Antioxidant and Nitric Oxide Inhibition Activities of Thai Medicinal Plants

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Nineteen Thai medicinal plants used in Thai traditional medicine preparation to treat colds, asthma and fever were studied for their antioxidant and NO inhibitory activities. Three extracts were obtained from each plant. First extract obtained by macerating the plant part in 95% ethanol (Et) residue was boiled in water, where water extract (EW) was obtained. The third extract (HW) was obtained by boiling each plant in water similar to that of Thai traditional medicine practice. These extracts were tested for their antioxidant activity using DPPH assay, and anti-inflammatory activity by determination of inhibitory activity on lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW 264.7 cell lines using Griess reagent. Results indicated that Et, EW and HW of Syzygium aromaticum showed the highest antioxidant activity (EC_{50} = 6.56, 4.73 and 5.30 μg/ml, respectively). Et of Atractylodes lancea exhibited the most potent inhibitory activity on lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW 264.7 cells, with IC_{50} value of 9.70 μg/ml, followed by Et of Angelica sinensis and Cuminum cyminum (IC_{50} = 12.52 and 13.56 μg/ml, respectively) but water extract (EW, HW) of all plants were apparently inactive. These results of anti-inflammatory activity of these plants correspond with the traditional use for fever, cold, allergic-related diseases and inflammatory-related diseases.

Keywords: *Antioxidant, RAW 264.7 cells, Nitric oxide, Lipopolysaccharide, Thai medicinal plants, Syzygium aromaticum, Atractylodes lancea, Angelica sinensis, Cuminum cyminum*

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Reactive nitrogen species (RNS) and reactive oxygen species (ROS) include both radical and non-radical molecules with unpaired orbital electrons derived from nitrogen, such as nitric oxide and oxygen, such as peroxyl radical. RNS and ROS play important roles in killing foreign organisms and in acute inflammation but their over-production may cause tissue damage and vascular leakage in septicemia, rheumatoid arthritis and inflammatory disease[1]. Furthermore, the interaction between RNS and ROS can also lead to the production of highly reactive non-radical species such as peroxynitrite, a product of nitric oxide and superoxide; commonly generated by macrophages under pathological conditions[2].

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Nitric oxide (NO) is one of the inflammatory mediators causing inflammation in many organs. This inorganic free radical has been implicated to involve in physiologic and pathologic processes. At low concentration NO has been shown to play a role as neurotransmitter and NO produced at high concentration is implicated in having a role in the pathogenesis of vasodilation, non-specific host defense, ischemic stroke, septic shock and acute or chronic inflammation[3,4]. NO is produced from L-arginine by a chemical reaction catalyzed by the enzyme inducible nitric oxide synthase (iNOS) in living systems. After stimulation with bacterial lipopolysaccharide (LPS), many cells including macrophages express the iNOS which is responsible for the production of large amount of NO[1]. Therefore, Thai medicinal plants used in the relevant aspect should be examined for their inhibitory activities against lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW 264.7 cell lines.

Nineteen Thai medicinal plants which have

been used in Thai traditional medicine preparation to treat fever, cold and asthma, were *Amomum testaceum* Ridl (Zingiberaceae), *Anethum graveolens* L (Umbelliferae), *Angelica dahurica* Benth (Umbelliferae), *Angelica sinensis* (Oliv) Diels (Umbelliferae), *Artemisia annua* L (Compositae), *Atractylodes lancea* (Thunb) DC (Compositae), *Cuminum cyminum* L (Umbelliferae), *Dracaena loureiri* Gagnep (Dracaenaceae), *Foeniculum vulgare* Mill var *dulce* (Mill) Thell (Umbelliferea), *Kaempferia galanga* L (Zingiberaceae), *Lepidium sativum* L (Cruciferae), *Ligusticum sinense* Oliv cv Chuanxiong (Umbelliferae), *Mammea siamensis* Kosterm (Guttiferae), *Mesua ferrea* L (Guttiferae), *Mimusops elengi* L (Sapotaceae), *Myristica fragrans* Houtt (Myristicaceae), *Nelumbo nucifera* Gaertn (Nelumbonaceae), *Nigella sativa* L (Ranunculaceae) and *Syzygium aromaticum* (L) Merr et Perry (Myrtaceae). They are commonly used as carminative, expectorant, tonic, cardiotonic, diuretic, antipyretic and cold treatment in Thai traditional medicine[5]. In spite of the fact that all of these plants have been reported for antioxidant activity (Table 1), but the NO-inhibitory activity of some plants were not reported. In the present study, the nineteen Thai medicinal plant extracts were tested for their antioxidant and NO-inhibitory activities. These results should be supported using these plants for anti-inflammatory in reducing fever or allergy in asthma or cold.

Material and Method

Animal cell lines and Reagents

RAW 264.7 murine macrophage leukemia cell lines were established and kindly provided by Assoc Prof Dr. Supinya Tewtrakul, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. RPMI Medium 1,640 (RPMI 1,640) powder with L-glutamine, Fetal Bovine Serum (FBS), Penicillin-Streptomycin (P/S), trypsin-EDTA and trypan blue were purchased from Gibco, USA. Phosphate Buffer Saline (PBS) was from Amresco (USA), sodium bicarbonate was from BDH, England, lipopolysaccharide (LPS, from *Escherichia coli*), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) were from Sigma. Ninety six well microplates were also purchased from Costar Corning, USA. DPPH and BHT were from Fluka, other chemicals were from Sigma and Merck.

Plant materials

The parts of plants, which were reported to be used as anti-allergy and anti-inflammation by folk doctors in Thailand, were collected and purchased from several regions of Thailand, China, India, Indonesia and Australia. The place of collection, plant parts, voucher specimens and biological activities of these plants are shown in Table 1. The voucher specimens are deposited at the herbarium of Southern Center of Thai Medicinal Plants at Faculty of Pharmaceutical Science, Prince of Songkla University, Songkhla, Thailand.

Preparation of the plant extracts

Plant materials were washed, sliced thinly, dried in an oven at 50°C, powdered and extracted similar to those practiced by Thai traditional doctors, *i.e.* each dried plant material (300 g) was macerated in 95% ethanol for 3 days, 2 times, filtered and dried using an evaporator. For decoction, each residue from maceration (100 g) or each dried plant material (100 g) was boiled in distilled water for 30 minutes, filtered and dried using a lyophilizer. The percentage of yield is shown in Table 2. The ethanolic extracts were dissolved in dimethyl sulfoxide (DMSO) and the water extracts were dissolved in sterile water, sterilized by filtration (pore size, 0.2 mm) before testing. Stock solutions (10 mg/ml) of the extracts were stored at -20°C until use.

DPPH radical scavenging assay

The antioxidant activity of these plant extracts were evaluated by DPPH radical scavenging assay using modified method[6]. Samples for testing were dissolved in absolute ethanol or distilled water to obtain the highest concentration of 200 µg/ml. Each sample was further diluted for at least 4 concentrations (two-fold dilutions). Each concentration was tested in triplicate. A portion of the sample solution (100 µl) was mixed with an equal volume of 6×10^{-5} M DPPH (in absolute ethanol) and allowed to stand at room temperature for 30 minutes. The absorbance (A) was then measured at 520 nm. BHT (butylated hydroxytoluene), a well known synthetic antioxidant, was used as a positive standard. The scavenging activity of the samples is the ability to reduce the color intensity of DPPH. Inhibition (%) was calculated using the following equation and EC_{50} values was calculated from the Prism program.

$$\% \text{ inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Assay for NO inhibitory effect

Inhibitory effect on NO production by murine macrophage-like RAW 264.7 cells was evaluated by the following method[4,7]: the RAW 264.7 cell lines were

Table 1. The ethnobotanical data and biological activities of plants

Species	Places for specimen collection	Voucher specimen number	Thai name	Plant part	Biological activities
A. testaceum	Chanthaburi	SKP206011101	Krawan	fruit	Antioxidant[8]
A. graveolens	India	SKP199010701	Thian ta takkataen	fruit	Antioxidant[8-10]
A. dahurica	China	SKP199010401	Kot so	root	Antioxidant[11,12], Anti-inflammation[13-16]
A. sinensis	China	SKP199010901	Kot chiang	root	Antioxidant[17-19], Analgesic[20,21], Anti-inflammation[20]
A. annua	China	SKP051010101	Kot chula lampha	all part	Antioxidant[17,22], Anti-inflammation[23]
A. lancea	China	SKP051011201	Kot kamao	rhizome	Antioxidant[17], Anti-inflammation[24]
C. cyminum	India	SKP199030301	Thian khao	fruit	Antioxidant[10,25-27], Antinociceptive[28]
D. loureiri	Ratchaburi, Kanchanaburi	SKP065041201	Chan daeng	stem	Antioxidant[29], Antinociceptive[30], Antipyretic[30], Anti-inflammation[31]
F. vulgare	India	SKP199062201	Thian khao plueak	fruit	Antioxidant[10,17,26,27]
K. galanga	Ratchaburi, Kanchanaburi	SKP206110701	Proh hom	rhizome	Antinociceptive[32]
L. sativum	India	SKP057121901	Thian daeng	seed	Analgesic[33], Antipyretic[33], Anti-inflammation[33]
L. sinense	China	SKP199121901	Kot hua bua	rhizome	Antioxidant[34]
M. siamensis	Ratchaburi, Kanchanaburi	SKP083131901	Saraphi	flower	Antioxidant[35]
M. ferrea	Ratchaburi, Kanchanaburi	SKP083130601	Bunnak	flower	n/a
M. elengi	Ratchaburi, Kanchanaburi	SKP171130501	Phikul	flower	Antioxidant[36]
M. fragrans	Australia	SKP121130601	Chan thet	stem	Antioxidant[29]
	Suratthani	SKP121130601	Mace	aril	Antioxidant[26], Analgesic[37], Anti-inflammation[37]
	Suratthani	SKP121130601	Nutmeg	seed	Antioxidant[26,38]
N. nucifera	Ratchaburi, Kanchanaburi	SKP125141401	Kasorn bua luang	pollen	Antioxidant[39,40]
N. sativa	India	SKP160141901	Thian dam	seed	Antioxidant[25], Analgesic[41], Anti-inflammation[41-43]
S. aromaticum	Indonesia	SKP123190101	Kan phlu	flower	Antioxidant[44]

n/a = not applicable

cultured in RPMI 1640 medium supplemented with 10% heated fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were maintained at 37°C in 5% CO_2 atmosphere with 95% humidity where cultured medium is changed three times a week. Cells were washed with phosphate buffer saline (PBS). PBS was decanted and cells were harvested with 0.25% trypsin-EDTA and fresh medium was added. Cell pellet, obtained by centrifugation (1,000 rpm, 6 min), was resuspended in 10 ml of medium to make a single

Table 2. Percentage of yield of extracts, antioxidant activity (EC_{50} µg/ml ± SEM) and inhibition of NO production (IC_{50} µg/ml ± SEM) by plant extracts (n = 3)

Plant name	Solvent	Code	% Yield of extract	Antioxidant activity EC_{50} ± SEM (µg/ml)	Inhibition of NO production	
					% Inhibition at conc 100 µg/ml	IC_{50} ± SEM (µg/ml)
A. testaceum	Et	AmTEt	2.42	64.45 ± 1.71	82.50 ± 4.54	81.42 ± 3.48
	EW	AmTEW	9.32	>100	40.93 ± 5.11	>100
	HW	AmTHW	2.41	>100	37.20 ± 3.18[a]	>100
A. graveolens	Et	AnGEt	4.34	>100	83.13 ± 3.20	84.97 ± 4.48
	EW	AnGEW	6.67	65.45 ± 3.88	27.93 ± 2.26	>100
	HW	AnGHW	9.66	>100	53.80 ± 9.93[a]	-
A. dahurica	Et	AnDEt	5.12	>100	88.33 ± 1.76[a]	44.23 ± 2.71
	EW	AnDEW	9.78	>100	13.17 ± 0.72	>100
	HW	AnDHW	2.54	>100	13.50 ± 0.35[a]	>100
A. sinensis	Et	AnSEt	15.05	>100	95.60 ± 0.71[a]	12.52 ± 2.31
	EW	AnSEW	17.83	>100	6.17 ± 0.23	>100
	HW	AnSHW	11.39	>100	42.13 ± 4.56	>100
A. annua	Et	ArAEt	4.27	72.40 ± 3.95	96.47 ± 2.23[a]	17.06 ± 2.69
	EW	ArAEW	11.16	32.75 ± 1.78	16.50 ± 0.80	>100
	HW	ArAHW	12.14	>100	35.17 ± 3.08[a]	>100
A. lancea	Et	AtLEt	16.89	>100	94.03 ± 3.22[a]	9.70 ± 0.54
	EW	AtLEW	19.29	>100	49.30 ± 0.35[a]	>100
	HW	AtLHW	18.22	>100	39.27 ± 4.41[a]	>100
C. cyminum	Et	CuCEt	8.73	>100	92.57 ± 2.05[a]	13.56 ± 0.59
	EW	CuCEW	10.83	>100	62.50 ± 0.20[a]	-
	HW	CuCHW	7.31	>100	27.40 ± 7.31	>100
D. loureiri	Et	DrLEt	17.87	17.28 ± 1.53	89.00 ± 2.89[a]	38.37 ± 1.66
	EW	DrLEW	0.58	44.97 ± 4.66	18.97 ± 1.84	>100
	HW	DrLHW	0.79	23.01 ± 1.72	47.63 ± 2.87[a]	>100
F. vulgare	Et	FoVEt	6.69	>100	89.57 ± 1.59[a]	40.81 ± 0.59
	EW	FoVEW	11.09	88.48 ± 4.10	48.00 ± 1.61[a]	>100
	HW	FoVHW	6.46	>100	14.33 ± 3.21	>100
K. galanga	Et	KaGEt	6.39	>100	94.60 ± 3.29[a]	30.30 ± 1.23
	EW	KaGEW	21.51	>100	12.13 ± 1.25	>100
	HW	KaGHW	3.38	>100	45.77 ± 4.13[a]	>100
L. sativum	Et	LeSEt	9.20	>100	44.93 ± 1.67	>100
	EW	LeSEW	0.87	>100	68.07 ± 1.05[a]	-
	HW	LeSHW	6.37	>100	5.30 ± 2.66	>100
L. sinense	Et	LiSEt	12.19	56.96 ± 3.85	92.00 ± 4.70[a]	16.48 ± 2.03
	EW	LiSEW	7.94	>100	43.57 ± 1.78[a]	>100
	HW	LiSHW	7.24	>100	20.43 ± 5.06	>100
M. siamensis	Et	MaSEt	32.78	8.54 ± 0.73	73.07 ± 3.65	74.62 ± 8.77
	EW	MaSEW	8.76	32.69 ± 1.95	12.77 ± 2.37	>100
	HW	MaSHW	10.42	8.70 ± 0.58	43.77 ± 2.59[a]	>100
M. ferrea	Et	MeFEt	23.17	16.12 ± 0.93	96.03 ± 1.82[a]	26.23 ± 3.42
	EW	MeFEW	15.45	7.49 ± 0.57	24.33 ± 2.25	>100
	HW	MeFHW	4.65	6.95 ± 0.27	41.30 ± 6.90	>100
M. elengi	Et	MiEEt	8.82	8.19 ± 0.40	83.33 ± 2.83[a]	69.24 ± 5.30
	EW	MiEEW	4.34	54.28 ± 3.40	35.90 ± 2.03	>100
	HW	MiEHW	3.97	>100	43.40 ± 6.05[a]	>100
M. fragrans	Et	MyFET	7.07	46.62 ± 2.08	93.83 ± 2.86[a]	25.14 ± 0.46
(Chan thet)	EW	MyFEW	0.79	48.93 ± 2.67	25.40 ± 1.83	>100
	HW	MyFHW	0.44	34.82 ± 3.99	40.50 ± 4.40[a]	>100

Table 2. Cont.

Plant name	Solvent	Code	% Yield of extract	Antioxidant activity $EC_{50} \pm$ SEM (μg/ml)	Inhibition of NO production	
					% Inhibition at conc 100 μg/ml	$IC_{50} \pm$ SEM (μg/ml)
M. fragrans	Et	MyFET(A)	18.97	18.02 ± 0.76	88.97 ± 2.71	78.38 ± 1.82
(Mace)	EW	MyFEW(A)	2.62	>100	46.37 ± 2.28	>100
	HW	MyFHW(A)	2.63	>100	44.13 ± 4.0	>100
M. fragrans	Et	MyFEt(S)	13.67	11.38 ± 0.64	78.38 ± 1.84[a]	47.23 ± 0.32
(Nutmeg)	EW	MyFEW(S)	7.14	>100	38.37 ± 3.03	>100
	HW	MyFHW(S)	4.70	>100	36.30 ± 2.87	>100
N. nucifera	Et	NeNEt	10.59	83.27 ± 2.98	44.20 ± 2.03	>100
	EW	NeNEW	6.11	32.72 ± 2.55	48.57 ± 0.37	>100
	HW	NeNHW	3.04	8.87 ± 0.01	42.23 ± 1.38[a]	>100
N. sativa	Et	NiSEt	32.29	>100	27.60 ± 7.97	>100
	EW	NiSEW	6.78	>100	7.20 ± 0.53	>100
	HW	NiSHW	6.02	>100	78.20 ± 3.02[a]	-
S. aromaticum	Et	SyAEt	31.24	6.57 ± 0.31	81.43 ± 1.74	81.34 ± 2.62
	E	SyAEW	6.53	4.73 ± 0.18	27.37 ± 1.59	>100
	HW	SyAHW	6.42	5.30 ± 0.35	24.43 ± 1.64	>100
BHT	-	-	-	11.66 ± 1.01	-	-
Indomethacin	-	-	-	-	63.70 ± 1.50[b]	20.32 ± 3.23

- = Not tested
[a] = Cytotoxic effect was observed
[b] = % Inhibition at concentration 100 mM

cell suspension. The viable cells were counted using trypan blue and diluted with medium to give a final concentration of 1 x 10^6 cells/ml for RAW 264.7. Volume of 100 μl/well of these cell suspensions was seeded in each 96-well microplates having 1 x 10^5 cells/well and allowed to adhere for 1 h at 37°C in 5% CO_2. The medium was then replaced with fresh medium containing 5 μg/ml of LPS together with test sample at various concentration and then incubated for 48 h. Each extract was initially dissolved in DMSO for ethanolic extract, or dissolved in sterile distilled water, for water extract. The extracts were diluted in medium to required concentrations. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess reagent. Cytotoxicity was determined using the 3-(4,5-dimethyl-2- thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) colorimetric method. Briefly, after 48 h incubation with test samples, MTT solution (10 μl, 5 mg/ml in PBS) was added to the wells. After 2 h incubation, the medium was removed, and isopropanol containing 0.04 M HCl was then added to dissolve the formazan production in the cells. The optical density of the formazan solution was measured with a microplate reader at 570 nm. The test sample was considered to be cytotoxic when the optical density of the sample-treated group was less than 70-80% of that in the control (vehicle-treated) group. Indomethacin was used as positive controls. Inhibition (%) was calculated using the following equation and IC_{50} values was calculated from the Prism program.

$$\text{Inhibition (\%)} = \frac{A - B}{A - C} \times 100$$

Value represents NO_2^- concentration (μM) in:
A: LPS (+), sample (-)
B: LPS (+), sample (+)
C: LPS (-), sample (-)

Results

The ethnobotanical data of the investigated plant species, which include botanical names, plant part used and biological activities showed in Table 1. The results of DPPH radical scavenging activity and inhibitory activity exerted by all plants extracts against LPS induced NO production in RAW 264.7 cell lines were shown in Table 2.

For DPPH radical scavenging activity, this

data showed that the ethanolic extract of *Syzygium aromaticum*, *Mimusops elengi*, *Mammea siamensis* and *Myristica fragrans* (Nutmeg) (EC_{50} = 6.57, 8.19, 8.54 and 11.38 µg/ml, respectively) exhibited higher activity than BHT (EC_{50} = 11.66 µg/ml), water extracts of *Syzygium aromaticum* (EW, HW), *Mesua ferrea* (EW, HW), *Mammea siamensis* (HW) and *Nelumbo nucifera* (HW) (EC_{50} = 4.73, 5.30, 7.49, 6.95, 8.70 and 8.87 µg/ml, respectively) exhibited stronger activity than BHT.

For nitric oxide inhibitory activity, this result showed that ethanolic extract of these plants exhibited the NO production inhibitory activity, where all the water extracts were apparently inactive (IC_{50} > 100 µg/ml). Six ethanolic extracts namely of *Atractylodes lancea*, *Angelica sinensis*, *Cuminum cyminum*, *Ligusticum sinense*, *Artemisia annua* and *Mesua ferrea* showed potent inhibitory activity, with IC_{50} values of 9.70, 12.52, 13.56, 16.48, 17.06, 26.23 µg/ml, respectively. Interestingly, these plant extracts exhibited NO production inhibitory effect higher than that of Indomethacin (IC_{50} = 56.78 mM or 20.32 µg/ml) which is a positive control except for *Mesua ferrea*. Particularly, ethanolic extract of *Atractylodes lancea* exhibited the most potent inhibitory activity having IC_{50} value of 9.70 µg/ml.

Discussion

The investigation of nineteen Thai medicinal plants based on their use by Thai traditional doctors for treatment of fever, cold, anti-allergy and anti-inflammation. Antioxidant activity of ethanolic and water extracts of these plants and their inhibitory effect on NO production from RAW 264.7 cells were determined. The results showed that the extracts of *Syzygium aromaticum, Mesua ferrea, Mimusops elengi, Mammea siamensis, Nelumbo nucifera* and *Myristica fragrans* exhibited strong antioxidant activity. Previous phytochemical studies on these plants, except for *Mesua ferrea* and *Mimusops elengi,* have indicated the presence of flavonoids and phenolic compounds[39,40,44-47]. It is possible that the chemical content of these plants, *i.e.*, the flavonoids and phenolic compounds are responsible for the strong antioxidant properties of these plants[48].

Anti-inflammatory activities of ethanolic and water extracts of these plants were tested by measuring their effects on the pro-inflammatory mediators NO in activated macrophages RAW 264.7 cells. The results showed that all water extracts of all plants are inactive. The ethanolic extract of *Atractylodes lancea* exhibited the most potent NO production inhibitory activity (IC_{50} = 9.70 µg/ml) which is higher than that of Indomethacin (IC_{50} = 20.32 µg/ml). This result is comparable to that study by Wang et al[49]. They isolated Atractylenolide I, from the rhizome of *Atractylodes lancea* where it inhibited LPS-induction of NO production in a dose-dependent manner, (IC_{50} = 7.5 µg/ml)[49]. Furthermore, the ethanolic extracts of *Angelica sinensis*, *Artemisia annua*, *Cuminum cyminum*, *Ligusticum sinense* and *Mesua ferrea* showed potent inhibitory activity (IC_{50} < 30 µg/ml). These results support previous study showing that water and ethanolic extracts of root of *Angelica sinensis* exhibited inhibitory effect on NO production in LPS activated RAW 264.7 macrophage in the concentration range of 20-200 µg/ml[18]. Ethyl acetate fraction of *A. sinensis* has been reported to decrease NF-kB luciferase activity and also the secretion of NO and PGE_2 in LPS/IFN-g stimulated mouse peritoneal macrophages[50].

In conclusion, the results obtained from the present study indicated that Thai medicinal plants, which were the ingredients of Thai folk medicine to treat fever and cold, possess strong antioxidant activity, as well as are active against LPS induced NO production in RAW 264.7 cell lines. *Syzygium aromaticum* and *Mimusops elengi* show strong antioxidant activity in both ethanolic and water extracts. The ethanolic extracts of *Atractylodes lancea, Angelica sinensis* and *Cuminum cyminum* exhibited strong NO-inhibitory activity. These results support using these plants by Thai folk medicine for treatment of fever, cold, allergic-related diseases and inflammatory-related diseases at least, through the inhibition of NO release. Further work should be on antioxidant and anti-inflammatory activities of isolated compounds from active extracts.

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ฤทธิ์ต้านอนุมูลอิสระและฤทธิ์ในการยับยั้งไนตริกออกไซด์จากสมุนไพรไทย

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ผู้นิพนธ์ได้ทำการศึกษาฤทธิ์ต้านอนุมูลอิสระ และฤทธิ์ในการยับยั้งการสร้างไนตริกออกไซด์ของสมุนไพร 19 ชนิด ซึ่งเป็นส่วนประกอบในการเตรียมยาที่ใช้รักษาอาการหวัด หอบหืด และแก้ไข้ ประกอบด้วยกระวาน เทียนตาตั๊กแตน โกฐสอ โกฐเชียง โกฐจุฬาลำพา โกฐเขมา เทียนขาว จันทน์แดง เทียนข้าวเปลือก เปราะหอม เทียนแดง โกฐหัวบัว สารภี บุนนาค พิกุล จันทน์เทศ เกสรบัวหลวง เทียนดำ และกานพลู โดยนำสมุนไพรมาสกัดได้สารสกัดด้วยเอทานอล (Et) กากที่เหลือต้มน้ำ (EW) และนำสมุนไพรแต่ละชนิดมาต้มน้ำตามตำราแผนไทย (HW) หลังจากนั้นนำสารสกัดที่ได้มาทดสอบฤทธิ์ต้านอนุมูลอิสระด้วยวิธี DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay และฤทธิ์ในการยับยั้งการสร้างไนตริกออกไซด์ในเซลล์ RAW 264.7 เมื่อถูกกระตุ้นด้วย LPS ด้วยวิธี Griess reagent จากผลการศึกษาฤทธิ์ต้านอนุมูลอิสระพบว่าสารสกัดชั้นเอทานอล (Et) และชั้นน้ำ (EW, HW) ของกานพลูมีฤทธิ์ต้านอนุมูลอิสระสูงที่สุด โดยมีค่า EC_{50} เท่ากับ 6.56, 4.73 และ 5.30 μg/ml ตามลำดับ จากผลการศึกษา ฤทธิ์การยับยั้งการสร้างไนตริกออกไซด์พบว่า สารสกัดชั้นเอทานอลของโกฐเขมามีฤทธิ์ในการยับยั้งการสร้างไนตริกออกไซด์ดีที่สุด โดยมีค่า IC_{50} เท่ากับ 9.70 μg/ml รองลงมาได้แก่ สารสกัดชั้นเอทานอลของโกฐเชียงและเทียนขาว โดยมีค่า IC_{50} เท่ากับ 12.52 และ 13.56 μg/ml ตามลำดับ ส่วนสารสกัดชั้นน้ำ (EW, HW) ไม่มีฤทธิ์ในการยับยั้งการสร้างไนตริกออกไซด์ (โดยมีค่า IC_{50} มากกว่า 100 μg/ml) ผลจากการศึกษาครั้งนี้สามารถใช้เป็นข้อมูลสนับสนุนการใช้พืชสมุนไพรไทยในการรักษาอาการไข้หวัดโรคที่เกี่ยวกับการแพ้ และโรคที่เกี่ยวข้องกับการอักเสบได้