Comprehensive extraction and NMR-based Metabolomics : novel approaches to natural products lead finding in drug discovery

Yuliana, N.D.

Chapter 2

Screening of traditional medicinal plants and selected Asian spices for anti obesity-related bioactivities

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Abstract

To investigate the potential health effects of several traditional medicinal plants and spices commonly used for daily consumption, we selected eight traditional medicinal plants and 32 spices for bioactivity screening in several anti-obesity related bioassays: adenosine A1 receptor binding, cannabinoid CB1 receptor binding, inhibition of TNF-α and induction of 3T3-L1 adipocytes lipolysis. *Benincasa hispida* seed, sesame seed and red chili show high binding activity to adenosine A1 receptor; nutmeg, mace, black pepper, and turmeric have high binding activity to the cannabinoid CB1 receptor; mulberry stem bark, temulawak, and temukunci have high binding activity for both receptors; piment and turmeric showed high inhibition of TNF-α accumulation, while black onion seed is the only spice having high activity for induction of 3T3-L1 adipocyte lipolysis. Several well known major compounds found in these active spices were tested in the respective bioassays but they did not show activity. Thus, the activity should be from other minor compounds or from synergistic effects among different compounds.

Keywords: traditional medicine, spices, obesity

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Introduction

The discovery of new drugs from plant sources may involve several approaches such as random screening of phytochemicals for biological activity; follow up biological activities reported; or follow up of the traditional use of the plants (283). The last approach, which can be referred to as the ethnopharmacological approach, has been successfully delivered a number of important drugs in the past, such as artemisinin, reserpine, atropine, codeine, and morphine (283). Information sources for the ethnopharmacological approach could be the documentation of ancient traditional medicines such as Ayurveda, Unani, Kampo, and traditional Chinese medicine; or from the information passed to the next generation by a herbalist, traditional healer, or shaman, which is usually unwritten (283).

Although the use of natural products particularly from plants as the source of new drugs has been neglected in the past few years, recently this invaluable source has emerged again as an important source of novel health affecting compounds. This is because of the failure of the new technologies in modern drugs discovery, such as combinatorial chemistry, and high throughput screening to deliver sufficient novel drugs in the past two decades to cover the costs of the next generation of medicines. Moreover, the rapid developing interest of Asian countries in their own heritage in medical knowledge is a further reason for the exponentially increasing number of studies in this field. In particular, several drugs discovered from the ethnopharmacological approach for treatment of obesity were able to reach the clinic, such as ephedrine, an alkaloid derived from traditional Chinese medicine Ma huang (*Ephedra sinica*) although it was banned by Food and Drugs Administration (1) due to its side effect; or P57, an appetite suppressant from *Hoodia gordonii*, a group of stem succulents traditionally used by the San people of South Africa as an appetite suppressant, thirst quencher, a cure for abdominal cramps, hemorrhoids, tuberculosis, indigestion, hypertension, and as anti-diabetes medicine (72).

Since ancient times, there is awareness that herbs and spices are more than a culinary condiment to improve sensory properties of food. The term herbs refer to the leaves of plant used in cooking, while spices refer to any other part (284). The history of how this knowledge developed from 5000 - 1000 BCE with human civilization (e.g. the

use of thyme by the Sumerians, coriander, fennel, juniper, cumin, garlic, cardamom, and cinnamon by the Egyptians, or juniper and saffron by the Assyrians), and subsequently how modern scientists tried to find evidence for various types of bioactivity of spices, and to identify responsible active compounds and their molecular targets, were reviewed (284). In the past years various bioactivities of herbs and spices used as food condiments have been reported especially those which are related to degenerative diseases. A reversed correlation between consumption of spices and risk of cancer in India, China, and USA, has been reported (285). Spices are also included as one of the main components in the Mediterranean diet, while studies showed that the Mediterranean people have a lower risk of several diseases such as cardiovascular diseases, metabolic disorders and certain types of cancer (286). For example, the addition of 1.5% (w/w) dose of lemon balm and marjoram herbs increased by 150% and 200%, respectively, the antioxidant capacity of a salad portion (287). Incorporation of star anise or clove at the dose of 1% (w/w) into rats' diet decreased the level of blood and liver lipids (288). Similarly, several studies on bioactivity of spices derived-compounds have been performed as well; capsaicin was found to have sympathomimetic thermogenic activity (267); some active spice-derived compounds such as allylisothiocyanate, zingerone, and curcumin, were found to inhibit the production of TNF-α, nitric oxide, and monocyte chemoattractant protein-1 (MCP-1), and mediators resulting from obesity induced-inflammation (289); the ginger-derived compounds 6-shogaol and 6-gingerol showed significant inhibition of downregulation of adiponectin expression mediated by TNF-α in 3T3-L1 adipocytes (183).

In our exploratory work to find sources for plant derived-compounds having anti-obesity activity, various traditional medicinal plants and spices commonly used in Asian cuisine were screened in several bioassays related to obesity at receptor and cellular levels. The following assays were used: adenosine A1 receptor, cannabinoid CB1 receptor, TNF-α suppression, and lipolytic activity on 3T3-L1 adipocyte. Blocking the adenosine A1 receptor by antagonist or inverse agonist has been reported to correlate with lipolytic activity (7, 8), while blocking cannabinoid CB1 receptor reduces the appetite and stimulates lipid metabolism (9). An increase level of TNF-α in adiposity was found to correlate with obesity related hyperleptinemia (10).

Our intention was to investigate spices commonly used for daily consumption and several traditional medicinal plants for possible effects on obesity. The advantage of spices being that there is already a long tradition of apparently safe use of these plants. We purchased fresh spices samples from a general supermarket, while the dried ones we purchased from major producers whose products are widely found at local retail stores in The Netherlands and Indonesia. Traditional medicinal plants used in this study were purchased from herbal drugstores in China, Korea, and The Netherlands.

Materials and Methods

Spices and medicinal plants

The following spices were obtained from TRS Co. Ltd, London, UK, in a dried form and identified by Mr. Anil Shah from TRS Co. Ltd; Anis (seed of *Foeniculum vulgare* P. Mill.), coriander (seed of *Coriandrum sativum* L.), cumin (seed of *Cuminum cyminum* L.), dill (seed of *Anethum graveolens* L.), lovage (seed of *Levisticum officinale* Koch.), annato (seed of *Bixa orellana* L.), brown mustard (seed of *Brassica juncea* (L.) Czern.), candle nuts (seed of *Aleurites moluccana* (L.) Willd.), fenugreek (seed of *Trigonella foenum-graecum* L.), star anise (fruit of *Illicium floridanum* Ellis*),* cinnamon (stem bark of *Cinnamomum verum*), cloves (flower bud of *Syzygium aromaticum* (L.) Merr. et Perry), piment (fruit of *Pimenta officinalis* Lindl.), mace (arillus seed of *Myristica fragrans* Houtt.), nutmeg (seed of *Myristica fragrans* Houtt.), poppy seeds (seed of *Papaver somniferum* L.*)*, sesame seeds (seed of *Sesamum indicum* L.), black pepper (seed of *Piper nigrum* L.), pomegranate seeds (seed of *Punica granatum* L.), black onion (seed of *Nigella sativa* L.), red chili (fruit of *Capsicum annuum* L.), and black cardamom (seed of *Amomum subulatum* Roxb.). Lemon grass, (stalk of *Cymbopogon citratus* (DC) Stapf), greater galangal (rhizome of *Alpinia galanga* (L) Willd.), ginger (rhizome of *Zingiber officinale* Rosc.), sand ginger (rhizome of *Kaempferia galanga* L.), onion (bulb of *Allium cepa* L.), garlic (bulb of *Allium sativum* L.), and kluwek nut (seed of *Pangium edule* Reinw. ex Blume) were purchased fresh from local supermarket in Leiden, The Netherlands. Temulawak (rhizome of *Curcuma xanthorrhiza* Roxb.) and temukunci (rhizome of *Boesenbergia rotunda* (L.) Mansf.) were purchased from traditional market in Bandung, Indonesia.

Orthosiphon stamineus Benth. leaves was purchased from drugstore van der Pigge, Haarlem, The Netherlands, and identified by Nancy Dewi Yuliana (Leiden University, Leiden, The Netherlands). *Astragalus membranaceus* (Fisch.) Bunge roots was purchased from Shanxi Hunyuan Hengshan Huangqi Company of Limited Liability (Hunyuan County, Shanxi Province, China). *Codonopsis pilosula* Franch roots were purchased from Beijintonrentang Lingchuandanshen Youxianzerendongsi (Linchuan County, Shanxi Province, China). Both were identified by Dr. Young Hae Choi (Leiden University, Leiden, The Netherlands) and Prof. Xue-Mei Qin (Shanxi University, Taiyuan, Shanxi Province, China). *Morus alba* L. stem bark and leaves were purchased from Korean Export and Import Federation of Drugs, Seoul, Korea, and identified by Dr. Young Hae Choi. *Curcuma kwangsiensis* S. G. Lee & C. F. Liang rhizome. *Plantago major* L. leaves, *Morus alba L.* fruit were purchased from TongRengTang TCM Pharmacy, Chengdu City, Sichuan province and identified by Dr. Henrie Korthout (Fytagoras BV Plant Science, Leiden, The Netherlands). *Urtica dioica* L. leaves was purchased from drug store van der Pigge, Haarlem, The Netherlands and identified by Dr. Henrie Korthout. *Hoodia gordonii* (Masson) Sweet ex Decne was provided by BZH exporters and Importers CC, Hermanus, South Africa, and identified by Mr. Adolf Joubert (BZH exporters and Importers CC, Hermanus, South Africa). All voucher specimens are stored in Pharmacognosy Department, Section Metabolomics, Leiden University, Leiden, The Netherlands.

Chemicals and reagents

Methanol, *n*-hexane, ethyl acetate, HCl, NaOH, and DMSO were purchased from Biosolve BV (Valkenswaard, The Netherlands). Tris buffer was purchased from Gibco BRL (New York, NY, USA), *n*-butanol was obtained from JT Baker BV (Deventer, The Netherlands), HEPES powder, bovine serum albumine, methanol-d_4 NMR solvents and CP55940 were from Sigma Aldrich (St. Louis, MO, USA). Human Cannabinoid CB1 receptor membrane and [^{3}H]CP55940 were from Perkin Elmer (Boston, MA, USA), EDTA and $MgCl_2.7H_2O$ were from Merck (Darmstadt, Germany), [^{3}H]DPCPX (8-cyclopentyl-1,3-dipropylxanthine) was from DuPont NEN (Boston, MA, USA), and CPA (N6 cyclopentyladenosine) was from RBI Inc. (Zwijndrecht, The Netherlands). Kieselguhr was bought from Fluka (Paesel, Germany). Fetal bovine

serum (FBS), penicillin, streptomycin and RPMI1640 were purchased from GIBCO (Grand Island, NY) and U937 cell lines were purchased from ATCC (CRL-1593.2). Lipopolysaccharide (Escherichia coli O111:B4) and phorbol 12-myristate 13-acetate (PMA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Human TNF-α ELISA kit was purchased from BioSource International Inc. (Camarillo, CA, USA). Dulbecco's Modification of Eagles Medium (DMEM), fetal bovine serum (FBS), penicillin and streptomycin solution, phosphate buffered saline, TrypLE Express and Hanks Balanced Salt Solution (HBBS) were supplied by GIBCO Netherlands BV (Breda, The Netherlands), 3-Isobutyl-1-methylxanthine (IBMX), dexamethasone, insulin from bovine pancreas, isoproterenol, glycerol, free glycerol assay reagent, 10% sterile BSA Solution and DMSO were supplied by Sigma Aldrich (St. Louis, MO, USA), 3T3-L1 pre adipocyte cell line was obtained from American Type Culture Collection (Rockville, Md., USA). Plastic ware for tissue culture was supplied by Greiner Bio-One GmbH (Frickenhausen, Germany). All solvents and reagents were analytical grade.

Extraction Method

Lemon grass, galangal, ginger, sand ginger, onion, garlic, and kluwek nuts were powdered then dried in freezedrier, while other spices and medicinal plants were powdered then directly used in this experiment. One gram of each dried powdered plant material was placed in a reaction tube, 2 mL of methanol 80% was added, vortexed, and then sonicated for 15 minutes. The solvent was separated by filtration and then the extraction was repeated two times. The solvent was evaporated by vacuum rotavapor. The dried extracts were dissolved in DMSO at 1.5 mg/mL concentration and ready for the assays.

Adenosine A1 receptor assay

The assay was performed as previously described by Chang *et al*. (290), except that the volume of the total mixture in the assay was 200 µL. The radioactive ligand used for the assay was 0.4 nM [3H] DCPCX (8-cyclopentyl-1,3-dipropylxanthine) (*K*d = 1.6 nM). Membranes were prepared from Chinese hamster ovary (CHO) cells stably expressing human adenosine receptors by a method previously described by Dalpiaz *et*

al. (291), and CPA (N6-cyclopentyladenosine) was used to determine non-specific binding. The mixture consisting of 50 µL [^{3}H]DPCPX, 50 µL CPA/50 mM Tris-HCl buffer/test compounds in different concentrations, 50 µL 50mM Tris-HCl buffer pH 7.4, and 50 µL of membrane was incubated at 25° C for 60 min and then filtered over a GF/B Whatman filter under reduced pressure. The filters were washed three times with 2 mL ice-cold 50 mM Tris/HCl buffer, pH 7.4, and 3.5 mL scintillation liquid was added to each filter. The radioactivity of the washed filters was counted by a Hewlett-Packard Tri-Carb 1500 liquid scintillation detector. Non-specific binding was determined in the presence of 10^{-5} M CPA. The bioactivity was described as percentage of inhibition of [^{3}H]DPCPX binding to the adenosine A1 receptor by the extracts and was calculated by using the software package Graphpad Prism (Graphpad Software Inc., San Diego,CA, USA).

CB1 receptor assay

The CB1 binding assay was a modification from the method described previously by Ross *et al*. (292). Incubation buffer was made from 20 mM Hepes buffer pH 7.4 containing 5 mM $MgCl_2$, 1 mM EDTA and 0.3% BSA. The CB1 membrane was diluted 200 times with assay buffer. The assay cocktail consists of 25 µL of incubation buffer, or extract, or CP 55,940 to determine unspecific binding at final concentration of 5 µM, 25 µL of 8.10^{-5} µM [^{3}H]CP55940, and 500 µL of diluted membrane. The mixture was incubated at 30^o C for 60 minutes and filtered over GF/B filter. The filters were washed 3 times with 2 mL ice-cold 20 mM Hepes pH 7.4 containing 0.01% BSA, then 3.5 mL scintillation liquid was added to each filter. The radioactivity of the washed filters was counted by a Hewlett-Packard Tri-Carb 1500 liquid scintillation detector. The bioactivity was described as percentage of inhibition of [^{3}H]CP55940 binding to the CB1 receptor by the extracts and was calculated using the software package Graphpad Prism (Graphpad Software Inc., San Diego,CA, USA).

3T3-L1 pre-adipocyte lipolysis assay

The assay was performed according to the assay description in Adipolysis Assay kit (Article number OB100) from Chemicon (Millipore BV, Amsterdam Zuidoost, The Netherlands).

Inhibition of LPS-induced TNF-α accumulation assay

Human monocyte-like histiocytic lymphoma cells U937 obtained from the ATCC (CRL-1593.2) were grown according to Sundstorm *et al*. (293). The TNF-α production *in-vitro* and cell viability determination after treatment of various plant extracts using a MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) reagent was performed according to Cho *et al*. (294). The cell suspension having a concentration of 10^5 cells/mL was plated in a 96 wells plate. After 48 h culture, various concentrations of test extracts and LPS (2 µg/mL) as positive control were added to each well and cultured for another 4 h. Finally, 20 µl of MTT solution (5 mg/mL in phosphate buffered saline) was added to each well and incubated further for 2.5 h at 37° C. After that the medium was discarded and the formazan blue, which is formed by reacting MTT with mitochondrial dehydrogenase in the living cells, was dissolved with 100 µL DMSO. The optical density (OD) was measured at 540 nm with a microplate reader.

Results and discussion

As presented in table 1, plants having medium inhibition for Adenosine A1 receptor binding are *O. stamineus*, nutmeg, poppy seed, pomegranate seed, and onion, while *M. alba* stem bark, *B. hispida*, *C. xanthorrhiza*, *B. rotunda*, sesame seed and red chili show very high activity (complete displacement of radioligand).

Sesame seed and oil is already known as a rich source of lignans with sesamin as the most abundant one. Sesamin was found to suppress hepatic fatty acid synthase expression in rats via suppression of the sterol regulatory element binding protein-1 mRNA expression (295). However, it is important to bear in mind that sesame seeds are also a rich source of oil (total oil content is 40% w/w) with linoleic acid and oleic acids as the two most abundant unsaturated fatty acids, representing 40% each of the total fatty acid content of the oil (296). It has been reported that unsaturated fatty acids bind unselectively to the adenosine A1 receptor (249).

Capsaicin, myristicin, and papaverine, major components of red chili, nutmeg, and poppy seed, respectively, were tested in order to determine if the high and medium binding activity of these spices could be attributed to their presence, but no significant binding for any of the pure compounds was detected (results not shown).

Many compounds have been isolated from *O. stamineus,* such as flavonoids and diterpenoids (297-299). Several flavonoids such as quercetin and kaempferol have been reported to be active in the adenosine A1 receptor assay (300). Anti-obesity effect of *B. hispida* in rats has been reported. After intraperitoneal injection of the *B. hipida* extract, food intake was reduced 27%, 38%, and 54% with extract dose of 0.2, 0.6. and 1 g/kg body weight respectively (76). There is no report on the possible active compounds. *Morus alba* bark is a traditional Chinese medicine used as kidney and liver protective, diuretic, hypotensive, sedative, and anti-coughing (301). Recently, it was reported to have hypolipidemic and hypoglycemic effects on rats (134, 302). The authors presumed that these bioactivities could be attributed to the flavonoids and prenyl-flavonoids present in this plant. Several prenyl flavonoids have been isolated from this plant, such as kuwanon C and leachianone (303).

Several sesquiterpenoids have been isolated from *C. xanthorriza*. The most popular one is xanthorrizol which has anti-bacterial activity (304). The hexane-soluble fractions from *C. xanthorriza* were found to decrease the level of serum and liver triglycerides in rats. The major compound in *C. xanthorriza* essensial oil, α-curcumene, was thought to be one of the active principles (305).

From the methanolic extract of *B. rotunda,* several prenylchalcones and prenylflavanones have been isolated. Among them are krachaizin B, 4-hydroxypanduratin A 4-hydroxypanduratin A, isopanduratin A, alpinetin, cardamonin, and 2,6-dihydroxy-4-methoxy dihydrochalcone (306). Most of the compounds isolated from *C. xanthorriza* and *B. rotunda*, and from *M. alba* have a prenyl substituent, which may be involved in activity, as it is thought to be important for protein-binding (307).

Plants with medium binding activity to the Cannabinoid CB1 receptor are *B. hispida*, annatto, kluwek nut, and sand ginger, while *M. alba* stem bark, *C. xanthorrhiza*, *B. rotunda*, nutmeg, mace, black pepper, and turmeric have high binding activity. As previously mentioned, *B. hispida* has shown anorexic effects in rats (76). Based on a previous report that *B. hispida* extract showed anti-depressant activity in rats

(77), the authors presumed that the mechanism is via central appetite regulation (76). This assumption seems to be a controversial since the reason that Rimonabant, a CB1 antagonist, was withdrawn from European market is due to the risk of psychiatric effects such as depression and anxiety. It is necessary to investigate further whether *B. hispida* extract binds to CB1 as an agonist or antagonist to see if the anorexic effect of this plant is independent from its binding activity to CB1 receptor.

Table 1. Bioactivity of spices and medicinal plants to adenosine A1 receptor binding, CB1 receptor binding, inhibition of LPS-induced TNF-α accumulation, and induction of lipolysis in 3T3-L1 adipocyte.

Spices	Common name	Family	Activity*			
			Adenosine A1[a]	CB1[b]	TNF-α[c]	3T3-L1 lipolysis[d]
Pangium edule	Kluwek nut	Achariaceae	Na	Medium	Na	Nd
Foeniculum vulgare	Anis	Apiaceae	Low	Na	Na	Na
Coriandrum sativum	Coriander	Apiaceae	Na	Na	Na	Na
Cuminum cyminum	Cumin	Apiaceae	Low	Na	Na	Na
Anethum graveolens	Dill	Apiaceae	Low	Na	Na	Na
Hoodia gordonii	-	Apocynaceae	Low	Na	Na	Nd
Allium sativum	Garlic	Alliaceae	Na	Na	Na	Na
Allium cepa	Onion	Alliaceae	Medium	Na	Na	Nd
Bixa orellana	Annato	Bixaceae	Low	Medium	Na	Na
Brassica juncea	Brown mustard	Brassicaceae	Na	Na	Na	Na
Codonopsis pilosula	Dang Shen, poor man's ginseng	Campanulaceae	Low	Na	Na	Nd
Benincasa hispida	Winter melon	Cucurbitaceae	High	Medium	Na	Nd
Aleurites moluccana	Candle nut	Euphorbiaceae	Low	Low	Na	Na
Trigonella foenum-graecum	Fenugreek	Fabaceae	Na	Na	Medium	Na
Astragalus membranaceus	Membranous milk-vetch root	Fabaceae	Na	Na	Na	Nd
Illicium verum	Star anise	Illiciaceae	Na	Na	Medium	Na
Orthosiphon stamineus	Cat whiskers	Lamiaceae	Medium	Na	Na	Nd
Cinnamomum verum	Cinnamon	Lauraceae	Na	Na	Medium	Na
Syzygium aromaticum	Cloves	Myrtaceae	Na	Low	Medium	Na
Pimenta officinalis	Piment	Myrtaceae	Low	Na	High	Na

Myristica fragrans	Nutmeg	Myristicaceae	Medium	High	Medium	Na
Myristica fragrans	Mace	Myristicaceae	Low	High	Medium	Na
Morus alba (leaves)	Mulberry leaves	Moraceae	Low	Low	Medium	Nd
Morus alba (fruit)	Mulberry fruit	Moraceae	Na	Low	Low	Nd
Morus alba (stem bark)	Mulberry stem bark	Moraceae	High	High	Medium	Nd
Papaver somniferum	Poppy seed	Papaveraceae	Medium	Low	Na	Na
Sesamum indicum	Sesame seed	Pedaliaceae	High	Low	Na	Na
Piper nigrum	Black pepper	Piperaceae	Na	High	Na	Na
Plantago major	Common Plantain	Plantaginaceae	Low	Na	Na	Nd
Punica granatum	Pomegranate seed	Punicaceae	Medium	Na	Na	Na
Cymbopogon citratus	Lemon grass	Poaceae	Na	Na	Low	Na
Nigella sativa	Black onion	Ranunculaceae	Na	Low	Na	High
Capsicum annuum	Red chilli	Solanaceae	High	Na	Na	Na
Urtica dioica	Stinging nettle	Urticaceae	Low	Na	Low	Nd
Curcuma xanthorrhiza	Temulawak	Zingiberaceae	High	High	Medium	Nd
Boesenbergia rotunda	Temukunci	Zingiberaceae	High	High	Na	Nd
Amomum subulatum	Black cardamom	Zingiberaceae	Na	Low	Medium	Na
Alpinia galanga	Great galangal	Zingiberaceae	Na	Na	Na	Na
Zingiber officinale	Ginger	Zingiberaceae	Na	Na	Low	Na
Kaempferia galanga	Sand ginger	Zingiberaceae	Na	Medium	Na	Na
Curcuma longa	Turmeric	Zingiberaceae	Low	High	High	Nd
Curcuma kwangsiensis	Ezhu	Zingiberaceae	Low	Low	Medium	Nd

*Determined base on the average of three independent replications, activity value is presented as High = 75% – 100%, Medium = 50% – 75%, Low = 30% – 50%, Not active (Na) ≤ 30%, Nd = not determined

[a]Percentage of acitivity represents the binding activity of extract to the receptor, concentration tested was 50 µg/mL in the assay

[b]Percentage of activity represents the binding activity of extract to the receptor, concentration tested was 70 µg/mL in the assay

[c]Percentage of activity represent the ability of the extract to inhibit TNF-α production in the medium, concentration tested was 15 µg/mL in the assay

[d]Percentage of activity represents the amount of glycerol released to the medium, concentration tested was 40 µg/mL in the assay

Curcuma longa is a rich source of curcuminoids and sesquiterpenoids which showed hypoglycemic effects in rats via PPAR-γ activation as one of the mechanisms

(308). Effect of this plant extract on food intake and body weight was not reported. Its main curcuminoid is curcumin which was reported to have potential anti-obesity activity although the reported dose is not reasonable; it suppressed 3T3-L1 differentiation at the dose of (5–20 μmol/L) *in-vitro* and reduced body weight gain of mice fed with a high-fat diet (22%) supplemented with 500 mg curcumin/kg diet for 12 weeks *in-vivo* (309). Whether curcumin is also the responsible active compound for the high CB1 binding activity needs to be investigated further. As discussed above sesquiterpenes, prenyl chalcones and prenyl flavonoids were isolated from *C. xanthorrhiza*, *B. rotunda,* and *M. alba.* It might be the active principles of these spices due to the presence of keton and prenyl substituents in their structures.

Nutmeg has been used since ancient times to cure many kinds of disorders such as digestion problems, fever, skin diseases, respiratory ailments, and it was also reported to have an effect on the central nervous system (CNS). Myristicin is a major compound in nutmeg essential oil (accounting for 70% of the essential oil). The pharmacological effects, however, cannot be attributed only to myristicin (310) as the authors showed a high binding activity to the CB1 receptor for both mace and nutmeg, but when myristicin was tested, no binding activities were detected (results not shown). It was suggested that the bioactivity, especially the one related to the CNS might result from a synergism between myristicin, saffrol, and elemicin (310). Individually, these 3 compounds are psychoactive, but the effect is potentiated when they are present together (310). The activity shown in this screening can thus probably be explained by a synergism between myristicin and other compounds found in the nutmeg essential oil.

Other spices which have high binding activity to CB1 are black pepper and turmeric. Black pepper alone is traditionally used to stimulate the appetite but piperine, (1-piperoylpiperidine), the primary pungent alkaloid in black pepper did not exhibit binding activity to CB1 (results not shown).

Additionally, the monoterpenes α-pinene, camphene, and borneol which are abundant in spices did not show any binding activity to both adenosine A1 and CB1 receptors.

Spices with medium inhibition to TNF-α accumulation are fenugreek, star anise, cinnamon, cloves, nutmeg, mace, and black cardamom. While piment and turmeric showed high inhibition. Although nutmeg shows medium activity to TNF-α

inhibition whereas its major compound, myristicin, did not show any activity (results not shown). Strong inhibition of TNF-α accumulation was found for piment and turmeric while fenugreek, star anise, cinnamon, cloves, nutmeg, mace, and black cardamom showed medium inhibition. Though nutmeg displayed a medium TNF-α inhibition activity, its major compound, myristicin, did not show any activity whatsoever (results not shown). Surprisingly, sesame seed did not show significant inhibition of TNF-α accumulation despite the high content of sesamin, its typical lignan with two fused tetrahydrofuran rings.

Although prenylchalcones and prenylflavanones isolated from *B. rotunda* were reported to significantly inhibit TNF-α-induced cytotoxicity in L929 cells at 10 μM concentration, the methanolic extract of *B. rotunda* did not show a significant inhibition of TNF-α activity in the present study.

Black onion seed is the only spice with high activity on induction of 3T3-L1 adipocyte differentiation. *In-vivo*, it has been reported to improve the lipid profile of albino rats by decreasing the level of triglyceride, total cholesterol and LDL cholesterol and increasing HDL cholesterol as compared to controls (311). Despite that an anorexic effect of its petroleum ether extract to rats has been reported (312), it has low binding activity to the CB1 receptor, indicating that a different pathway maybe involved. It will be interesting to perform further work to identify the active principles.

Conclusion

Several medicinal plants and spices tested in the various obesity related bioassays used in this study showed strong activity. *Benincasa hispida*, sesame seed and red chili show very high activity to the adenosine A1 receptor. Nutmeg, mace, black pepper, and turmeric expressed high binding activity to Cannabinoid CB1 receptor. Three medicinal plants showed high binding activity to both receptors, those are *Morus alba*, *Curcuma xanthorrhiza*, and *Boesenbergia rotunda.* Several compound previously isolated from these plants were thought to be the active compounds based on the structure similarity with the known receptors ligands but further studies are required to confirm this. Piment showed strong inhibition to TNF-α accumulation, while black onion is the only spice having high activity in the induction of 3T3-L1 adipocyte

lipolysis. Several reference compounds which are found as major compounds in the spices studied were tested in the respected bioassays but none of them was found as the responsible compound for the activity. Either the active principles are other, minor compounds, or the bioactivities are from synergism between different compounds. This will be an interesting topic for further studies.