al., 2009).

P. amboinicus is a large and widespread perennial herb found throughout Thailand. Leaves of this species are prescribed for acute edematous otitis acuta (MRD, 1998). The species has also been recorded for the treatment of nervous disorders including epilepsy, convulsions, and meningitis (Lukhoba et al., 2006). The activity observed from the leaf extract of *P. amboinicus* in this study could be due to various flavonoids such as quercetin, apigenin, and luteolin, which were isolated previously from this species and frequently shown to be implicated in antibacterial activity (Devi & Periyanayagam, 2010).

P. pentandra, Thai name “Kob-chanang-dang”, is used in Thai herbal remedies for the treatment of several diseases. Stem barks are traditionally used against menstrual disorders, skin inflammation, periodentitis, cancer, and as diuretic (MRD, 1998). A previous study has scientifically reported the antibacterial activity from stems and leaves of this species against *S. aureus* and *Escherichia coli*. Various bioactive chemical classes including phenolics, triterpenes, and phytosteryl glycosides have been described from these plant parts (Trakulsomboon et al., 2006).

The preliminary phytochemical analysis showed that the active medicinal plant extracts contain various classes of phytochemicals including alkaloids, flavonoids, terpenoids, and tannins (Table 2). This could be attributed to the diversity of chemical constituents in the individual plants. These compounds were demonstrated previously to have bacteriostatic activities and serve as a natural defense of certain plants against phytopathogenic bacteria (Clark, 1981; Mather & Gonzalez, 1982). Tannins and flavonoids were the phytochemical components found in all of the active extracts tested. It could be possible that these bioactive compounds are responsible for the observed growth-inhibitory activity of the Thai herbal formulas against the tested bacteria. Previous reports are available on antibacterial activity of these bioactive ingredients. Flavonoids are known to have antibacterial activity against a broad range of pathogens. The mode of action is probably due to their ability to form complexes with bacterial surface proteins and consequently cause bacterial membrane dysfunction (Ikigai et al., 1993; Tsuchiya et al., 1996). The capacity of flavonoids to inhibit glycosyltransferase enzyme promoting the firm adherence of *S. mutans* to the tooth surface had been described previously (Iio et al., 1984). In addition, a number of biological activities have been reported for plant tannins. The anti-*S. mutans* mechanism of tannins appears to be associated with the ability to reduce the attachment of cariogenic bacteria to the tooth surface by interacting with bacterial surface proteins and changing enzyme structure (Hogg & Embery, 1982; Wolinsky & Sote, 1984). Furthermore, there have been current studies reporting antibacterial activity of other bioactive phytochemicals including alkaloids and terpenoids (Samy & Gopalakrishnakone, 2008). The mechanism of actions of these metabolites is not fully understood but it might be partly associated with membrane disruption due to their lipophilic characteristic.

Conclusion

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. Numerous single medicinal plants have been reported to be potential sources of antimicrobial agents but not many have been studied when combined in herbal formulas. This study suggests that the results of anti-*S. mutans* activity of THF-DC tested and the phytochemicals found in the active medicinal plants could be used as a preliminary scientific evidence for the traditional uses of these Thai herbal formulas as a remedy for dental caries. Again, our study also reported the significant result of the bacteriostatic property of *A. myriophylla* against *S. mutans*, responsible for dental caries. Further studies may be required for the isolation and chemical characterization of the active ingredients in this plant extract for the development of a novel agent against dental caries in the future.

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Declaration of interest

The authors report no conflicts of interest.

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