The black garlic

J. Sasaki

Department of Emergency Medical Technology, Hirosaki University of Health and Welfare Junior College, 2/5 Ohgimachi, Hirosaki 036/8104, Japan

Black garlic (*Allium sativum*) was a novel vegetable created by controlling humidity and temperature for a month without any additives. Under these conditions fresh garlic gradually changed color from white, brown and eventually became deep black at the end of processing. It is a chemical reaction called "*Mailard-Browning Reaction*". The final product is soft in texture, sweet with no pungent smell and directly edible like fruit. Water soluble amino acid *S-ally-L-cysteine* that is a trace in amount in fresh garlic increased by processing. Seventy percent amino acids (14/18) increased in amount along with elevation of carbohydrate contents. The black garlic extracts demonstrated a strong anti-tumor potency in a mouse tumor model accompanied with enforcement of NK cells activity. Bacteria-killing and anti-oxidant activities were also intensified by the black garlic extracts. Thus the multi-functional black garlic made sensationally debut armed with strong bio-functions in a field of vegetable world.

Keywords: black garlic (*Allium sativum*); *Mailard-Browning Reaction; S-ally-L-cysteine*; anti-oxidant potency; anti-tumor activity; anti-bacterial potency; immune enforcement

1. Introduction

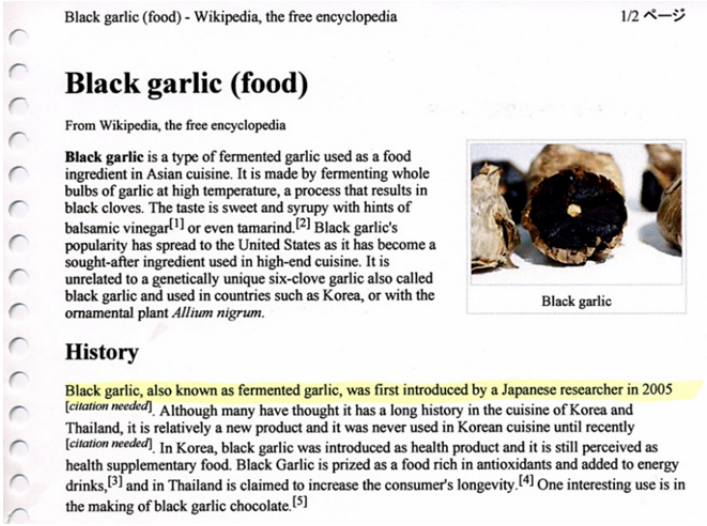
In the past garlic has been utilized as the most effective remedy against health disorders such as common cold, malaria, typhus, dysentery [1], and microbiologist *Louis Pasteur* scientifically studied the bactericidal properties of garlic. During the Second World War garlic was called "Russian penicillin" because the Russian government turned to this ancient treatment for soldiers when antibiotic supplies had been exhausted. More experimental data have been piling up against bacterial infection, atherosclerosis, high total cholesterol, hypertension, free radicals and blood coagulation [2]. Further the National Cancer Institute USA nominated garlic as the strongest cancer preventive vegetable on their "Designer Foods Project" [3].

In the vegetable world an extraordinary vegetable 'Black Garlic' made debut, which was developed by processing ordinary fresh garlic under the control of temperature and humidity [4]. The processed garlic was black in color with less irritating odor, fruity tasty, and readily edible without any further treatments like cooking. In this chapter, I will introduce extraordinary faces of the black garlic based on our experimental data.

2. History of the black garlic

On the history of the black garlic, people who read Wikipedia are understanding that it was created first in Korea. Whereas we Japanese know that the black garlic had been developed in our country. I will present herein a precise documents preserved in the Japanese company that was the first black garlic produced company. His name is Mr. *Hamasuke Hamano* who is still doing the black garlic business Mie Prefecture in Japan.

According to his documents he had initiated experiments in 1990 and succeeded in development of the black garlic in 1998. Wikipedia in the first edition had written the black garlic introduction following;



Black garlic (food) - Wikipedia, the free encyclopedia 1/2 ページ

Black garlic (food)

From Wikipedia, the free encyclopedia

Black garlic is a type of fermented garlic used as a food ingredient in Asian cuisine. It is made by fermenting whole bulbs of garlic at high temperature, a process that results in black cloves. The taste is sweet and syrupy with hints of balsamic vinegar[1] or even tamarind.[2] Black garlic's popularity has spread to the United States as it has become a sought-after ingredient used in high-end cuisine. It is unrelated to a genetically unique six-clove garlic also called black garlic and used in countries such as Korea, or with the ornamental plant *Allium nigrum*.

Black garlic

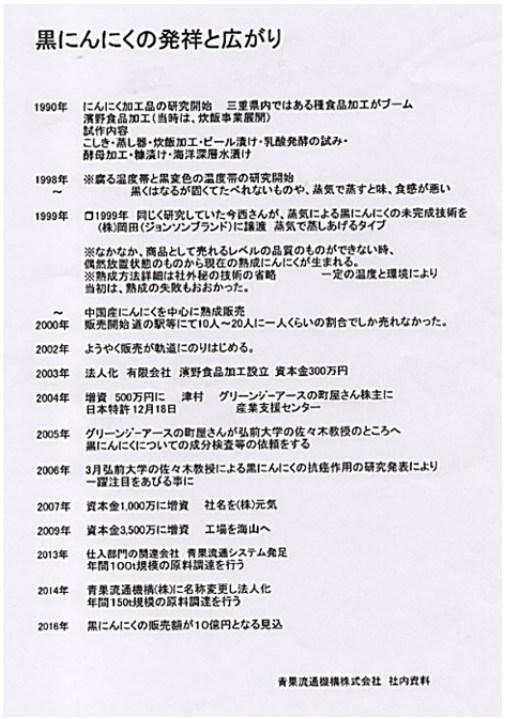
History

Black garlic, also known as fermented garlic, was first introduced by a Japanese researcher in 2005 [*citation needed*]. Although many have thought it has a long history in the cuisine of Korea and Thailand, it is relatively a new product and it was never used in Korean cuisine until recently [*citation needed*]. In Korea, black garlic was introduced as health product and it is still perceived as health supplementary food. Black Garlic is prized as a food rich in antioxidants and added to energy drinks,[3] and in Thailand is claimed to increase the consumer's longevity.[4] One interesting use is in the making of black garlic chocolate.[5]

Fig. 1 Black garlic descriptions in Wikipedia first edition. Pay attention to the first sentence of History saying Japanese researcher in 2005, it was my lab. work.

The above text said “It was first introduced by a Japanese researcher in 2005”. It indicates our laboratory works. Later Wikipedia deleted this part to start from likely: “In Korea, black garlic was developed as a health product and it is still perceived as health supplementary food. Black garlic is prized as a food rich in antioxidants and added to energy drinks, and in Thailand is claimed to increase the consumer's longevity. It is also used to make black garlic chocolate.”

This writing made people misunderstand the history of the black garlic. Here is the document by Mr. Hamasuke Hamano showing the date when the black was created in Japan (Fig. 2).


黒にんにくの発祥と広がり

1990年　にんにく加工品の研究開始　三里県内ではある種食品加工がブーム
演野食品加工（当時は、炊飯事業展開）
試作内容
こしき・蒸し器・炊飯加工・ビール漬け・乳酸発酵の試み・
酵母加工・糠漬け・海洋深層水漬け

1998年～　※腐る温度帯と黒変色の温度帯の研究開始
黒くはなるが固くてたべれないものや、蒸気で蒸すと味、食感が悪い

1999年　□1999年 同じく研究していた今西さんが、蒸気による黒にんにくの未完成技術を（株）岡田（ジョンソンブランド）に譲渡 蒸気で蒸しあげるタイプ

※なかなか、商品として売れるレベルの品質のものができない時、
偶然放置状態のものから現在の熟成にんにくが生まれる。
※熟成方法詳細は社外秘の技術の省略　一定の温度と環境により
当初は、熟成の失敗もおおかった。

～2000年　中国産にんにくを中心に熟成販売
販売開始 道の駅等にて10人～20人に一人くらいの割合でしか売れなかった。

2002年　ようやく販売が軌道にのりはじめる。

2003年　法人化 有限会社 演野食品加工設立 資本金300万円

2004年　増資 500万円に　津村　グリーンジーアースの町屋さん株主に
日本特許 12月18日　産業支援センター

2005年　グリーンジーアースの町屋さんが弘前大学の佐々木教授のところへ
黒にんにくについての成分検査等の依頼をする

2006年　3月弘前大学の佐々木教授による黒にんにくの抗癌作用の研究発表により
一躍注目をあびる事に

2007年　資本金1,000万に増資　社名を(株)元気

2009年　資本金3,500万に増資　工場を海山へ

2013年　仕入部門の関連会社 青果流通システム発足
年間100t規模の原料調達を行う

2014年　青果流通機構(株)に名称変更し法人化
年間150t規模の原料調達を行う

2016年　黒にんにくの販売額が10億円となる見込

青果流通機構株式会社 社内資料

Fig. 2 Chronological description of the black garlic development (by the HAMANO Co. Ltd. document Mie Prefecture in Japan).

1990: The Hamano Food Processing Business initiated experiments to develop a direct edible garlic.

1998: Prototype of the black garlic was created but incomplete.

1999: The black garlic was developed using rice cooker. Mr. Hamano started a new business.

2003: Founded the Seino Food Processing Corporation.

2004: Acquired the Japanese Patent Right –Japan Patent Office– (December 18th).

2005: Bio-functional studies of the black garlic were carried out in our Laboratory at Hirosaki University School of Health Sciences.

2006: Research Group at Hirosaki University confirmed a strong anti-tumor potency, which was featured by the mass media. It became a trigger to establish the black garlic companies in Japan.

2007: The Hamano Company increased capital to 10 million yen and changed name to The Genki.

2014: The Vegetable & Fruit Distribution System was found.

According to Mr. Hamano’s data, he was invited from Korea to give them technical advice for the black garlic production. They exported their products to California.

3. Experimental design

3.1 Creation of the black garlic

The black garlic is an ordinary garlic (*Allium sativum)* and not belongs to a special species. It is easily created under control of temperature (70ºC) and humidity (75%) for around 30 days without any additional treatments and additives. Fresh garlic gradually changes color from white, brown and eventually becomes black a month later as seen in Fig. 3 due to chemical reaction called “*Maillard and Browning Reactions*” between carbohydrate and amino acid. Final product is soft and sweet like fruit in taste with a non-irritating odor.

Fig. 3 Color changes during processing of ordinary garlic. Fresh garlic gradually changes color from white (left), light (5 days) and deep brown (10 days), and becomes deep black after 30 days treatment in the humidity and temperature controlled room.

3.2 Extraction of the black garlic compounds for bio-functions tests

The smashed black garlic was mildly heated in distilled water at 100ºC for 2 hours, and filtrated. Centrifuged supernatant (2,220×g for 20 min) was frozen at -80ºC overnight for lyophilization (BFD-2, Nihon Freezer Co. Ltd, Japan).

3.3 Chemical analysis

Amino acid, *S-allyl-L-cysteine* (SAC) and *γ-glutamyl-S-allyl-L-cysteine* (GSAC) were respectively analyzed by amino acid analyzer (JLC-500/v, Nihon-Denshi Co. Ltd, Japan), and liquid chromatography (Alliance 2696, Japan).

3.4 Anti-oxidant test

Anti-oxidant activity of the black garlic extracts was determined by the DPPH method, and expressed by mg used to reduce 50% of 1.1-diphenyl-2-picrylhydrazyl (RS50%).

3.5 Anti-tumor test

Meth A *fibrosarcoma* in BALB/c mouse model was used for anti-tumor test of the extracts. Briefly, tumor cells adjusted at 5.0×10^6/0.5 ml per mouse in GIT cell culture medium (Nihon-Seiyaku Co. Ltd, Tokyo, Japan) were transplanted into intra-dermal, and the extracts solution in disinfected saline was injected into tumor transplanted site on days 2, 4, 6 after transplantation. Anti-tumor potency of the black garlic extracts was evaluated three weeks later from tumor transplantation by measuring tumor size (longitude × latitude mm).

3.6 Toxicity test of the black garlic extracts against tumor cells

Toxicity of the black garlic extracts was tested against Meth A tumor cells at 5.0×10^7/ml suspended in 1% extracts contained-GIT medium. Followed by incubation in 5% CO_2-air, cell viability was microspcopically examined by trypan blue dye exclusion test at 3, 6, 9, and 24 hours cultivation interval.

3.7 Anti-bacterial test

Nutrient agar plate method [4] was adopted against the representative pathogens as MRSA (methicillin-resistant *Staphylococcus aureus*), enterohemorrhagic *Escherichia coli* O157:H7, *Pseudomonas aeruginosa* and *Candida albicans*. They are all troublesome organisms as drug resistance/food poisoning in the medical field and in the society.

3.8 NK (Natural Killer) cells activity test by spleen cells cultivation

Spleen cells culture system was adopted for NK cells activity test prepared from the black garlic extracts-fed mice [5]. Triplicate experiments were carried out for statistic evaluation.

3.9 Cytokine measurement in spleen cells cultivation system arranged by the extracts-fed mice

Cytokines amount of IL-2, TNF-α, IL-4, IFN-γ, and NO were measured in spleen cells culture supernatant after 48 hours incubation under 5% CO_2-air condition [5]. Triplicate experiments were conducted for statistic evaluation.

3.10 Statistic analysis

Chi-square (χ^2) was applied to evaluate the statistic significance between experimental and control groups.

4. Results

4.1 Chemical composition of the black garlic

Chemical constituents in the processed and fresh garlic were listed in Table 1. Carbohydrate in the black garlic increased in amount by processing, probably associated with enforced sweetness, but others did not change much in amount compared with those of fresh garlic.

Table 1 Chemical constituent of the black garlic and fresh garlic

	Black garlic	*Fresh garlic*
Energy (kcal/100 g)	227.1	138
Water (%)	45.1	60.3
Protein (%)	9.1	8.4
Lipid (%)	0.3	0.1
Carbohydrate (%)	47.0	28.7
Ash (%)	2.1	ND
Na (mg	4.0	ND
Ca (mg)	24.0	ND
Lactobacillus (No./g)	<300	ND

*ND; not determine

Carbohydrate amount rose by processing probable associated with black garlic sweetness.

Amino acid as cysteine, phenylalanine, tyrosine, leucine, isoleucine, methionine, valine, alanine, glycine, proline, glutamic acid, serine, threonine, and aspartic acid increased by processing (Table 2).

Table 2 Amino acid comparison between the black garlic and fresh garlic

Amino acid	*Black garlic (mg/100 g)*	*Fresh garlic (mg/100 g)*
Cysteine	260	100
Ricin	230	290
Histidine	110	130
Phenylalanine	300	190
Tyrosine	340	170
Leucine	460	260
Isoleucine	250	150
Methionine	90	70
Valine	410	250
Alanine	410	220
Glycine	360	180
Proline	210	180
Glutamic acid	1670	960
Serine	330	210
Threonine	270	190
Aspartic acid	930	630
Tryptophan	80	94
Arginine	970	1300

*Seventy percent of amino acid (14/18) increased during processing.

Organic-sulfur in the black garlic is considered to play a key role in bio-activity and *S-allyl-L-cysteine* (SAC) is one of the representative elements. SAC increased during processing, and its amount reached 194.3μg/g after 40 days processing to compare with 23.7μg/g before aging began. By contrast *γ-glutamyl-S-allyl-L-cysteine* (GSAC) was decreased from 748 to 248 μg/g after aging (Table 3).

Table 3 Relevancy between *S-allyl-L-cysteine* (SAC) and *γ-glutamyl-S-allyl-L-cysteine* (GSAC) in processing

	SAC (μg/g)	*GSAC (μg/g)*
Black garlic	194.3	248.7
Fresh garlic	23.7	748.7

SAC amount increased by processing but GSAC decreased, suggesting GSAC converted into SAC by processing.

4.2 Bio-activity potency of the black garlic

4.2.1 Anti-oxidant activity

Super-oxide elimination potency was strengthened in the black garlic and reached 28-fold more compared with that of fresh garlic in Japanese products and 12 times in Chinese products (Table 4), suggesting that activity depends on garlic producing area.

Table 4 Anti-oxidant potency of the black garlic (DPPH method).

	RS50%*
Japanese black garlic	4.1 – 28 times
Japanese fresh garlic	114.9
Chinese black garlic	7.3 – 12 times
Chinese fresh garlic	88.5

*mg used to reduce 50% of 1.1-diphenyl-2-picrylhydrazyl
Increasing anti-oxidant potency was observed in both Japanese and Chinese black garlic.

4.2.2 Anti-tumor activity

Tumor curative effect of the black garlic was more than expected. Fifty percent of tumor was deleted by the extracts treatments and fresh garlic used as a reference failed in eradication of tumor (Table 5). Average tumor size in non-cured mice in the black garlic treated was half to that of control group.

Table 5 Anti-tumor potency of the black garlic against Meth A *fibrosarcoma.*

	Dosage treated	***Cured/Used***	***Tumor size against control (%)***
Exp.1 Black garlic	1 mg (three shots)	2/5	40
Control	(-)	0/5	
Exp.2 Black garlic	1 mg (three shots)	3/5	55
Control	(-)	0/5	
Total		5/10 ($p<0.05$)	47.5
Fresh garlic	5 mg (three shots)	0/5	64
Control	(-)	0/5	

Extracts were injected into tumor transplanted site on day 2, 4, 6 after tumor transplantation, and activity was evaluated three weeks later from tumor transplantation.
Fifty percent of cure rate was obtained by the black garlic treatment, and no cure in the fresh garlic treated group.

4.2.3 Toxicity test of the black garlic extracts against Meth A tumor cells

One percent of the black garlic extracts was no toxic against Meth A tumor cells in mixture cultivation test (Fig. 4). This result suggests that the extracts probably enhance immune system first, which will play later for tumor eradication *in vivo*.

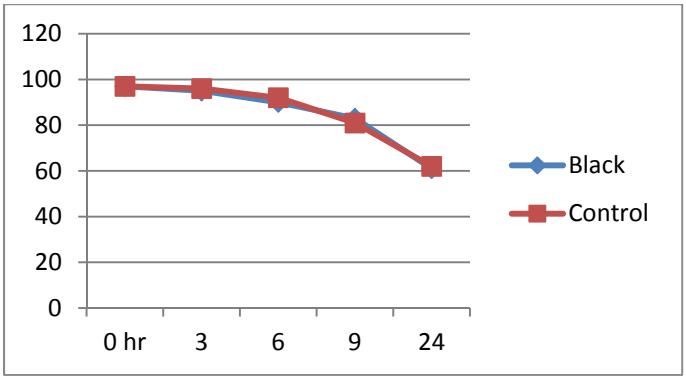


Fig. 4 Toxicity test of the black garlic extracts against Meth A tumor cells.

One percent of the black garlic extracts was mixed with tumor cells and incubated in 5% CO_2-air condition. Viability rate of tumor cells (%) was determined by the dye exclusion test counting 200 cells. Non toxic action was observed in the black garlic extracts.

4.2.4 Anti-bacterial test

MRSA (methicillin-resistant *Staphylococcus aureus*), *Pseudomonas aeruginosa,* and enterohemorrhagic *Escherichia coli* O157:H7 were sensitive to the extracts, and could not grow on the extracts included site. While *Candida albicans* resisted somewhat to the extracts and faintly grew as seen in Fig. 5.

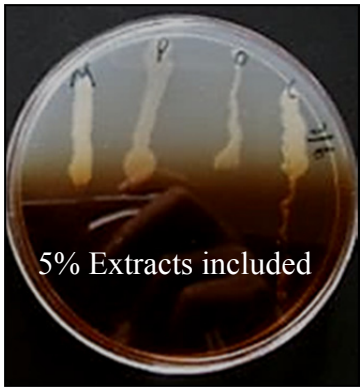


Fig. 5 Bacteria killing potency of the black garlic extracts. Upper control site allowed bacteria growth but no growth at lower part with the black garlic extracts. From left; MRSA, *Pseudomonas*, O157, *Candida* (faint growth)

4.2.4 Enhancement of NK cells activity

As seen in Fig. 6, NK cells were activated and it reached maximum 10 days after the experiments beginning (p<0.001).

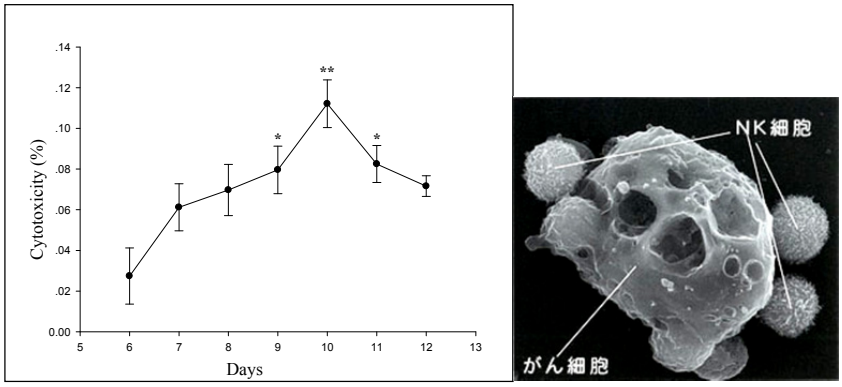


Fig. 6 Enforcement of NK cells activity in the black garlic extracts-fed mice. NK cells activity gradually increased and reached a maximum on day 10 (**P*<0.05, ***P*<0.001 against control value). Tumor cell was attacked by NK cells by creating holes (Right picture, by Hasumi Institite in USA).

4.2.5 Increased cytokine generation

Cytokine TNF-α, and IFN-γ reached a maximum generation on day 8, and IL-2 and NO were on day 11 (Table 6). Time lag was observed on reaching maximum depending on the types of cytokine. While IL-4 decreased, implying less production of *IgE* from B lymphocytes (allergy suppression).

Table 6 Cytokine amount generated in the extracts-fed mice spleen cells culture.

Days	***IL-2 (pg/ml)***	***IL-4 (〃)***	***TNF-α (〃)***	***IFN-γ (〃)***	***NO (μM)***
Control	20.1± 3.9	20.0±2.1	27.3±8.2	17.4±1.5	11.6±0.9
6	15.9 ± 2.3	16.6±2.8	47.0±5.9*	24.5±1.7*	11.2±2.3
7	15.5±1.8*	17.7±1.7	60.7±3.9*	34.7±4.3**	10.4±1.2*
8	18.2±4.0	15.5±1.0*	68.7±0.6**	105±55.7*	26.9±1.0**
9	18.0±2.6	15.9±1.3*	56.5±3.4**	30.0±4.9*	26.1±1.1**
10	16.2±1.8	15.9±1.6*	51.0±8.7*	18.1±0.7	26.3±1.8**
11	28.0±3.6*	16.5±1.0*	44.0±5.4*	32.2±4.3*	28.8±2.9**
12	18.6±3.0	17.2±1.2*	29.0±5.4	19.7±1.4	26.0±2.5*

Mice were treated with the black garlic extracts for 5 days. Non-treatment period was from day 6 to 12. (**P*<0.05, ***P*<0.001 against control).

5. Prospect in the future

Reputation of the novel vegetable, black garlic, is steadily raising up in both domestic and overseas due to beneficial bio-functions against tumor, super-oxide, bacteria, and immune enhancement. Citizens are also interested in their health problems and trying to strengthen their health conditions. The most hopeful candidate is the black garlic, which still covered with unknown nature (bio-activities).

One of the biggest concerns is for instance how this product works against a modern disease allergy. We are experiencing the black garlic effectiveness against allergy as improvement of clinical symptoms among the patients who daily ingested the black garlic. Another concern is affection to the brain. Because the black garlic involves much γ-*amino-butyric acid* (GABA), which is a necessary element to brain work as a neurotransmitter in central nervous system [6]. Deficiency of GABA in brain causes various mental diseases as anxiety disorders likely to be panic attacks, Parkinson disease, and also depression. Likely the black garlic bio-functions are still unexplored and remained for future's works.

Acknowledgments Author acknowledges Mr H. Hamano and Y. Osanai for providing information on the history of the black garlic in Japan.

References

[1] Bratman S. Your Complete Guide to Herbs. Prima Publishing. California, pp 67-75, 2000
[2] Sasaki J. Bioactive Phytocompounds and Products Traditionally Used in Japan. In Modern Phytomedicine -Turning Medical Plants into Drug- Edited by Iqbal Ahmad et al. WILEY-VCH. pp79-96, 2006
[3] Articles.latimes.com/1991-12.../food/fo-1157_1_cancer-research
[4] Sasaki J, Lu C, Machiya E, Tanahashi M, Hamada K. Processed black garlic (*Allium sativum*) extracts enhance anti-tumor potency against mouse tumors. Medicinal and Aromatic Plant Science and Biotechnology. Global Science Books. 1 (2), 278-281, 2007
[5] Wang D. Feng Y. Liu J. Yan J. Wang M. Sasaki J. Lu C. Black garlic (*Allium sativum*) extracts enhance the immune system. Medicinal and Aromatic Plant Science and Biotechnology. Global Science Books. 4 (1), 37-40, 2011
[6] Sasaki J. Overview of the Black Garlic Movement in the Fields of Research and Marketing. J Life Sciences. 9 (2), 65-74, 2015