EVALUATION OF BLACK GARLIC AS POTENTIAL MEDICINAL PRODUCT THROUGH ANALYSIS OF ITS NUTRITIVE CONTENT, CHEMICAL PROPERTIES AND ANTI-CANCER ACTIVITIES

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I. ABSTRACT

Black garlic (BG) is prepared under high temperatures and humidity for several days. It has a light, savory, sweet taste with a hint of tartness, and it is claimed to have many therapeutic properties. In other countries, BG can demand a high selling price and is known for its health and culinary benefits. Since garlic is one of the flagship commodity crops of Ilocos Norte, it is important to study the potential of our own black garlic as a functional food and evaluate its medicinal properties so that our local garlic may increase its value and help our local garlic farmers to increase their sales and profits. Results will also serve as baseline information for its further evaluation as a wonder all-natural medicine against cancer, high blood pressure, and diabetes, among others.

To determine the potential of black garlic, its nutrient, chemical, and anti-cancer properties were evaluated based on existing protocols. Mineral contents such as sodium calcium, iron, potassium, and zinc increased from MMSU raw to black garlic, but most components were higher with the Japan black garlic. However, chemical properties such as S-allyl cysteine, Y-aminobutyric acid (GABA), and pyroglutamic acid were found to be higher in MMSU black garlic. Black garlic contains phytochemicals such as alkaloids, flavonoids, phenol, tannins, steroids, and terpenoids. The DPPH scavenging activities were higher in control, but the result indicates that raw and black garlic also have antioxidant activities. Results also showed that black garlic is cytotoxic to sample colon and lung cancer cells but Fluorouracil is more potent against colon cancer while there are no significant effects of the garlic extracts and Fluorouracil in the cytotoxicity against lung cancer.

Keywords: Black garlic, phytochemicals, cycloallicin, S-allyl cysteine, Y-aminobutyric acid (GABA) and pyroglutamic acid, DPPH, Cytotoxicity, HCT116 colon cancer line, A549 lung cancer cell line

II. INTRODUCTION

Garlic is the flagship commodity crop in the entire province of Ilocos Norte. The province is considered a champion garlic supplier in the Philippines (Ilocosnorte.gov.ph, 2016) for contributing 67 to 70 percent of the total garlic production of the country annually. According to the records of the office of the Provincial Agriculturist of Ilocos Norte as reported by Lazaro of balita.ph, the whole province produced at least 6,855 metric tons of garlic during the harvest season in the first quarter of 2014. It contributes to forty percent (40%) of the province's gross domestic product and is an effective tool for poverty alleviation of the farmers.

Garlic is known for its many health benefits. Garlic is widely used for several conditions linked to the blood system and heart, including atherosclerosis (hardening of the arteries), high cholesterol, heart attack, coronary heart disease and hypertension. It is also used today by some people for the prevention of lung cancer, prostate cancer, breast cancer, stomach cancer, rectal cancer, and colon cancer (Nordqvist, 2015). What makes raw garlic such a healthful and potent vegetable is the compound allicin, which accounts for its pungent odor. However, according to Mercola (2014), allicin is a very unstable compound which is decomposed into simpler molecules in less than an hour when garlic is cooked, crushed, aged or processed.

Recently, a processed garlic called black garlic is becoming popular for its unique appearance, taste and most specially, its health benefits. Black garlic is produced by processing ordinary garlic under constant high temperature and humidity up to 30 days. It is not a variety of garlic that grows and produces black cloves. During processing, the garlic changes its color from white to dark brown due to Maillard Reaction, a non-enzymatic browning reaction that also causes the change in flavor, texture, smell and nutrient content (Bae, et al., 2014).

In Japan, black garlic is already established in the market with the number of black garlic companies reaching to 500 (Sasaki, 2015). These companies produce in bulk using processing machines can control the temperature and relative humidity in the chamber. In MMSU, in the absence of commercial black garlic processing machine and optimized

condition for Ilocos White to process to black garlic, several experimental trials were conducted to come up with the condition that favor the transformation of white to black garlic. Black garlic of MMSU was produced using a convection oven. However, the oven is used for drying which is contradictory to the condition requirement in making black garlic as it requires maintenance of moisture inside the chamber to maintain the tenderness and fruitiness of the texture of the resulting black garlic. To address this, a two-layer aluminum foil was used to wrap the test bulb garlic to avoid excessive loss of moisture.

Studies have also shown that the compound s-allyl cysteine is higher in concentration in black garlic than raw garlic. This compound, compared to allicin is absorbed more easily by the body (Hobson, 2012). Other articles have claimed that antioxidants of black garlic increased (Choi, et al., 2014 and Sato, et al., 2006) which may also increase the medicinal benefits of black garlic that include anticancer.

Cancer, which has been regarded as one of the leading causes of death worldwide, is a type of disease related to uncontrolled or abnormal growth of cells or tissues in our bodies. With the growing number of evidence reported for the anticancer effect of black garlic in recent decades, some researchers suggested that black garlic could be used as a dietary product for preventing and treating cancers (Tran et al., 2018). People who include garlic and other *Allium* herbs in their diet have lesser chances of developing cancer than those who do not, as extensively observed in many studies. Bollinger (2016) said that the reduced development of tumor cells was mainly found in organs located along the gastrointestinal tract, such as the mouth, larynx, esophagus, intestines, kidneys, and reproductive parts, *i.e.,* breast and ovary.

Ilocos white garlic is said to have efficient medicinal properties, and because it is very pungent, this seems to indicate a high amount of organosulfur. Mariano Marcos State University (MMSU) has recently produced black garlic from the local variety. Considering the high selling price and health benefits of black garlic, it is important to study the potential of our own black garlic as a functional food and evaluate its medicinal properties so that our local garlic may increase its value and help our local garlic farmers increase their sales and profits.

III. OBJECTIVES

Generally, this study aims to investigate the medicinal potential and health properties of black garlic. Specifically, it aims to:

1. Determine the nutrient and phytochemical contents of black garlic;
2. Compare the chemical compounds present in MMSU black garlic and Japan black garlic;
3. Evaluate the preventive and cytotoxic effect of white and black garlic (Ilocos white variety) against representative human lung (A549) and colon (HCT116) cancer cell lines.

IV. REVIEW OF RELATED LITERATURES

Traditionally, the medical properties of garlic were recognized as early as 3000 BC. The functional benefits of garlic are its antimicrobial activity, anticancer activity, antioxidant activity, ability to reduce cardiovascular diseases, improving immune functions, and anti-diabetic activity. Garlic consumption decreases the risk of oral, stomach, esophageal, colon, and prostate cancers (Rahman, 2007). In addition, the biological activities of garlic, including antibacterial, antithrombotic, antioxidant, immunomodulatory, and antidiabetic actions and modulation of drug metabolism, have been extensively investigated (Tsai, et al, 2011). White garlic contains at least 33 sulfur compounds responsible for the pungent odor and many of its medicinal effects. It also contains enzymes, 17 amino acids and minerals, and phytochemical constituents such as saponins, tannins, and alkaloids (Philippine Medicinal Plants, 2015).

Phytochemicals are a large group of plant-derived compounds that may have protective or disease-preventive properties. Some of the benefits derived from phytochemicals are antioxidant, inhibit cancer, and have anti-bacterial effect (Heneman and Zidenberg-Cherr, 2006). Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents but chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Mojab et al., 2003).

The chemical composition of black garlic with its white counterpart was compared by an analysis conducted by ALS Laboratory Group and they found out that black garlic has higher amounts of nutrients compared to white garlic. Wrench, S. (2013), moreover, stated that black garlic is packed with twice as many antioxidants and seven times the disease-fighting polyphenols as its pale counterpart [white garlic]. Choi I.S. et al. (2014) have also pointed out

that several studies have reported that BG extracts have antioxidative, anti-allergic, antidiabetic, anti-inflammatory, hypocholesterolemic, hypolipidemic, and anti-carcinogenic effects.

However, the referenced studies were done in other countries using commercial black garlic. This study used black garlic from the local Ilocos White variety to investigate its nutritive, chemical, and anti-cancer properties.

V. METHODOLOGY

A. Production of Black Garlic

The MMSU black garlic was processed locally using the Ilocos White variety. Whole bulbs were processed in continuous heat in a convection oven for 30 days, maintaining moisture by wrapping the bulbs in foil. The Japanese garlic and black garlic were obtained from our industry partner, Takara, Inc., a company in Takko-machi, Aomori, Japan. The pH, % moisture content, and color (via visual test using Munsell Color Chart done by Intertek Testing Services Philippines in Makati City) were taken to determine if the MMSU INBG produced had the same characteristics as the commercial one.

B. Analysis of Nutrient and Mineral Content

Analysis was done by Intertek Testing Services Philippines in Makati City. Peeled 500 g of raw and black garlic from MMSU and Japan were submitted to the laboratory. The following are the parameters tested and the methods used:

PARAMETERS	METHOD
Crude protein	Kjeldahl Method
Ash	Gravimetric method
Total fat	Acid Hydrolysis
Carbohydrates	By computation
Total Calories	By computation
Total Sugars	Munson-Walker Method

Sodium	Atomic Absorption Spectrophotometry
Calcium	
Iron	
Potassium	
Zinc	Inductively Coupled Plasma-OES

C. **Qualitative Screening of Phytochemicals**

Cloves of raw Ilocos White garlic (RIWG) and INBG were peeled and soaked in 95% ethanol with a solid to liquid ratio of 1:3 for 72 hours at room temperature. After soaking, the solution was filtered to obtain the ethanolic extract. The ethanolic extract was used for the qualitative phytochemical analysis of the garlic samples.

D. Analysis of Chemical Properties

The determination for chemical properties of the MMSU and Japan black garlic was done at the Aomori Prefectural Industrial Technology Research Center (AITC) in Rokunohe, Aomori, Japan. The samples were submitted through Takara, Inc. The chemical properties analyzed were cycloallicin, S-allyl cysteine, Y-aminobutyric acid (GABA) and pyroglutamic acid.

E. Test for Antibacterial Properties

Standard microbiological procedures were followed for the sterilization of glass wares that were used in the study. Aseptic technique was also observed in every procedure.

1. Collection and Isolation of Microbes

Adolescents within the age bracket of 15-20 were used in the sampling population. Acnes of approximately equal sizes and stages were pricked aseptically using a sterile applicator stick to collect the pus. The pus was placed in nutrient broth and was mixed using a vortex mixer. It was then streaked on previously prepared NA plates (Gonzales, K., 2006).

The bacteria were allowed to grow inside the incubator at a controlled temperature. Bacterial colonies under aerobic incubation were characterized and counted after 24 hrs. incubation, while bacterial colonies under anaerobic incubation were characterized and

counted after 120 hrs. Bacterial cultures were also sent for identification using Vitek analysis at Mariano Marcos Memorial Hospital & Medical Center (MMMH & MC), Batac, Ilocos Norte.

2. Preparation of Garlic Extracts

The garlic and black garlic samples were peeled and ground using an electric blender. Pure extract was obtained by squeezing and filtering ground samples.

Ethanolic extracts were obtained by soaking grounded samples in 90% ethanol (Onyeagba, R.A., Ugbugo, O.C., Okeke, C.U. & Iroakasi, O., 2004) in Erlenmeyer flask sealed with a stopper. It was left to stand for 72 hrs. and was filtered to obtain crude ethanolic extract.

3. In-Vitro Antimicrobial Bioassay

Kirby-Bauer method was used for the bioassay. Filter disks with 6mm diameter was sterilized in separate beakers. Each beaker containing filter disks was poured with equal amounts of each treatment and allowed to stand for 24 hours.

The following treatments were used:

Treatment 1: Pure white garlic extract (WGE)

Treatment 2: Pure black garlic extract (BGE)

Treatment 3: Ethanolic white garlic extract

Treatment 4: Ethanolic black garlic extract

Treatment 5: 90% Ethanol (Negative control)

Treatment 6: Clindamycin Phosphate (Positive Control)

0.1 ml of pure bacteria isolates were placed in MHA plates and spread using a sterile spreader. Three (3) filter disks were positioned equidistant from each other aseptically using sterile forceps in each MHA plate.

Petri dishes were labelled respectively and were placed in the incubator in an inverted manner. The development of clear inhibition zones around each disk indicated the antibacterial activity of the treatments. This was an indication of the presence of certain active ingredients in the extracts which inhibited the growth of microorganisms.

The inhibition zones were interpreted using Guevera's (2005) inference (as cited by Ibe, et. al. (2014):

Interpretation of inhibition zones.

DIAMETER OF INHIBITION ZONE (mm)	ANTIBACTERIAL ACTIVITY
6 6<x<10	Resistant Inactive
10-13	Partially Active
14-19	Active
>19	Very Active

Antioxidant Activities

The antioxidant activity was evaluated using the DPPH radical scavenging activity.

For preparation of the methanolic extract, 100 g each of freshly peeled raw and black garlic cloves were crushed and soaked in methanol with solid to liquid ration of 1:3. These were stored in an oven at controlled temperature (70° C) for 30 days.

Each extract was then filtered and concentrated to dryness under reduced pressure and controlled temperature (40-50°C) in a rotary evaporator (rotavap). To accurately produce the needed concentration for the DPPH, the percent moisture of the rotavap extracts were obtained by drying them in the oven at 100°C until they had a constant mass.

Different concentrations of extracts (4000, 2000, 1000, 500 and 250 µg/mL, in methanol) were each loaded into a 96-well microplate at an equal volume (10 µL). Afterwards,

140 µL of 6.85x10^{-5} M of methanolic DPPH solution was added to each well and incubated in the dark at room temperature for 30 mins. the absorbance (Ab) values was measured at 517 nm on a spectrophotometer and converted into the percentage antioxidant activity using the following equation:

$$DPPH\ antiradical\ scavenging\ capacity\ (\%) = 1 - \frac{Ab\ of\ sample - Ab\ of\ blank}{Ab\ of\ contro} x100$$

Test for Cytotoxicity

The procedure adapted from Moreno et al. (2015) with some modifications, was performed for each of the cancer cell lines, A549 and HCT116. The cancer cell lines were grown at University of the Philippine, Manila, College of Medicine, Department of Biochemistry and Molecular Biology (UPCM-DBMB). A549 cancer cell line, McCoy's 5A (Modified) Medium was used. On the other hand, Ham's F12 Nutrient Mixture was used for HCT116.

The concentration used for the test extracts 500, 166.67, 55.57, 18.52 and 6.17 µg/mL. For the negative control, a culture medium was used.

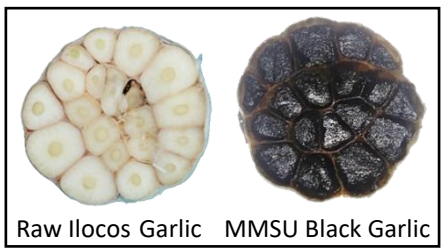
To determine the % cytotoxicity, the previously seeded plates were washed with phosphate-buffered saline (PBS) and then stimulated with the A. sativum extracts (500, 166.67, 55.57, 18.52 and 6.17 µg/mL) for 24 hours in complete culture medium.

The culture medium was then removed and 100 µL PBS plus 10 µL MTT solution (5 mg/mL) was added to each well and incubated for 3h at 37°C in the dark. The medium was aspirated and 100 µL DMSO was added to each of the wells. The plate was again incubated for 10 min at 37°C and the absorbance at 570 nm was measured using a microplate spectrophotometer.

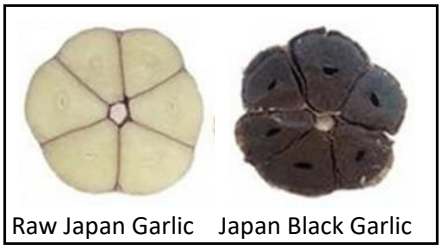
VI. RESULTS AND DISCUSSION

1. Production of Black Garlic

Ilocos garlic variety was used for the processing of MMSU black garlic. The Japanese garlic and black garlic were obtained from our industry partner, Takara, Inc., a company in Takko-machi, Aomori, Japan. Morphologically, the two garlic varieties are different. The local garlic is of the soft neck kind with 18-20 cloves while Takara, Inc. used hard neck kind with 5-6 cloves. The size of the Japan garlic is also larger than the Ilocos White.



Raw Ilocos Garlic MMSU Black Garlic



Raw Japan Garlic Japan Black Garlic

Table 1. Changes in pH, moisture and color of garlic after processing.

SAMPLES	PROPERTIES			
	pH	Moisture (%)	Color	Ave. # of Cloves
RING	6.44	62.64	Pale yellow	23*
INBG	3.78	49.61	Dark brown	23*
Japan BG	3.74	44.35	Dark brown	6

Table 1 shows some physical and other chemical characteristics of garlic and black garlic. A commercial black garlic from Japan was added as comparison whether INBG is the same as other black garlic in the market in terms of their characteristics. pH of the garlic bulbs decreased from 6.44 of the white garlic to 3.78 as the garlic bulbs age after a 30-day period. The color of INBG and Japan BG are the same.

2. Nutrient and Mineral Content

Table 1 show the nutrient and mineral information of raw and black garlic. Some properties have increase in value such as crude protein, total sugars, sodium, calcium, iron and potassium in MMSU black garlic when compared to the raw Ilocos White. The result of the analysis also shows that the local black garlic contains the same constituent as the Japanese black garlic but most of the constituents were higher in Japan black garlic than in MMSU black garlic. This may be attributed to the nutrient management during crop production.

Table 1. Nutrient and mineral content of garlic and black garlic (per 100 g).

PARAMETERS	Ilocos Garlic		Japan Garlic	
	Raw	Black	Raw	Black
Moisture (g)	67.26	68.04	65.74	55.7
Ash (g)	1.58	1.52	1.27	1.92
Crude Protein (g)	7.91	9.43	5.22	8.9
Total Fat (g)	0.24	0.37	0.36	0.16
Carbohydrates (g)	23.01	20.64	27.41	33.32
Total Calories (Cal)	125.84	123.61	133.76	170.32
Total Sugars (g)	93.58	151.22	101.48	189.09
Sodium (mg)	5.70	15.43	9.01	19.07
Calcium (mg)	1.94	3.31	1.16	0.90
Iron (mg)	9.23	19.51	11.26	19.88
Potassium (mg)	427.88	466.03	503.00	642.00
Zinc (mg)	1.32	1.54	1.26	1.07

3. Screening for Phytochemicals

For this study, 11 phytochemicals were screened. Result of the phytochemical screening are shown in Table 2. It shows that 9 phytochemicals are present in both black garlic samples. These are alkaloids (Mayer's, Wagner's), flavanoid (Alkaline reagent, Lead acetate), phenol (Lead acetate) tannins, steroids, terpenoids, protein, carbohydrates and cardiac glycocides. Anthraquinones and saponins were absent in both garlic samples. The presence of active phytochemicals in garlic gives the plant its medicinal value and its use for alternative medicine and phytotherapy. Flavanoids is reported to exhibit a wide range of biological activities such as antioxidant, anti-inflammatory, anti-angiogenic, anticancer and anti-allergic properties (Hossain, et al, 2013). Phenols and alkaloid are also found to have anti-microbial properties as well as anti-diarrheal and anthelmintic capabilities (Tiwari, et al, 2011)

Table 2. Phytochemical screening of garlic and black garlic.

Phytochemical	Test	Ilocos Garlic		Japan Garlic	
		Raw	Black	Raw	Black
Alkaloids	*Mayer's*	-	+	-	+
	Dragendorff's	-	-	-	-
	Wagner's	-	+	-	+
Flavonoid	*Shinoda's*	-	-	-	-
	Alkaline Reagent	+	+	+	+
	Lead Acetate	-	+	+	+
Phenol	*Ferric Chloride*	-	-	-	-
	Lead Acetate	+	+	+	+

Tannins	*Ferric Chloride*	-	+	-	+
Steroids	*Salkowski*	+	+	+	+
	Liebermann-Burchard	+	+	+	+
Terpenoids	*Salkowski*	+	+	+	+
	Liebermann-Burchard	+	+	+	+
Saponins	*Froth*	-	-	-	-
	Foam	-	-	-	-
Anthraquinones	*Modified Bomtrager's*	-	-	-	-
Protein	*Xanthopoteic*	+	+	+	+
Carbohydrates	*Molisch*	+	+	+	+
Cardiac glycoside	*Keller-Killiani*	+	+	+	+

+ Present; - Absent

3. Test for other chemical properties

The test for other chemical properties was done to determine the other health benefits that can be derived from black garlic. The result of the analysis (Table 3) shows that the local black garlic has higher S-allyl cysteine (34 mg/100g), Y-aminobutyric acid (154 mg/100g) and pyroglutamic acid (1153 mg/100g) than that of the Japanese black garlic. Meanwhile, Cycloallicin was found higher in Japanese black garlic (450 mg/100g).

Table 3. Chemical compound content of MMSU and Japanese black garlic.

Chemical Compound	**MMSU**	**Japan**
Cyclo alliin (mg)	48	450
S-allyl cysteine (mg)	34	26
Y-aminobutyric acid (GABA) (mg)	154	85
Pyroglutamic acid (mg)	1,153	480

Like other sulfur-containing compounds in garlic, cycloallicin is known to be associated with many health benefits of garlic. Previous studies have shown that cycloallicin is responsible for reducing the risk of both cancer and cardiovascular disease, as well as playing an important role in the reduction of serum triglyceride in rats (Lee, et al, 2016).

S-allyl cysteine (SAC), a sulfur containing amino acid derived from garlic, has been reported to have antioxidant, anti-cancer, antihepatotoxic and neurotrophic activity (Javed, et al, 2011). Studies have also shown that the compound s-allyl cysteine is higher in concentration in black garlic than raw garlic. This compound, compared to allicin is absorbed more easily by the body (Hobson, 2012).

Y-aminobutyric acid (GABA), on the other hand is a naturally occurring amino acid that works as a neurotransmitter in the brain. When GABA attaches to a protein in the brain known as a GABA receptor, it produces a calming effect. This can help with feelings of anxiety, stress, and fear. It may also help to prevent seizures (https://www.healthline.com/health/gamma-aminobutyric-acid#side-effects).

Pyroglutamic acid is a natural brain- boosting nutrient for a better mental health. It has brain-stimulating effects that help the brain cell to work efficiently enhancing one's mental skills and capabilities. It also improves blood circulation in the small arteries of the brain, keeping the brain cells healthy. Pyroglutamic acid has memory boosting capabilities making it a highly studied nutrient as possible cure for Alzheimer's disease and dementia (Wang-Tan, 2011).

5. Test for Antibacterial Properties

Table 4. Antibacterial activities of garlic samples against *Staphylococcus epidermis*.

Treatment	24 Hours		48 Hours	
	Inhibition zone (mm)	Activity	Inhibition zone (mm)	Activity
Pure WGE	17.44	Active	7.33	Inactive
Pure BGE	16.78	Active	7.33	Inactive
Ethanolic WGE	13.11	Partially Active	7.70	Inactive

Ethanolic BGE	11.15	Partially Active	8.33	Inactive
Clindamycin Phosphate	21.33	Very Active	6.89	Inactive

Result of the antimicrobial analysis (Table 4) against *Staphylococcus epidermis* show that pure white garlic extract and pure black garlic extract were more active in inhibiting growth compared to their ethanolic extract after 24 hours of exposure. After 48 hours, all the sample were inactive against the bacteria.

4. Antioxidant Activities

Table 5. Percent (%) DPPH Scavenging activity of raw and black garlic at each level of concentration.

Source of Extract	**Concentration of Extract (µg/mL)**				
	4000	**2000**	**1000**	**500**	**250**
Black garlic	20.43 b	7.68 b	6.29 b	5.19 b	4.23 c
Raw garlic	3.77 c	4.87 b	7.01 b	9.13 b	10.96 b
Ascorbic Acid	96.64 a	96.38 a	96.00 a	93.91 a	64.03 a

Means in a column with the same letter are not significantly different from each other at 0.1% level using LSD.

Based on the result of the assay (Table 5), ascorbic acid (control) had the highest DPPH scavenging activities in all level of concentrations. Black garlic, when compared to raw garlic at concentration level 4000 µg/mL was significantly higher with 20.43% while raw garlic was higher at concentration level 250 µg/mL with 10.96%. Ascorbic acid and black garlic had the same trend where the % scavenging activity increases when the level of concentration of extract also increases. The raw garlic had the opposite reaction where the scavenging activity increases as the concentration decreases.

5. Test for Cytotoxicity

Table 6. Percent (%) cytotoxicity of raw and black garlic at each level of concentration against HCT116 colon cancer line.

Source of Extract	Concentration of Extract (µg/mL)				
	500.00	**166.67**	**55.57**	**18.52**	**6.17**
Black garlic	44.51 b	51.21 a	49.53 a	45.10 a	45.81b
Raw garlic	45.25 b	45.81 a	48.42 a	52.14 a	55.68 a
Fluorouracil	61.45 a	50.09 a	48.04 a	46.18 a	41.53b

This means in a column with the same letter is not significantly different from each other at 0.1% level using LSD.

The percent cytotoxicity of the different concentrations of black garlic on HCT116 cells is shown in Table 6. Fluorouracil (control) had the highest % toxicity at the level of 500 µg/mL. At levels 166.67, 55.57, and 18.52 µg/mL, the % cytotoxicity was comparable in all the extracts, but raw garlic had the highest (55.68%) at level 6.17 µg/mL. For black garlic, from the lowest to the second highest concentration, 6.17 to 166.67 µg/mL, the trend is increasing. However, there was already a decline of 6.7 percent in the 500 µg/mL concentration. Unlike the former, the trend for raw garlic is decreasing and the relationship is inversely proportional. The lowest concentration, 6.17 µg/mL, obtained the highest percent cytotoxicity, 55.68 percent. For the control, there was a sharp decline as the concentration went down. At 166.67 µg/mL, 50.28 percent cytotoxicity was observed. This was followed by the concentrations 55.57 and 18.52 µg/mL with 48.23 and 46.37 percent cytotoxicity, respectively. The lowest cytotoxicity, 41.71 percent, was seen from the 6.17 µg/mL concentration

Table 7. Percent (%) cytotoxicity of raw and black garlic at each level of concentrations against A549 lung cancer cell line.

Source of Extract	Concentration of Extract (µg/mL)				
	500.00	**166.67**	**55.57**	**18.52**	**6.17**
Black garlic	22.55	22.16	16.97	4.59	15.57
Raw garlic	5.79	1.40	-6.39	-6.79	-10.58
Ascorbic Acid	23.35	22.75	8.38	8.98	-1.20

Table 7 shows the Percent (%) cytotoxicity of raw and black garlic at each level of concentrations against A549 lung cancer cell line. Statistical analysis did not show significant differences among the treatments at different level of concentrations, although numerically, raw garlic showed the lowest cytotoxicity.

For black garlic, the highest concentration, 500µg/mL, showed 22.55 percent cytotoxicity. This was followed by the concentrations 166.67 and 55.57 µg/mL with 22.16 and 16.97 percent cytotoxicity, respectively. The results showed a direct relationship between the concentrations and the percent cytotoxicity.

The relationship of the cytotoxicity and concentration in raw garlic is directly proportional as in the black garlic. Interestingly, only the two highest concentrations, *i.e.* 166.67 and 500 µg/mL, showed cytotoxicity effect against this cancer cell line, giving 1.6 and 5.99 percent, respectively.

As for the control, the relationship between cytotoxicity and concentration is also directly proportional. The efficacy of Fluorouracil against cancer cells is on account of its ability to inhibit the thymidylate synthetase, resulting in an imbalance of deoxyribonucleotides, which causes damage in the DNA.

VII. CONCLUSION AND RECOMMENDATION

1. In terms of some chemical components that were analyzed, INBG contains the same nutrient and phytochemical constituent as the Japan black garlic.
2. It also showed that it contains cyclo-alliin, s-allyl cysteine, Y-aminobutyric acid (GABA) and pyroglutamic acid which are bioactive compounds with health benefits.
3. Black garlic and garlic extract both showed cytotoxic activity against HCT116 colon cancer line which is even comparable to the control drug fluorouracil at concentration of 166.67, 55.57 and 18.52 µg/mL.

Based on the results, the researchers recommend the following:

1. A more comprehensive study on the uses of the chemical properties of black garlic for potential pharmaceutical products.
2. A more comprehensive and elucidative studies should be conducted to unravel the full potential of black garlic as an antioxidant and an anticancer drug.
3. Test other varieties of garlic, especially those from Ilocos Norte.
4. Conduct advocacy on the economics and health benefits of black garlic.

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