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Original Article

BIOACTIVITIES OF THE THAI MEDICINAL AND EDIBLE PLANTS *C. CAJAN*, *M. CITRIFOLIA* AND *O. AMERICANUM*

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ABSTRACT

Objective: Inflammation and oxidative stress are closely related and play a role in various diseases. If an infectious component plays a role, an antibacterial effect is of advantage. Thus, natural remedies which combine different bioactivities have a broader range of application.

Methods: Here we elucidate the anti-inflammatory, antioxidant and antibacterial effects of three edible and traditionally used Thai plants including leaves of *Cajanus cajan*, *Morinda citrifolia* and *Ocimum americanum*.

Results: The extracts exerted significant anti-inflammatory activity in lipopolysaccharide (LPS)-stimulated macrophages. *C. cajan* extract shows a broad spectrum of antibacterial activity against Gram positive and negative, aerobic and anaerobic bacteria, whereas *M. citrifolia* and *O. americanum* possess antibacterial activity only against anaerobic bacteria. Extracts of all three plants showed significant antioxidant effects.

Conclusion: The three plants are potential herbal remedies or supplements for functional food for the treatment and prevention of inflammation, oxidative imbalance, and bacterial infections or associated diseases.

Keywords: *Cajanus cajan*, *Morinda citrifolia, Ocimum americanum*, Anti-inflammatory, Antibacterial.

INTRODUCTION

Inflammation and oxidative stress play a role in various diseases with high prevalence. Since conventional treatments show several side effects, there is a need for alternative or complementary treatment or prevention with natural remedies or functional food.

Cajanus cajan (L.) Millsp. (pigeon pea) is an edible plant in Thailand consumed fresh, streamed or blanched for example in chili paste and up to 50-100 g/meal. In some areas like Ethiopia, not only the pods but also the young shoots and leaves are consumed [1]. In Thailand it is commonly used as medical plant to treat diabetes and food poisoning or as an energy stimulant, analgesic, and antihelminthic agent [2-4]. Moreover the leaves have been used to relieve pain, heal wounds, and kill worms in traditional Chinese medicine [5] and for the treatment of diarrhea in India [3].

Morinda citrifolia or so-call "Thai noni" grows all-around in Thailand. The leaves are used for daily cooking in a recipe called "Hor Mhok Bai Y or" and "Bai Yor Rice" and the fruits are used to prepare "Tum Yor" [6]. The fruits of *M. citrifolia* are traditionally used for treatment of a wide range of diseases and disorders including diabetes, hypertension, inflammation [7], headache, fever, arthritis, gingivitis, respiratory disorders, infections, tuberculosis or to enhance wound healing [8].

Ocimum americanum (*O. basilicum* L. f. var. *citratum* Back.) leaves are used in many Thai remedies such as curried soups and fried curried meat. Local people in Thailand believe that eating food with the leaves of this plant leads to carminative action and relieves cold [9]. The daily dietary intake of each Thai is about 0.01 g/day. Furthermore, *O. americanum* is widely used as medicinal plant in the form of its essential oil which exerts antimicrobial activity [10]. The fresh plant has been traditionally used as mosquito-repellent [11]. The leaves are traditionally used for treatment of diabetes, constipation, diarrhea, and dysentery [12]. In India, the leaf juice of this plant is traditionally used for relieving cold and bronchitis [13].

However, there is a lack of scientific elucidation of the beneficial effects of the mentioned plants. All plants are traditionally used for various diseases where inflammation or oxidative processes play a major role such as diabetes, bronchitis or cancer. The plants are also used for healing where infection by bacteria is involved. Therefore an elucidation of these bioactivities is of high interest. Some biological activities of *C. cajan* leaves have been shown previously, including the antioxidant effect and the antibacterial activity [14, 15]. Anti-inflammatory effects were only found in peas or whole plants; there is a lack of knowledge on the bioactivity of leaves [16]. *M. citrifolia* leaves extract was reported to possess antioxidant activities and to contain anti-inflamatory compounds [17]. The leaves of *O. americanum* showed an antioxidant effect [12]. However, the anti-inflammatory and antibacterial effects of the leaves are still unknown. Thus, the aim of this study was the scientific elucidation of different bioactivities of these plants.

MATERIALS AND METHODS

Materials

Macrophages (RAW 264.7) were from American Type Culture Collection, ATCC-TIB-71. Dulbecco's minimum essential medium (DMEM), foetal bovine serum (FBS), L-glutamine, penicillin-streptomycin and Brain Heart Infusion agar were purchased from Life Technologies (Paisley, UK). Tryptic Soy Agar was obtained from Criterion (St. Maria, CA, USA). Enzyme Linked Immunosorbent Assay (ELISA) kit was obtained from eBioscience. All remaining chemicals were purchased from Sigma–Aldrich (St. Louis, MO USA) or Merck (Darmstadt, Germany).

Preparation of the extracts

Fresh leaves of *C. cajan, M. citrifolia, O. americanum* were collected from the area of Chiang Mai province, Thailand. The plant voucher specimens (no. 023173, 023174, and 023175, respectively) were deposited at the Herbarium of Faculty of Pharmacy, Chiang Mai University (CMU), Thailand authenticated by the CMU staff botanist. After washing, the plants were cut into small pieces, dried in a circulating oven at 55°C and ground into fine powders.

For the antioxidant and antibacterial assays, the powders were extracted 3 times for 48 h at room temperature using ethanol and

the 3 macerates were pooled and filtered. The solvent of the filtrate was removed using a rotary evaporator at 45 °C under vacuum to obtain crude extracts.

For the anti-inflammatory assay, the dried powder of the plants was extracted with 3 different solvents: 100% water, 100% ethanol, and 50% ethanol for 24 h under stirring at room temperature. The extracts were filtered and the ethanol of the 100% and 50% ethanol extract were evaporated in a rotary evaporator, then the remaining water part of 50% ethanol and 100% water was removed by freeze-drying.

Antibacterial activity test

Plant extracts in dimethyl sulfoxide (DMSO, 200 mg/ml, 20 µl) were gradually added onto a 5.5 mm-diameter paper disc until the entire extract was absorbed. The disc was placed on a petri dish containing the test bacteria in the respective agar-medium. Standard amoxicillin (20 µg/disc) was used as a positive control. Tryptic Soy Agar was used for *Staphylococcus aureus, Staphylococcus epidermidis*, *Escherichia coli* and *Salmonella spp*. and Brain Heart Infusion Agar for *Streptococcus suis* and *Corynebacterium diphtheriae.* The concentration of the bacteria was calibrated as equivalent to a McFarland standard No. 0.5. As a criterion for the antibacterial activity of the extracts the diameter of the inhibition zone was used.

Antioxidant activity test

The antioxidant activity of the extracts in the present study was investigated by two antioxidant mechanisms; the free radical scavenging mechanism and the reducing powder mechanism.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was determined using the method previously described [18] with some modification. In brief, the extract solution (20 µl, 0.1 mg/ml) and the DPPH radical stock solution in ethanol (180 uL, 100µM) were mixed, shaken and incubated at room temperature for 30 min. The decrease in absorbance was monitored at 520 nm. The results were expressed as Trolox equivalent antioxidant activity (TEAC) in mM trolox per mg of the extract.

Another assay based on the reducing power of a potential antioxidant compound which reduces the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}) was performed. The later reacts with 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ) to obtain a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 595 nm as described previously [19]. The extract samples (0.1 mg/ml, 20 µl) were combined with the ferric reducing antioxidant reagent (FRAP; 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3$ and 0.3 M acetate buffer, pH 3.6; 180 µl) in a well. After 5 min of mixing, the absorbance was measured at 595 nm. The reducing power was expressed as equivalent concentration (EC) which was defined as the concentration of antioxidant having a ferric reducing ability equivalent of 1 mM $FeSO_4$.

Anti-inflammatory assay

RAW 264.7 macrophages were cultured in DMEM supplemented with 10% FBS and 4 mM L-glutamine. Anti-inflammatory assay was performed as described previously [20]. In brief, cells were seeded at a density of 2×10^6cells/ml into 12 well plates. After 24 h of incubation, the samples (in DMSO) were added at a final concentration of 100 µg/ml. After 3 h of incubation, the cells were stimulated with 1 µg/ml lipopolysaccharide (LPS) from *E. coli* and incubated for another 24 h. The supernatants were harvested and used for ELISA. The cell viability of the attached cells at the plate bottom was determined using MTT (Thiazolyl Blue Tetrazolium Bromide) assay.

Cells were incubated with MTT solution for 2 h and lysed using 0.01N HCl containing 10% SDS. The absorbance of the lysed cells was measured at 570 nm with a reference at 690 nm using Infinite M200 microplate reader (Tecan, Austria).

The secretion of interleukin (IL)-6, tumor necrosis factor (TNF)-α, and IL-10 in the cell supernatants was quantified with ELISA kits according to the manufacturer's instructions (eBioscience). The variation of cell density was reduced by using MTT values to normalize the cytokine concentrations. The amount of cytokines of the positive control (LPS only) was defined as 100%. Dexa-methasone (10^{-7} M) was tested in parallel as positive control.

All the assays (anti-inflammatory, antibacterial and antioxidant) were performed in triplicates on independent days.

Table 1: Inhibition zone of the crude extracts by disk diffusion method

Plant name	Plant part	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*
C. cajan	Leaves	18.3±0.3	13.4±0.2	7.2±0.3	NZ	7.8±0.3	14.0±0.0
M. citrifolia	Leaves	NZ	NZ	NZ	NZ	7.0±0.0	7.8±0.3
O. americanum	Aerial part	NZ	NZ	NZ	NZ	8.0±0.0	8.3±0.3
Amoxicillin	-	30.0±0.0	32.0±0.0	11.0±0.0	10.0±0.0	7.0±0.1	8.0±0.0

*mean±SD (n = 6), NZ = no zone

Table 2: Antioxidant activity of the crude extracts

Plant name	Plant part	Antioxidant activity*	
		TEAC (mM/mg)	EC (mM/mg)
C. cajan	Leaves	4.6 ± 0.9	14.0 ± 2.2
M. citrifolia	Leaves	2.8 ± 1.2	6.0 ± 0.7
O. americanum	Aerial part	3.0 ± 2.7	8.6 ± 0.3
Butylated hydroxytoluene	–	1.0 ± 0.2	22.4 ± 1.8

*mean±SD (n = 3)

Table 3: Influence of the plant extracts on the secretion of cytokines in LPS-stimulated macrophages

Plant name	Plant part	Solvent for extraction*	IL-6 (%)*	TNF-α (%)*	IL-10 (%)*
C. cajan	Leaves	Ethanol	31±8	62±10	77±15
		Water	95±10	74±12	110±8
		50% Ethanol	62±7	84±7	80±8
M. citrifolia	Leaves	Ethanol	54±8	62±4	92±2
		Water	48±17	64±7	81±8
		50% Ethanol	45±12	76±17	77±4
O. americanum	Aerial part	Ethanol	53±17	56±2	77±5
		Water	115±15	111±37	175±6
		50% Ethanol	48±17	63±18	70±9
Dexamethasone 10^{-7}M			35±6	64±9	96±16

*mean±SD (n = 3)

RESULTS AND DISCUSSION

Antibacterial activity

The antibacterial activity exhibited by the extracts at different levels is shown in table 1 as diameters of inhibition zones. *M. citrifolia* and *O. americanum* possess antibacterial activity only against Gram positive and Gram negative anaerobs. Interestingly, the extracts of *C. cajan* show a broad spectrum of activity against both Gram positive and Gram negative (except *E. coli*) aerobic and anaerobic bacteria.

A significant inhibitory effect of *C. cajan* leaves extracts against *S. epidermidis, S. aureus* and *Bacillus subtilis* was found previously [15]. Recently the antibacterial effect of cajanol, isolated from the roots of this plant on six different strains was shown [21].

The methanol extract of *M. citrifolia* leaves from previous studies presented no antibacterial activity on Gram negative *E. coli*, similar to our results in the present study [22]. However, there are some reports that show that the plant extract possessed activity against the Gram positive *S. aureus* as well as the Gram negative, anaerobic *Aeromonas hydrophila* [17].

To the best of our knowledge this is the first study to show the antibacterial effect of *O. americanum* leaf extract. The antimicrobial activity of the essential oil extracted from the fresh leaves of *O. americanum* was previously reported against Gram positive *S. aureus* but not against Gram negative *E. coli* [23]. Other research groups reported that the essential oil of *O. americanum* has antimicrobial activity against *S. mutans* and *Candida albicans* [10] as well as certain Gram negative facultative anaerobic bacteria like *Klebsiella pneumonia* [23].

Antioxidant activity

All three extracts showed significant antioxidant effects in the two test systems used (table 2). Our study on the antioxidant effect of *C. cajan* are consistent with previous studies. The aqueous and ethanolic extract of *C. cajan* leaves exerted antioxidant effects in various systems including DPPH radical-scavenging assay and a beta-carotene-linoleic acid test [14, 24].

The results in the present study reveal that the leaf extract of *M. citrifolia* showed scavenging ability against DPPH and ABTS free radicals. This is consistent with literature reports where a leaves extract exhibited a significant antioxidant activity, which was similar to Trolox, an antioxidant standard. The extract was highly effective in inhibiting hydroxyl damage, lipid peroxidation and nitric oxide formation. The aqueous extract from leaves of *M. citrifolia* showed significant antioxidant effects *in vivo*, as tested in mice with carrageenan induced peritonitis [17].

The antioxidant activity of the extract of *O. americanum* leaves is consistent with previous studies where potent antioxidant activity was shown using various methods [12].

Anti-inflammatory activity

The secretion of the pro-inflammatory cytokine, IL-6, was significantly reduced by at least 25% when adding the extracts with all solvents of *M. citrifolia* leaves and the ethanolic extracts of leaves from *C. cajan* and *O. americanum* (table 3, Fig.1). The secretion of the pro-inflammatory TNF-α was significantly reduced by at least 25% when adding the extracts of *M. citrifolia* leaves, the ethanolic extracts of *O. americanum* leaves or the ethanol and water extracts of *C. cajan* leaves. We found that *O. americanum* leaf extracts showed anti-inflammatory activity in respect to all cytokines used in this study since the water extract also increased the anti-inflammatory IL-10 production.

To the best of our knowledge this is the first study which elucidates the anti-inflammatory effect of the leaves of *C. cajan*. Our results of the anti-inflammatory effect of *C. cajan* leaves extract was quite similar to a previous study, in which ethanol extracts of the whole plants of *C. cajan* extracts suppressed the production of inflammatory cytokines in RAW 264.7 cells [16]. In another study, pinostrobin and cajanus lactone were identified as main active anti-inflammatory compounds of the methanolic extracts from leaves as indicated by a significant inhibition of TNF-α and IL-1β in LPS-stimulated macrophages in rats [25].

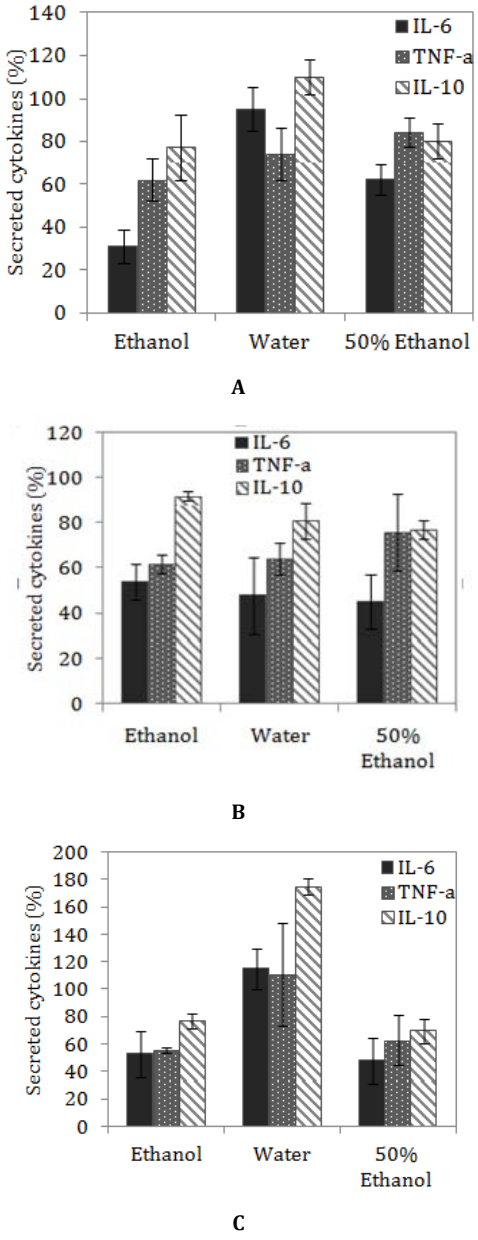

Fig. 1: Influence of extracts from A) *C. cajan*, B) *M. citrifolia*, C) *O. americanum* on the secretion of IL-6, TNF-α, and IL-10

The leaves of *M. citrifolia* extracts reduced both the IL-6 and TNF-α secretion. In previous studies, the aqueous extract from *M. citrifolia* leaves showed anti-inflammatory effects in mice [17]. More studies on the anti-inflammatory effects were reported on the fruit juice of the plant [26].

To the best of our knowledge, no previous study has been reported on the anti-inflammatory effect of *O. americanum.*

When comparing water and ethanol as solvent, ethanol dissolves more bioactive compounds from *C. cajan* and *O. americanum*. For *M. citrifolia*, water and ethanol are similarly effective as solvents.

CONCLUSION

Inflammation and oxidative stress are closely related and play a role in a broad range of diseases. If the disease includes an infectious component, an antibacterial effect of a natural drug is of advantage. The

findings of this study suggest that the tested plants can be developed as effective herbal remedies for the treatment and prevention of inflammation, oxidative imbalance or bacterial infections. As the plants are also common edible food, *C. cajan, M. citrifolia*, and *O. americanum* may be of use to the functional food industry.

CONFLICT OF INTERESTS

Declare None

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