i-Sabi™: Nature's Master Switch to Wellness™

Nrf-2 Activator

A white paper on a Nutraceutical derived From *Wasabia japonica.*

By: Joosang Park, Ph.D

i-Sabi™
Wasabia Japonica
Nature's Master Switch to Wellness

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CONTENTS

OVERVIEW

I-Sabi™ is a 100% natural, and science-backed, dietary ingredient made from the plant rhizome of *Wasabia japonica*, more commonly known as, wasabi. This paper discusses its background as traditional health food, and data from basic and clinical research that supports i-Sabi™ as a novel nutraceutical ingredient with multiple health benefits. Suggested usage and dosage of i-Sabi™ will also be noted.

This paper will familiarize the reader with this exciting nutraceutical, provide a snapshot of current research, and discuss the potential of i-Sabi™ to become a new stand-alone product or as a novel addition to an existing formulation.

WASABIA JAPONICA BACKGROUND

Wasabia japonica (wasabi) is best known as an ingredient in Japanese food, in particular, as a sushi condiment. Its characteristic bright green color can be immediately recog - nized, and its spicy flavor, similar to horseradish, can quickly clear out the sinuses. But what many people don't know is that wasabi harbors a wide spectrum of bioactivities. The ingredient derived from its rhizome includes potent anti-oxidant, liver-protective, anti-inflammatory, anti-proliferative, immunomodulatory, neuritogenic (enhancing neurite growth), and anti-bacterial activities and scientific research is underway to how these bioactivities are relevant to human health.

The discovery of health and medicinal properties of many common botanicals and foods often comes from epidemiologic studies that report on differences in disease prevalence and health statistics across populations and countries. For many traditional Asian foods and botanicals such as soy, green tea, and now wasabi, it is this important epidemiologic data demonstrating differences in cancer incidence, cardiovascular health benefit, and even longer life expectancies that spawn an extraordinary amount of basic and clinical research on the foods and their ingredients.[1-4] Such is the case for wasabi, a customary Japanese food ingredient, which is only now being uncovered as a potent, health-promoting nutraceutical.

Some of the epidemiologic studies focusing on the Asian diet and in particular, the Japanese diet, include a recent study of nutrition effects on mortality in the Japan Collaborative Cohort Study (JACC). In this analysis, multivitamin and vitamin E use was found to be associated with lower mortality from cerebrovascular disease and intake of the Japanese-style breakfast was associated with lower mortality from all causes for men. Other analysis of the JACC study demonstrated that the intake of fresh fish was associated with lower mortality from all causes for both men and women and intake of fish paste products and condiments such as wasabi was beneficial.[5]

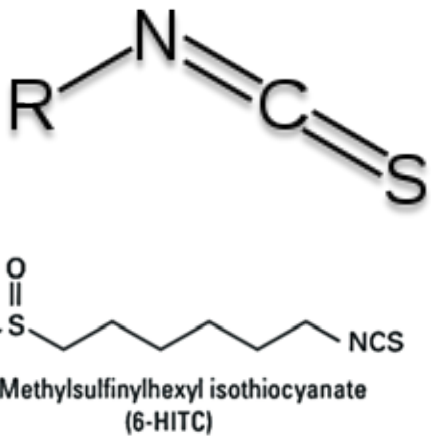
Figure 1: *Wasabia japonica* rhizomes

Wasabia japonica is a member of the *Brassicacea* or cruciferous vegetable family which includes broccoli, cauliflower and brussel sprouts, many of which are already valued for anti-proliferative activity and various healing potential. In general, all parts of the wasabi plant are harvested but it is the wasabi rhizome that has the most flavor, and is the storehouse for most of its active properties (Figure 1).

Chemical analysis of wasabi reveals the presence of a variety of bioactive compounds such as isothiocyanates (ITCs), vitamins, essential oils, and minerals. However, it seems that ITCs are the most potent and active components and the primary reason for a multitude of physiological effects of wasabi.

WHAT ARE ISOTHIOCYANATES?

Figure 2: General structure of an isothiocyanate



6-Methylsulfinylhexyl isothiocyanate (6-HITC)

Isothiocyanates (ITCs) are sulfur-containing phytochemicals that have the general formula R-N=C=S (Figure 2). There are many different molecules that belong to ITC group and most of its members are shown to have strong anti-proliferative activities in vitro. Some of the common ITCs include phenylethyl isothiocyanate (PEITC), benzyl isothiocyanate (BITC), 3-phenylpropyl isothiocyanate (PPITC), and sulphoraphane. 6-methylsulfinylhexyl isothiocyanate (6-HITC) has been identified as a major form of ITC in wasabi extract. ITCs occur naturally as glucosinolate conjugates in many of the *Brassicaceae* vegetables including wasabi, broccoli, cauliflower, kale, brussel sprouts, cabbage, and radishes. ITCs are also responsible for the typical flavor of these easy-to-identify vegetables.

Wasabi contains a spectrum of beneficial biological activities.

RECENT RESEARCH ON WASABI

Since wasabi is known to harbor a high concentration of ITCs as well as a plethora of other potentially beneficial compounds, extensive research has been done and much more is in progress. Scientific data strongly suggests that wasabi stimulates Nrf-2, a master switch to induce a series of cytoprotective genes, offering a variety of potential health benefits, including:

- √ Potent liver-detoxifying activity
- √ High antioxidant activity
- √ Promoting healthy immune responses by COX-2 modulation
- √ Enhancing brain health via neurite growth
- √ Immunomodulatory effect via activation of natural killer cells

Below, we will highlight research on wasabi and also on i-Sabi™, a freeze-dried powder of *Wasabia japonica* rhizome, manufactured by BioCell Technology LLC (Newport Beach, CA), and potential mechanisms leading to a variety of health benefits.

WASABI ACTIVATES NRF-2, A MASTER SWITCH TO WELLNESS

The Wasabi plant has a naturally activated defense system called the glucosinolate-myrosinase system, which produces isothiocyanates (ITCs). ITCs naturally protect the plant from dangerous pathogens and plant-destroying fungi. ITCs from the cruciferous plants have been extensively researched and have been found to be greatly beneficial for human health.

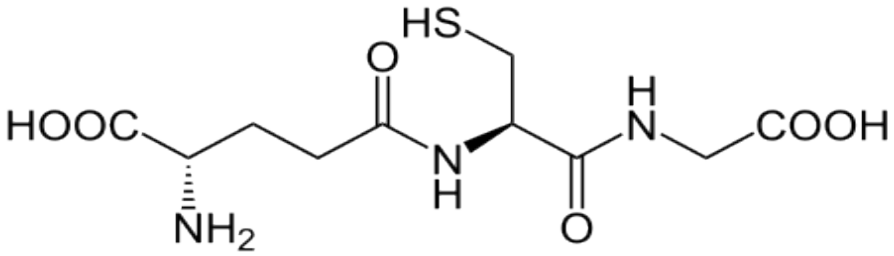
Wasabi's key active compound called 6-methylsulfinylhexyl isothiocyanate (6-HITC) has been shown to possess various biological properties including induction of a master redox switch, Nrf2. Nrf2 induction is required to turn on many genes such as glutathione S-transferases (GST) and NQO-1 that are involved in stress-responses and cytoprotection.

BioCell Technology has taken careful measures to ensure that i-Sabi™ is made from the finest harvest available. The leaves are carefully removed from the rhizome which is concentrated and freeze dried to preserve the maximum level of beneficial constituents in i-Sabi™.

LIVER PROTECTIVE ACTIVITY OF 6-HITC

Liver is the primary detoxification site in our body. It has recently been reported that a variety of cruciferous vegetables including wasabi may have potent liver protective activities which include induction of a detoxifying enzyme, glutathione S-transferases (GST). GST converts oxidized glutathione disulfide (GSSG) to its reduced state (GSH) (Figure 3), leading to an overall reduction of systemic oxidative stress. This activity can be both liver-protective and anti-proliferating, likely through affecting levels of reactive oxygen species.[6]

Figure 3. The structure of reduced glutathione



Morimitsu et al. screened vegetable extracts extensively searching for chemoprotective compounds that conferred resistance to carcinogenesis through induction of the phase II detoxification enzymes. Figure 4 shows that 6-HITC abundant in *Wasabia japonica* is a potent inducer of both class alpha and class pi GST isozymes (GST A1 and GST P1) in rat liver epithelial cells RL34.7

They showed further that 6-HITC was quickly absorbed after oral intake into the circulatory system reaching maximum concentration in plasma within 30 minutes, and that 6-HITC was more potent than sulforaphane (from broccoli) in inducing hepatic phase II detoxification enzymes such as GST and a quinone reductase, NQO1 (NAD(P)H:(quinone-acceptor) oxidoreductase) (Figure 5).

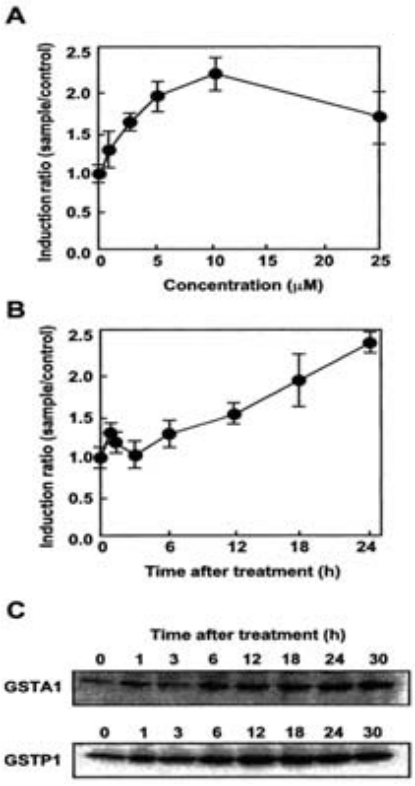
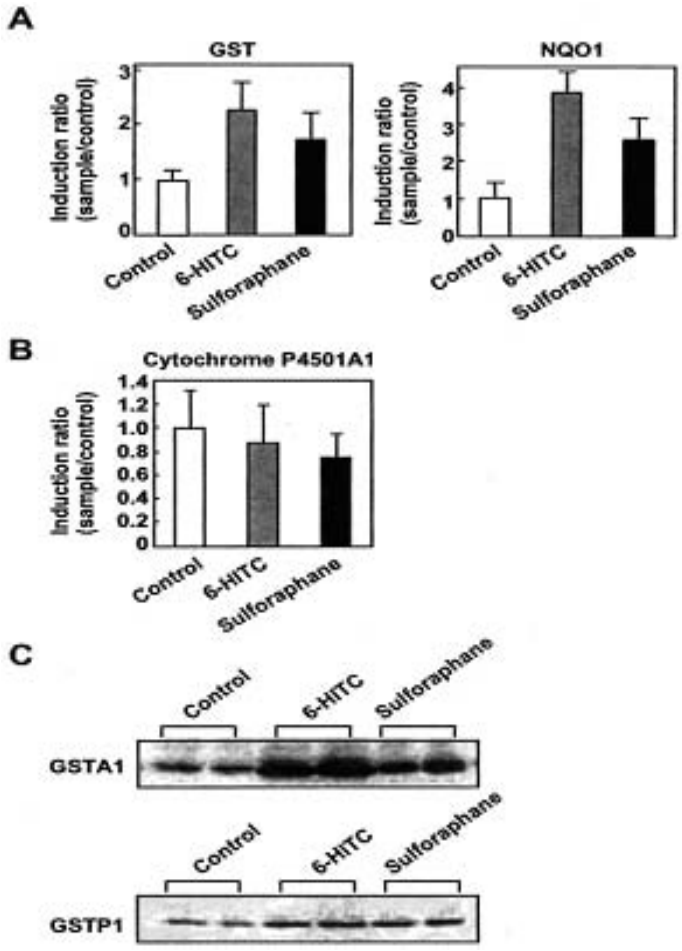


Figure 4. Induction of GST by 6-HITC. Dose-(A) and time-(B) dependent induction of GST activity. (C) Immunoblot analysis of GST isozymes. (RL 34 cells were treated with 25 uM 6-HITC). (Morimitsu et al., J Biol Chem 2002)

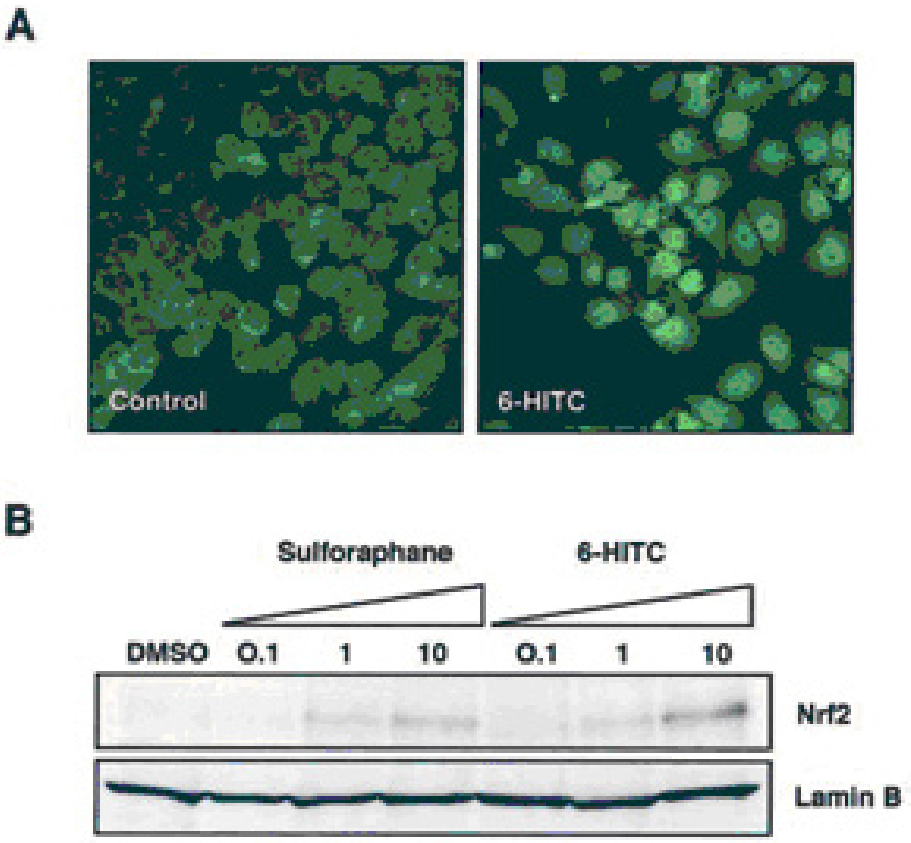
Figure 5. Effect of 6-HITC compared to sulforaphane on mouse hepatic detoxification enzyme activities. (A) GST and NQO1 activities. (B) cytochrome P450 1A1 activity. (C) Immunoblot analysis of GST isozymes.



(Morimitsu et al., J Biol Chem 2002)

Possible molecular mechanism for 6-HITC's bioactivities including liver detoxification was proposed. It has been established that antioxidant response element (ARE) or electrophile response element in the promoters of the phase II enzyme genes are required for the induction of the enzymes. Nrf2 (NF-E2-related factor 2) is considered to be involved in the activation of the element. Figure 6 shows that 6-HITC stimulated the activation of the antioxidant response element through the induction and translocation of Nrf2 into the nucleus.

Figure 6. Activation of Nrf2 by 6-HITC. (A) 6-HITC induces nuclear translocation of Nrf2. (B) 6-HITC stimulates induction of Nrf2.
(Morimitsu et al., J Biol Chem 2002)

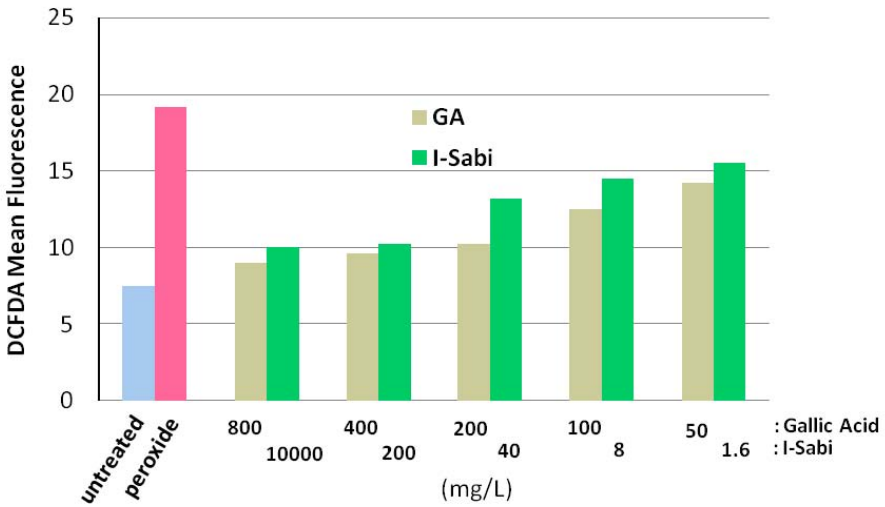


ANTIOXIDANT ACTIVITY OF i-Sabi™

Free-radicals such as superoxide and hydroxyl radical are formed in our body to play an important role in many physiological processes. However, uncontrolled formation or insufficient removal of these unstable and highly reactive organic molecules generated by stress, environmental factors, and consumption of smoke and alcohol is closely associated with aging process and development and progress of various health problems. Excessive amount of free radicals leads to oxidative damage on proteins, lipids, and DNA, interfering with normal cellular metabolism.

Antioxidant activity of i-Sabi™ has been examined by pre-treating human red blood cells (RBCs) with varying doses of i-Sabi™ extract and then exposed to hydrogen peroxide. The in vitro cell-based test showed that molecules in the i-Sabi™ extract were able to enter into the oxidized RBCs to reduce oxidative damage in a dose-dependent way as compared to untreated blood cells ($p < 0.016$) (Figure 7).8 It is noted that i-Sabi's™ antioxidant activity was similar to that of the proven antioxidant gallic acid used as a positive control. Our analysis showed that i-Sabi™ contains 15,000 ppm of glucosinolate/6-HITC, which was a significantly higher amount than other wasabi products in the market.

Figure 7. Dose-dependent antioxidant effect of i-Sabi™ extract in a cell-based system as measured by DCFDA mean fluorescence intensity



HEALTHY IMMUNE RESPONSES THROUGH MODULATION OF COX-2

Another important mechanism for wasabi's diverse health benefits is associated with promotion of healthy immune responses specifically through the modulation of cyclooxygenase-2 (COX-2).

Cyclooxygenases (COX-1 and COX-2) are responsible for formation of important biological mediators called prostanoids including prostaglandins. Pharmacological inhibition of COX provides relief from the symptoms of inflammation and pain as NSAIDs such as aspirin and ibuprofen exert their

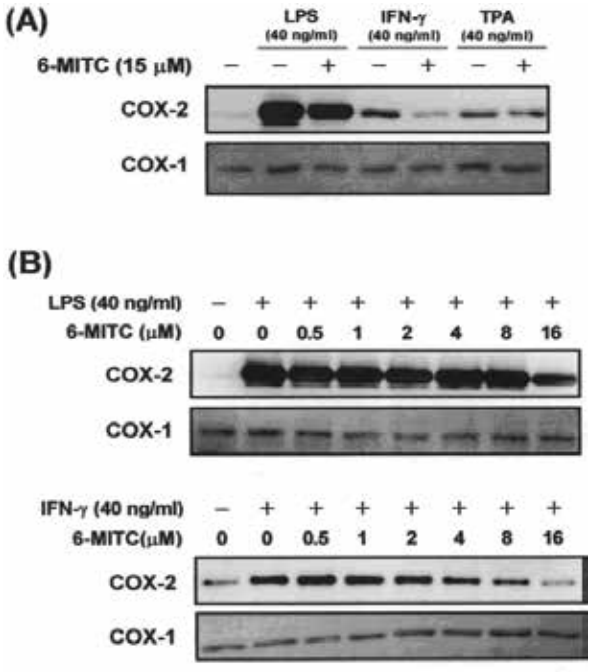
effects through inhibition of COX. Thus, the COX enzymes may be an important target for controlling unhealthy, persistent inflammation.

COX-2 is rapidly induced in activated macrophages and other cells at sites of inflammation in the presence of various inflammatory stimulants. Normally, COX-2 activity decreases to its pre-activation level after the removal of the stimulant. However, chronic activation of COX-2 can lead to a range of harmful effects on liver, kidney, gastrointestinal tract, and central nervous systems.

Wasabi extract contains an activity to modulate COX-2

A number of studies have shown that wasabi modulates COX-2 activity and may be beneficial in restoring healthy immune response. Uto et al. reported that an active compound from wasabi suppressed COX-2 expression. They isolated 6-HITC (also called 6-MITC) from wasabi rhizome and examined its effect on COX-2 expression in macrophage RAW264 cells in the presence of pro-inflammatory molecules such as LPS, IFN-gamma, or 12-O-tetradecanoylphorbol-13-acetate (TPA). They showed that 6-HITC suppressed COX-2 over-expression induced by LPS and IFN-gamma, but not by TPA, and the suppression was in a dose-dependent manner (Figure 9). These findings suggest that wasabi ITCs may be potent anti-inflammatory agents.

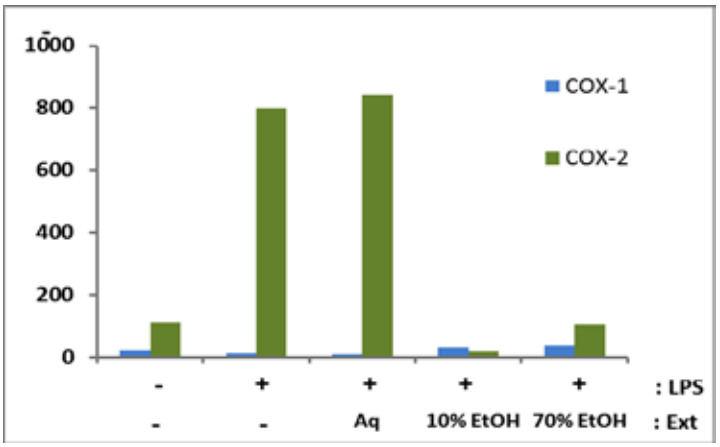
Figure 9. Dose-dependent suppression of COX-2 expression by 6-HITC (6-MITC). (Uto et al., Oncology Report, 2007)



i-Sabi™ strongly suppressed LPS-induced COX-2 expression

Recently, BioCell Technology LLC (Newport Beach, CA), developed and tested i-Sabi™, a freeze-dried powder of *Wasabia japonica* rhizome, for its ability to modulate COX-2 in vitro. As shown in Figure 10, i-Sabi™ strongly suppressed COX-2 expression.[8] Human cells were initially stimulated with lipopolysaccharide (LPS) and then treated with 0.1 mg/ml extracts of i-Sabi™. The effect of the wasabi extract on COX-1 and COX-2 over-expression was then measured by assessing mean fluorescence intensity in the human cells. While LPS stimulated induction of COX-2 expression almost 8-fold, 10% and 70% ethanol extract of i-Sabi™ caused the strong down-regulation of COX-2 expression even below untreated levels. It is intriguing that expression of COX-1 was not affected by i-Sabi™.

Figure 10. Effect of i-Sabi™ on LPS-induced COX-1 and COX-2 expression.



ENHANCING BRAIN HEALTH VIA NEURITE GROWTH

As discussed above, there has been a growing body of scientific evidence supporting diverse health benefits of wasabi. Recent report from Shibata el al. shed a unique light on wasabi's potential benefits towards neurodegenerative disorders.

Neurotrophins such as nerve growth factor (NGF), a molecule for the Nobel Prize in Physiology or Medicine in 1986, induce the growth, survival, differentiation, and functional maintenance of nerve cells in both the central and peripheral nervous systems. They help to prevent neuronal cell death. NGF is critical for the survival and maintenance of target neurons, and its binding and activation of its receptor, TrkA (neurotrophic tyrosine kinase receptor, type 1), is required for NGF-mediated neuronal survival and differentiation.

Shibata et al. investigated the effect of wasabi extract on growth attenuation in rat PC-12 cell line, a well-established and useful cell model for the investigation of signal transduction pathways of neuronal differentiation. They tested if NGF-dependent neuritogenesis of PC-12 could be enhanced by any of the ITCs present in *Brassicaceae* vegetables. First, they screened a number of these vegetables and found that wasabi was the richest source of ITCs. They identified 6-HITC as one of the major neuritogenic enhancers present in wasabi.

Figure 11A demonstrates that wasabi was one of the most potent neuritogenic enhancer for PC-12 cells among the vegetables when the PC-12 cells were treated with 1 μg/mL of each extract in the presence of NGF for 72 hours. Figures 11C and 11D illustrate the reverse-phase high performance liquid chromatography profile of the acetone fraction utilized in the panel, and the chemical structure of the isolated isothiocyanate, 6-HITC, respectively.

Figure 11. Enhanced neuritogenesis of PC-12 cells by wasabi extract and other Brassica vegetables and the chemical structure of 6-HITC
(Shibata et. al., J. Neurochem 2008)

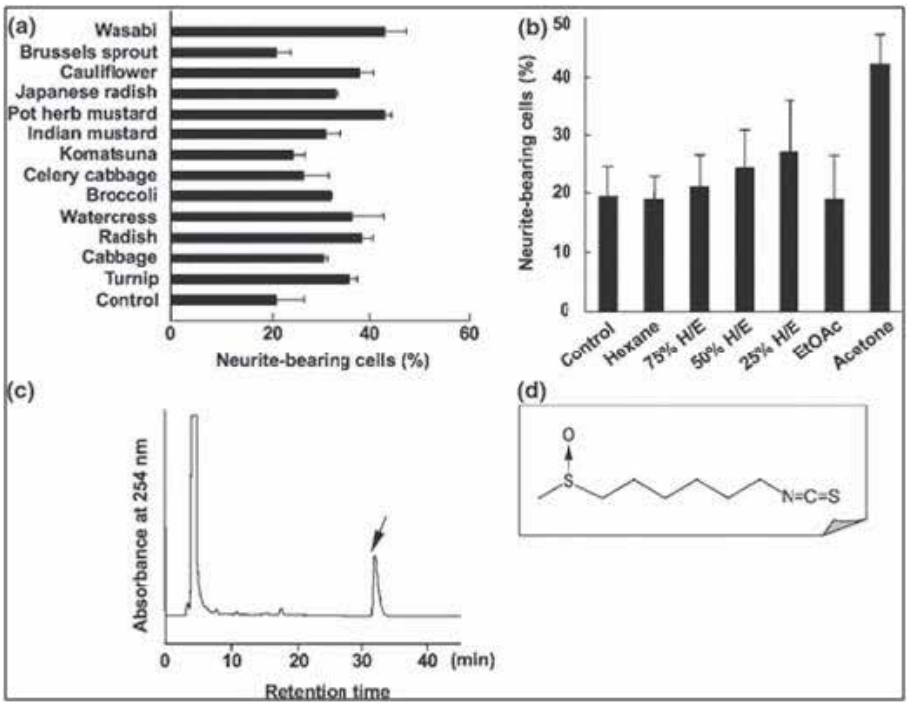
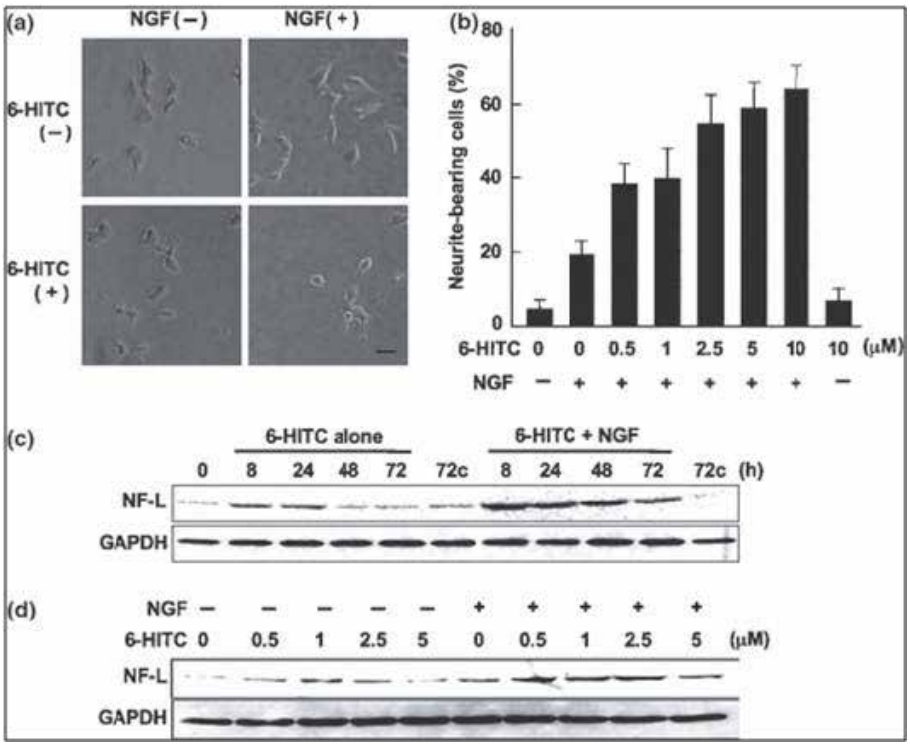


Figure 12. Enhanced neurite growth and change in NF-L of PC-12 cells treated with 6-HITC.



Figures 12A and 12B show that the 6-HITC fraction could enhance NGF-induced neurite outgrowth in the presence of NGF in a dose-dependent manner. In addition, expression of light neurofilament-L (NF-L), a neuronal differentiation marker, was enhanced and/or affected by the wasabi extract, as shown by Western blot analysis (Figures 12C and 12D).

Interestingly, the investigators identified the protein tyrosine phosphatase (PTP) 1B as a key regulator of the NGF receptor-initiated signal transduction, and its ability of dephosphorylating Tyr-490 of TrkA appeared to be inactivated by 6-HITC. This implies that the wasabi extract containing 6-HITC may facilitate the sustained phosphorylation of the extracellular signal-regulated kinase and the autophosphorylation of the NGF receptor TrkA, resulting in enhanced neuritogenesis. The potential effects of 6-HITC on brain health needs further investigation particularly involving animals and human subjects. However, enhancing neurite growth stimulated by 6-HITC and delineation of molecular mechanism involving the protein tyrosine phosphatase implies the utility of wasabi and i-Sabi™ as a beneficial dietary supplement to promote brain health.

IMMUNOMODULATORY ACTIVITY OF i-Sabi™

The immunomodulatory activity of various *Brassicaceae* family members, including wasabi, has been demonstrated using various studies and the effect of i-Sabi™ on human immune cells has been tested by Schauss et al. Table 1 showed that human mononuclear cells treated with i-Sabi™ in the presence of interleukin-2 (IL-2) displayed significantly enhanced expression of CD69, an activation marker of natural killer cells (NK), while CD25, another marker for a different functional state of NK cells, was not significantly induced by either IL-2 or i-Sabi™. Furthermore, i-Sabi™ also enhanced CD69 induction on NKT and T cells in the presence of IL-2, although the effect was moderate. This in vitro study implies a potential role of bioactive compounds in wasabi in stimulating the immune responses against virus-infected cells and aberrant cells, because NK cells are the effector cells for first-line immune defense causing the abnormal cells to undergo apoptosis and/or necrosis.

Table 1. Activation of natural killer cells by i-Sabi™ as measured by the induction of CD69 or CD25 mean fluorescence intensity.

	Untreated	IL-2	i-Sabi	i-Sabi + IL-2
CD69	1.00	**3.15**	**1.63**	**4.35**
CD25	1.00	1.07	1.07	1.19

i-Sabi™ AS A NUTRACEUTICAL

In summary, recent studies on wasabi and its bioactive component, isothiocyanates (ITCs), suggest that consumption of wasabi and other cruciferous vegetables may be highly protective for the human body. It is likely that wasabi among these vegetables is on the fast-track to becoming the next prime example of how a traditionally used herb turns out to have powerful phytochemical components with a variety of potential health benefits.

BioCell Technology's i-Sabi™, a freeze-dried powder derived from *Wasabia japonica* rhizome, harbors a higher amount of the key compound, 6-HITC, than other commercially available wasabi products in the market. As discussed above, our initial studies demonstrated that i-Sabi™ possessed strong antioxidant anti-inflammatory ,and immunomodulatory activities, while published studies suggest that 6-HITC promotes liver and brain health. Further research on i-Sabi™ in human subjects will reveal how much nutraceutical potential of wasabi has been hiding behind the unique flavor and taste.

CURRENT USE OF WASABI: DOSAGE FORM AND SUGGESTED USE

Although dosage varies depending on its intended use, the general recommendation for daily intake of stand-alone wasabi supplements is 200 mg taken 1-3 times per day. It is noted that i-Sabi™ can be formulated in various forms including capsules, tablets, and soft-gels. With a wide spectrum of biological activities, i-Sabi™ has broad applications, depending on whether it is used as a single supplement or a main ingredient in a combination formula.

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Joosang Park possesses a Ph.D. in cancer biology from Stanford University, he performed projects as Research Fellow on cancer vaccine development at Harvard Medical School. He acquired his MBA from Cornell University. Dr. Joosang Park has been serving BioCell Technology as VP of Scientific Affairs.

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