Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 11, 2015

Original Article

ANTI-INFLAMMATORY, ANTIBACTERIAL, AND ANTIOXIDANT ACTIVITIES OF THAI MEDICINAL PLANTS

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Received: 11 Jul 2015 Revised and Accepted: 22 Sep 2015

ABSTRACT

Objective: *Acacia farnesiana* (L.) Willd*, Senna alata* (L.) Roxb.*, Sesbania grandiflora* (L.) Pers.*, Syzygium cumini* (L.) Skeels and *Tabernaemontana divaricate* (L.) R. Br. ex Roem. & Schult. are used in Thai traditional remedies to treat various disorders ranging from fever and pain to inflammation or microbial infections. However, there is a lack of scientific data on some of the biological activities.

Methods: The present study was designed to compare the antibacterial, antioxidant, and anti-inflammatory effects of the five plants. Ethanolic extracts of *A. farnesiana, S. alata, S. grandiflora, S. cumini*, *T. divaricata* were firstly compared for antioxidant activity using free radical scavenging of 2,2-diphenyl-1-picrylhydrazyl and ferric reducing antioxidant power tests. Antibacterial activity indicated by minimum bactericidal concentration (MBC) was determined using broth and agar dilution tests against aerobic and anaerobic pathogenic bacterial strains. The anti-inflammatory activity was evaluated *in vitro* using a lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage model.

Results: All the tested extracts exerted antioxidant, antibacterial and anti-inflammatory effects. *S. cumini* and *S. grandiflora* extracts showed the highest free radical scavenging activities. *S. cumini* extract showed the highest activity against *Staphylococcus aureus, S. epidermidis,* and *Corynebacterium diphtheriae*. All extracts exerted anti-inflammatory activity as indicated by a reduction of interleukin (IL)-6 secretion and/or tumor necrosis factor (TNF)-α production.

Conclusion: Taken together, these findings suggest that the tested plants can be developed as effective herbal remedies for the treatment and prevention of inflammation or associated diseases as well as against bacterial infections.

Keywords: Antioxidant, Anti-inflammatory, Antibacterial, Medicinal plants.

INTRODUCTION

Inflammation plays a role in various diseases with high prevalence globally. Conventional anti-inflammatory treatments show several side effects. Thus there is a need for alternative or complementary treatment such as natural remedies. Plants which combine anti-inflammatory, antioxidant and antibacterial effects may have a broader range of applications.

In this study five different, traditionally used plants were tested for antibacterial, antioxidant and anti-inflammatory activity. These include *Sesbania grandiflora* (L.) Pers. (bark), *Syzygium cumini* (L.) Skeels (root), *Acacia farnesiana* (L.) Willd (stem, root), *Senna alata* (L.) Roxb. (flower, leaves, and stem) and *Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult. (stem).

S. grandiflora is commonly found in tropical regions. The leaves and flowers are edible food in Thailand. The juice of the bark or root is traditionally used for the treatment of cancer in India [1]. Moreover, this plant has been used to treat multifactorial diseases like leprosy, gout, rheumatism, cancer, liver disorders, inflammation, ocular diseases, epilepsy and anemia [2-4]. Scientific investigations confirmed several biological activities of the extract such as antioxidant, anti-inflammatory, antimicrobial, antitumor, and cytotoxic effects [5-8]. *S. cumini* possesses hypoglycemic, antibacterial and antispermatogenic effects [9-11]. The antioxidant properties of extracts from seed and fruit were evaluated in recent years [12, 13].

A. farnesiana stems have astringent properties [14] which contribute to cure wound, bleeding, diarrhoea, vomiting and stomachache [15, 16]. Moreover, Taiwanese people use the bark for bathing to relieve common cold [17]. In India, bark is applied in paste formulation for treatment of swollen neck glands [18]. The root is reputed to be active against snake bite venoms [19]. In Bangladesh, the root is administered to treat fever, and to calm down children [20].

S. alata is traditionally used to treat ringworm infections in Nigeria [20, 21]. Moreover, the root, stem and leaves are used by practitioners of herbal medicine to treat diarrhoeal diseases, gastrointestinal and upper respiratory tract infections, asthma, bronchitis, and parasitic skin diseases [22, 23]. The leaves were reported to possess anti-inflammatory, antimutagenic, analgesic and antimicrobial properties [24-26]. *T. divaricata* stem is traditionally used for treatment of fever, pain and dysentery in China, Ayurveda and Thailand [27, 28] or against infectious diseases such as syphilis, leprosy, and gonorrhoea, as well as against diarrhea or malaria [28, 29]. We found previously that the alkaloid extract has potent acetylcholinesterase inhibitory activity [30, 31].

Since there is a lack of scientific evaluation of the antibacterial, antioxidant and anti-inflammatory activity of the five plants, this study was designed to elucidate their potential beneficial effects.

MATERIALS AND METHODS

Materials

Tryptic soy agar (TSA) and broth (TSB) were obtained from Criterion (St. Maria, CA, USA). Brain heart infusion (BHI) agar and broth, Penicillin Streptomycin, Dulbecco's minimum essential medium (DMEM), foetal bovine serum (FBS) and L-glutamine were obtained from Life Technologies (Carlsbad, CA, USA). Macrophage RAW 264.7 cells were from an American Type Culture Collection (ATCC-TIB-71). Enzyme Linked Immunosorbent Assay (ELISA) kits were purchased from eBioscience (San Diego, CA, USA). All remaining chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA) or Merck (Darmstadt, Germany).

Preparation of the crude extract

The plant materials of *A. farnesiana, S. alata, S. grandiflora, S. cumini,* and *T. divaricata* were collected in Chiang Mai province in the north

of Thailand in January 2012. The plant voucher specimens (no. 023178, 023179, 023176, 023177, and 0010115, respectively) were deposited at the Herbarium of the Faculty of Pharmacy, Chiang Mai University and authenticated by a staff botanist. The plant materials were washed cut into small pieces, dried in a circulating oven at 55°C and ground into fine powder.

For the antioxidant and antibacterial assays, plants were macerated (48 h × 3) at room temperature in ethanol (500 ml). The extracts from 3 macerations were pooled, filtered and subjected to a rotary evaporation to obtain crude extracts. Those were stored at 4°C until further study. For the anti-inflammatory assay, powders of the 3 plants, *S. grandiflora* bark, and *S. cumini* roots were extracted with water, ethanol, or 50% ethanol for 24 h at room temperature. After filtration of the extracts, ethanol was removed using a rotary evaporator and water was removed by freeze-drying, affording the crude extracts. In addition, crude extracts from other plants were analyzed for anti-inflammatory activity: ethanol extract of *A. farnesiana* root and stem, ethanol extract of *S. alata* flower, stem, and leaves, ethanol extracts of *T. divaricata* stem, hexane extract of *T. divaricata* stem, and ethyl acetate extract of *T. divaricata* stem.

Antibacterial activity test

Six pathogenic bacterial strains *S. aureus*, *S. epidermidis, Escherichia (E.) coli*, *Streptococcus (S.) suis, Salmonella spp.* and *C. diphtheriae* were used. The extracts were diluted to 200 mg/ml using DMSO and a solution of 20 µl was gradually added onto a 5.5 mm-diameter paper disc. Subsequently, the disc was placed on the nutrient containing the test bacteria using TSA for *S. aureus, S. epidermidis*, *E. coli* and *Salmonella spp*. and BHI for *S. suis* and *C. diphtheriae.* The concentration of the bacteria was calibrated to McFarland standard No. 0.5. The antibacterial activity of the extracts was investigated by measuring the diameter of the inhibition zone.

The minimum bactericidal concentration (MBC) of the extracts was determined as previously described. Briefly, the extracts were dissolved in 10-20% DMSO at a concentration of 0.05 to 80 mg/ml and added into an equal volume of nutrient broths (TSB and BHI) containing the amount of bacteria corresponding to McFarland standard No. 0.5 and incubated for 24 h at 37°C. After that the broth was streaked onto agar plates and further incubated for 24 h. The MBC was defined as the lowest concentration of the extracts at which the microorganism was completely killed. Each test was performed in triplicates.

Antioxidant activity test

The antioxidant activity was investigated by two antioxidant mechanisms; the free radical scavenging mechanism and the reducing powder mechanism using butylated hydroxytoluene (BHT) as positive control.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was determined as previously described [32] with some modification. Briefly, the ethanolic extract (0.1 mg/ml) was incubated with DPPH radical stock solution for 30 min. The decrease in absorbance of the solution monitored at 520 nm indicated the free radical scavenging activity of the extract.

The results were expressed as Trolox equivalent antioxidant activity (TEAC) which was obtained by comparing the absorbance change at 520 nm in a reaction mixture containing a sample of plant extract or test material with that containing Trolox. This index is defined as the millimolar concentration of a Trolox solution whose antioxidant capacity is equivalent to 1.0 mg of the extract. All determinations were performed in triplicates .

In the second assay, the antioxidant compound reduces the ferric ion (Fe^{3}+) into the ferrous ion (Fe^{2}+); the later reacts with 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ) to form a blue complex which increases the absorption at 595 nm. The ferric reducing antioxidant power (FRAP) reagent was prepared by mixing TPTZ solution with $FeCl_3$ solution. The extract solutions were mixed with FRAP reagent and the absorbance was taken at 595 nm after 5 min. The standard curve of Fe(II) was constructed using $FeSO_4$ solution (50-500 mM). The reducing power was expressed as equivalent concentration (EC). This parameter is corresponding to the concentration of the extract having a reducing ability equivalent to that of 1 mM $FeSO_4$. Butylated hydroxytoluene (BHT) was used as a positive control.

Anti-inflammatory assay

RAW 264.7 macrophages were cultured in DMEM supplemented with 10% FBS, 4 mM L-glutamine, and 100 units penicillin-streptomycin. The cells were seeded at 2×10^6 cells/ml into 12 well plates, and incubated at 37 °C for 24 h. Subsequently, 100 µg/ml standards and samples were added and incubated for 3 h. Macrophages were activated with 1 µg/ml LPS and incubated for another 24 h. The positive control was treated with LPS without any test samples and negative control was not treated with LPS. The supernatants were harvested and stored at -20 °C until usage for ELISA.

Cell viability was assessed by MTT (Thiazolyl Blue Tetrazolium Bromide) assay. 100 µl of 5 mg/ml MTT was added into the remaining supernatants and cells. After 2 h, the cells were lysed with 1 ml of lysis buffer which consisted of 10% SDS in 0.01N of HCl. The absorbance was recorded at 570 nm with reference at 690 nm.

IL-6, TNF-α, and IL-10 in the cell supernatants were quantified using ELISA kit according to the manufacturer's instructions. The absorbance was recorded at 450 nm with a reference at 570 nm using the Infinite M200 reader. The variation from cell density was reduced by using MTT values for normalization. The amount of cytokines of the positive control was defined as 100%. The results of the samples were calculated as a percent of this value. The inflammation assay was repeated in triplicate on independent days. The influence of the test compounds on cytokine secretion was defined as significant if the level of the positive control was changed by at least 25%.

RESULTS AND DISCUSSION

Extraction of the plants

Among the extracts, obtained using 100% ethanol, *S. cumini* root gave the highest yield of 28% (table 1). *T. divaricata* bark gave the lowest yield with 5%.

Table 1: Extraction yields of the plants

Plant	Solvent	Yield (%)*
A. farnesiana	100% ethanol	19
S. alata	100% ethanol	34
T. divaricata	100% ethanol	5
	100% water	30
S. grandiflora	100% ethanol	25
	50% ethanol	26
	100% water	20
S. cumini	100% ethanol	28
	50% ethanol	13

* yield (%) = dry weight of extracts/raw weight of sample × 100%

Antibacterial activity

The extracts effected antibacterial inhibition at different levels (table 2). Most of them showed antibacterial activity only for Gram positive aerobic bacteria. All of them showed a significant inhibition for Gram negative anaerobic bacteria. *S. cumini, T. divaricata,* and *S. alata* showed antibacterial activity against Gram positive and Gram negative anaerobic bacteria.

Further investigation found that some extracts are highly bactericidal as indicated by the MBC values (table 3). *S. cumini* root extract showed high activity against *S. aureus* and *S. epidermidis* as well as against the Gram negative anaerobic *C. diphtheriae* with MBC values of 2.0, 1.5, and 2.5 mg/ml, respectively. The extract of *S. grandiflora* showed slightly lower inhibitory activity for these three bacteria with the MBC values of 2.5, 2.5, and 10 mg/ml, respectively. *S. alata* leaf extract produced a significant inhibition zone against Gram negative aerobic *Salmonella spp.* and Gram positive anaerobic *S. suis*.

Table 2: Inhibition zone of the crude ethanol extracts at a concentration of 200 mg/ml

Plant name	Part used	Diameter of inhibition zone* (mm)					
		Aerobic bacteria				Anaerobic bacteria	
		G+ve		G-ve		G+ve	G-ve
		S. aureus	*S. epidermidis*	*Salmonella sp.*	*E. coli*	*S. suis*	*C. diphtheriae*
S. cumini	root	9.5±0.0	11.7±0.4	NZ	NZ	7.0±0.0	12.1±0.4
T. divaricata	stem	13.5±0.4	11.3±0.4	NZ	NZ	8.5±0.5	11.5±0.0
S. grandiflora	bark	11.0±0.0	13.8±0.3	NZ	NZ	NZ	8.3±0.3
A. farnesiana	root	NZ	16.3±0.3	NZ	NZ	NZ	9.3±0.3
A. farnesiana	stem	7.0±0.0	7.0±0.0	NZ	NZ	NZ	12.3±0.3
S. alata	leaf	7.3±0.3	NZ	10.2±0.3	NZ	8.2	12.5±0.5

= mean±SD (n = 6), NZ = no zone

Table 3: MBC (mg/ml) of the crude 100% ethanol extracts

Plant name	Part used	Aerobic bacteria				Anaerobic bacteria	
		G+ve		G-ve		G+ve	G-ve
		S. aureus	*S. epidermidis*	*Salmonella sp.*	*E. coli*	*S. suis*	*C. diphtheriae*
S. cumini	root	2	1.5	>40	>40	>40	2.5
T. divaricata	stem	2.5	>40	>40	>40	>40	2.5
S. grandiflora	bark	2.5	2.5	>40	40	>40	10
A. farnesiana	root	40	20	>40	>40	>40	2.5
A. farnesiana	stem	>40	>40	>40	>40	>40	2.5
S. alata	leaf	20	>40	>40	>40	>40	>40

In a previous study, ethyl acetate and butanol extracts of *S. grandiflora* bark also showed strong antibacterial activity against *Enterococcus faecalis, S. aureus, Salmonella typhi***,** and *Shigella sonnei* [33]. Our results are in good agreement with a previous study which showed that *S. cumini* root ethanolic extracts are more active against Gram-positive than Gram-negative bacteria [34].

To the best of our knowledge, this is the first study which shows that the root of *A. farnesiana* exerts antibacterial activity against *S. epidermidis*. It was previously reported that the extract shows antimicrobial activity against *Bacillus subtilis* and *Saccharomyces cerevisiae* [35]. Furthermore, the methanolic extract of *A. farnesiana* bark had antibacterial activity against Gram negative *Vibrio cholera* [36].

S. alata exerts antibacterial effects against a broad range of strains which is consistent with the literature. In previous studies, the extract of the leaves showed antimicrobial activities against several microorganisms including bacteria, yeasts, and fungi [37]. Regarding antibacterial activity, *S. alata* leaf extracts acts against *S. aureus, S. epidermidis, S. typhi*, and *S. aureus* [25, 38].

For the bark of *S. alata*, it was previously reported that the methanol extracts showed antibacterial activity against *S. aureus*, *S. epidermidis*, *S. typhi*, and *E. coli* [24]. Meanwhile, some studies which used ethanol and water extracts showed conflicting results which indicated no antibacterial activity against either *S. aureus* or *E. coli* [25]. When comparing the leaves, flowers, stem, root and barks of *S. alata,* the dichloromethane fraction of the flower methanolic extract was reported to exert the most effective antibacterial activity with a broad spectrum [24].

As reported previously, *T. divaricata* leaf extracts exerted bactericidal effects against *Bacillus cereus, E. coli, S. aureus*, and *Klebsiella sp.* [39]. Similar results have been reported for the leaf extracts with high potency against various infectious pathogens [40]. Moreover extracts of the leaves were found to possess high activity against pathogenic bacteria causing clinical and subclinical mastitis [41] and extracts from the flower inhibited the growth of *S. aureus* and *E. coli* [42].

Antioxidant activity

To demonstrate antioxidant activity by a free radical scavenging mechanism, two commonly used methods were applied [32]. All of the extracts showed significant antioxidant effects in the two test systems used (table 4). *S. cumini* and *S. grandiflora* possess the highest free radical scavenging property with TEAC values of 13.1±1.6 mM/mg and 12.6±0.5 mM/mg, respectively.

These values are approximately 10 times higher than those obtained with the standard BHT. The extract of *S. grandiflora* exerts high reducing power with an EC value of 127.9 ± 2.9 mM/mg which is more than 5 times higher than BHT followed by that of *S. cumini* with the EC value of 76.5 ± 2.0 mM/mg, which is about 3 times higher than BHT.

Table 4: Antioxidant activity of the crude 100% ethanol extracts

Plant name	Part used	Antioxidant activity	
		TEAC (mM/mg)	EC (mM/mg)
S. cumini	Root	13.1 ± 1.6	76.5 ± 2.0
T. divaricata	Stem	2.5 ± 0.3	9.2 ± 0.8
S. grandiflora	Bark	12.6 ± 0.5	127.9 ± 2.9
A. farnesiana	Root	6.0 ± 0.2	16.0 ± 0.6
A. farnesiana	Stem	0.9 ± 0.6	19.8 ± 1.1
S. alata	Leaf	3.3 ± 0.4	9.1 ± 1.0
BHT	–	1.0 ± 0.2	22.4 ± 1.8

The high antioxidant activity *S. grandiflora* bark corresponds well to previous reports where the antioxidant activity of *S. grandiflora* methanolic bark extract was high with an EC_{50} of 40 µg/ml, compared to quercetin whose EC_{50} was 3.6 µg/ml [43]. The high effect of *S. cumini* is also consistent with a previous study which showed a significant antioxidant activity of the butanolic extract of the root of *S. cumini* using reducing power and DPPH assays when compared to standard ascorbic acid [44].

This is the first study to show the antioxidant activity of both root and stem extracts of *A. farnesiana.* A previous study reported the antioxidant activity of the leaf extract of this plant [35]. Similar to a previous report, our present data show that the ethanolic extract of *S. alata* leaves possesses moderate antioxidant capacity. In previous studies, the antioxidant effect of *S. alata* leaves were shown in various assays [22, 45]. To the best of our knowledge, we are the first who report the antioxidant activity of the stem of *T. divaricata*. An antioxidant effect of ethanolic leaf extracts was reported previously as indicated by superoxide scavenging activity [46]. The methanol leaf extract also displayed potent antioxidant property which was shown in the total antioxidant capacity, in the assay of nitric oxide scavenging activity and the reducing power test [47].

Anti-inflammatory activity

All tested plant extracts did not exert a significant cytotoxic effect towards macrophages as determined using MTT assay. Thus all samples could be tested at this concentration. The secretion of the pro-inflammatory cytokine, IL-6, was significantly reduced by at least 25% when adding the extracts with all solvents of *S. cumini* root and *S. grandiflora* bark, *A. farnesiana* root, *A. farnesiana* stem, *S. alata* leaves, *S. alata* flower, and *S. alata* stem and both of ethyl acetate and hexane extracts of *T. divaricata* stem (table 5). The secretion of the pro-inflammatory cytokine, TNF-α, was significant reduced by at least 25% when adding 100 % ethanol extract of *A. farnesiana* root and stem or the ethyl acetate and hexane extracts of *T. divaricata* stem.

Table 5: Anti-inflammatory effect of extracts (100 µg/ml) in LPS-stimulated macrophages; Secreted cytokines were related to the positive control (%)

Species	Plant part	Solvent for extraction	IL-6 (%)	TNF-α (%)	IL-10 (%)
S. grandiflora	Bark	Ethanol	32±13	95±5	120±1
		Water	38±10	96±18	67±9
		50% Ethanol	22±1	99±2	58±4
S. cumini	Root	Ethanol	41±7	120±24	120±1
		Water	42±13	131±24	67±9
		50% Ethanol	31±6	111±16	58±4
A. farnesiana	Stem	Ethanol	32±2	42±4	45±4
	Root		41±5	48±5	49±4
S. alata	Flower	Ethanol	28±4	97±23	62±10
	Leaves		24±4	81±9	50±1
	Stem		74±16	85±24	74±12
T. divaricata	Stem	Ethyl acetate	44±6	38±3	39±7
		Hexane	56±5	52±1	43±12

The aqueous extracts of the bark of *S. grandiflora* extracts reduced the IL-6 secretion, did not significantly change the secretion of TNF-α and reduced the IL-10 secretion. Thus, this result shows a significant anti-inflammatory effect; a previous study confirms *in vivo* anti-inflammatory effects of extracts of the bark of *S. grandiflora* which reduced carrageenan induced inflammation [48]. The aqueous and ethanolic extracts of *S. cumini* root showed a slight anti-inflammatory effect as indicated by a reduced IL-6 production. No previous study tested the anti-inflammatory activity of *S. cumini* root. In a previous study, the ethanolic extract of the bark of *S. cumini* has a potent anti-inflammatory action against different phases of inflammation without any side effect on gastric mucosa [49]. *A. farnesiana* stem and root extracts reduced IL-6 and TNF-α secretion. To the best of our knowledge, this is the first study which investigates the anti-inflammatory effect of an extract from the stem of this plant. Our results are consistent with a previous study where some compounds isolated from the roots of *A. farnesiana* presented slight inhibition of superoxide anion generation or elastase release by human neutrophils, indicating moderate anti-inflammatory activities [17]. In further previous reports, the anti-inflammatory activity of *A. farnesiana* leaves showed significant anti-inflammatory effects in both acute and chronic inflammation models studied [50].

All of the *S. alata* extracts reduced IL-6 secretion; leaves also showed a slight decrease of the TNF-α secretion. In previous studies, the hexane and ethyl acetate extracts of *S. alata* exhibited anti-inflammatory effects in a carrageenan-induced inflammation model [26]. The ethyl acetate and hexane extracts of *T. divaricata* stem strongly reduced IL-6 and TNF-α production. The ethanol extract was cytotoxic in the concentration tested. The anti-inflammatory effect of *T. divaricata* was found previously in mice [51]. The extract of the leaves also showed *in vivo* anti-inflammatory effects as indicated by a reduction of croton oil-induced edema in the mouse ear after topical application [52].

CONCLUSION

In this study, different parts of plants and also solvents for extraction were used. Thus, the results presented different values even from the same plant, depending on the solubility of the beneficial substances and the part of the plant. In conclusion, all tested plants exerted significant antibacterial, antioxidant and anti-inflammatory effects to different extents. The findings of this study suggest that the tested plants or their combinations can be developed as effective herbal remedies for the treatment and prevention of inflammation, bacterial infections or associated diseases.

CONFLICT OF INTERESTS

Declared None

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