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Investigation of Antimicrobial Activity of 13 Thai Medicinal Plants against Bacteria and Fungi

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Thirteen medicinal plants used in Thai traditional medicine for the treatment of inflammatory diseases were screened for *in vitro* antibacterial and antifungal activities. The agar disc diffusion method was employed against five strains of bacteria (*Bacillus cereus*, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi*) and four strains of fungi (*Candida albicans, Candida tropicalis, Candida glabrata* and *Candida guilliermondii*). The 13 plants were extracted with methanol and the antimicrobial results were evaluated as the diameter of the inhibition zone of microbial growth. The results showed that the extract with the greatest activity against the gram-positive bacteria was *Garcinia mangostana* (peri-carb). The extract with the greatest activity against the gram-negative bacteria was *Psidium guajava* (leaves). The extracts with the greatest activity against *Candida* was *Psidium guajava* (leaves) and *Allium sativum* (bulbs). The results support the ethnopharmacological uses of the tested plants, especially *G. mangostana*, *P. guajava* and *A. sativum*.

Keywords: Antibacterial, Antifungal, *Garcinia mangostana*, *Psidium guajava*, *Allium sativum*.

Since ancient times, plants have been used by Thai communities to treat a large number of diseases, including inflammation and infections. The 13 plants used in this study exist in many Thai traditional books. Plants have been known to be an important source of new structures and starting materials for the discovery of new therapeutic agents since bacteria resistant to conventional medicines started to increase. Medicinal plants may enhance the effects of conventional antimicrobials that will probably decrease costs and shorten the duration of sickness in some aspects. However, several plants may interfere with antibiotics when they are used together[1].

Natural products and/or natural product structures continue to play a highly significant role in the drug discovery and development process[2]. About 25% of drugs in the modern pharmacopoeia are derived from plants, including several anticancer drugs currently in clinical use[3]. Besides, about 12.5% of the 422,000 plant species documented worldwide are reported to have medicinal value[4]. Many prescription drugs originate from chemicals found in plants and today are simple synthetic modifications or copies of the naturally obtained substances.

In developing countries, high throughput screening for new drugs or new compounds from plants is rather difficult as the budget is limited. Therefore, a multidisciplinary approach is a tool that can help in discovering new compounds. Ethnobotanical data can help to find a link between the ancient usage and the activities of such plant products. In addition, the long history of use may prove that the plant is safe to consume. The 13 plant species in this study were selected based on their anti-inflammatory use or anti-infectious use from

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some Thai ancient textbooks. It is expected that the long history of their usage might offer opportunities for the discovery of novel antimicrobial agents.

EXPERIMENTAL

Collection of plant materials

All 13 plant materials were collected in Thailand, 100–200 g per sample. *Basella alba, Basella rubra, Gynura pseudochina* and *Rhinacanthus nasutus* were collected from farmland in Buriram Province. *Oroxylum indicum* and *Cayratia trifolia* were collected in suburban areas. *Pouzolzia indica* and *Muehlenbeckia platyclada* were collected from the Sirirukhachart Botanical Garden, Mahidol University. *Morus alba* and *Psidium guajava* were collected from Roi-Et Province. *Garcinia mangostana* was collected from Chantaburi Province and *Vernonian einerea* was collected from Mahasarakham Province. Bulbs of *Allium sativum* were purchased from a local market in Mahasarakham Province.

Their scientific names, local names and medicinally used parts are detailed in Table 1. Their macroscopic characteristics were identified according to Thai herbal pharmacopeia guidelines. The plants were gathered during August 2015. The fresh and dried plants were identified by comparison with the plant specimens at the Forest Herbarium of the Thai Royal Forest Department, Bangkok, Thailand.

Preparation of extracts

All plant materials were collected, washed with water, dried at about 35–40 ºC for several days and then ground to a powder using a laboratory scale mill. Twenty grams of dried powder of each plant was extracted with 90% methanol and dried under pressure using a rotary evaporator. All dried extracts were then kept in tightly fitting stopper bottles in a freezer (-4 ºC) until used. Thereafter, the extracts were re-dissolved in 10 % dimethyl sulfoxide (DMSO) at a concentration of 100 mg/ml and then cold sterilized by filtration through a mini-disk filter (0.45 µm) and stored in amber glass bottles for antimicrobial testing.

Microorganisms and media

The five bacterial strains used (*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonalla typhi*) were cultured on nutrient agar at 35ºC for 18 hours. The bacterial colonies were placed into test tubes containing sterile normal saline solution (0.85% NaCl) to make a suspension equal to the turbidity of McFarland standard No. 0.5 (approximately 1-5 x 10^8 CFU/ml of bacteria) and spread on Mueller Hinton agar using a sterile cotton swab.

The four yeast strains used (*Candida albicans*, *Candida tropicalis*, *Candida glabrata* and *Candida guilliermondii*) were cultured on Sabouraud dextrose agar at 35ºC for 24-48 hours. The yeast colonies were placed into test tubes containing a sterile normal saline solution (0.85% NaCl) to make a suspension equal to the turbidity of McFarland standard No. 0.5 (approximately 1-5 x 10^6 CFU/ml of yeast) and spread on Sabouraud dextrose agar using a sterile cotton swab.

Antimicrobial screening

The agar disc diffusion method [10,11] was used to screen the antimicrobial activity of the herb extracts. Sterile 6-mm diameter paper discs (Schleicher and Schuell, Germany) were impregnated with 10 µl of herb extracts (100 mg/ml) and placed on the inoculated Mueller Hinton agar (MHA) and Sabouraud dextrose agar (SDA) plates. Lincomycin (5 mg/mL) antibiotic discs were used as antibacterial agents. Amphotericin B (5 mg/mL) and garlic extract were used as antifungal agents. Discs impregnated with 10 µl of DMSO were used as negative controls. Plates were incubated at 35ºC for 18 h for bacteria and 48 h for yeasts. The inhibition zone diameter was measured. The tests were performed in triplicate and the results were averaged.

RESULTS

The results, evaluated as the diameter of the inhibition zone of microbial growth, showed that most extracts tested were active against gram-positive bacteria (both *B. cereus* and *S. aureus*). The extracts with the greatest activity against both gram-positive bacteria tested were *G. mangstana* and *P. guajava* with similar inhibition zone diameters that ranged from 11.7-14.8 mm.

For gram negative bacteria, four plant species (*C. trifolia, G. mangostana, P. indica* and *P. guajava*) were active against *E. coli*. However, only *C. trifolia* was active against *S. typhi*. The extracts that showed the strongest inhibition against gram negative *P. aeruginasa* were *O. indicum* followed

Table 1. Plants species, their names, family, local names, parts used and traditional uses.

Plant species	Local names	Parts used	Traditional uses
FAMILY *Allium sativum* L. AMARYLLIDACEAE	Kra-tiem	Bulbs	Regulating blood pressure, lowering blood sugar and cholesterol levels, effective against bacterial, viral, fungal and parasitic infections[5].
Basella alba L. BASELLACEAE	Pak-pang -kaow	Leaves	Treatment of infectious disease, e.g., appendicitis, small pox, skin infections, eye infections and mouth ulcers[6].
Basella rubra L. BASELLACEAE	Pak-pang-daeng	Leaves	Similar to *B. alba* but more for urticarial, skin burns and tumors[6].
Cayratia trifolia (L.) Domin. VITACEAE	Thow-kan-kaow	Leaves	Used externally for nose ulcers, muscle pain, abscess, fever and asthma and used as expectorant, carminative and blood purifier[6].
Garcinia mangostana L. GUTTIFERAE	Mang-kood	Peri-carb	Treatment of anti-inflammatory diseases and skin infections, wounds, dysentery, different urinary disorders, cystitis and gonorrhoea[7].
Gynura pseudochina (L.) DC. ASTERACEAE	Wan-hua-nuam	Underground root/stem	Treatment of herpes, fevers, pimples, sore throat, bruises and used as homeostatic and against breast tumors[6].
Morus alba L. MORACEAE	Mon	Leaves	Cancer preventative, relieves fever, indigestion and flatulence[8].
Muehlenbeckia platyclada (F. Muell.) Meisn. POLYGONACEAE	Tra-kab-bin	Leaves	Applied externally for skin swelling, sores and insect bites, poisonous snake bites and fracture injuries, alleviating fever and detoxification[6].
Oroxylum indicum (L.) Kurz. BIGNONIACEAE	Pae-ka	Stem bark	Used against abscesses, skin inflammation, purifying blood and expectorant, fevers, tongue inflammation, bruises and swellings, vomiting, diabetes, diarrhea, rheumatism, stomachache, arthritis, antimalarial, antibacterial and antiviral[6].
Pouzolzia indica (L.) *Gaudich.* URTICACEAE	Kob-cha-nang-daeng	Leaves	Applied as emmenagogue, galactogogue, diuretic, insecticide, used against stomachache, sores, ulcers, gangrene, gonorrhoea, syphilis and wounds[6].
Psidium guajava L. MYRTACEAE	Fa-rang	Leaves	Used for diarrhea, skin infections, head lice, parasites and as mouthwash to relieve bad smell.
Rhinacanthus nasutus (L.) Kuntze. ACANTHACEAE	Thong-pan-chang	Leaves	Treatment of ringworm, eczema, tinea, herpes infections and many skin disorders, antipyretic, anti-inflammatory, detoxicant, cancers, hepatitis, diabetes, hypertension, mental disorders, rheumatism, circulatory problems, asthma and bronchitis, epilepsy and immune system deficiencies[6].
Vernonian einerea L. ASTERACEAE	Mo-noi	Aerial parts	Used as aid in smoking cessation for relieving cigarette craving. Treatment of asthma, cough, fever, malaria[9]

Table 2. Antimicrobial activities of Thai herb extracts against bacteria and fungi.

Extract	Inhibition zone (mm)								
	B. cereus.	*S. aureus*	*E.coli*	*S. typhi*	*P. aeruginosa*	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. guilliermondii*
Allium sativum	ND	ND	ND	ND	ND	10.5	17.4	7.7	9.0
Basella alba	-	-	-	-	-	8.8	-	-	9.3
Basella rubra	-	7.5	-	-	-	-	-	-	-
Cayratia trifolia	12.0	11.3	12.3	9.0	-	12.6	14.1	11.3	12.0
Garcinia mangstana	14.5	11.8	10.5	-	-	14.4	11.4	11.0	12.3
Gynura pseudochina	-	-	-	-	10.0	9.5	-	7.7	9.0
Morus alba	9.7	8.8	-	-	-	9.8	10.6	-	9.0
Muehlenbeckia platyclada	10.2	9.0	-	-	-	11.9	10.9	7.7	10.5
Oroxylum indicum	-	8.0	-	-	12.0	10.4	15.8	7.0	9.5
Pouzolzia indica	10.5	9.0	10.5	-	-	11.9	11.8	-	-
Psidium guajava	11.7	14.0	13.3	-	10.0	13.2	17.4	9.0	14.3
Rhinacanthus nasutus	-	-	-	-	-	-	-	-	-
Vernonian einerea	9.5	8.5	-	-	-	9.9	8.0	-	11.0
Lincomycin	11.0	12.0	12.0	11.0	12.0	-	-	-	-
Amphotericin B	ND	ND	ND	ND	ND	11.0	10.0	10.0	9.3

Note: Lincomycin was a control for bacteria, Amphotericin B was a control for fungi and *Allium sativum* Linn. (garlic) was a control for microbes. ND = not determined

by *G. pseudochina* and *P. guajava.* The extract with the greatest activity against the gram-negative bacteria overall was *P. guajava* (diameter of the inhibition zone was 10-13.3 mm).

For fungi, the extract with the greatest activity against *C. albicans* was *G. mangostana,* while *P. guajava* had the greatest activity against *C. tropicalis* and *C. guilliermondii*. *A. sativum* was also active against *C. tropicalis* but was not good for other fungal strains. For *C. grabrata*, only *C. trifolia* and *G. mangostana* were active, while the other plants showed mild activity.

DISCUSSION

A. sativum, or garlic, has been widely used in Thai traditional medicine as a potent antifungal remedy. Thus, it was selected to be a positive control for the natural products in this study with the hope that it would show the greatest activity against all the *Candida* strains tested. It was the best against *C. tropicalis* but not the other strains tested, including *C. albicans,* while previous studies found that allicin from garlic has antifungal activity particularly against *C. albicans*[12]. However, the concentration of allicin can vary depending on the location that the garlic grows. The allicin compound is also instable and decomposes[13].

C. trifolia, which is commonly known as fox grape in English, in a previous study, was found to possess the most potent DPPH free radical scavenging activity and strongly inhibited lipid peroxidation as well as containing the highest amount of phenolic compounds[6]. The bark extract showed antiviral, antibacterial, antiprotozoal, hypoglycemic, anticancer and diuretic activities[14]. In this study, its methanolic extract showed good antibacterial activity against both gram positive and gram negative bacteria. In addition, it showed potency against all *Candida* spp., especially *C. tropicalis*. *C. trifolia* has been reported to contain yellow waxy oil, steroids, terpenoids, flavonoids and tannins[15].

G. mangostana, or mangosteen, is the queen of fruit in Thailand, which has a wide range of medicinal properties including antibacterial and antifungal[7]. In this study, its extract showed the greatest activity against *B. cereus, S. aureus* and *C. albicans,* which is in agreement with many previous studies. Recently, it was found that the crude chloroform extract of mangosteen pericarp showed an effective zone of inhibition against *Streptococcus mutans, Streptococcus sanguis, Streptococcus salivarius, Streptococcus oralis* and *Lactobacillus acidophilus*[16]. Thus, the mangosteen pericarp extract showed promising activity against dental pathogens and would aid in designing a novel drug for the treatment of dental infections.

P. guajava, or guava leaves, tea is commonly used as a medicine against gastroenteritis and diarrhea in children by those who cannot afford or do not have access to antibiotics[17]. Of the bacteria tested in this study, the *S. aureus* strain was inhibited the most by the extracts followed by the *E. coli*. This result is in agreement with many previous studies in which the guava extracts were very active antibacterially and that the ethanol and methanol were better than n-hexane and water for the extraction of the antibacterial properties of guava[18]. One study found that the essential oil extracted from guava showed inhibitory activity against *S. aureus*, *Salmonella* spp. and *E. coli* isolated from seabob shrimp, which caused diarrhea[17]. These data supported the use of guava leaf-made medicines in diarrhea cases where access to commercial antibiotics is restricted.

CONCLUSION

The findings of this study generally support the popular use of some plant species that show great activity against bacteria and fungi as antibiotic folk medicines, especially *A. sativum, C. trifolia, G. mangostana* and *P. guajava.* In addition, this paper also provides preliminary information about thirteen plant species. Such information may serve as basic knowledge for further insight for future pharmacological, phytochemical and clinical investigations.

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