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Extracts of black garlic exhibits gastrointestinal motility effect

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ABSTRACT

In this studied, extracts of black garlic on the improvement of gastrointestinal function, antioxidant activity, total polyphenols, total flavonoids and total polysaccharides were evaluated. Results showed that the black garlic *n*-butanol fraction extract (BA) had significantly increased effect within small intestine *in vitro*, while the ethyl acetate fractions had no significant effect on small intestine *in vitro*. Increase of 5-HT_4 content effectively stimulated the gastrointestinal peristalsis, which enhanced its gastrointestinal tract emptying, and promoted defecation. As for antioxidant activity test, the water extract was more effective in SOD activity test, DPPH radical scavenging rates, ferric reducing antioxidant power and reducing power. In addition, the water fraction was simulated by gastric acid digestion and hydrolysis, and the small intestine was isolated after acid hydrolysis (AW). It was found that the water fraction extract after acid hydrolysis did significantly improve the intestinal contraction rate. In short, extract of black garlic could effectively promote gastrointestinal motility and promote defecation. The active compounds were highly polar ingredients since water extract of black garlic exhibits most significant effect on improving gastrointestinal function.

1. Introduction

Black garlic is a processed food produced and aged under controlled conditions in a high-temperature, humid environment with reduced spicy and irritating qualities compared to raw garlic (Bae, Cho, Won, Lee, & Park, 2014; Chen, Kao, Tseng, Chang, & Hsu, 2014; Lu, Li, Qiao, Qiu, & Liu, 2017). Due to the oxidation of γ-glutamyl cysteine, it produces no irritating and spicy water-soluble sulfur-containing compounds, and the organic lipid-sulfur-containing compounds are reduced with thermal cracking or volatilization caused by the high-temperature environment (Amagase, Petesch, Matsuura, Kasuga, & Itakura, 2001; Kodera et al., 2002; Maria, Feliciano, & Maria, 2017). Moreover, it inhibits the transformation of alliin into allicin with the heat-inactivation of alliinase (Corzo-Martinez, Corso, & Villamiel, 2007; Montano, Casado, Castro, Sánchez, & Rejano, 2004).

The flavor and nutritional properties of the garlic are also changed during the thermal processing of black garlic by the Maillard reaction (Kim, Kang, & Gweon, 2013; Shunsuke et al., 2017). Reports have shown that black garlic extract demonstrates several functions, such as antioxidation, antiallergy, antidiabetes, anti-inflammation, and anticancer effects (Banerjee, Mukherjee, & Maulik, 2003; Jeong et al., 2016).

The gastrointestinal tract is a vital organ essential for digestion and absorption of nutrients and excretion of waste. The effective functioning of gastrointestinal tract is not only essential for adequate nutrition but also for protection from ingested pathogens, allergens and toxins (Natasha, Kaarunya, & Say, 2017). The human gut is particularly susceptible to lifestyle influences. Obesity, smoking, excessive alcohol intake, stress, fatty foods, and binge eating could also affect problems from the top (reflux/dyspepsia) to the middle (irritable bowel) to the bottom (constipation/diarrhea) of the gut (Hansen, 2017). Gastrointestinal tract is a sophisticated system, composed of several functional fractions, including serosa, longitudinal muscle, myenteric plexus, circular muscle, submucosa, submucosal plexus and mucosal epithelium. The interact to enable simultaneous contraction and relaxation of the gut, facilitating adequate absorption and sensation (Goldstein, Hofstra, & Burns, 2013; Jonathan, Rachel, Vanesa, & Kulmira, 2017). Serotonin,

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also known as 5-hydroxytryptamine (5-HT), is a neurotransmitter that has been implicated in the regulation of diverse physiological processes, including cellular growth and differentiation, neuronal development, and regulation of blood glucose concentrations. The multiple 5-HT receptor subtypes have been cloned. The 5-HT_4 (5-hydroxytryptamine receptor 4) subtype is a high-affinity receptor located in the myenteric plexus of the gastrointestinal tract, which accelerates the release of acetylcholines from intestinal cholinergic neurons, contracts the smooth muscle, and thus participates in gastrointestinal motility (Concepción, Marta, Jose, & Maria, 2013; Shinichi et al., 2012). 5-HT_4 receptor mRNA is found in vascular smooth muscle. 5-HT_4 receptor is also located on neurons of the alimentary tract, for example the myenteric plexus of the ileum, and on smooth muscle cells and secretory cells of the gastrointestinal tract, where they evoke secretions and the peristaltic reflex. 5-HT_4 receptor agonists are used therapeutically in the treatment of constipation-predominant irritable bowel syndrome, and in functional motility disorders of the upper gastrointestinal tract. For example the cisapride and prucalopride are common receptor agonists (Julie, 2012; Granato, Calado, & Jarvis, 2014).

In addition, garlic is a long-use pharmaceutical/food resource which can regulate the gastrointestinal tract and promote digestion (Miron, Mironchik, Mirelman, Wilchek, & Rabinkov, 2003; Seki et al., 2008). Garlic also reported to exhibit laxative effect, but only few studies discuss the effects of improving the gastrointestinal tract function resulting in merely no information on the effective compounds in black garlic (Ning, Xuesong, Yanhua, Xiyang, & Xichun, 2013). In this study, five solvents: *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol and water, are used to harvest compounds with different polarity in black garlic. The physiologically active components in different fractions are evaluated for their ability toward gastrointestinal motility and 5-HT_4 receptor contents enhancement.

2. Materials and methods

2.1. Materials

Black garlic was provided by the All Wealth (Xiamen) Biotech Co., Ltd. as a gift. The Sprague Dawley (SD) rats were purchased from the Bio LASCO animal center (Taipei, Taiwan, R.O.C.). The *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol were purchased from Honeywell Burdick & Jackson (Korea). All of the other chemicals used in these studied were of analytical grade and were obtained commercially.

2.2. Preparation of black garlic extract

The black garlic was crushed and then extracted with different polar solvents as described earlier (Li, Li, Wu, & Tan, 2017). It was extracted using the 1:5 *n*-hexane by weight of the black garlic at least 3 times. The filtrates were collected and concentrated. After extraction, the residues were extracted in sequence with *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol and water as explained above. The partition extraction rate (E) was calculated as follows:

$$E = \frac{V_t}{C_t} \times 100\% \quad (1)$$

V_t was the extraction weight in the phase, and C_t was the sample weight phase volume.

2.3. Total polyphenol content, total flavonoids and antioxidant activity

The total polyphenol concentration of the black garlic fraction extract samples was determined by the Folin–Ciocalteu method (Padhi, Liu, Hemandez, Tsao, & Ramdath, 2016; Singleton, Orthofer, & Lamuela-Raventos, 1999). Some 50 μL of the extract, 50 μL of Folin–Ciocalteu reagent and 125 μL of carbonate sodium 20% w/v were placed in a volumetric 25 mL flask. The tubes were vortexed for 15 s and allowed to stand for 20 min at room temperature for color development. Samples were cooled with water and OD was red in the spectrophotometer at 765 nm (Metertech SP8001, Taipei, Taiwan, R.O.C.). The reading was compared to a calibration curve prepared with different gallic acid solutions.

Table 1
Raw garlic and black garlic partition extraction rate of different solvents (n = 3).

	Raw garlic	Black garlic
n-Hexane	2.45 ± 0.18%	0.04 ± 0.02%
Dichloromethane	6.21 ± 0.42%	0.34 ± 0.12%
Ethyl acetate	18.38 ± 0.12%	0.56 ± 0.09%
n-Butanol	32.67 ± 2.15%[b]	28.82 ± 1.52%[b]
Water	40.29 ± 1.42%[a]	70.24 ± 3.18%[a]

[a] The reported values are the mean ± SEM. Mean values with different letters are significantly different ($p < 0.05$).

The total flavonoid content was measured by aluminum chloride colorimetric assay. Aliquots of 100 μL of the extracts were added to equal volumes of a solution of 2% $AlCl_3$-$6H_2O$ 100 μL. The mixture was vigorously shaken and absorbance at 430 nm was read after 10 min of incubation. Rutin was used as a standard, made with a standard curve, and the standard curve equation was then used to calculate the sample's total flavonoid content (Chen et al., 2015).

The DPPH radical scavenging abilities were determined by the standard method with minor modifications (Athukorala, Kim, & Jeon, 2006). DPPH solution (0.1 mM, in 50% alcohol solution) was used for incubating, with varying concentrations of the sample. The reaction mixture was shaken for 20 min. The DPPH radical scavenging ability was determined by measuring the absorbance at 517 nm using a spectrophotometer. Distilled water was used as a blank, and the L-ascorbic acid was used as a positive control. The DPPH radical scavenging activity was calculated as follows:

$$Scavenging\ Capacity\ (\%) = \{1 - (A_s - A_c \mid A_0)\} \times 100\% \quad (2)$$

A_c, A_s and A_o represented the absorbance of the control, the sample and the blank, respectively. The antioxidant capacity of the test compounds was expressed as IC50, the concentration necessary for a 50% reduction of DPPH.

2.4. Experimental animals

SD male rats (200–300 g) were purchased from Bio LASCO Taiwan Co., Ltd. (Taipei, Taiwan). They were housed in standard cages at a constant temperature of 22 ± 2 °C, relative humidity 55 ± 5% with a 12 h/12 h light-dark cycle for at least one week, and fed food and water *ad libitum*. The protocol was approved by the Committee on Animal Research, Da-Yeh University, under code 104007.

2.5. In vitro bowel shrinkage test

For the *in vitro* gastrointestinal motility test, 1–2 cm of the stomach or intestine was cut and was then placed in the organ bath and ventilated (95% O_2, 5% CO_2) for 2 h to stabilize it. Then the same isometric contraction (0.5–1 g) was performed. Testing was then conducted to determine the contraction of the food in the stomach or intestine (Pozzoli & Poli, 2010).

2.6. In vivo gastrointestinal motility test and emptying test of intestinal activated carbon

An animal-configured irrigation solution (containing 10% activated carbon and test materials) was used on the male SD rats. The calculation of the emptying rate was then tracked; activated carbon made it easy to observe the characteristics of the black color as a further

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