



Flavonoids and Phenolics Contents, Antioxidant and Antibacterial Potential of Folk Medicinal Plants Used in Northeastern Thailand

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Abstract

Background and objectives: Thailand has abundant traditional medicinal plant species which are efficacious for many illnesses, but most of them still lack the supportive scientific information for their healing properties. The aim of this study was to evaluate and compare the constituents and antioxidant and antibacterial activities of some of these plants. **Methods:** The medicinal plant extracts were assessed for their flavonoids and phenolics composition and tested for antibacterial activity using disk diffusion method. In vitro antioxidant capacity was evaluated by DPPH, ABTS, and FRAP assays. **Results:** Major flavonoids present in the medicinal plants were naringenin, (+)-catechin and quercetin. The highest contents of naringenin, quercetin and (+)-catechin were observed in *Tinospora crispa* (896.15 mg/100 g dw), *Betula alnoides* (521.57 mg/100 g dw) and *Albizia procera* (430.28 mg/100 g dw), respectively ($P < 0.05$). Naringenin was first reported from *T. crispa*, quercetin and (-)-epicatechin were also found in this plant. The lowest EC_{50} value based on the DPPH assay was found in *Capparis micracantha* extracts (9.10 mg/mL). The strongest antioxidant capacities, examined by the DPPH, FRAP and ABTS assays, were found in *Capparis micracantha* (EC_{50} 9.10 mg/mL), *Zingiber cassumunar* (334.00 mg Fe(II)/100 g dw) and *Plumbago indica* (61.56 mg TE/100 g dw), respectively ($p < 0.05$). The extract of *Plumbago indica* root exhibited the highest antibacterial activity mainly against *Bacillus subtilis* (MIC = 1.56 mg/mL), *Bacillus cereus* (MIC = 0.39 mg/mL), *Streptococcus faecalis* (MIC = 0.19 mg/mL), *Salmonella sp.* (MIC = 0.39 mg/mL) and *Salmonella typhi* (MIC = 0.19 mg/mL). **Conclusion:** The results provided significant scientific data on phytochemical constituents and biological activities of Thai medicinal plants use in traditional medicine and the relation to their therapeutic properties.

Keywords: antioxidant activity; antibacterial activity; flavonoids; folk medicinal plants

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Introduction

Within the recent years, human infections caused by antibiotic-resistant bacteria have become an increasing therapeutic problem. Natural active ingredients of medicinal plants might be new sources of bactericidal agents with potential novel antibiotic activity [1,2]. They can be very

effective in the treatment of infectious diseases. Thailand has abundant biodiversity of medicinal plants. Thai folk medicine has included 1800 medicinal plant species. Medicinal plants have been proved to be effective, inexpensive, relatively less toxic and possess fewer side

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effects compared to synthetic drugs. The health beneficial properties of medicinal plants have been related to the secondary metabolites, especially phenolic substances with high biological activities [3].

Phenolic compounds are plant secondary metabolites that possess high antioxidant capacity and can protect against cell and tissue damages caused by free radicals resulting in delay or inhibition of numerous diseases and degenerative conditions such as diabetes, central nervous system disorders, cardiovascular and respiratory diseases, atherosclerosis, cancer, Parkinson's, and Alzheimer's diseases [4]. In addition, the major significant effect of phenolic compounds is antimicrobial activity allowing their use in treatment of various diseases caused by pathogenic microbes [1].

Phenolic compounds are the common antioxidant natural products present in medicinal plants. Thus, it is of very interest to undertake phytochemicals and antibacterial screening of medicinal plants for the purpose of approving their use in folk medicine. The systematic investigation of folk medicinal plant should be done to identify novel active substances for further application in pharmaceutical and food industries. The aim of this study was to investigate the phenolic constituent and antibacterial and antioxidant properties of some selected Thai folk medicinal plants, which currently there is little information available about their bioactivity.

Materials and Methods

Ethical considerations

The research proposal was approved by the Committee of Mahasarakham University (Code; 6308022/2563). All investigators have considered ethics of biosafety throughout the research.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid, 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and FeCl₃ were purchased from Sigma-Aldrich (USA); Folin-Ciocalteu's phenol reagent and TPTZ (2,4,6-tripyridyl-S-triazine) were purchased from Fluka (Switzerland); phenolic standards [(+)-catechin, (-)-epicatechin, quercetin-3-O-rutinoside, *trans*-resveratrol, myricetin, quercetin, naringenin, and kaempferol] were of HPLC grade from Sigma company

(USA); acetonitrile and milli-Q water were of HPLC grade and other chemicals were of analytical grade.

Instruments

The individual phenolic profile was measured on Apollo C-18 column (Alltech Associates, USA) (4.6 mm x 250 mm, 5 μm), protected by a Inertsil ODS-3 (4.0 mm x 10 mm, 5 μm; GL Science Inc., Japan) guard column. The HPLC system consisted of a Shimadzu LC-20AC pumps combined with a SPD-M20A diode array detector (Shimadzu, Japan). A microplate reader (Synergy HT, Biotek instruments, USA) was used for all spectrophotometric measurements (TPC, TFC, DPPH, FRAP, ABTS, MIC).

Plant collection

Medicinal plant samples were collected from their natural habitats or gained from the regional market of Mahasarakham (Thailand) in 2020 (Table 1).

The identity of each plant was confirmed by authors and specimens have been deposited at the Natural Antioxidant Innovation Research Unit (NAIRU) Laboratory, Faculty of Technology, Mahasarakham University, Thailand.

Plant extraction

The samples were dried and ground to powders. Five grams (5 g) of each plant sample was extracted with 50 mL of 80% aqueous methanol and immersed in the sonicator bath (Temperature: 20±1°C, 35 min) and then filtered. The filtrate of each plant was concentrated using rotary evaporator at 40 °C. All extracts were stored at 4 °C for further study.

Bacterial strains

Antibacterial activity was measured against four Gram-positive (*Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, *Streptococcus faecalis* TISTR 459, *Staphylococcus aureus* ATCC 13150) and three Gram-negative strains (*Salmonella* sp. TISTR 96, *Salmonella typhi* ATCC 43579 and *Escherichia coli* ATCC 29214).

Disc diffusion method

Antibacterial activity of tested medicinal plants was examined by disc diffusion method [23]. Briefly, sterile paper discs (6 mm diameter) were placed onto the agar and 10 μL of each medicinal plant extracts solutions (1 mg/disc) were dispensed into the discs and incubated for 24 h at

37±2 °C. Antibacterial activity was estimated by measuring inhibition zone diameters in mm (including disc). Neomycin (5 µg/ disc) and DMSO were used as positive and negative controls, respectively.

Determination of minimum inhibitory concentration (MIC)

A serial dilution method using 96-well microtiter plates with minor modification [24] was used to determine the MIC of medicinal plant extracts. The medicinal plant extracts were dissolved in 10% DMSO (50-0.097 mg/mL). Bacterial suspension at 10⁶ CFU/mL was added to each medicinal plant extract dilution. The optical density of solutions containing the medicinal plant extract and bacteria was measured at 630 nm after incubation at 37±2 °C for 24 h using a microplate reader. The lowest concentration of each plant extract, which with no bacterial growth was defined as MIC value.

HPLC analysis of phenolics and flavonoids

The quantification of individual phenolics and flavonoids was performed by HPLC. The HPLC system consisted of Shimadzu LC-20AC pumps, SPD-M20A diode array detector set at 254 nm. The solvents were as following: A, acetonitrile/deionized water (2/97.8, v/v) containing 0.2% phosphoric acid and B, acetonitrile/deionized water (97.8/2, v/v) containing 0.2% phosphoric acid at a flow rate of 0.6 mL/min. The elution program was as follows: 0–30 min 20–50% B, 30–35 min 50–60% B, 35–40 min 60–20% B [25]. Column was equilibrated with 20% B for 15 min prior to the next run.

Total phenolics content (TPC) determination

The amount of TPC was measured by Folin-Ciocalteu reagent [26]. Diluted sample (12.50 µL) or gallic acid was mixed with Folin-Ciocalteu reagent (12.50 µL, 1:10 diluted with deionised water) and deionised water (12.50 µL). After 6 min, 7% sodium carbonate solution (Na₂CO₃) (125 µL) was added and allowed to stand in the dark at room temperature for 90 min. The absorbance of all samples was measured at 760 nm using a microplate reader spectrophotometer and the results were expressed as mg gallic acid equivalents per 100 g dry weight (mg GAE/100 g dw).

Total flavonoids content (TFC) determination

The TFC was performed according to colorimetric assay [27]. Diluted sample or

catechin (25 µL) was mixed with 7% NaNO₂ (125 µL) and deionised water (125 µL). After 5 min, 10% AlCl₃ (10 µL) was added and allowed to stand for 5 min. Subsequently, 50 µL of 1 M NaOH and 27.5 µL of distilled water were added. The absorbance was measured at 510 nm. Catechin was chosen as a standard and expressed as mg CE/100 g dw.

Determination of the free radical scavenging activity

The free radical scavenging activity was evaluated using 1,1-diphenyl-2-picryl-hydrazil (DPPH) according to the method of Shimada et al. [28] with some modifications. Diluted sample (100 µL) was mixed with 0.2 mM DPPH solution (100 µL) and allowed to stand in the dark at room temperature for 1 h. The absorbance was measured at 520 nm using a microplate reader and expressed as EC₅₀ (mg/mL), the extract dose required to decrease the absorbance of DPPH by 50%.

Determination of the ferric reducing antioxidant power (FRAP)

The FRAP assay was modified from Benzie and Strain [29]. An aliquot of 30 µL medicinal plant extracts was mixed with 270 µL of freshly FRAP reagent (300 mM acetate buffer (pH 3.6), 20 mM FeCl₃.6H₂O, 10 mM TPTZ in 40 mM HCl in the proportion of 10:1:1 (v/v), respectively and allowed to stand for 30 min. The absorbance was measured at 595 nm. Ferrous sulfate (FeSO₄.7H₂O) was used as a standard and expressed as mg Fe (II)/100g dw.

Determination of the ABTS scavenging activity

Measurement of the ABTS scavenging activity was performed with minor modifications from Re et al. [30]. The diluted sample (10 µL) was mixed with of ABTS^{•+} radical cation solution (190 µL) and allowed to stand at room temperature for 2 h. The absorbance at 750 nm was read using a microplate reader spectrophotometer. Trolox was used as a standard and expressed as mg TE/100g dw.

Statistical analysis

The experiments of the study were performed in triplicates. The results were express as mean± SD. The significant differences between means were calculated by one-way analysis of variance (ANOVA) using Duncan post hoc test at p< 0.05.

Table 1. Botanical, folk or traditional uses of tropical Thai medicinal plants

Scientific name	Common name	Local name	Family	Part tested ^a	Voucher number	Collection location	Medicinal uses /Traditional uses	Ref.
<i>Albizia procera</i> (Roxb.) Benth.	White siris, sit	Thingthon	Fabaceae	Heartwood	NAIRU-2020-0154	Community forest, Khok Phra Subdistrict, Kantharawichai, District, Maha Sarakham Province, Thailand	Anticancer, for pain, convulsions, delirium, and septicemia, for treating pregnancy problems, as stomach-ache, used for sinus, wounds, and ulcers	[5]
<i>Betula alnoides</i> Buch-Ham. ex G. Don	Birch	Kamlangseukkhong	Betulaceae	Heartwood	NAIRU-2020-0155	Community forest, Khok Phra Subdistrict, Kantharawichai, District, Maha Sarakham Province, Thailand	Antidote in the treatment of snake bites, for dislocated bones, treatment of fatigue	[6]
<i>Capparis micracantha</i> DC.	-	Saemthalai	Capparaceae	Roots	NAIRU-2020-0156	Community forest, Khok Phra Subdistrict, Kantharawichai, District, Maha Sarakham Province, Thailand	Antipyretic, relief of fever, common cold and influenza, treatment of skin disorders	[7]
<i>Cryptolepis dubia</i> (Burm.f.) M.R.Almeida	-	Thao-en-on	Apocynaceae	Stem	NAIRU-2020-0157	Community forest Na Si Nuan, Na Si Nuan Sub district, Kantharawichai district, Mahasarakham Province, Thailand	Treatment of muscle and tendon pains, bone fracture; the latex is applied topically on wounds, boils or sore	[8]
<i>Curcuma longa</i> L.	Turmeric	Khamin	Zingiberaceae	Rhizomes	NAIRU-2020-0164	Market of Borabue, Borabue Subdistrict, Borabue District, Mahasarakham Province, Thailand	Stomachache, carminative, appetizer, blood purifier, tonic, treatment of cancer, high cholesterol level, dermatitis, and AIDS	[9]
<i>Cyperus rotundus</i> L.	Nut grass, coco grass	Haewmoo	Cyperaceae	Rhizomes	NAIRU-2020-0163	Thakhon Yang Village, Thakhon Yang Sub district, Kantharawichai District, Mahasarakham Province, Thailand	Treatment of dysmenorrhea and menstrual irregularities	[10]
<i>Ficus foveolata</i> Wall	-	Ma-Kra-Tuep-Rong	Moraceae	Stem, Heartwood	NAIRU-2020-0165	Market of Borabue, Borabue Subdistrict, Borabue District, Mahasarakham Province, Thailand	Rejuvenating agent, tonic	[11]
<i>Phyllanthus emblica</i> L.	Amla, malacca tree	Makhampom	Phyllanthaceae	Fruit	NAIRU-2020-0158	Community forest Na Si Nuan, Na Si Nuan Sub district, Kantharawichai District, Mahasarakham Province, Thailand	Folk remedies for numerous ailments, Ayurvedic medicine, as analgesic and anti-pyretic	[12]
<i>Piper interruptum</i> Opiz	-	Thaosakhan	Piperaceae	Stem	NAIRU-2020-0166	Market of Borabue, Borabue Sub district, Borabue District, Mahasarakham Province, Thailand	Treatment of choke, as carminative, anti-flatulent, and tonic	[13]
<i>Piper nigrum</i> L.	Pepper	Prigthai	Piperaceae	Seeds	NAIRU-2020-0167	Market of Borabue, Borabue Sub district, Borabue District, Mahasarakham Province, Thailand	Colic disorder, fever, diarrhea, cold and gastric conditions	[14]

Table 1. Continued

Scientific name	Common name	Local name	Family	Part tested ^a	Voucher number	Collection location	Medicinal /traditional uses	Ref.
<i>Piper retrofractum</i> Vahl	Long pepper	Deepee	Piperaceae	Fruit	NAIRU-2020-0168	Market of Borabue, Borabue Sub district, Borabue District, Maharakham Province, Thailand	Treat asthma, bronchitis, hemorrhoids, fever, abdominal pain, has stimulant effects	[15]
<i>Piper sarmentosum</i> Roxb	Wild betal leaf bush	Chaphlu	Piperaceae	Leaves	NAIRU-2020-0169	Market of Borabue, Borabue Sub district, Borabue District, Maharakham Province, Thailand	Treat cough, flu, rheumatism, fever, tooth pain, foot dermatitis, asthma, pleurisy	[16]
<i>Plumbago indica</i> L.	Rose-colored leadwort, rosy leadwort, fire plant, official leadwort	Cetamulpherngdaeng	Plumbaginaceae	Roots	NAIRU-2020-0159	Community forest Na Si Nuan, Na Si Nuan Sub district, Kantharawichai District, Maharakham Province, Thailand	Hepatitis, dyspepsia, flatulence, piles, leukoderma, leprosy, anasarca, rheumatism, paralytic affections, enlarged glands, ophthalmia, scabies, as germicidal, abortifacient, in treatment of fever, body pain, inflammation, liver diseases, and cancer	[17]
<i>Pueraria candollei</i> Graham ex Benth var. <i>mirifica</i>	Pueraria mirifica	kwawkuakhao	Fabaceae	Rhizomes	NAIRU-2020-0160	Community forest Na Si Nuan, Na Si Nuan Sub district, Kantharawichai District, Maharakham Province, Thailand	Menopausal syndrome, to increase sexual desire for women	[18]
<i>Strobilus asper</i> Lour.	Siamese rough bush, Toothbrush tree	Khoi	Moraceae	Stem, bark	NAIRU-2020-0161	Community forest Na Si Nuan, Na Si Nuan Sub district, Kantharawichai District, Maharakham Province, Thailand	Diarrhea, leprosy, dysentery, piles, cancer, elephantiasis	[19]
<i>Tinospora crispa</i> L.	Heart-leaved moonseed	Boraphed	Menispermaceae	Stem	NAIRU-2020-0162	Community forest Na Si Nuan, Na Si Nuan Sub district, Kantharawichai District, Maharakham Province, Thailand	Enhancing hunger, as antipyretic, for decreasing thirst, cooling down body temperature	[20]
<i>Zingiber officinale</i> Roscoe	Ginger	Khing	Zingiberaceae	Rhizomes	NAIRU-2020-0169	Market of Borabue, Borabue Sub district, Borabue District, Maharakham Province, Thailand	Arthritis, sore throats, cramps, rheumatism, sprains, pains, muscular aches, dementia, constipation, vomiting, indigestion, hypertension, fever, and infectious diseases	[21]
<i>Zingiber cassumunar</i> Roxb.	Cassumunar ginger, Bengal root	Phlai,	Zingiberaceae	Rhizomes	NAIRU-2020-0170	Market of Borabue, Borabue Sub district, Borabue District, Maharakham Province, Thailand	Treatment of joint pain, rheumatism, inflammation, asthma, wounds	[22]

Results and Discussion

Thailand is well known for its biodiversity of many tropical medicinal plants grown wildly in the region of Northeast. Phenolic compounds found in medicinal plants are related to their various pharmacological activities. The phenolic contents of medicinal plants are affected by both environmental factors (for example, UV light, heavy metals, and pathogens) and endogenous factors (for example, genetic) [31]. The flavonoid and total phenolic contents of medicinal plants studied range from 84.11 to 2875.04 mg CE/100 g dw and 154.39 to 1940.40 mg GAE/100 g dw, respectively (Table 2). The highest total flavonoid and phenolic contents (2875.04 mg CE/100 g dw and 1940.40 mg GAE/100 g dw, respectively) were observed in *P. nigrum* extract. From the report of Nahak and Sahu [32], the total phenolic content of *P. nigrum* L. extract (6.23 mg/100g dw) had lower content than those of this report. On the other hand, our result was lower than those reported by Akbar et al. [33] which was 17,493 mg GAE/100 g dw.

Table 3 reveals the flavonoids detected from medicinal plants. There were variations in terms of flavonoids contents of Thai medicinal plants. The presence of these flavonoids has roughly described the significant pharmacological potency reported by previous researchers. Many

kinds of flavonoids and phenolics included naringenin, (+)-catechin, (-)-epicatechin, quercetin, quercetin-3-*O*-rutinoside, *trans*-resveratrol, kaempferol and myricetin were found (Figure 1). Quercetin, naringenin, and (+)-catechin were the main flavonoids present in the selected medicinal plants. The highest contents of naringenin, quercetin and (+)-catechin were observed in *T. crispa* (896.15 mg/100 g dw), *B. alnoides* (521.57 mg/100 g dw) and *A. procera* (430.28 mg/100 g dw), respectively. Flavone glycosides namely, genkwanin, diosmetin, luteolin 4'-methyl ether 3'-glucoside, luteolin 4'-methyl ether 7-glucoside, and genkwanin 7-glucoside have been reported in the stem of *T. crispa* or "Boraphed" [34]. To the best of our knowledge, naringenin was first reported from *T. crispa* and quercetin and (-)-epicatechin were also found in this plant. The highest content of kaempferol was observed in *Ph. emblica* or "Makhampom" with value of 100.81 mg/100 g dw higher than those reported by Ruangchakpet and Sajjaanantakul [35] in Indian gooseberry (*Ph. emblica* L.) with the content of 0.2 mg/100 g fresh weight. The content variations of kaempferol might be due to various factors including genetic variability, growth conditions, degree of maturity of the medicinal plants and postharvest storage conditions.

Table 2. Total phenolics and flavonoids contents and antioxidant activities of folk Thai medicinal plant extracts

Scientific name	Total phenolics content (mg GAE/100g dw)	Total flavonoids content (mg CE/100g dw)	DPPH (EC ₅₀ , mg/mL)	FRAP (mg Fe(II)/100g dw)	ABTS (mg TE/100g dw)
<i>Albizia procera</i>	155.68±10.26 ^{fg}	524.88±186.02 ^{ef}	12.06±0.18 ^h	106.29±0.86 ^{jk}	21.75±0.09 ^d
<i>Betula alnoides</i>	347.57±43.87 ^{ef}	787.41±123.63 ^{de}	11.65±0.14 ^{hi}	105.98±1.25 ^{jk}	32.83±1.03 ^c
<i>Cryptolepis dubia</i>	595.55±72.21 ^{bc}	849.70±105.33 ^{de}	12.70±0.35 ^g	178.43±3.32 ^f	33.81±1.51 ^c
<i>Curcuma longa</i>	556.03±98.18 ^{bcd}	1522.18±235.79 ^b	10.80±0.21 ^j	294.00±3.25 ^c	32.44±1.02 ^c
<i>Capparis micracantha</i>	644.28±6.13 ^{bc}	529.45±40.07 ^{ef}	9.10±0.37 ^k	152.54±2.04 ^g	32.83±2.23 ^c
<i>Cyperus rotundus</i>	203.75±14.21 ^{fg}	481.31±98.94 ^{ef}	13.89±0.06 ^f	133.53±0.95 ^h	31.95±1.89 ^c
<i>Ficus foveolata</i>	127.61±3.91 ^g	286.40±25.38 ^{fg}	11.96±0.25 ^h	119.36±6.08 ^{ij}	32.25±1.03 ^c
<i>Pueraria candollei</i>	160.40±20.43 ^{fg}	84.11±13.71 ^g	72.94±0.07 ^a	127.91±1.43 ^h	30.09±6.12 ^c
<i>Phyllanthus emblica</i>	194.28±11.27 ^{fg}	472.11±17.23 ^{ef}	25.65±0.13 ^c	296.34±17.85 ^c	27.74±3.61 ^{cd}
<i>Plumbago indica</i>	723.62±6.41 ^b	1422.75±174.40 ^{bc}	13.07±0.17 ^g	312.18±4.83 ^c	61.56±0.00 ^b
<i>Piper interruptum</i>	154.39±1.71 ^{fg}	593.92±40.97 ^{ef}	11.70±0.21 ^{hi}	168.90±6.85 ^{fg}	34.21±3.11 ^c
<i>Piper nigrum</i>	1940.40±187.18 ^a	2875.04±29.73 ^a	11.44±0.33 ⁱ	89.05±2.64 ^k	28.52±0.95 ^c
<i>Piper retrofractum</i>	392.67±30.04 ^{de}	556.02±61.14 ^{ef}	11.28±0.10 ⁱ	227.7±9.97 ^e	31.56±1.35 ^c
<i>Piper sarmentosum</i>	486.92±22.40 ^{cde}	741.42±43.07 ^{de}	13.07±0.16 ^g	130.46±7.26 ^h	31.85±0.59 ^c
<i>Streblus asper</i>	176.16±21.26 ^{fg}	247.31±108.36 ^{fg}	11.74±0.18 ^{hi}	89.26±0.18 ^k	29.21±0.88 ^c
<i>Tinospora crispa</i>	561.97±34.92 ^{bcd}	574.02±98.18 ^{ef}	30.26±0.07 ^c	295.04±5.19 ^c	32.54±1.67 ^c
<i>Zingiber cassumunar</i>	467.17±81.09 ^{cde}	1017.52±148.33 ^d	22.88±0.11 ^b	334.00±5.33 ^b	34.01±1.72 ^c
<i>Zingiber officinale</i>	526.58±19.68 ^{cde}	1102.28±175.81 ^{cd}	16.69±0.06 ^d	251.55±0.18 ^d	33.52±0.45 ^c
Ascorbic acid	-	-	0.005±0.0001	816.63±66.29 ^a	275.24±7.69 ^a

Values are expressed as mean values±SD, n=3. Values in the columns with different superscript letters are significantly different (p<0.05).

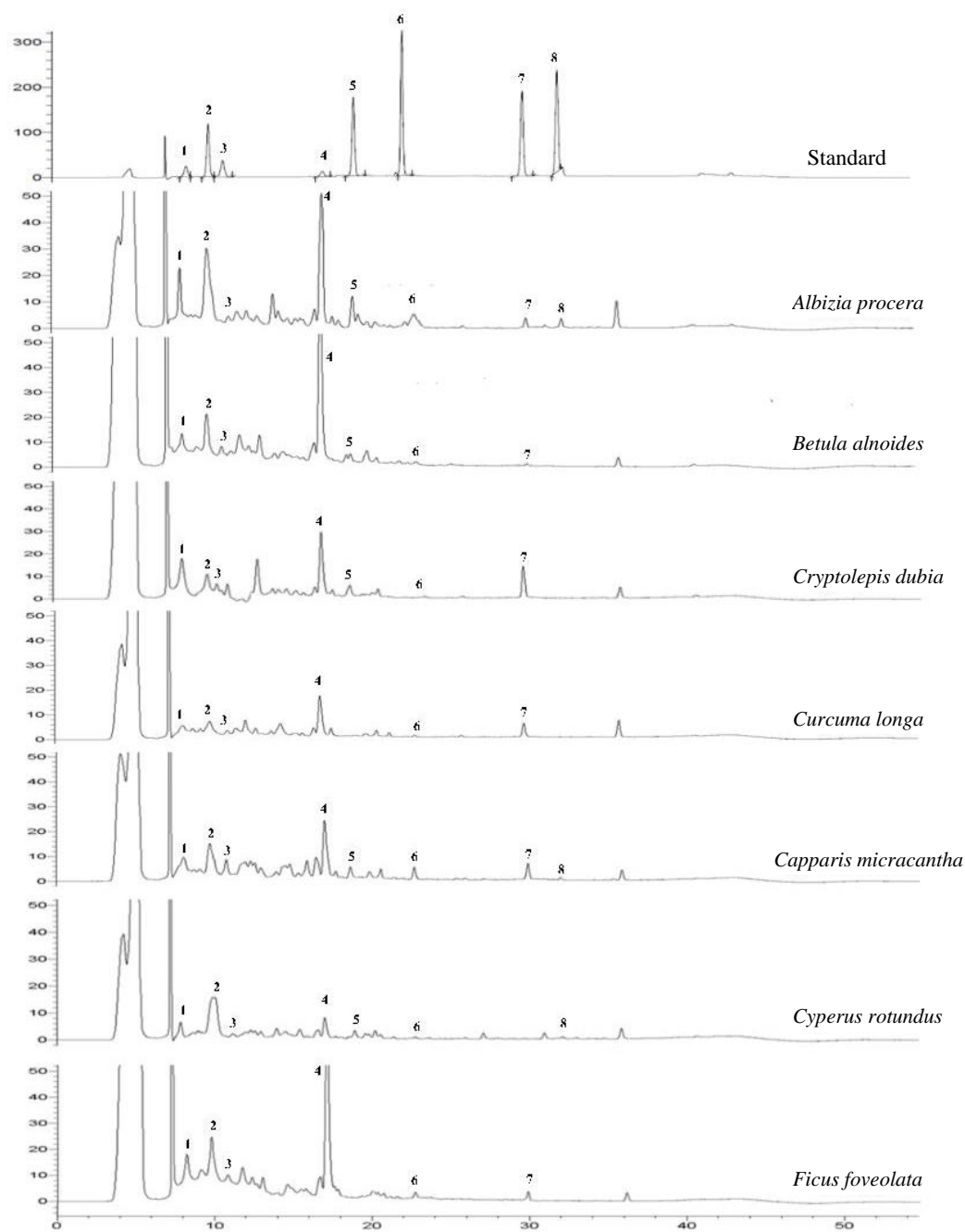


Figure 1. HPLC chromatograms of flavonoids of folk Thai medicinal plants. Peaks: (1) (+)-catechin, (2) (-)-epicatechin, (3) quercetin-3-O-rutinoside, (4) quercetin, (5) myricetin, (6) trans-resveratrol, (7) naringinin, and (8) keampferol

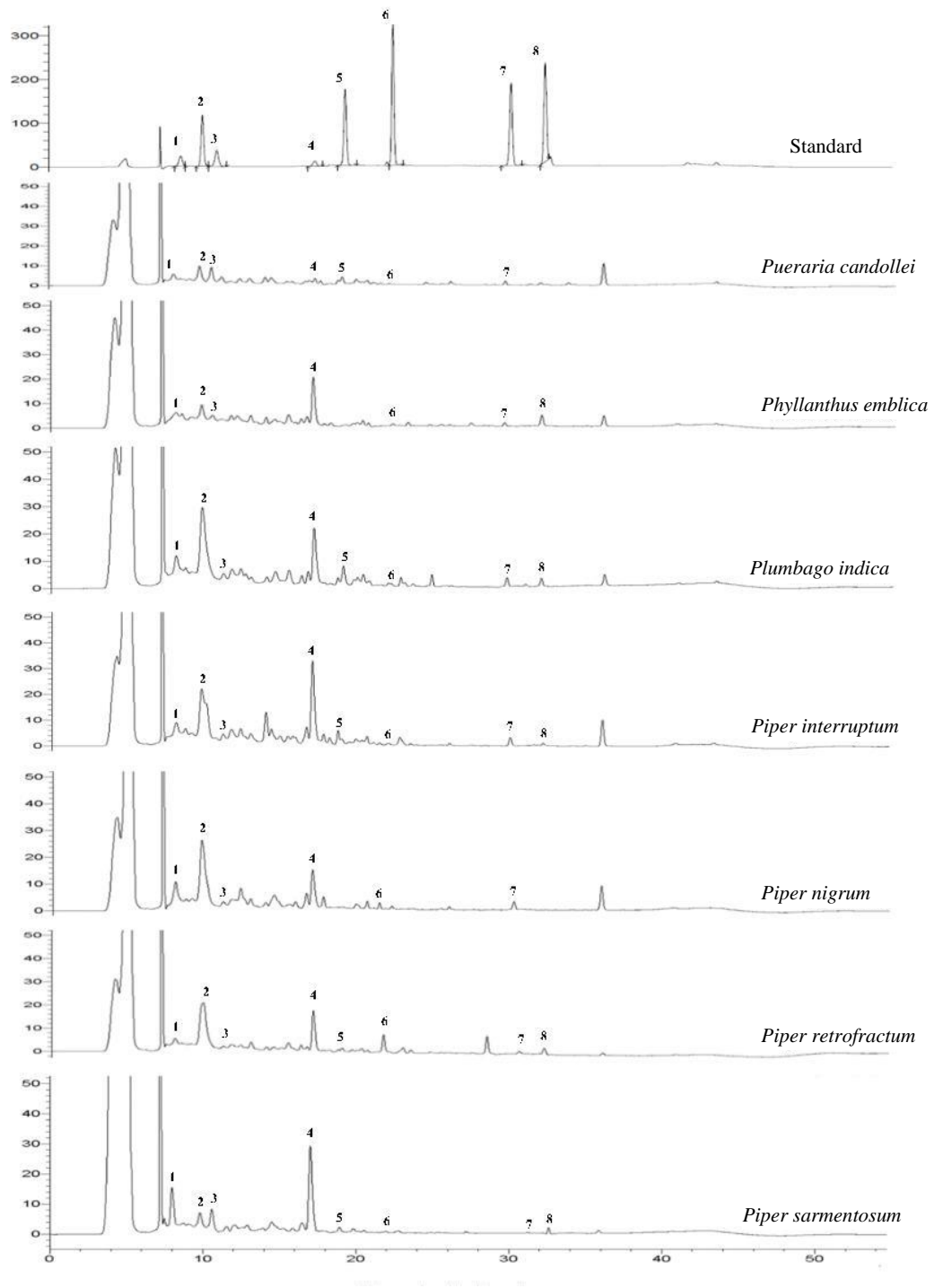


Figure 1. Continued

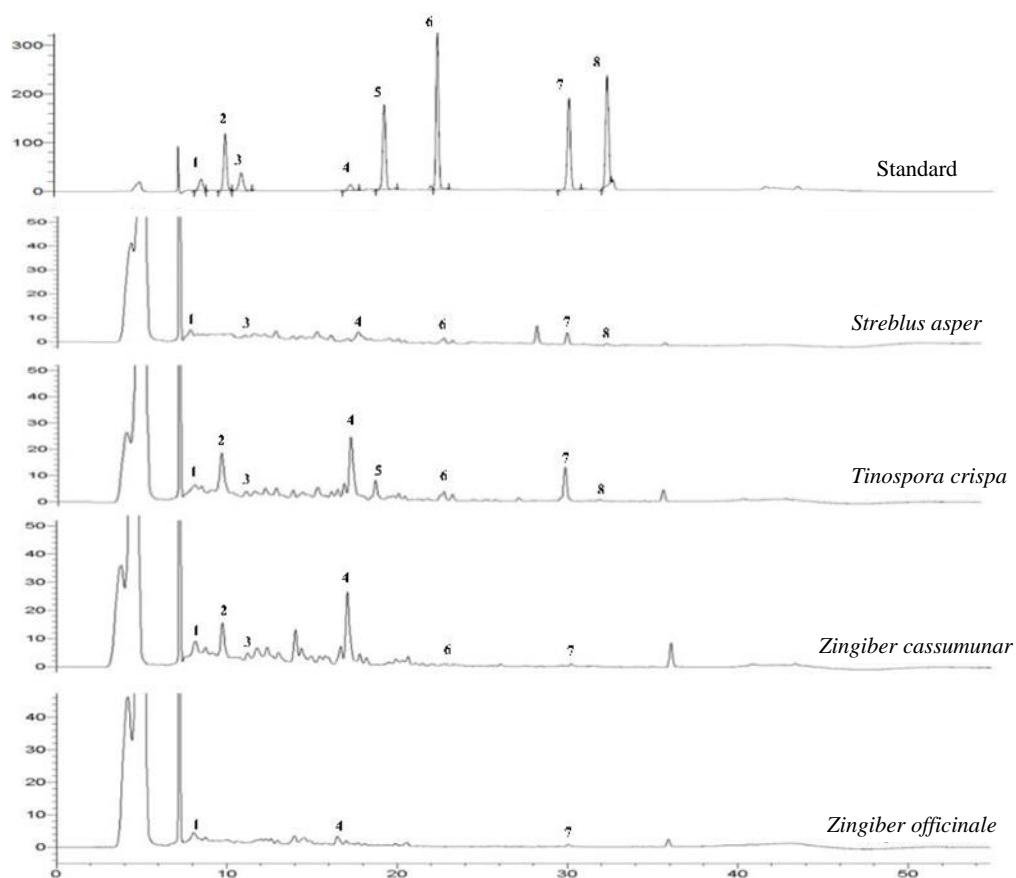


Figure 1. Continued

The potential health risk of some chemical antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) have been reported [36]. The search for other alternatives to synthetic antioxidants has triggered a significant research interest on the discovery of bioactive compounds from natural sources that can reverse, ameliorate, or inhibit diseases caused by oxidative stress. In this quest, we investigated the antioxidant potentials of 18 Thai methanol extracts. Table 2 revealed the comparison of the EC_{50} values of methanol extracts against 0.2 mM of DPPH radical. Most of the extracts showed strong free radical scavenging activity with EC_{50} values lower than 13 mg/mL including the extracts of *A. procera* (12.06 mg/mL), *B. alnoides* (11.65 mg/mL), *C. dubia* (12.70 mg/mL), *C. longa* (10.80 mg/mL), *C. micracantha* (9.10 mg/mL), *F. foveolata* (11.96 mg/mL), *P. interruptum* (11.70 mg/mL), *P. nigrum* (11.44 mg/mL), *P. retrofractum* (11.28 mg/mL) and *S. asper* (11.74 mg/mL). These results suggested that medicinal

plant extracts with higher phenolic contents also showed higher scavenging capacity.

Antioxidant activity of medicinal plants measured by FRAP assay was highly variable ranging from as low as 89.26 mg Fe (II)/100 g dw in *S. asper* to as high as 334.00 mg Fe (II)/100 g dw in *Z. cassumunar* (Table 2). This is probably because oxidative stress in medicinal plants depends on their growing environments and that plants synthesize various secondary metabolites including phenolics, alkaloids and terpenes with different proportions for protecting themselves [37].

Antioxidant activity determined by the ABTS assay, varied from 21.75 to 61.56 mg TE/100 g dw. For example, *Plumbago indica* or “Cetamulpherngdaeng” from Plumbaginaceae exhibited the strongest antioxidant activity (61.56 mg TE/100 g dw), followed by *P. interruptum* (34.21 mg TE/100 g dw) (Table 2).

Ten out of the 18 medicinal plant species contained more than 32 mg TE/100 g dw. The extract of *P. indica* contained high levels of

flavonoids and demonstrated antioxidant capacity. The roots of *P. indica* L. are abundant of the alkaloid called pumbagin. The plant is traditionally used as germicidal, abortifacient, and in treatment of fever, body pain, inflammation, liver diseases, and cancer [17]. The present report supports the medicinal and traditional uses of this plant.

Due to concomitant rise of resistant to antibiotics of human pathogens and the world's urgent need for new antibiotics and chemotherapeutic agents, exploration for antimicrobial drugs increased in the last few decades [38]. Research conducted in many countries have revealed that using active compounds from medicinal plants may be supportive in the antibiotic production [39]. Screening of medicinal plants for phytochemicals and antibacterial activities is important for finding potential new compounds for therapeutic use. Normally, the antibacterial capacities of plant extracts are evaluated using in vitro antimicrobial susceptibility testing (AST). Numerous plants were chosen on the basis of initial ethnobotanical study and the micro dilution, agar diffusion and disc diffusion methods, that are most widely used for screening plant extracts were applied [40]. Assessment of the antibacterial capacity of methanol extracts of Thai medicinal plants was measured initially by the disc diffusion method against different bacteria including four Gram-positive; *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus faecalis* and three Gram-negative; *Escherichia coli*, *Salmonella* sp. and *Salmonella typhi*. The initial screening helped us identify medicinal plants that can be used in future studies. The results of the diameters of inhibition zones are presented in Table 4. Among the 18 medicinal plant extracts, 12 plants revealed the most antibacterial activity with diameter of inhibition zones of more than 12 mm presented by the methanol extracts of *C. longa*, *F. foveolata*, *Ph. emblica*, *P. indica*, *P. interruptum*, *P. nigrum*, *P. retrofractum*, *P. sarmentosum*, *S. asper*, *T. crispa*, *Z. cassumunar* and *Z. officinale*. The extract of *P. indica* exhibited the highest activity mainly against *B. cereus*, *B. subtilis*, *S. faecalis*, *Salmonella* sp. and *E. coli* (14, 16, 16, 14 and 10 mm, respectively). The present study agreed with the results of Valsaraj et al. [41] and Saha and Paul [42]. Their results showed that the *P. indica* root extracts exhibited antibacterial activity against *E. coli*, *B. subtilis* and *B. cereus*.

P. indica root has long been used as antiseptic and for treatment of ringworm and dermatitis. The results obtained suggest that the crude extract of *P. indica* root is efficacious in treating microbial-caused ailments (Table 4).

The high antimicrobial capacity of *P. indica* root extracts could be explained by the high contents of phenolics and also high antioxidant activity. It seems likely that hydroxyl groups of phenolics with high protein binding affinity may inhibit microbial enzymes, thus increasing the antibacterial capacity [43]. The extract of *F. foveolata* showed high activity with diameter of about 12 mm mainly against *E. coli*. *Salmonella* species are intracellular pathogens that cause diarrhea, abdominal cramps, and fever. Traditional Thai medicine uses *Z. officinale* rhizomes to treat these fever and infectious diseases. In the present study, the extracts *Z. officinale* rhizomes and *P. retrofractum* fruit showed a relatively high activity mainly against *Salmonella* sp. and *S. typhi* (14 and 12 mm, respectively) which is consistent with the traditional use of this medicinal plant. The extracts of *C. longa* rhizomes showed a relatively moderate activity mainly against *B. subtilis*, *S. faecalis*, *E. coli* and *Salmonella* sp. (10, 14, 10 and 10 mm, respectively). Generally, gram negative bacteria are in more resistant compared to Gram positive ones. It was interesting to note that *Salmonella* sp. and *E. coli* showed more sensitivity to the investigated medicinal extracts than other antibiotic susceptible bacteria.

Antibacterial activity was assessed by determining the minimum inhibitory concentration (MIC) using microtiter dilution broth method. The MICs of methanol extracts of medicinal plants varied against different tested bacterial strains. As shown in Table 5, *P. indica* root revealed promising activity as it inhibited the growth of five tested bacterial cultures, namely *B. cereus* (MIC 0.39 mg/mL), *B. subtilis* (MIC 1.56 mg/mL), *S. faecalis* (MIC 0.19 mg/mL), *Salmonella* sp. (MIC = 0.39 mg/mL) and *S. typhi* (MIC 0.19 mg/mL) at a very low concentration.

The present study indicated that *P. indica* root showed higher antibacterial activity than other extracts ($p < 0.05$). The extracts of *P. nigrum* seeds also showed high antibacterial activity against three bacterial tests, namely *B. subtilis* (MIC 1.56 mg/mL), *S. faecalis* (MIC 0.19 mg/ml) and *S. typhi* (MIC 0.19).

Table 3. Phenolic constituents (mg/100 g dw) in the folk Thai medicinal plants

Scientific name	Flavonols				Flavan-3-ols			Flavanone		Total polyphenols	
	Quercetin	Quercetin-3-O-rutinoside	Myricetin	Kaempferol	Total flavonols	(+)-Catechin	(-)-Epicatechin	Total flavan-3-ols	Naringenin		trans-Resveratrol
<i>Albizia procera</i>	465.52±10.92 ^b	25.50±1.24 ^c	44.55±3.78 ^a	19.72±0.49 ^e	555.29±13.85 ^a	430.28±15.23 ^a	321.16±15.39 ^b	751.44±18.06 ^a	366.36±17.65 ^d	154.43±6.99 ^d	1827.52±32.60 ^a
<i>Benula adnoides</i>	521.57±25.23 ^a	14.86±0.17 ^{ef}	21.39±1.41 ^d	ND	557.82±15.91 ^a	202.92±15.74 ^e	124.62±6.49 ^{ab}	327.54±10.65 ^g	10.15±1.02 ^j	22.11±0.95 ⁱ	917.62±29.63 ^g
<i>Cryptolepis dubia</i>	222.45±19.77 ^c	42.55±2.09 ^b	11.65±1.05 ^e	ND	276.65±12.42 ^c	399.97±14.71 ^b	55.50±1.23 ⁱ	455.47±16.46 ^d	845.00±17.31 ^b	35.09±1.63 ^b	1612.21±63.70 ^c
<i>Curcuma longa</i>	91.86±5.39 ^e	5.76±0.33 ^b	ND	ND	97.62±9.74 ^j	11.18±1.12 ^j	17.85±0.20 ^{kl}	29.03±1.57 ^{kl}	338.05±15.88 ^c	13.25±0.89 ^{ij}	477.95±11.84 ^{kl}
<i>Capparis micracantha</i>	205.54±12.76 ^c	78.18±1.68 ^a	34.32±1.19 ^b	14.41±0.79 ^{ef}	332.45±16.35 ^d	109.79±0.85 ^{ab}	259.37±12.60 ^d	369.16±18.31 ^f	584.66±19.06 ^c	173.94±3.89 ^e	1460.21±38.79 ^d
<i>Cyperus rotundus</i>	79.75±5.97 ^f	8.22±0.52 ^{ab}	7.68±0.16 ^f	12.13±1.02 ^f	107.78±14.84 ^j	125.82±7.16 ^g	196.83±6.28 ^f	322.65±10.08 ^g	ND	34.08±2.09 ^b	464.51±23.39 ^{kl}
<i>Ficus foveolata</i>	444.88±8.68 ^b	21.17±1.61 ^d	ND	ND	466.05±12.74 ^b	367.27±15.73 ^c	135.13±5.87 ^g	502.40±32.40 ^e	299.40±15.05 ^f	116.29±4.36 ^e	1384.14±20.79 ^e
<i>Pueraria candollei</i>	43.93±2.49 ^g	41.86±1.70 ^b	6.85±0.17 ^f	ND	92.64±4.48 ^j	80.27±2.50 ^j	61.88±2.06 ⁱ	142.15±6.70 ^j	184.41±4.20 ^g	9.14±0.31 ^j	428.34±24.01 ^{kl}
<i>Phyllanthus emblica</i>	92.28±4.47 ^{ef}	7.23±0.17 ^b	ND	100.81±9.14 ^a	200.32±19.66 ^{fg}	115.43±6.02 ^g	29.05±1.65 ^j	144.48±7.85 ⁱ	11.37±1.43 ^j	6.78±0.11 ^j	362.95±18.02 ^m
<i>Plumbago indica</i>	83.01±4.61 ^f	28.97±1.37 ^c	29.07±1.50 ^e	38.81±2.91 ^c	179.86±7.57 ^{gh}	204.87±10.62 ^e	378.49±17.99 ^a	583.36±21.24 ^b	294.85±9.08 ^f	49.20±1.51 ^g	1107.27±53.66 ^f
<i>Piper interruptum</i>	224.36±11.05 ^c	11.65±0.98 ^{fg}	20.20±1.10 ^d	13.04±1.03 ^f	269.25±16.04 ^e	34.89±2.21 ^{kl}	108.70±5.12 ^h	143.59±9.08 ⁱ	320.00±10.44 ^{ef}	100.92±10.93 ^f	833.76±32.22 ^h
<i>Piper nigrum</i>	93.73±6.43 ^e	21.11±4.20 ^d	ND	ND	114.84±6.36 ^j	148.87±4.98 ^f	287.54±16.55 ^e	436.41±13.86 ^c	120.26±7.25 ^h	12.78±1.57 ^{ij}	684.29±20.44 ⁱ
<i>Piper retrofractum</i>	95.45±3.76 ^e	15.90±0.61 ^e	7.06±0.08 ^f	29.11±2.83 ^d	147.52±12.43 ^j	19.65±1.32 ^{kl}	269.13±14.74 ^{cd}	288.78±7.45 ^b	95.63±5.95 ^h	192.28±9.01 ^b	724.21±29.91 ⁱ
<i>Piper sarmentosum</i>	223.86±8.44 ^c	81.33±4.57 ^a	13.41±0.81 ^e	55.84±3.04 ^b	374.44±21.11 ^c	253.69±18.55 ^d	226.28±8.29 ^e	479.97±14.01 ^{cd}	108.18±7.30 ^h	125.49±5.49 ^e	1088.08±15.80 ^f
<i>Streblus asper</i>	ND	8.12±0.47 ^{ab}	ND	9.20±0.24 ^f	17.32±1.30 ^k	44.27±1.54 ^j	ND	44.27±2.74 ^k	369.72±13.51 ^d	99.09±7.89 ^f	530.40±36.60 ^g
<i>Tinospora crispa</i>	107.49±10.91 ^e	15.59±1.10 ^{ef}	43.00±2.25 ^a	42.75±1.78 ^c	208.83±11.87 ⁱ	92.57±2.04 ^{hi}	248.19±14.37 ^d	340.76±10.41 ^{fg}	896.15±21.31 ^a	245.44±11.55 ^a	1691.18±48.14 ^b
<i>Zingiber cassumunar</i>	158.60±6.53 ^d	12.78±0.33 ^{ef}	ND	ND	171.38±12.01 ^{hi}	19.76±1.82 ^k	59.52±2.12 ^j	79.28±6.04 ^j	57.63±3.18 ⁱ	24.75±2.23 ^{hi}	333.04±12.86 ^m
<i>Zingiber officinale</i>	16.79±0.96 ^b	ND	ND	ND	16.79±2.05 ^k	10.98±0.33 ^j	ND	10.98±1.23 ^j	30.24±1.47 ^j	ND	58.01±6.12 ⁿ

Values are expressed as mean values±SD, n=3. Values in the columns with different superscript letters are significantly different (p < 0.05); ND: not detected

Table 4. Antibacterial activity of folk Thai medicinal plants by disc diffusion

Scientific name	Inhibition zone diameter (mm)						
	<i>Bacillus cereus</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus faecalis</i>	<i>Escherichia coli</i>	<i>Salmonella sp.</i>	<i>Salmonella typhi</i>
<i>Albizia procera</i>	7±0.42 ^b	8±0.35 ^c	-	10±0.31 ^c	9±0.06 ^c	8±0.30 ^f	8±0.30 ^f
<i>Betula alnoides</i>	11±0.86 ^d	8±0.30 ^c	8±0.21 ^f	10±0.44 ^c	8±0.21 ^f	9±0.21 ^e	-
<i>Cryptolepis dubia</i>	7±0.28 ^b	-	7±0.42 ^g	8±0.21 ^{fg}	10±0.48 ^d	8±0.20 ^f	-
<i>Curcuma longa</i>	9±0.57 ^f	10±0.74 ^d	8±0.28 ^f	14±0.51 ^c	10±0.30 ^d	10±0.34 ^d	7±0.16 ^g
<i>Capparis micracantha</i>	8±0.21 ^g	-	7±0.27 ^g	8±0.18 ^{fg}	11±0.51 ^c	8±0.21 ^f	11±0.28 ^c
<i>Cyperus rotundus</i>	7±0.14 ^b	10±1.05 ^d	9±0.30 ^c	8±0.07 ^{fg}	10±0.20 ^d	8±0.03 ^f	10±0.95 ^d
<i>Ficus foveolata</i>	8±0.45 ^g	-	-	10±0.91 ^c	12±0.52 ^b	9±0.24 ^c	10±0.31 ^d
<i>Pueraria candollei</i>	8±0.31 ^g	7±0.45 ^c	8±0.31 ^f	9±0.11 ^{ef}	8±0.34 ^f	8±0.31 ^f	8±0.20 ^f
<i>Phyllanthus emblica</i>	6±0.38 ⁱ	12±1.15 ^c	7±0.07 ^g	12±0.62 ^d	10±0.25 ^d	10±0.48 ^d	8±0.33 ^f
<i>Plumbago indica</i>	14±0.75 ^b	16±0.54 ^b	8±0.13 ^f	16±0.48 ^b	10±0.20 ^d	14±0.45 ^b	8±0.07 ^f
<i>Piper interruptum</i>	9±0.34 ^f	-	10±0.48 ^d	12±0.25 ^d	8±0.40 ^f	10±0.35 ^d	10±0.25 ^d
<i>Piper nigrum</i>	10±0.11 ^c	12±0.64 ^c	9±0.11 ^c	10±0.28 ^c	10±0.38 ^d	8±0.27 ^f	8±0.31 ^f
<i>Piper retrofractum</i>	11±0.58 ^d	10±0.47 ^d	10±0.14 ^d	14±0.21 ^c	8±0.24 ^f	10±0.47 ^d	12±0.51 ^b
<i>Piper sarmentosum</i>	10±0.13 ^c	-	12±0.58 ^b	12±0.52 ^d	8±0.13 ^f	11±0.20 ^c	8±0.24 ^f
<i>Streblus asper</i>	12±0.25 ^c	10±0.41 ^d	11±0.42 ^c	12±0.55 ^d	8±0.21 ^f	10±0.49 ^d	10±0.13 ^d
<i>Tinospora crispa</i>	11±0.51 ^d	12±1.19 ^c	10±0.30 ^d	8±0.06 ^{fg}	10±0.44 ^d	8±0.13 ^f	10±0.34 ^d
<i>Zingiber cassumunar</i>	7±0.10 ^b	8±0.45 ^c	12±0.44 ^b	7±0.13 ^g	10±0.27 ^d	8±0.10 ^f	9±0.07 ^c
<i>Zingiber officinale</i>	10±0.16 ^c	10±0.51 ^d	8±0.20 ^f	8±0.31 ^{fg}	8±0.45 ^f	14±0.30 ^b	11±0.41 ^c
neomycin	18±0.27 ^a	20±1.22 ^a	20±1.03 ^a	22±1.23 ^a	22±1.05 ^a	20±1.16 ^a	20±1.07 ^a

(-): no inhibition at the concentration tested. Values are expressed as mean values±SD, n=3. Values in the columns with different superscript letters are significantly different (p<0.05).

Table 5. Minimum inhibitory concentration (MIC) of folk Thai medicinal plants

Scientific name	MIC (mg/mL)						
	<i>Bacillus cereus</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus faecalis</i>	<i>Escherichia coli</i>	<i>Salmonella sp.</i>	<i>Salmonella typhi</i>
<i>Albizia procera</i>	12.50 ^b	25.00 ^b	nt	0.19 ^c	1.56 ^d	12.50 ^b	12.50 ^b
<i>Betula alnoides</i>	3.12 ^d	50.00 ^a	0.39 ^f	1.56 ^c	0.39 ^f	0.78 ^f	nt
<i>Cryptolepis dubia</i>	12.50 ^b	nt	0.39 ^f	0.19 ^c	12.50 ^b	12.50 ^b	nt
<i>Curcuma longa</i>	3.12 ^d	50.00 ^a	50.00 ^a	1.56 ^c	25.00 ^a	1.56 ^c	1.56 ^c
<i>Capparis micracantha</i>	3.12 ^d	nt	0.78 ^c	0.39 ^d	0.19 ^g	0.78 ^f	6.25 ^c
<i>Cyperus rotundus</i>	12.50 ^b	25.00 ^b	0.39 ^f	0.39 ^d	0.39 ^f	25.00 ^a	3.12 ^d
<i>Ficus foveolata</i>	6.25 ^c	nt	nt	0.19 ^c	0.19 ^g	12.50 ^b	0.78 ^f
<i>Pueraria candollei</i>	3.12 ^d	50.00 ^a	3.12 ^d	0.39 ^d	25.00 ^a	25.00 ^a	25.00 ^a
<i>Phyllanthus emblica</i>	25.00 ^a	50.00 ^a	50.00 ^a	0.19 ^c	0.39 ^f	3.12 ^d	3.12 ^d
<i>Plumbago indica</i>	0.39 ^f	1.56 ^d	25.00 ^b	0.19 ^c	6.25 ^c	0.39 ^g	0.19 ^g
<i>Piper interruptum</i>	12.50 ^b	nt	0.39 ^f	0.39 ^d	0.78 ^c	0.39 ^g	25.00 ^a
<i>Piper nigrum</i>	1.56 ^c	1.56 ^d	0.78 ^c	0.19 ^c	6.25 ^c	6.25 ^c	0.19 ^g
<i>Piper retrofractum</i>	6.25 ^c	nt	6.25 ^c	0.39 ^d	0.78 ^c	3.12 ^d	1.56 ^c
<i>Piper sarmentosum</i>	0.39 ^f	25.00 ^b	6.25 ^c	0.19 ^c	0.39 ^f	3.12 ^d	6.25 ^c
<i>Streblus asper</i>	6.25 ^c	1.56 ^d	0.39 ^f	0.19 ^e	25.00 ^a	1.56 ^e	1.56 ^c
<i>Tinospora crispa</i>	6.25 ^c	12.50 ^c	3.12 ^d	1.56 ^c	0.19 ^g	3.12 ^d	0.19 ^g
<i>Zingiber cassumunar</i>	25.00 ^a	12.50 ^c	0.19 ^g	6.25 ^b	1.56 ^d	1.56 ^e	6.25 ^c
<i>Zingiber officinale</i>	3.12 ^d	1.56 ^d	0.39 ^f	25.00 ^a	6.25 ^c	12.50 ^b	25.00 ^a
Neomycin (µg/mL)	2.00 ^e	1.00 ^e	1.00 ^c	0.50 ^d	0.50 ^f	1.00 ^f	1.00 ^f

nt: not tested. Values in the columns with different superscript letters are significantly different (p<0.05).

In addition, *S. asper* bark extract exhibited the highest antibacterial activity against three bacterial tests, namely *S. aureus* (MIC = 0.39 mg/mL) and *S. faecalis* (MIC = 0.19 mg/mL). The present study was in agreement with the results of Prasansuklab et al. [44]. Their results revealed that the *S. asper* extracts exhibited antibacterial activity against *S. aureus* with MIC value range from 0.13-1.00 mg/mL. Traditional Thai medicine uses *S. asper* extracts in the form of infusion or decoction to treat diarrhea, abdominal pain, diabetes, relief of toothache and remedy for fever. The results obtained suggest that the crude extract of *S. asper* was effective against abdominal pain and diabetes, pneumonia, and skin infection that could be attributed to *S. aureus* infection [45]. Finally, the extracts of *B. alnoides*, *C. dubia*, *C. micracantha*, *F. foveolata*, *P. sarmentosum* and *T. crispa* showed higher values of MICs varying from 0.19 to 12.50 mg/mL. Some of the selected Thai traditional medicinal plants are commonly used as spices in Thai cuisine. The results of this study suggest that the use of these medicinal plants may improve food safety. Consequently, there are increasing consumer trends for more natural alternatives to chemical bactericides.

Conclusion

The present study validates and documents, in a systematic way, the antioxidant and antibacterial properties of various medicinal plants used from long time ago by the Thai people. Both biological activities have relation to phytochemical constituents of medicinal plants. It also provides valuable information for further application in development of new drugs and alternative natural active ingredients for food industries.

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Author contributions

Wanee Samappito was the main study investigator and contributed to the collection of the data; Sujitar Jorjong was the study investigator and contributed to the collection of the medicinal plants; Luchai Butkhup was the

study investigator, contributed to the preparation of manuscript and critically revised the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

DPPH: 1,1-diphenyl-2-picrylhydrazyl; Trolox: 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid; ABTS: 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid; HPLC: high performance liquid chromatography; DMSO: dimethyl sulfoxide; IC₅₀: median inhibitory concentration; FRAP: ferric reducing antioxidant power; TPTZ: 2,4,6-tripyridyl-s-triazine; TPC: total phenolic content; TFC: total flavonoid content, CE: catechin equivalents; TE: trolox equivalents; GAE: gallic acid equivalents; EC₅₀: half maximal effective concentration; MIC: minimum inhibitory concentration; CFU: colony forming unit; ND: not detected; nt: not tested