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Antioxidant and antimicrobial activities of *Saraca thaipingensis* Cantley ex Prain

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ABSTRACT

Objective: To investigate antioxidant and antimicrobial activities of *Saraca thaipingensis* Cantley ex Prain; and isolation of its flower extracts. **Methods:** The plant species (flowers, leaves, and twigs) were extracted by hexane, dichloromethane, ethyl acetate and methanol; and tested for antioxidant activity (DPPH assay) and antimicrobial activity (agar dilution method) against twenty-seven strains of microorganisms; gram positive and gram negative bacteria, and diploid fungus. Bioactive constituents were isolated by column chromatography. **Results:** The plant extracts has been firstly reported to display strong antioxidant activity and antimicrobial activity selective against gram positive bacteria (*Corynebacterium diphtheriae* NCTC 10356 and *Streptococcus pyogenes*) with MIC of 256 μg/mL. Stigmasterol and a mixture of triterpenoids and phenolic compounds were isolated from the flower extracts. **Conclusions:** The study revealed that the *S. thaipingensis* is a new source of natural antioxidants and antimicrobials with potential for medicinal uses.

1. Introduction

A number of medicinal plants have been studied worldwide, focusing on their isolated bioactive constituents and pharmacological activities as well as phytochemical screening of the plant extracts. However, in searching for new sources of bioactive metabolites for drug discovery development and/or alternative medicine, there are still many plants or herbs remain to be explored.

In this regard, *Saraca thaipingensis* Cantley ex Prain is of interest plant to be investigated. *S. thaipingensis* (Leguminosae) is a popular ornamental medium tree species native to southeast Asia, grown for its floral effect in the moisture area and in the rain forest or mountain land. It has yellow flowers, borne on old wood, and has been considered as highly valued medicinal plant with fragrant bright flowers blooming in January-April of the year. Its flowers have been used as traditional medicine for anticough and antiexpectorant. It is commonly known as Yellow-Saraca, and in Thai as Sok lueang, Asok lueang and Asok yai[1]. Herein, antioxidant and antimicrobial activities of flowers, leaves and twigs of *S. thaipingensis* as well as some isolates from the plant species have been reported.

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2. Materials and methods

2.1. General

Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. 1H-NMR spectra were recorded on a Bruker AVANCE 300 NMR spectrometer operating at 300 MHz. Infrared spectra (IR) were obtained on a Perkin Elmer System 2000 FTIR. Column chromatography was carried out using silica gel 60 (0.063-0.200 mm). Analytical thin layer chromatography (TLC) was performed on silica gel 60 PF254 aluminium sheets (cat. No. 7747 E., Merck). Solvents were distilled prior to use. Reagents for cell culture and assay were the following: RPMI-1640 (Rosewell Park Memorial Institute medium from Gibco and Hyclone laboratories, USA; MTT (3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid), L-glutamine, penicillin, streptomycin, sodium pyruvate and glucose from Sigma, USA, Ham′s/F12 (Nutrient mixture F-12), DMEM (Dulbecco′s Modified Eagle′s Medium) and FBS (fetal bovine serum) from Hyclone laboratories, USA and gentamicin sulfate from Government Pharmaceutical Organization, Thailand.

2.2. Plant material

Flowers, leaves and twigs of *Saraca thaipingensis* were collected from Bangkok, Thailand. The specimens have been identified (BKF 105896) by The Forest Herbarium, Royal Forestry Department, Bangkok.

2.3. Extraction

The dried flower of *S. thaipingensis* (312 g) was extracted with hexane 5 L (3×3 days), the filtrate was evaporated in vacuo to provide a hexane extract (14.5 g). Similarly, the plant was further extracted by dichloromethane, ethyl acetate and methanol to give the corresponding extracts 4.3 g, 12.5 g and 53 g, respectively.

2.4. Isolation

The plant extracts were isolated by a silical gel column chromatography using gradient elution with increasing polarity of the solvents. The colleted fractions were combined based on TLC chromatograms and evaporated in vacuo.

Hexane extract (9.5 g) was chromatographed on the silica gel (200 g) column, eluted with hexane, hexane:dichloromethane, dichloromethane, dichloromethane:ethyl acetate to give six fractions of pale yellow solid; H1 (0.93 g), H2 (0.052 g), H3 (0.63 g), H4 (0.07 g), H5 (2.68 g) and H6 (0.89 g).

Dichloromethane extract (2.5 g) was separated by silica gel (60 g) column. Elution with hexane:ethyl acetate, ethyl acetate and ethyl acetate:methanol provided seven fractions of pale yellow to dark brown solid of D1 (0.07 g), D2 (0.14 g), D3 (0.11 g), D4 (0.14 g), D5 (0.40 g), D6 (0.52 g) and D7 (0.34 g). Fraction D6 was further re-isolated by the silica gel (15 g) column to give stigmasterol (1, 18 mg) from hexane:ethyl acetate (1:1) as shown in Figure 1.

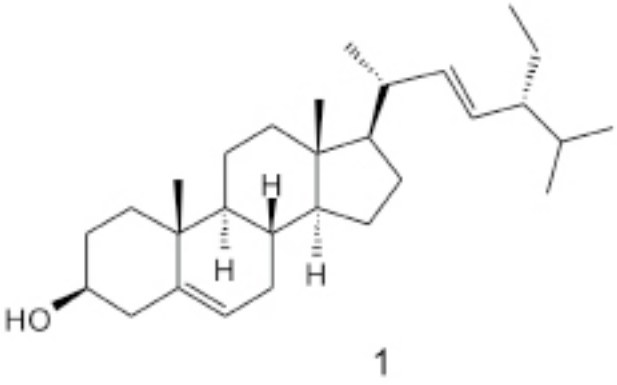

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Figure 1. Chemical structure of stigmasterol 1.

Ethyl acetate extract (8.10 g) was isolated by silica gel (220 g) column, eluted by hexane:ethyl acetate, then ethyl acetate and ethyl acetate:methanol to give seven fractions of dark viscous oil; E1 (0.45 g), E2 (0.95 g), E3 (0.67 g), E4 (0.21 g), E5 (1.07 g), E6 (0.23 g, stigmasterol) and E7 (0.52 g).

Methanol extract (25 g) was placed on the silica gel (600 g) colum, elution with hexane:ethyl acetate, ethyl acetate and ethyl acetate:methanol to afford eight fractions of dark brown oil of M1 (0.39 g), M2 (0.76 g), M3 (0.55 g), M4 (2.93 g), M5 (1.05 g), M6 (4.02 g), M7 (12.41 g) and M8 (0.27 g).

2.5. Bioactivities

2.5.1. Radical scavenging activity (DPPH) assay

A stable purple color radical (DPPH) reacts with an antioxidant to form a light-yellow colored of diphenylpicrylhydrazine which is the reduced product that can be spectrophotometrically recorded. Briefly, a solution of DPPH (0.1 mM) was prepared in methanol. After an incubation of the DPPH solution and sample for 30 min, an absorbance was measured using UV-Visible spectrophotometer (UV-1610, Shimadzu) at 517 nm. The percentage of radical scavenging activity (RSA) was calculated from the following equation:

RSA (%)

where Abs.control is the absorbance of the control reaction and Abs.sample is the absorbance of the tested compound. α-Tocopherol was used as a control[2].

2.5.2.Antimicrobial assay

Antimicrobial activity was assayed using the agar dilution method as previously described[3]. The tested compounds dissolved in DMSO were individually mixed with 1 mL Mueller Hinton (MH) broth, the MH broth was used as a negative control. The solution was then transferred to the MH agar solution to give the final concentrations of 256 μg/mL. Microorganisms were cultured in MH broth at 37 ℃ for 24 h and diluted with 0.9% normal saline solution to adjust

the cell density of 1× 10^8 cell/mL. The microorganisms were inoculated onto each plate and further incubated at 37 ℃ for 24-48 h. Cell growth inhibition of the compounds were determined. Twenty-seven tested microorganisms were gram negative bacteria: Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 700603, Serratia macescens ATCC 8100, Salmonella typhimurium ATCC 13311, Shewanella putrefaciens ATCC 8071, Achromobacter xylosoxidans ATCC 2706, Pseudomonas aeruginosa ATCC 15442, Pseudomonas stutzeri ATCC 17587, Shigella dysenteriae, Salmonella enteritidis, Morganella morganii, Aeromonas hydrophila, Citrobacter freundii, Plesiomonas shigelloides; gram positive bacteria: Staphylococcus aureus ATCC 29213, Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis ATCC 12228, Enterococcus faecalis ATCC 29212, Enterococcus faecalis ATCC 33186, Micrococcus lutens ATCC 10240, *Corynebacterium diphtheriae* NCTC 10356, Bacillus subtilis ATCC 6633, *Streptococcus pyogenes*, Listeria monocytogenes, Bacillus cereus and diploid fungus (yeast): Candida albicans ATCC 90028, Saccharomyces cerevisiae ATCC 2601.

2.5.3. Cytotoxic assay

Cytotoxicity was evaluated as described[4]. Cancer cells were grown in Ham′s/F12 medium containing 2 mM L-glutamine supplemented with 100 U/mL penicillin, streptomycin and 10% FBS. Except for HepG2 cell was grown in DMEM. Briefly, cell lines suspended in RPMI-1640 containing 10% FBS were seeded at 1×10^4 cells (100 μL) per well in a 96-well plate. The incubation was performed at 37 ℃ under humidified atmosphere (95% air, 5% CO_2) for 24h, Additional medium (100 μL) containing the test compound and vehicle was added to a final concentration of 50 μg/mL, 0.2% DMSO, and further incubated for 3 days. Cells were subsequently fixed with 95% EtOH, stained with crystal violet solution, and lysed with a solution of 0.1 N HCl in MeOH. The absorbance was measured at 550 nm. Whereas HuCCA-1, A549 and HepG2 cells were stained by MTT. Doxorubicin and/or etoposide were used as the reference drugs. IC50 values were determined as the drug and sample concentrations at 50% inhibition of the cell growth.

3. Results

3.1. Isolation

The flower extracts of hexane, dichloromethane, ethyl acetate and methanol were isolated by column chromatography. It was found that the hexane and dichloromethane extracts afforded a mixture of triterpenoids and fatty esters as shown by 1H-NMR and IR spectra (data not shown). Stigmasterol was obtained from D6 fraction. Its structure was confirmed by comparison of 1H-NMR and IR data with the authentic sample.

The ethyl acetate extract gave a mixture of triterpenoids and fatty esters including aromatic constituents in fractions 5E and 6E. The stigmasterol was also contained in fraction E6. The most polar methanol extract yielded aromatic compounds in fractions M2 and M3.

3.2. Bioactivities

Several parts of the *S. thaipingensis* extracts include flowers, leaves and twigs were tested for their antioxidant (DPPH assay) and antimicrobial (the agar dilution method) activities as well as cytotoxicity. Results (Table 1) showed that all the tested ethyl acetate and methanol extracts displayed the DPPH activity with IC_{50} range of 10.76-24.24 μg/mL. The corresponding IC_{50} of both (ethyl acetate and methanol) extracts for flowers were 21.20 and 22.26 μg/mL; for leaves were 24.24 and 10.76 μg/mL; and for twigs were 23.24 and 17.52 μg/mL, respectively. On the other hand, the hexane and dichloromethane extracts of the tested plants were found to be inactive antioxidants.

Table 1

Antioxidant activity of *Saraca thaipingensis*.

Plant extract	IC_{50} (μg/mL)			
	hexane	dichloromethane	ethyl acetate	methanol
Flower	NA	NA	21.20	22.26
Leaf	NA	NA	24.24	10.76
Twig	NA	NA	23.24	17.52

α-tocopherol with IC50 of 5.35 μg/mL.

NA: not active

Antimicrobial activity of the plant extracts was tested against twenty-seven strains of microorganisms; gram negative and gram positive bacteria, and diploid fungus. It was found that (Table 2) the hexane and dichloromethane extracts of flowers and twigs exhibited antigrowth activity against C. diphtheriae NCTC 10356 and *S. pyogenes* with MIC value of 256 μg/mL. But, the ethyl acetate and methanol extracts were inactive antimicrobials. In case of the leaves extracts, only the methanol extract showed growth inhibition against C. diphtheriae NCTC 10356 and *S. pyogenes* with MIC of 256 μg/mL.

Table 2

Antimicrobial activity of *Saraca thaipingensis*.

Plant extract	Microorganism, MIC 256 μg/mL			
	hexane	dichloromethane	ethyl acetate	methanol
Flower	+	+	-	-
Leaf	-	-	-	+
Twig	+	+	-	-

+: *C. diphtheriae* NCTC 10356, *S. pyogenes*; -: inactive.

MIC: Minimum inhibitory concentration was the lowest concentration that inhibited the growth of microorganisms. Ampicillin at 10 μg/mL was used as a control of antibacterial testing system; it showed 100 % inhibition against S. typhimurium ATCC 13311, *P. stutzeri* ATCC 17587, *C. diphtheriae* NCTC 10356, *S. aureus* ATCC 29213, S. aureus ATCC 25923, *S. epidermidis* ATCC 12228, *M. lutens* ATCC 10240, *B. subtilis* ATCC 6633, *S. pyogenes*, L. monocytogenes and *P. shigelloides*.

Cytotoxicity of the flowers; hexane, dichloromethane, ethyl acetate and methanol extracts were performed toward four

human cancer cell lines. These include HuCCA-1 (Human cholangiocarcinoma cancer cell); HepG2 (Human hepatocellular carcinoma cell line); A549 (Human lung carcinoma cell line) and MOLT-3 (Human lymphoblastic leukemia cell line). The results showed that all the tested flower extracts exhibited no cytotoxic activity at 30 μg/mL.

4. Discussion

The flower extracts of the plant species were selected for chromatographic isolation, due to its uses in the folk remedy as mentioned. Nonpolar hexane extract, and polar dichloromethane and ethyl acetate extracts constitute mainly a mixture of triterpenoids and fatty esters together with aromatics as noted by IR and 1H-NMR spectra (data not shown). Phenolic compounds were found in the most polar methanol extract as observed by IR and 1H-NMR spectra. Bioactive stigmasterol was isolated from dichloromethane extract by repeated silica gel column. Such compound was also found in the ethyl acetate extract fraction E6.

Up to date, diverse medicinal plants have been reported to possess antimicrobial and antioxidant activities, some of which are belong to Annonaceae, Polyalthia cerasoides[5]; Compositae, Eclipta prostrata Linn.[6] and Spilanthes acmella Murr.[7]; and Rubiaceae, Hydnophytum formicarum Jack.[8].

This study showed that all the investigated parts of *S. thaipingensis* extracts exhibited antioxidant and antimicrobial activities. Strong radical scavenging activity with IC_{50} <30 μg/mL[9] was observed for ethyl acetate and methanol extracts. Apparently, the strongest antioxidant activity was noted for the leaves methanol extract with IC_{50} of 10.76 μg/mL, followed by the twigs methanol extract (IC50 of 17.52 μg/mL). The flower extracts of ethyl acetate and methanol had comparable antioxidant activity with IC_{50} of 21.20 and 22.26 μg/mL, respectively. The antioxidant property of the *S. thaipingensis* could be resulted from the containing triterpenoid and phenolic compounds[6] and the stigmasterol which was previously reported to be the antioxidant[8, 10].

Interestingly, the plant extracts selectively inhibited the growth of gram positive bacteria, *C. diphtheriae* NCTC 10356 and *S. pyogenes* with the same MIC value of 256 μg/mL. The antimicrobial activity was observed for hexane and dichloromethane extracts of the flowers and twigs. For the leaves, only the methanol extract was shown to be antimicrobials. The antimicrobial activity of these plant extracts is possibly due to the triterpenoid constitutents[6]. Previously, a mixture of stigmasterol and β-sitosterol was reported as antimicrobials[11].

In conclusion, this study provides the first report of *Saraca thaipingensis* with antioxidant and antimicrobial activities that is important to medicinal applications.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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References

[1] Smitinand T. *Thai plant names*. (Botanical names-Vernacular names). Bangkok: Royal Forest Department; 1980, p. 296.

[2] Worachartcheewan A, Prachayasittikul S, Pingaew R, Nantasenamat C, Tantimongcolwat T, Ruchirawat S, et bl. Antioxidant, cytotoxicity, and QSAR study of 1-adamantylthio derivatives of 3-picoline and phenylpyridines. *Med Chem Res* 2011. doi: 10.1007/s00044-011-9903-y.

[3] Prachayasittikul S, Worachartcheewan A, Nantasenamat C, Chinworrungsee M, Sornsongkhram N, Ruchirawat S, et al. Synthesis and structure-activity relationship of 2-thiopyrimidine-4-one analogs as antimicrobial and anticancer agents. *Eur J Med Chem* 2011; **46**(2): 738-742.

[4] Prachayasittikul S, Worachartcheewan A, Pingaew R, Suksrichavalit T, Isarankura-Na-Ayudhya C, Ruchirawat S, et al. Metal complexes of uracil derivatives with cytotoxicity and superoxide scavenging activity. *Lett Drug Des Discov* 2012; **9**(3): 282-287.

[5] Treeratanapiboon L, Worachartcheewan A, Suksrichavalit T, Kiatfuengfoo R, Prachayasittikul S, Ruchirawat S, et al. Bioactive 4-hydroxycinnamide and bioactivities of Polyalthia cerasoides. *EXCLI J* 2011; **10**: 16-22.

[6] Prachayasittikul S, Wongsawatkul O, Suksrichavalit T, Ruchirawat S, Prachayasittikul V. Bioactivity evaluation of *Eclipta prostrata* Linn : A potential vasorelaxant. *Eur J Sci Res* 2010; **44**(2): 167-176.

[7] Prachayasittikul S, Suphapong S, Worachartcheewan A, Lawung R, Ruchirawat S, Prachayasittikul V. Bioactive metabolites from *Spilanthes acmella* Murr. *Molecules* 2009; **14**(2): 850-867.

[8] Prachayasittikul S, Buraparuangsang P, Worachartcheewan A, Isarankura-Na-Ayudhya C, Ruchirawat S, Prachayasittikul V. Antimicrobial and antioxidative activities of bioactive constituents from *Hydnophytum formicarum* Jack. *Molecules* 2008; **13**(4): 904-921.

[9] Ahmad R, Mahbob ENM, Noor ZM, Ismail NH, Lajis NH, Shaari K. Evaluation of antioxidant potential of medicinal plants from *Malaysian Rubiaceae* (subfamily Rubioideae). *Afr J Biotechnol* 2010; **9**(46): 7948-7954.

[10] Ramadan MF, Zayed R, El-Shamy H. Screening of bioactive lipids and radical scavenging potential of some solanaceae plants. *Food Chem* 2007; **103**: 885-890.

[11] Prachayasittikul S, Buttrawong K, Worachartcheewan A, Ruchirawat S, Prachayasittikul V. Isolation of bioactive metabolites from root extracts of *Polyalthia debilis*. *Eur J Sci Res* 2009; **38**(4): 596-603.