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NF-κB Inhibitory Activities of the Methanol Extracts and some Constituents therein of some Vietnamese Medicinal Plants

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Abstract

Eighty-seven methanol extracts of medicinal plants, most of them are currently used in Vietnamese oriental medicine, were screened for NF-κB inhibitory activity. Seven methanol extracts showed strong to moderate inhibitory activity. These include the extracts of *Crinum latifolium* (leaves), *Evodia rutaecarpa* (fruits), *Polygonum cuspidatum* (rhizoma), *Perilla ocymoides* (leaves), *Rubia cordifolia* (leaves), *Scutellaria barbata* (leaves) and *Sparganium stenophyllum* (leaves). The NF-κB inhibitory activities of several metabolites isolated from some of these plants were also reported.

Keywords

NF-κB Inhibitory Activity • Screening • Medicinal Plants

Introduction

Nuclear factor-kappa B (NF-κB) is an inducible and ubiquitous transcriptional factor required for the gene expression of many inflammatory mediators [1]. Inappropriate and prolonged activation of NF-κB has been linked to several diseases associated with inflammatory events, including septic shock, acute respiratory distress syndrome, ischemia, and reperfusion injury [1]. Because of the pathophysiological importance of an enhanced production of inflammatory mediators through NF-kB activation, selective inhibitors of NF-κB activation may have broad application as novel therapeutics, for example, antiinflammatory agents. A number of other scientific evidences have also linked

the role of NF-κB activation to several oncogenic processes. Thus, NF-κB inhibitors may also have a potential for the treatment of some types of malignancy. In deed, a bulk of evidences has validated NF-κB as a target for antiinflammatory and anticancer agents.

In the past decade, extensive efforts have been devoted to discover selective NF-κB inhibitors. Several reports have documented a few NF-κB inhibitors, naturally occuring[2–6] or synthetic [7]. However, to date, no NF-κB inhibitor has been approved for use in clinics. Thus, the development of NF-κB inhibitors continues to be an appealing need. Medicinal plants have been and continue to be a powerful source of novel structures with diverse biological activities. Vietnamese flora in particular is still relatively under-investigated. From these perspectives, we have chosen Vietnamese medicinal plants as a source for screening of NF-κB inhibitory activity in our continuous program to discover novel and potent compounds as NF-κB inhibitors. As a part of this program, we have screened eighty-seven kinds of herbal drugs used in Vietnamese oriental medicine for NF-κB inhibitory activity. This paper describes and discusses the results obtained from this screening and some preliminary results from phytochemical studies carried out on some active plants found in the preceding screening.

Results and discussion

The results of the screening of eighty-seven Vietnamese herbal medicine and medicinal plants for NF-κB inhibitory activity are presented in table 1. This NF-κB inhibition assay used RAW264.7 cells and was carried out as detailed in the Experimental part. To choose a non-cytotoxic concentration of each sample for the NF-κB assay, an *in vitro* cytotoxicity assay was also performed and the ED_{50} values (the concentration of the sample that inhibits the growth of cells by 50%) were included in this table. Based on the results of the cytotoxicity assay we have chosen a noncytotoxic concentration for each plant or herbal medicine sample. These concentrations were used in the NF-κB inhibition assay. Seven plant samples were found to exhibit strong to moderate inhibitory activity. These samples include *Crinum latifolium* (folium), *Evodia rutaecarpa* (herba), *Perilla ocymoides* (folium), *Polygonum cuspidatum* (radix), *Rubia cordifolia* (folium), *Scutellaria barbata* (folium) and *Sparganium stenophyllum* (folium). The list of the active medicinal plants in the NF-κB inhibition screening is extracted from table 1 and presented in table 2.

Chemically, the plant *Evodia rutaecarpa* has been reported to contain evodiamine, an alcaloid which exhibits antiproliferative, antimetastatic, and apoptotic activities. In a recent study Takada and colleagues have demonstrated that evodiamine inhibits both constitutive and induced NF-κB activation and NF-κB-regulated gene expression [8]. Thus, the NF-κB inhibitory activity of the *Evodia rutaecarpa* sample might well be attributable to that of evodiamine.

Regarding *Polygonum cuspidatum*, it was postulated that the NF-κB inhibitory activity might be resulted from resveratrol and emodin. Emodin is one of the main chemical constituents of *Polygonum cuspidatum*. Resveratrol has also been widely reported to be present in this plant. In our separate study on the Vietnamese medicinal plants we have also isolated both resveratrol and emodine from *Polygonum cuspidatum* growing in Vietnam [9]. Especially, the content of resveratrol therein was determined to be 0.28%, relatively high [10]. In our NF-κB inhibition assay system, resveratrol and emodin showed IC_{50} values of 5.2 and 5.9 μM, respectively.

Tab. 1. NF-κB inhibitory activity of methanol extracts of some medicinal plants

Medicinal Plants No	Scientific name	Family name	Vernacular name	Part*	Code	ED_{50}**	NF-κB inhibitory activity***
1	*Acanthopanax aculeatus*	*Araliaceae*	Ngugiabi	W	N22	36.96	–[2]
2	*Acorus gramineus*	*Araceae*	Xuongbo	H	N36	75.34	–[2]
3	*Aconitum sinensis*	*Ranunculaceae*	Odau	Ra	N23	58.25	–[2]
4	*Achyrantes bidentata*	*Amaranthaceae*	Tancuu	F	N47	> 100	–[1]
5	*Adenosma caeruleum*	*Crophulariaceae*	Nhantran	H	N61	> 100	–[1]
6	*Ageratum conyzoides*	*Asteraceae*	Hoacutlon	H	N75	>100	–[1]
7	*Alpinia officinalum*	*Zingiberaceae*	Rieng	Ra	N67	53.85	–[2]
8	*Alpinia oxyphyllium*	*Zingiberaceae*	Ichchinhan	S	N68	>100	–[1]
9	*Alpinia katsumadai*	*Zingiberaceae*	Thaodaukhau	Rh	N69	77.21	–[2]
10	*Alocasia odora*	*Araceae*	Ray	Ra	N76	>100	–[1]
11	*Anemarrhena aspheloides*	*Liliaceae*	Trimau	Ra	N97	>100	–[1]
12	*Angelica laxiflora*	*Apiaceae*	Dochoat	Ra	N19	> 100	–[1]
13	*Arctium lappa*	*Asteraceae*	Nguubang	H	N10	>100	–[1]
14	*Aristolochia westlandii*	*Aristolochiaceae*	Phongky	Rh	N24	> 100	–[1]
15	*Areca catechu*	*Arecaceae*	Cau	S			
16	*Argyreia acuta*	*Convolvulaceae*	Bacthau	H	N77	>100	–[1]
17	*Aquilaria agalocha*	*Thymelaceae*	Tram	W	N54	>100	–[1]
18	*Aristolochia balancae,*	*Aristolochiaceae*	Phongky	Rh	N58	>100	–[1]
19	*Astemisia vulgaris*	*Asteraceae*	Ngaicuu	H	N78	>100	–[1]
20	*Atractylodes macrocephala*	*Asteraceae*	Bachtruat	Rh	N79	>100	–[1]
21	*Blumea lacera*	*Asteraceae*	Caitroi	H	N80	>100	–[1]
22	*Bombax ceiba*	*Bombacaceae*	Gao	W	N70	>100	–[1]
23	*Bupleurum falcatum*	*Apiaceae*	Saiho	Ra	N81	>100	–[1]
24	*Caryota urens*	*Arecaceae*	Dungdinh	Fm	N82	>100	–[1]

Tab. 1. (Cont.)

Medicinal Plants No	Scientific name	Family name	Vernacular name	Part*	Code	ED_{50}**	NF-κB inhibitory activity***
25	*Cebotium barometz*	*Dicksoniaceae*	Cautich	Rh	N04	>100	−[1]
26	*Ceiba pentandra*	*Bombacaceae*	Gongai	St	T05	>100	+[1]
27	*Ceiba pentandra*	*Bombacaceae*	Gongai	Fr	T01	>100	+[1]
28	*Chromolaena odorata*	*Asteraceae*	Colao	H	N83	>100	+[1]
29	*Chrysanthemum sinensis*	*Asteraceae*	Cuc	H	N53	>100	−[1]
30	*Cissus modeccoides*	*Ampelidaceae*	Chia voi	F	T06	>100	−[1]
31	*Coix lachryma jobi*	*Poaceae*	Ydi	S	N35	>100	−[1]
32	*Crinum asiaticum*	*Amaryllidaceae*	Nanghoatrang	F	N84	>100	+[1]
33	*Crinum ensifolium*	*Amaryllidaceae*	Nanghoado	F	N85	>100	+[1]
34	*Crinum latifolum*	*Amarylidaceae*	Trinhnuhoang cung	F	N71	36.12	++[2]
35	*Cuscuta sinensis*	*Convolvulaceae*	Tohong	H	N86	>100	+[1]
36	*Dendrobium sp.*	*Orchidaceae*	Thach hoc	H	N26	41.45	−[2]
37	*Dioscorea tokoro*	*Dioscoreaceae*	Ty giai	W	N55	>100	−[1]
38	*Drynaria fortunei*	*Polipodiaceae*	Cottoaibo	Rh	N39	> 100	−[1]
39	*Elephantopus scaber*	*Asteraceae*	Chithien	H	N87	>100	+[1]
40	*Ephedra sinica*	*Ephedraceae*	Mahoang	H	N40	>100	−[1]
41	*Euryale ferox*	*Nymphaeacea*	Khiemthuc	S	N44	>100	−[1]
42	*Evodia rutaecarpa*	*Rutaceae*	Ngothudu	H	N46	45.98	++++[2]
43	*Ficus glomerata*	*Moraceae*	Sung	F	D05	44.86	−[2]
44	*Haliotis sp*	*Haliotidae*	Thachquyet minh	Wh	N13	65.97	−[2]
45	*Heliotropium indicum*	*Borraginaceae*	Voivoi	F	N17	>100	−[1]
46	*Hibiscus sinensis*	*Asteraceae*	Dambut	Ra	N52	> 100	−[1]
47	*Homalomena romatica*	*Araceae*	Thiennienkien	Rh	N14	88.32	−[2]
48	*Illicium verum*	*Illiciaceae*	Hoi	C	T09	51.67	−[2]
49	*Illicium verum*	*Illiciaceae*	Hoi	Fr	N66	39.22	−[2]

Tab. 1. (Cont.)

Medicinal Plants No	Scientific name	Family name	Vernacular name	Part*	Code	ED_{50}**	NF-κB inhibitory activity***
50	*Impomoea hederacea*	*Convolvulaceae*	Bimbim	H	N88	>100	+[1]
51	*Kaemferia galanga*	*Zingiberaceae*	Dialien	Rh	N03	55.34	–[2]
52	*Lactuca indica*	*Asteraceae*	Boconganh	H	N60	74.28	–[2]
53	*Lawsonia inermis*	*Lythraceae*	Lamongtay	F	N08	>100	–[1]
54	*Mimosa pudica*	*Mimosaceae*	Cayxauho	H	N34	27.37	+[3]
55	*Momordica charantia*	*Cucurbitaceae*	Muopdang	Fr	N89	>100	+[1]
56	*Morinda officinalis*	*Rubiaceae*	Bakich	Rh	N37	>100	–[1]
57	*Myristica fragrans*	*Myristicaceae*	Nhucdaukhau	S	N11	32.54	–[3]
58	*Notopterygium incisum*	*Umbeliferaceace*	Khuonghoat	Rh	N05	21.75	–[3]
59	*Ophiopogon japonicus*	*Liliaceae*	Machmon	Ra	N59	>100	–[1]
60	*Perilla ocymoides*	*Lamiaceae*	Tiato	F	N29	67.34	++++[2]
61	*Peonia suffruticosa,*	*Ranunculaceae*	Maudonbi	W	N07	>100	–[1]
62	*Piper lolot*	*Piperaceae*	Lalot	F	N20	63.66	–[2]
63	*Pistia stratiotes*	*Araceae*	Beocai	H	N90	>100	–[1]
64	*Pharbitis nil*	*Convovulaceace*	Khiennguu	S	N65	>100	–[1]
65	*Phyllanthus acidus*	*Euphorbiaceae*	Tamduot	F	T02	>100	–[1]
66	*Phyllanthus reticulatus*	*Euphorbiaceae*	Phenden	F	T03	>100	–[1]
67	*Pleomele cochinchinensis*	*Liliaceae*	Huyetgiac	H	N56	>100	–[1]
68	*Polygonum cuspidatum*	*Polygonaceae*	Cotkhicu	Ra	N30	>100	++++[1]
69	*Polygonum multiflorum,*	*Polygonaceae*	Hathuodo	Rh	N57	>100	–[1]
70	*Polyscias fruticosa*	*Araliaceae*	Dinhlang	F	N91	>100	–[1]
71	*Pseuderanthemun sinensis*	*Acanthaceae*	Xuanhoa	F	T04	>100	+[1]
72	*Ricinus communis*	*Euphorbiaceae*	Thaudautia	F	K02	62.32	–[2]
73	*Ricinus sinensis*	*Euphorbiaceae*	Thaudautia	S	K01	55.56	–[2]

Tab. 1. (Cont.)

Medicinal Plants No	Scientific name	Family name	Vernacular name	Part*	Code	ED_{50}**	NF-κB inhibitory activity***
74	*Rubia cordifolia*	*Rubiaceae*	Thienthao	H	N72	45.30	++++[2]
75	*Sambucus javanica*	*Caprifoliaceae*	Comchay	F	N92	>100	−[1]
76	*Saussurea lappa*	*Asteraceae*	Vanmochuong	Ra	N96	>100	−[1]
77	*Scutellaria barbata*	*Lamiaceae*	Banchilien	H	N73	53.80	++++[2]
78	*Sophora flavescens*	*Fabaceae*	Khosamchore	Ra	N93	>100	−[1]
79	*Sparganium stenophyllum*	*Typhaceae*	Tamlang	H	N74	>100	++++[1]
80	*Smilax glabra*	*Liliaceae*	Thophuclinh	Rh	K03	>100	+[2]
81	*Siegesbeckia orientalis*	*Asteraceae*	Hythiem	H	N15	19.23	−[1]
82	*Thuja orientalis*	*Cupressaceae*	Tracbach	F	N94	>100	−[1]
83	*Tinospora sinensis*	*Tinosporaceae*	Daydauxuong	H	N32	>100	−[3]
84	*Zanthoxylum nitidum*	*Rutaceae*	Hoatieu	S	N42	> 100	−[1]
85	*Zizania dahuruca*	*Poaceae*	Nieng	F	D03	>100	−[1]
86	*Zizyphus jujuba*	*Rhamnaceae*	Taota	F	D04	>100	−[1]
87	*Wedelia calendulacea*	*Asteraceae*	Saidat	H	N95	>100	−[1]

* F: folium, S: semen; St: stem, W: wood, Wh: whole organism, H: herba, Ra: radix, Rh: rhizome, Fr: fructus, C: cortex. ** The concentration that causes 50% reduction in RAW264.7 cell growth. *** To simplify the results for quick comparison of the activity, the inhibition is denoted as follows: −, no inhibition at all; +, NF-κB inhibition < 25%; ++, ≥ 25%, < 50%; +++, ≥ 50%, < 75% and ++++, > 75%.
Test concentrations; [1] 100 μg/mL; [2] 30 μg/mL; [3] 10 μg/mL; these are non-cytotoxic concentrations.

From *Perilla ocymoides* we have isolated two compounds which structurally were determined to be (2*E*,4*E*)-5-(1,3-benzodioxol-5-yl)-1-(piperidin-1-yl)penta-2,4-dien-1-one (piperine) and 4-hydroxycinnamic acid [11]. Piperine is a well-known alkaloid that has been found naturally in plants belonging to the *Piperaceae* family, such as *Piper nigrum* L, commonly known as black pepper, and *Piper longum* L, commonly known as long pepper. However, this was the first report on the presence of this compound in *Perilla ocymoides*. Piperine has been reported to be an NF-κB inhibitor [12]. Reexamination of the NF-κB inhibitory activity of piperine revealed that this compound had an IC_{50} value of 2.7 μM in our assay. Compound p-hydroxycinnamic acid also exhibited moderate inhibitory effects on the activation of NF-κB with an IC_{50} value of 14.5 μM.

From *Crinum latifolium*, in our study on non-alcaloidal composition of this plant we have previously isolated a number of metabolites, including (3β,9β)-9,19-cyclolanostan-3-ol (cycloartenol), 4-[(senecioyloxy)methyl]-6,7-dimethoxycoumarin, 4',7-dihydroxyflavan,

4',7-dihydroxy-3'-methoxyflavan, 2',4',7-trihydroxydihydrochalcone, 3',5,6-trihydroxy-4',7,8-trimnethoxyflavone and 4',7-dihydroxy-3'-vinyloxyflavan [13, 14] from the DCM fraction of its methanol extract. Unexpectedly, none of these compounds showed significant inhibition of NF-κB activation even at the concentration of 30 μM. It is likely that other compound(s) might be accountable for the bioactivity detected with the methanol extract of *Crinum latifolium*.

Aiming at the investigation of the chemical constituents responsible for the NF-κB inhibitory activities of the remaining three plants including *Rubia cordifolia, Scutellaria barbata,* and *Sparganium stenophyllum* we have fractionated the corresponding methanol extracts against dicloromethane (DCM), ethyl acetate (EA), and n-butanol to give the DCM, EA, and BuOH fractions, respectively. The remaining water layers were concentrated to give the AQ fractions. Subsequent assays located the NF-κB inhibitory activity of *Rubia cordifolia* in the DCM soluble part, meanwhile the NF-κB inhibition of the other two methanol extracts of *Scutellaria barbata,* and *Sparganium stenophyllum* was mainly caused by the AQ fractions. Efforts have been undertaken to isolate the active metabolite(s) from the DCM fraction of *Rubia cordifolia* and the results will be reported in due course.

Experimental

Plant materials

Most of the plant materials were purchased from an oriental herbarium in Hanoi, Vietnam. Some medicinal plants were provided by Professor Tran Cong Khanh, Department of Botany, Hanoi University of Pharmacy, Vietnam.

Reagents and instruments

Unless otherwise stated, all materials, chemicals and solvents were of reagent grade and obtained from commercial sources; RPMI 1640 medium, Dulbecco's modified Eagles medium (DMEM), medium 199 (M-199), fetal bovine serum (FBS), penicillin, streptomycin, DMSO, sulforhodamine B (SRB), PBS (phosphate buffer saline), trypsin-EDTA solution (100 mL), tris(hydroxymethyl)aminomethane (Tris-base) and other reagents used for cell culture and assay were purchased from GIBCO Co., Ltd. (Grand Island, NY). Optical density was read using ELISA reader (Spectra Max 250, USA), An incubator purchased from Shellab Co., Ltd., (USA) was used for cell culture.

Cells and cell culture

Cell lines were obtained from a Cancer Cell Bank at Korea Research Institute of Bioscience and Biotechnology (KRIBB) and cultured in DMEM supplemented with FBS in 10% and L-glutamine (0.2 mM/ mL).

Tab. 2. Summary of the NF-κB inhibitory activity of methanol extracts of the active medicinal plants

Medicinal Plants No	Scientific name	Family name	Vernacular name	Part*	Code	ED_{50}**	NF-κB inhibitory activity***
1	*Crinum latifolum*	*Amarylidaceae*	Trinhnu hoangcung	F	N71	36.12	++[2]
2	*Evodia rutaecarpa*	*Rutaceae*	Ngothudu	H	N46	45.98	++++[2]
3	*Perilla ocymoides*	*Lamiaceae*	Tiato	F	N29	67.34	++++[2]
4	*Polygonum cuspidatum*	*Polygonaceae*	Cotkhicu	Rh	N30	>100	++++[1]
5	*Rubia cordifolia*	*Rubiaceae*	Thienthao	H	N72	45.30	++++[2]
6	*Scutellaria barbata*	*Lamiaceae*	Banchilien	H	N73	53.80	++++[2]
7	*Sparganium stenophyllum*	*Typhaceae*	Tamlang	H	N74	>100	++++[1]

* F: folium, S: semen; St: stem, W: wood, Wh: whole organism, H: herba, Ra: radix, Rh: rhizome, Fr: fructus, C: cortex. ** The concentration that causes 50% reduction in RAW264.7 cell growth. *** To simplify the results for quick comparison of the activity, the inhibition is denoted as follows: −, no inhibition at all; +, NF-κB inhibition < 25%; ++, ≥ 25%, < 50%; +++, ≥ 50%, < 75% and ++++, > 75%.
Test concentrations; [1] 100 μg/mL; [2] 30 μg/mL; [3] 10 μg/mL; these are non-cytotoxic concentrations.

Tab. 3. Summary of the NF-κB inhibitory activity of solvent fractions from methanol extracts of the active medicinal plants

Medicinal Plants No	Scientific name	Family name	Used part	Code	NF-κB inhibitory activity* of fractions** DCM	EA	BuOH	AQ
1	*Rubia cordifolia*	*Rubiaceae*	Herba	N72	+++[3]	+[2]	–[2]	–[2]
2	*Scutellaria barbata*	*Lamiaceae*	Herba	N73	–[2]	+[2]	+[2]	+++[3]
3	*Sparganium stenophyllum*	*Typhaceae*	Herba	N74	+[2]	+[2]	+[2]	+++[3]

*To simplify the results for quick comparison of the activity, the inhibition is denoted as follows: -, no inhibition at all; +, NF-κB inhibition < 25%; ++, ≥ 25%, < 50%; +++, ≥ 50%, < 75% and ++++, > 75%. Test concentrations; [1] 100 μg/mL; [2] 30 μg/mL; [3] 10 μg/mL; these are non-cytotoxic concentrations. **Fractions: DCM, dichloromethan fraction; EA, ethyl acetate fraction; BuOH, butanol fraction; AQ, aqueous fraction.

Extraction, fractionation and sample preparation

Dried plant materials (100 gr each) were extracted with MeOH (3 times x 200 mL) under reflux. The combined methanol extracts were concentrated to dryness *in vacuo*. In some cases, the MeOH extracts were suspended in water and partitioned with dichloromethane (DCM), ethyl acetate (EA) and butanol (BuOH) successively to give DCM, EA, BuOH fractions, respectively. The remains in water suspension were concentrated to give corresponding water fractions (WTR). For assays, the fractions were dissolved in dimethyl sulfoxide (DMSO) or ethanol at the initial concentration of 100, 30 or 10 mg/mL, respectively and serially diluted into 30, 10, 3, and 1 mg/mL concentrations. The insoluble parts were filtered off. The filtrates were stored at 4 °C and used as stock solutions.

NF-κB inhibition test

The assay was carried out using the procedure described in literature [4]. Briefly, NF-κB mediated reporter plasmid vector was constructed as previously described with minor modification [15]. This reporter vector has 9x binding site for the NF-κB and secretable alkaline phosphatase (SEAP) as a reporter. The RAW264.7 cells stably transfected with the reporter construct were seeded in a 96-well plate at 5 x 104 cells/200 µL/well and incubated for 3 h to let the cells attach. The cultures were treated with compounds tested at a predetermined concentration (non-cytotoxic) and stimulated with 1 µg/mL of LPS. After 6 h incubation, the medium was replaced with 200 µL of fresh DME medium containing 0.5% FBS followed by twice washing with serum-free medium, and then the cultures were incubated for 24 h. One hundred µL of each culture supernatant were transferred a new 96-well plate, heated at 65 °C for 5 min, and then mixed with equal volume of 2 x SEAP assay buffer (2M diethanolamine, 1 mM $MgCl_2$, 20 mM L-homoarginine). The reaction was initiated by the addition of 20 microL of 120 mM p-nitrophenyl phosphate dissolved in 1 x SEAP assay buffer and incubated at 37 °C. The absorbance (A) of the reaction mixture was measured at 405 nm with a microplate reader (Molecular Devices Co., Menlo park, CA, USA). The inhibition percentage (IP) was calculated using the following equation: $IP = [(A_{blk} - A_{spl})/A_{blk}] \times 100\%$, in which A_{blk} = average absorbance of the blank wells; A_{spl} = average absorbance of the sample wells.

In vitro cytotoxic assay

To choose a noncytotoxic concentration for the above NF-κB inhibition test, a cytotoxicity assay was conducted using SRB method [16]. The ED_{50} values (the concentration of the sample that inhibits the growth of cells by 50%) were calculated and included in Table 1.

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Authors' Statement

Competing Interests

The authors declare no conflict of interest.

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