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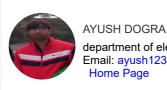
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Author(s): Fathul Djannah, Anny Setijo Rahaju, Hamsu Kadriyan, Eva Triani, Heru Fajar Trianto, Rahadian Zainul DOI: 10.52711/0974-360X.2024.00201

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RESEARCH ARTICLE

Black Garlic for the treatment of Tuberculosis and Diabetes mellitus

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ABSTRACT:

Black garlic is made from fresh garlic by thermal processing. It is produced under certain temperatures and humidity to generate a product with greater content, taste, and texture. Additionally, black garlic has many benefits, such as being an antioxidant, antibacterial, anti-inflammatory, antihypertensive, anticancer, antidiabetic, and cardiovascular protective. Thus, black garlic can be utilized as a treatment for tuberculosis and diabetes mellitus. Moreover, the ingredients in black garlic, such as SAC, polyphenols, flavonoids, tannins, and ajoene, can reduce levels of Mycobacterium TB, lessen plasma glucose levels, and increase plasma insulin in diabetes mellitus.

KEYWORDS: Black garlic, Tuberculosis, Diabetes mellitus.

INTRODUCTION:

Garlic is believed to originate from Central Asia, particularly Kazakhstan, Uzbekistan, and western China. It later spread to the Mediterranean in ancient times. Presently, garlic is grown all over the world¹. It is utilized not only as a cooking spice but also as a natural treatment for various diseases. Despite the numerous health benefits of garlic, some people are reluctant to eat raw garlic because of its pungent taste and smell. Raw garlic can cause gastrointestinal discomfort in some people². For this reason, black garlic has been developed. Processing black garlic at a certain temperature produces a sweet and sour taste, a mild aroma, and a jelly-like texture²⁻⁴.

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Black garlic is generally processed at different temperatures and humidity ranges. Processing conditions vary greatly depending on regional traditions and the specific features desired in the ultimate product. In addition, producing black garlic at 700C can facilitate the formation of good black garlic quality and taste compared to other temperatures^{3,5}.

Phytochemical compounds in black garlic are higher than in fresh garlic. Several bioactive compounds increasing in black garlic include flavonoids, pyruvate, thiosulfate, phenols, and S-allycysteine (SAC). The SAC content in black garlic varies depending on the analytical method used. Representative antioxidant compounds in black garlic include phenols and flavonoids, such as SAC, S-allylmercaptocysteine (SAMC), Diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS)^{2,6}. The bioactive compounds in black garlic can increase antioxidant activity, antibacterial activity, antiinflammatory activity, antihypertensive activity, anticancer activity, antidiabetic activity, cardiovascular protection, and so forth⁷. Thus, it is used as an alternative medicine for various diseases. This article discusses the process of making black garlic and its benefits for tuberculosis (TB) and diabetes mellitus (DM).

Data Collection:

Researchers conducted a literature search regarding the process of producing black garlic and its benefits for TB and DM from various sources, such as relevant articles, official books, and national and international journals published until August 2022. Additionally, researchers sought sources from various platforms, such as Google scholar, Medline (PubMed), NCBI, ScienceDirect, and trusted journal publishers.

DISCUSSION:

Black garlic:

Black garlic is a type of garlic product produced by heating raw garlic at a controlled temperature and humidity without any additional treatment or additives. After being processed into black garlic, the pungent odor of fresh garlic becomes mild. During the heating process, the unstable and unpleasant compounds in raw garlic are converted into stable and tasteless compounds. As a result, black garlic generally has a sweet-sour taste instead of a pungent odor and taste. Moreover, black garlic does not cause abdominal pain or other gastrointestinal discomfort^{3,5,8}.

Black garlic first appeared in 1999 and was produced by Japanese Kamimura in Mie prefecture, Japan, who also filed a manufacturing patent of the black garlic in the Japan Patent Office. Black garlic is also called Aged garlic, which is produced at controlled temperature and humidity⁸.

The benefits of black garlic for TB disease:

Tuberculosis (TB) is caused by the bacterium called Mycobacterium tuberculosis (MTB)⁹⁻¹¹. Most of the droplets containing MTB from infected patients are trapped in the upper respiratory tract and expelled by ciliated mucosal cells; only a small portion reaches the alveoli. Mycobacterium then binds to alveolar macrophage cells via complement receptors, mannose receptors, or type A scavenger receptors. After phagocytosis, mycobacterium reduces the acidity in phagosomes and cell wall components (i.e., lipoarabinomannan) and damages the Ca+/calmondulin thereby inhibiting phagosome-lysosome pathway, fusion. After successfully stopping phagosome maturation, bacilli multiplication begins, and the macrophage eventually even ruptures to release its bacilli, which are taken up by the macrophages and continue the cycle of infection that further expands the spread. During primary infection, MTB bacilli undergo hematogenous and lymphatic spread, involving the hilar and mediastinal lymph nodes to form the primary Ghon complex. Eventually, the bacilli enter the bloodstream and reach various organs¹²⁻¹⁸.

The long duration (6–12 months) and complexity of TB treatment lead to non-adherence in patients, which results in incomplete treatment and the development of drug resistance. This situation is called multidrug-resistant (MDR) and extensively drug-resistant (XDR)^{12,19-21}. In addition, TB drugs have side effects, such as hepatotoxicity, nephrotoxicity, exanthema, arthritis, and hyperuricemia. This has led scientists to seek new alternative treatments that are effective against all forms of MTB with minimal side effects^{22,19,23}.

Garlic is known as an alternative treatment for tuberculosis. The content in garlic can reduce the number of *Mycobacterium tuberculosis*^{22,24,25}. Therefore, the following researches examine the benefits of garlic against tuberculosis.

Table 1: Research on	the henefits of garlie	against tuberculosis
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Researchers	Preparation/dose	Research subject	Results
Nair. S.S, Gaikwad S.S, Kulkarni S.P, MukneA.P ¹⁹ .	Garlic extract (GE)	RAW 264.7 mouse macrophage cells infected with MTB H37Rv	 Anti-TB activity was better than isolate The anti-TB activity of rifampicin, isoniazid, and ethambutol was still better than GE
Rajani S.D, Desai P.B, & Rajani D.P ²⁶ .	Ethanol Garlic Extract	Sputum from 230 TB patients	 MTB MDR was inhibited at an EGE concentration of 2.0 mg/ml
Hanan A, Ullah MI, Usman M, Hussain S, Absar M, &Javed K ²⁷ .	Ethanol Garlic Extract (EGE)	Cultural isolates (15 MDR and five non-MDR MTB)	 Most of the MDR isolates were inhibited at an EGE concentration of 2.0 mg/dl. Non-MTB MDR was inhibited at an EGE concentration of 1.5 mg/dl The minimum inhibitory concentration (MIC) of garlic extract ranged from 1 to 3 mg/ml; showing the inhibitory effect of garlic against both non-MDR and MDR M. tuberculosis isolates

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Lindawati NY & Hartono H ²⁸ .	Garlic dry extract (capsule)	Inoculum Mycobacterium (sputum culture of TB patients type BTA+2)	 Garlic extract at a dose of 240 mg/ml (600 mg of garlic extract in capsules) had the potential to inhibit the growth of mycobacterium TB In addition, higher concentrations (medium dose of 300 mg/ml and 400 mg/ml) were not sensitive to MTB.
Dwivedi VP, Bhattacharya D, Singh M, et all ²⁹ .	Allicin, garlic extract	MTB strain H37Rv, C57BL/6 mice, aged 6-8 weeks	Allicin and garlic treatment significantly reduced the bacterial burden in macrophages Allicin inhibited infected cells in macrophages, reduced bacteria, cleared MTB within 45 days after treatment. Allicin/garlic extract could act as an immunomodulator.
Shukla P & Sharma A ³⁰ .	Ethanol garlic extract	Mycobacterium culture	 Growth of MTB and MDR-MTB was inhibited Having no inhibitory effect on mycobacterium other than tuberculosis (MOTT) species
Nasab F, Valizadeh M, Beigomi M, &saeidi S ³¹ .	Ethanol garlic extract	50 strains of mycobacterium	- Ethanol garlic extract was highly effective in inhibiting the growth of <i>Mycobacterium tuberculosis</i> .

Nair, S.S., et al. (2017) conducted in vitro evaluation of the anti-tubercular activity of garlic extract by using the Resazurin Microtiter Plate Assay (REMA) method¹⁹. As a result, the anti-tubercular activity of garlic extract demonstrated better results than other samples/isolates when evaluated by the REMA method. Additionally, garlic extract had high anti-tuberculosis activity in macrophages and low cytotoxicity. The standard drugs, such as rifampicin and ethambutol, indicated significantly lower MICs as compared to GE by the REMA method. However, when tested in RAW 2648. mouse macrophages infected with MTB, the extract at 50µg/ml showed activity comparable to rifampicin (10 µg/ml) and significantly better than isoniazid (10µg/ml) and ethambutol (10µg/ml). Rifampicin, isoniazid, and ethambutol showed higher cytotoxicity as compared to GE at all concentrations tested. It can thus be inferred that GE had the advantage of having lower cytotoxicity than standard TB drugs. E-ajoene showed anti-TB activity at concentrations of 125-250gr/dl, and Z-ajoene was inactive at the highest concentration of 250mg/dl. This might be due to the degradation of Z-ajoene after incubation at 37°C for seven days. Mycobacteria remained in the phagosomal compartment of the macrophages. Therefore, macrophages should be activated to kill intracellular mycobacteria. In this research, the maximum concentration of GE was 150 ml/dl. Ge demonstrated better anti-tubercular activity with increasing concentrations¹⁹.

In another research, ethanol garlic extract could inhibit the growth of MTB and MDR MTB with a minimum concentration of 0.5mg/dl. EGE inhibited all MDR MTB isolates that were resistant and sensitive to second-line drugs at concentrations of 0.5 to 2.0mg/dl. Garlic showed the same effect on MDR MTB and was sensitive to second-line drugs. However, garlic had no effect on mycobacterium other than tuberculosis (MOTT)^{26,30}. Garlic had a multi-factorial mode of action because the constituents exert their effects simultaneously. The difference in results between studies was due to differences in garlic species in the concentrations of active constituents 26 .

An in vitro study conducted by Ahmad Hasan et al. revealed that Ethanol garlic extract (EGE) could inhibit MDR and MDR MTB with different concentrations of EGE (1-3mg/ml). Most of the MDR isolates were inhibited at an EGE concentration of 2.0mg/dl. Non-MTB MDR was inhibited at an EGE concentration of 1.5 mg/dl. The minimum inhibitory concentration (MIC) of garlic extract ranged from 1 to 3mg/ml; showing the inhibitory effect of garlic on Mycobacterium isolates²⁷.

The test results for the antibacterial potency of dry garlic extract against *mycobacterium tuberculosis* showed that garlic extract at a medium dose of 240μ g/ml (equivalent to 600mg garlic extract/capsule) had the potential to inhibit MTB growth, on top of that, it was more sensitive than rifampicin at a medium dose of 40μ g/ml (equivalent to 100mg rifampicin/capsule). At higher doses (300μ g/ml and 400μ g/ml), it was even less sensitive to MTB. The higher the dose, the more resistant it was. It was suspected that other compounds dissolved in the garlic extract actually supported the proliferation of MTB bacteria. Garlic dry extract fulfilled the requirements as an ingredient in traditional medicine capsules²⁸.

Allicin treatment significantly reduced the bacterial burden on macrophages, inhibited infected cells, lessened the percentage of infected cells, decreased MTB internalization in macrophages, reduced bacteria, and cleared MTB within 60 days after treatment. In addition, the combination treatment of isoniazid and garlic extract showed an additive effect and could clear MTB within 45 days. Meanwhile, isoniazid treatment alone could be effective for more than 60 days. These data were confirmed by histological analysis of the lungs, which revealed a reduction in the number of granulomas after garlic extract treatment. The effect of garlic extract on MDR and XDR drastically reduced the bacterial burden, prevented skin lesions, and extended survival by more than 90 days until the end of the experiment. Meanwhile, the untreated group indicated severe skin lesions and passed away within 15 days after the MDR infection. Garlic extract promoted the generation of a protective Th1 immune response against MTB infection. Besides, allicin and garlic extracts had immunomodulatory properties. Garlic has strong antimycobacterial agents and inhibits bacterial growth either directly or through immunomodulation²⁹. The lowest minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against MTB were 3.25 and 7.5ppm, respectively. Meanwhile, the highest MIC and MBC were 60 and 120ppm, respectively. Ethanol garlic extract was highly effective inhibiting the growth of *Mycobacterium* in tuberculosis³¹.

Garlic extract could inhibit the growth of MTB and MDR TB. This process was associated with the antioxidant and antimicrobial in garlic. To increase the active content, fresh garlic was processed to get better quality for treatment.Several studies have revealed that the processing of garlic into black garlic could change the chemical composition and bioactive compounds. In general, black garlic contains better bioactive than fresh garlic. The content in black garlic showed an increase in bioactive properties^{8,32,33}. In addition, black garlic contains higher antioxidants than fresh garlic. The increase in antioxidant capacity could be caused by an increase in polyphenols and S-allyl cysteine, which was a derivative of alliin. In black garlic, the antioxidant content increased 2-3 times compared to fresh garlic^{34,32}.

S-allyl cysteine is a derivative of alliin. In black garlic, S-allyl cysteine increased 2.5 to 8 times through the heating process. Sulfur oxidation of S-allyl cysteine by flavin-containing monooxygenases formed S-Allyl-I-Cysteine Sulfoxide (ACSO). ACSO could be converted to allyl sulfenic acid (2-propene-1-sulfenic acid) and amino acrylic acid by alliinase. The acrylic amino acid was then spontaneously converted to pyruvic acid and ammonia. Two molecules of allyl sulfenic acid produced diallyl thiosulfinate⁸.

Allicin contained in black garlic could reduce *Mycobacterium tuberculosis* and inhibit MDRMTB²⁶. It

also suppressed the inflammatory process in TBC by increasing the performance of the glutathione peroxidase enzyme and stopping the Antigen 85B transcription process, thereby reducing the production of Reactive Oxygen Species (ROS) and inflammatory mediators³⁵. Besides, allicin could act as an immunomodulator by increasing host immunity and reducing the side effects of taking TB drugs²⁹. Ajoene in black garlic activated the IRE1a-JNK-ROS pathway stimulating macrophage release to carry out phagocytosis and autophagy against *Mycobacterium tuberculosis*²².

Black garlic contains tannins that may interrupt the permeability of bacterial cell walls, thereby preventing MTB growth. Similarly, alkaloid compounds contained in black garlic can inhibit bacterial growth and cause damage to peptidoglycan in bacteria²⁰.

Black garlic has been proven to have higher antioxidant and antimycobacterial levels than fresh garlic. However, the advantages of black garlic as anti-*mycobacterium tuberculosis* have not been extensively studied. Therefore, further study is required to determine how black garlic affects the growth of MTB and MDR MTB.

The benefits of black garlic for diabetes:

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia resulting from impaired insulin secretion, insulin action, or both³⁶. Diabetes mellitus is classified as type 1 diabetes (DMT1) caused by beta cell dysfunction and leading to insulin defect. Meanwhile, type 2 diabetes (DMT2) is generally due to progressive insulin defects against a background of insulin resistance^{37,38}. DMT1 is considered a chronic autoimmune disorder caused by the progressive destruction of T-cells mediated by insulin-producing pancreatic B-cell. The gradual loss of B-cells' glucose sensitivity contributed to the degree of insulin deficiency, leading to hyperglycemia³⁹⁻⁴¹.

Chronic hyperglycemia constitutes a major source of oxidative stress, and increased free radicals play a key role in DM pathogenesis and complications. Therefore, antioxidants are required to neutralize free radicals. Black garlic is one of the traditional treatments for DM containing antioxidants and antidiabetic agents⁵.

Table 2: Research or	the benefits of garlic for Diabe	tes Mellitus
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Researchers	Preparation/dose	Subjects	Results
Pangestu, TYI &Setyawan, AB ⁴² .	Two black garlic cloves/day	patients with DMT2	Decreasing blood sugar levels
Kim JH, Yu SH, Cho YJ, Pan JH, et al ⁴³ .	200mg/kg black garlic juice	C57BL/6J male rats	Neutralizing damage due to diabetes, B-cell apoptosis, and insulin deficiency; improving glutathione antioxidant system; increasing leptin and adiponectin secretions; inhibiting hepatic gluconeogenesis; and suppressing NF- kB-mediated inflammatory signals

Saryono, Nani D, Proverawati A, &Sarmoko ⁴⁴ .	Steeping black garlic	6-7 weeks-old male Wistar rats	BG at doses of 13.5 g/kg and 26 g/kg could decrease the levels of pro-inflammatory cytokines (IL-18, IL-6, and TNF-a) and significantly boost IFN-y levels.
Lee YM, Gweon OC, et al ⁴⁵ .	Freeze-dried black garlic powder	3-weeks-old cstbl/ksl male rats	Decreasing levels of Thiobarbituric acid reactive substances (TBARS) and increasing the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxide (GSH-px)
Prihanti GS, Isnaini F, Yudistia R, et al ⁴⁶ .	Black garlic extract with a dose of 1.5 mg/200 gBW/day, 3 mg/200 gBW/day, and 6 mg/200 gBW/day	2-3 months-old male Wistar rats	Decreasing blood sugar level by 94%, total cholesterol by 79.1%, TG by 69.5%, LDL by 81.3%, SGPT by 91.4%, and SGOT by 70.6%
Ha AW, Kim WK ⁴⁷ .	0.5%, 1.0%, and 1.5% black garlic extract	4-weeks-old male Sprague-Dawley rats.	Decreasing plasma glucose, plasma insulin, HOMA-IR, plasma TBARS levels, and TAC concentrations, increasing catalase activity, glutathione peroxidase, hepatic Nrf2, NQ01, HO-1, and GSTS2 mRNA expression levels

Research conducted by Pangestu and Setyawan in patients with DMT2 by applying treatment using two black garlic cloves indicated to have effects on reducing blood sugar levels from an average of 300.87 mg/dl to 251.47mg/dl⁴². S-allyl cysteine (SAC), flavonoids, and tannins act as antioxidants in black garlic, which are proven to be anti-glycemic and prevent DM complications. SAC garlic can reduce plasma glucose levels and increase plasma insulin levels^{42,48}.

Administering black garlic juice to male rats at a dose of 200 mg/dl could neutralize damage caused by diabetes, B-cell apoptosis, and insulin deficiency. The SAC performance in this study could increase glutathione, leptin, and adiponectin secretions, inhibiting hepatic glucogenesis and suppressing Nf-kB-mediated inflammatory signals⁴⁹.

Another study suggested that the administration of steeping black solo garlic to diabetic Wistar rats (STZ) induced by streptozotocin at doses of 13.5g/kg and 26 g/kg decreased IL-1, IL-6, and TNF- α as well as increased TNF- α in the experimental animals. These findings suggested the possibility of using black solo garlic as an immunomodulator and an anti-inflammatory in diabetes to avoid complications⁴⁴. STZ induction produced free radicals that oxidize pancreatic cells by alkylating DNA, damaging mitochondria, and inhibiting the O-GlcNAcase enzyme. The oxidation process induced toxicity through free radical chain reaction causing an inflammatory process for organ damage triggering the release of pro-inflammatory cytokines, such as IL-1 β and TNF- α^{39} . SAC and polyphenols could neutralize free radicals and decrease TBARS levels while increasing SOD, CAT, and GSH-px activities^{45,48}.

Black garlic extract at a dose of 1.5mg/200gBW/day, 3mg/200gBW/day, and 6mg/200gBW/day in male Wistar rats could reduce blood sugar levels by 94%, total cholesterol by 79.1%, TG by 69.5%, LDL by 81.3%, SGPT by 91.4%, and SGOT by 70.6%. In

addition, polyphenols and flavonoids could reduce oxidative stress and prevent damage to pancreatic beta cells by inhibiting the chain reaction, converting superoxide to hydrogen superoxide, and supplying hydrogen atoms to bind free radicals and dispose of them through the excretory system⁴⁶.

Administering black garlic extract to white male rats at the doses of 0.5%, 1.0%, and 1.5% could reduce plasma glucose, plasma insulin, and HOMA-IR levels as well as plasma TBARS and TAC concentrations, increasing catalase activity and glutathione peroxidase, hepatic Nrf2, NQ01, HO-1, and GSTS2 mRNA expression levels⁴⁷.

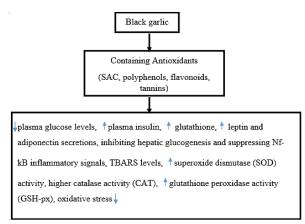


Figure 1: The benefits of black garlic for diabetes.

CONCLUSION:

Previous studies conclude that the ideal temperature to produce black garlic with good taste and quality is 70°C. Black garlic has several benefits over raw garlic, including a greater taste and smell, as well as the ability to decrease mycobacterium levels and drug resistance in TB. In addition, black garlic is also beneficial for DM sufferers, including reducing blood sugar levels, increasing plasma insulin, and preventing complications. Therefore, black garlic is recommended as an additional supplement for patients with TB andDM.

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