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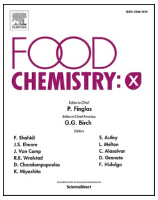

# Bioactive properties and organosulfur compounds profiling of newly developed garlic varieties of Bangladesh

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2,3,5-Triphenyltetrazolium Chloride (PubChem CID: 9283)
1,1-Diphenyl-2-picrylhydrazine-DPPH (PubChem CID: 74358)
Anhydrous $MgSO_4$ (PubChem CID: 24083)
Folin-Ciocalteu reagent
Gallic Acid (PubChem CID: 370)
Methanol (PubChem CID: 9283)
*N*-hexane (PubChem CID: 8058)
Sodium Carbonate (PubChem CID: 10340)

## ABSTRACT

Studies are being carried out on achieving the maximum quality of garlic through various approaches. In Bangladesh, new garlic varieties (BARI 1–4, BAU-1, BAU-2, BAU-5) have been recently developed by artificial selection to enhance their quality. The present study aimed to evaluate their potency in terms of bioactive properties and organosulfur compounds content using different bioassay and GC–MS techniques while comparing them with other accessible varieties (Chinese, Indian, Local). The new variety, BARI-3 showed the highest antioxidant activity and total phenolic content. It was also found with the highest level of a potent blood pressure-lowering agent, 2-vinyl-4H-1,3-dithiine (78.15 %), which is never reported in any garlic at this percentage. However, the local variety exhibited greater inhibitory properties against the tested organisms including multidrug-resistant pathogens compared to other varieties. This study primarily shows the potential of these two kinds of garlic for their further utilization and development.

## Introduction

Garlic, known as *Allium sativum***,** is a popular spice in many cuisines around the world including Southeast Asia (Rivlin, 2001). Although known for culinary purposes, its potential health benefits have made it an important ingredient of traditional and modern medicine (Lanzotti, Scala, & Bonanomi, 2014). These benefits include lowering blood cholesterol and lipid in the serum, decreasing high blood pressure, and preventing cancer and cardiovascular diseases, etc. (Iciek, Kwiecień, & Włodek, 2009). It also possesses other biological activities including antibacterial, antiviral, antifungal, and antioxidant properties (Phan, Netzel, Chhim, Netzel, & Sultanbawa, 2019). Therefore, there is considerable interest in garlic all over the world for its major bioactive properties. Additionally, a variety of pre-harvest factors (genotype,

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*Abbreviations:* MIC, Minimum Inhibitory Concentration; MBC, Minimum Bactericidal Concentration; DPPH-2, 2,2-diphenyl-1-picrylhydrazyl; TPC, Total Phenolic Content; GAE, Gallic Acid Equivalent.

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diverse growing techniques) and post-harvest conditions (storage conditions as well as processing treatments) have a significant impact on the quality of garlic, which is leading to the efforts of continuous improvement of garlic worldwide (Martins, Petropoulos, & Ferreira, 2016).

The antimicrobial activity of garlic is recognized for centuries and has been used in many cultures and traditions throughout the world to ward off bacterial infections (Lawson, 1998). Numerous studies evaluated different garlic preparations such as crude or fresh garlic extract, garlic powder, garlic paste, garlic oil, etc, and found that they were effective against many enteric and foodborne pathogens including *Escherichia coli*, *Escherichia coli 0157:H7*, *Salmonella typhi*, *B. cereus*, *K. pneumoniae*, *S. aureus*, *Shigella species*, *Proteus vulgaris*, *Proteus mirabilis*, *Listeria monocytogens* etc. (Bhatwalkar, Mondal, Krishna, Adam, Govender, & Anupam, 2021). Furthermore, other investigations found substantial variations in the antibacterial activity of garlic with different origins (Petropoulos et al., 2018; Phan, Netzel, Chhim, Netzel, & Sultanbawa, 2019). These antibacterial activities are mainly due to a potent antimicrobial compound known as allicin that has been confirmed by invitro test, although not established with clinical studies (Petropoulos et al., 2018). The antibacterial effect is also attributed to other organosulfur compounds such as diallyl polysulfide and ajoenes (Nakamoto, Kunimura, Suzuki, & Kodera, 2020).

Garlic is also known for its strong antioxidant properties. It has a protective effect against oxidative DNA damage and prevents endothelial dysfunction. Hence, it is widely used in the protection of humans against multiple disorders such as oxidative stress, chronic diseases, cancer, atherosclerosis, etc. (Chen et al., 2013). Recently, there has been a surge of interest in natural antioxidants, particularly the dietary antioxidants found in vegetables that help humans fight off oxidative stress. Garlic is a great source of natural antioxidants that has significant health-promoting properties (Nuutila, Puupponen-Pimiä, Aarni, & Oksman-Caldentey, 2003). Regarding per capita consumption, it is one of the highly regarded vegetables that is consumed as one of the best sources of total phenolic compounds in the human diet (Lanzotti, Scala, & Bonanomi, 2014). However, a wide range of variations regarding the phenolic content has been found among the different garlic genotypes and ecotypes, while the cultivation practices, as well as growing conditions, were different (Volk & Stern, 2009).

Typically, the bioactive properties of garlic including its derived products are mostly ascribed to organosulfur compounds (Lanzotti, 2006). The biochemical composition of fresh garlic varies widely, particularly in terms of the concentration of organosulfur compounds. As a result, numerous studies have been conducted to estimate the level of organosulfur compounds in different types of garlic from around the world, where novel compounds were found in some garlic (Liu et al., 2020). The sulfur-based organic substances in garlic are mainly volatile and are derived from the precursor alliin (S-allyl-cysteine-S-oxide) compound which is known as the principal component of garlic. This precursor Allin is frequently released upon the breakage of garlic tissues during the crushing and hydrolyzed into a mixture of both volatile and non-volatile organosulfur compounds. The volatile sulfur-based compounds include thiosulphinates which are very unstable and converted into multiple compounds such as Sulphides-di-allyl-sulphide (DAS), di-allyl-disulphide (DADS), di-allyl-trisulphide (DATS) or 'Vinyldithiins' (cyclic sulfur-containing compounds) or other constituents. All of these components contribute to the distinctive flavour of garlic as well as its cardiovascular disease prevention abilities (Bonasia, Conversa, Lazzizera, Loizzo, Gambacorta, & Elia, 2020). Therefore, these chemicals have recently attracted a lot of attention.

In Bangladesh, three varieties of garlic, including Local, Chinese and Indian are commonly found in local markets across the country. Recently, new high-quality garlic varieties including BARI-1, BARI-2, BARI-3, BARI-4, BAU-1, BAU-2, and BAU-5 known as local landraces have been developed via artificial selection from local cultivars of various districts throughout the country (Digital Herbarium of Crop Plants, 2016). BARI 1–4 varieties were developed by Bangladesh Agriculture Research Institute (BARI), whereas BAU-1, BAU-2, and BAU-5 varieties were introduced by Bangladesh Agriculture University (BAU). They seem promising due to their high storage quality and resistance to pests and diseases. However, their bioactive properties and organosulfur compound profiling have not been reported so far, despite their promising qualities. Therefore, this study was designed to explore these garlic varieties as well as determine their variability in terms of these properties for their further development and effective utilization, while comparing them with the varieties available in local markets around the country.

## Material and Methods

### Plant Materials

Three types of garlic varieties including Local, Indian and Chinese were purchased from the local market of Chattogram, Bangladesh. They were then identified by Bangladesh National Herbarium (Accession No: DACB 64495, DACB 64494, DACB 64496) and their specimen were deposited at the herbarium. Newly developed varieties such as BARI-1, BARI-2, BARI-3, and BARI-4 were brought from Bangladesh Agriculture Research Institute, Gazipur. Other developed garlic including BAU-1, BAU-2, and BAU-5 were collected from Bangladesh Agriculture University, Mymensingh. All of these varieties were categorized into five distinguished groups for their different growth and cultivation conditions and denoted as Local Cultivar, Chinese Garlic (Imported Variety), Indian Garlic (Imported Variety), BARI Varieties, and BAU Varieties (Supplementary Table). After collection, the individual fresh garlic cloves were washed, sliced, and sun-dried for about seven days. Then, cloves were ground to a fine powder using an electric blender and maintained at room temperature (22–25 °C) until needed for analysis.

### Bacterial Strains

Common five different bacterial strains such as *Escherichia coli* ATCC25922*, Salmonella enterica vs Typhimurium* ATCC14028*, Bacillus cereus* ATCC14574*, Klebsiella pneumonia* ATCC 13883*, Staphylococcus aureus* ATCC6538 were used in the study. Five multidrug-resistant *E. coli* and five *Proteus* spp isolates were collected from the Bangladesh Council of Scientific and Industrial Research (BCSIR). The strains were maintained at −80 °C before the study.

### Antibacterial Assay

The antibacterial assay of investigated garlic was performed according to the procedure previously described (Petropoulos et al, 2018). The assay was carried out by broth microdilution method as recommended by a CLSI protocol (CLSI, 2021). The bacterial suspensions were adjusted spectrophotometrically at 625 nm (UV 1800 spectrophotometer, Shimadzu, Japan) to a concentration of $10^8$ CFU/mL, then diluted to a concentration of $10^6$ CFU/mL. 1 % garlic powder in distilled water was prepared and immediately added to the first well of the microtitre plate containing Mueller Hinton Broth (100 μL). It was then serially diluted with an equal volume of broth in the next consecutive wells. Finally, each well was loaded with the corresponding bacterial inoculum ($10^6$ CFU/mL). The minimum concentration without visible bacterial growth was termed MIC (Minimum Inhibitory Concentration). The MIC values of garlic extract were also confirmed by a colourimetric microdilution assay (Veiga et al., 2019) based on the reduction of TTC (2,3,5-triphenltetrazolium chloride) and positive control of each microbial strain was used for comparison. The optical density of each well was measured at a wavelength of 540 nm by a high-performance microplate reader (CYT5MFA, Biotek Instruments. Inc. USA). The MBC (Minimum Bactericidal Concentration) of garlic extract was determined by transferring inoculum (2 μL) serially from the microtitre plate containing 100 μL of broth, corresponding organisms, and garlic extract concentration into Nutrient Agar Media. The culture media was then kept at a 37°C incubator for 24 h to check their growth. The lowest concentration at which no visible growth of bacteria was observed, indicative of 99.5 % killing of the bacterial inoculum was defined as MBC. Ciprofloxacin (Oxoid, UK) was used as the positive control whereas autoclaved distilled water was used as the negative control.

### *Antioxidant Activity Assay*

The Antioxidant activity of garlic powder was ascertained using the methanolic extract. Briefly, 1. 25 g garlic powder was dissolved in 25 mL methanol at a final concentration of 50 mg/mL.Then, it was mixed continuously in an orbital shaker incubator (New Brunswick Scientific, USA) for 30 min at 25°C with 150 rpm. The extracts were then centrifuged at 4000 rpm for 10 min (Universal Centrifuge, Taiwan). After centrifugation, the supernatant was collected and further diluted to different concentrations used for DPPH Radical Scavenging Assay. The result was shown in $EC_{50}$values (concentration of sample exhibiting 50 % of DPPH free radical scavenging activity) whereas ascorbic acid was used as a standard control.

### *Measurement of Total Phenolic Content*

Total phenolic content was measured from the methanolic extract of garlic powder by the Folin-Ciocalteu colourimetric method as described by Tawaha et al, 2007 (Tawaha, Alali, Gharaibeh, Mohammad, & El-Elimat, 2007). For the extraction, 250 mg garlic powder was mixed with 10 mL of 80 % methanol and kept in a shaker water bath at 37 °C for 3 h. After that, the extract was centrifuged at 3500 rpm for 10 min and the supernatant was collected for the assay. In brief, 0.5 mL of garlic extract was mixed with 2.5 mL of Folin-Ciocalteu Reagent previously diluted with water at a 1:10 ratio and 2.0 mL of sodium carbonate (75 g/L). Then, the tubes were vortexed for 20 s and kept at 40°C for 30 min for colour development. The absorbance of the blue-coloured solution was then measured at 765 nm (UV-1800 Spectrophotometer, Shimadzu, Japan). Gallic acid was used as a standard control where five different concentrations (100, 50, 25, 12.25, 6.125 μg/mL) were taken (Fig. 1). The total phenolic content was expressed as mg of gallic acid equivalents (GAEs) per gram of dry extract.

### *GC–MS Analysis*

Organosulfur compounds of studied garlic were analyzed by a GC–MS system (GCMS-QP2020, Shimadzu, Japan). Briefly, 5 g of fresh garlic was weighed and crushed using a mortar and pestle. Then, crushed garlic was mixed with 15 mL *n*-hexane and kept in a shaking water bath at 45 °C for 2 h. Then, the mixture was centrifuged at 4000 rpm for 10 min. After the centrifugation, 10 mL supernatant was recovered and approximately 3g anhydrous $MgSO_4$ was added to the supernatant. Again, the mixture was centrifuged at 4000 rpm for 10 min at 15 °C. Finally, 1 mL supernatant was separated for the analysis.

The analysis was carried out with an RTX-5 MS capillary column having a cross band of 95 % diphenyl-95 % dimethylpolysiloxane (30 m × 0.25 mm × 0.25 μm). Helium was used as a carrier gas with a flow rate of 1.72 mL/min. The initial oven temperature was set to 80 °C (isothermal for 2 min) and then raised to 150 °C at the rate of 5 °C where it was maintained for 3 min. The final oven temperature obtained was 280 °C at the raised rate of 5 °C/min with a 5 min holding time. The garlic sample (1 μL) was injected in splitless mode at 50:0 where the injection temperature was 220 °C. The total run time was 50 min. The ion source was adjusted at 280°C and the electron-impact ionization method was ensured with an ionizing potential of 70 ev. The mass spectra were found within the scan range of 45–350 (*m/z*) and the separated components present in the mass spectrum were identified by comparing the spectrum of known compounds stored in NIST08.LIB program library using a probability-based algorithm.

### *Statistical Analysis*

The antimicrobial assay was carried out in triplicate by analyzing three samples for each treatment, while for all the antioxidant properties and total phenolic content analyses, tests were run in duplicate. The results were expressed as mean values and standard deviations (SD). IBM SPSS Version 22.0 (IBM, USA) was used to perform statistical analysis of data and one-way analysis of variance (ANOVA) with Post Hoc Tukey's Test at alpha = 0.05 was applied to check for any significant differences among the garlic varieties for their antimicrobial, antioxidant activity and total phenolic content. Principal Component Analysis was performed to examine the variability among the garlic varieties for the distribution of major organosulfur compounds using Minitab Statistical Software Version 21.1. 0.

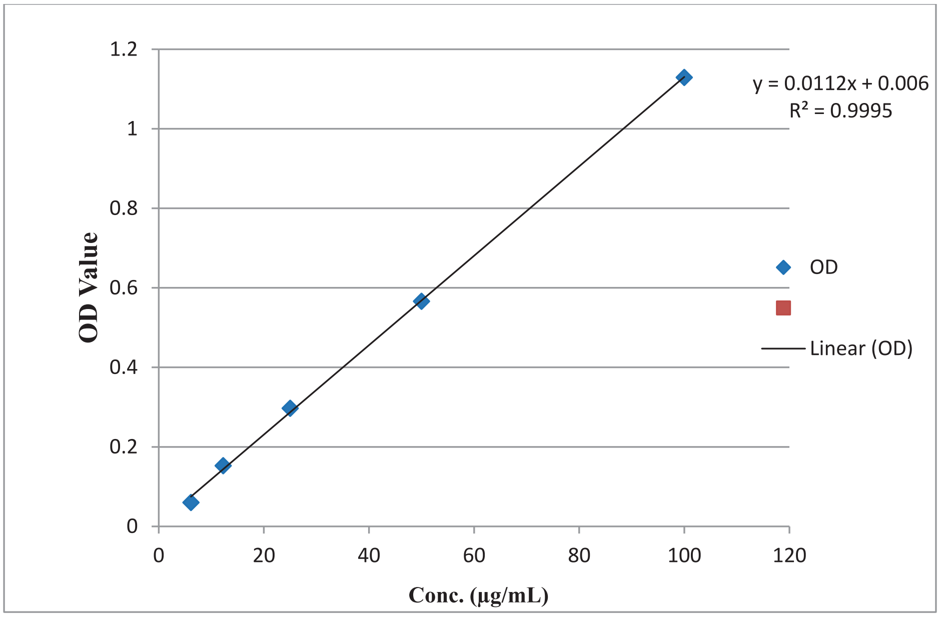

**Fig. 1.** Standard Curve of Gallic Acid with the five concentrations (100, 50, 25, 12.25, 6.125 μg/mL).

## Results & Discussion

The present study firstly reports the antimicrobial and antioxidant activities of newly developed garlic varieties of Bangladesh (BARI 1–4, BAU-1, BAU-2, BAU-5) with their total phenolic and organosulfur compounds content. It also compares their activity with the varieties (Local, Chinese, Indian) found in local commercial markets around the country.

The antimicrobial activity of these garlic varieties against the studied bacteria was determined by the broth microdilution method and is presented in Table 1. Common pathogenic bacteria (*E. coli, S. typhimurium, B. cereus, K. pneumonia*, *S. aureus*) and multidrug-resistant *E. coli*, *Proteus* spp isolates respond differently to various concentrations of garlic powder prepared from studied varieties. There is a significant variation of bacteriostatic and bactericidal activity against the tested pathogens including multidrug-resistant bacteria among the different groups of garlic varieties. The MIC and MBC values of local garlic against these organisms ranged from 1.25 to 5 mg/mL and 5 to 20 mg/mL respectively were lower than that of other's garlic, representing its high potency. The other varieties such as BARI-2, BARI-3, BARI-4, BAU-1, BAU-2, and BAU-5 had almost similar bacteriostatic (MIC value 1.25 to 10 mg/mL) and bactericidal activity (MBC value 5 to 40 mg/mL) against the tested pathogens with some exception. Furthermore, they were more effective than commercial Indian and Chinese garlic as their MIC and MBC value were slightly lower compared to the value of both two imported garlic (MIC 2.5 to 10 mg/mL; MBC 5 to > 40 mg/mL). In the case of multidrug-resistant *E. coil* and *Proteus* spp isolates, their MIC and MBC values were also mostly lower than the value of Indian and Chinese Garlic. Interestingly, significant inhibitory properties (MIC value 1.25 mg/mL) against multi-drug resistant *Proteus* spp were observed for local garlic. BARI-1 was the least active among the garlic varieties against all of the tested organisms in terms of their bacteriostatic and bactericidal activity (MIC value 5 to > 10 mg/mL; MBC value 20 to > 40 mg/mL). A very low level of variation of antibacterial activity within different BARI and BAU varieties was also observed. However, the positive control of Ciprofloxacin showed MIC values against the tested bacteria ranging between 0.0039 and 1.0 mg/mL, which was much lower than the examined garlic varieties.

The antimicrobial activity of garlic against a broad range of pathogenic microorganisms including the emerging multidrug drug-resistant pathogens has been reported by various studies (Magryś, Olender, & Tchórzewska, 2021). In our study, the studied garlic varieties had a significant inhibitory effect against all the tested bacteria including multi-drug resistant bacterial isolates. In particular, the local garlic was more potent than that of others. The antibacterial activity of some Chinese garlic was examined by Chen et al. (2018) and they reported that among a variety of speculated antibacterial compounds, 3-vinyl-1,2-dithiacyclohex-5-ene and 3-vinyl-1,2-dithiacyclohex-4-ene—were the major compounds and mostly responsible for the inhibition of microbes. Therefore, it might be possible that some compounds are responsible for the enhanced antibacterial property of local garlic which can be identified by comparing its extract to other garlic extracts demonstrating antimicrobial potency. The Column Chromatography as well as other analysis techniques including TLC (Thin Layer Chromatography), HPLC (High Performance Liquid Chromatography), and GC–MS (Gas Chromatography Mass Spectrometry) can be utilized in this regard. The local garlic also exhibited better antimicrobial activity than other garlic of UK origin against human enteric pathogens such as

**Table 1**
Antimicrobial Activities of various garlic species.

| Bacterial Species | MIC/MBC value (mg/mL) of garlic powder | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | BARI-1 | BARI-2 | BARI-3 | BARI-4 | BAU-1 | BAU-2 | BAU-5 | Chinese | Indian | Local | Control |
| *E. coli* | 10 | 2.5 | 5 | 2.5 | 2.5 | 2.5 | 2.5 | 10 | 5 | 2.5 | 0.0039 |
| | >40 | 5 | 20 | >10 | >10 | >10 | >10 | >40 | >20 | >10 | >0.0156 |
| *K. pneumoniae* | 10 | 2.5 | 2.5 | 2.5 | 2.5 | 5 | 2.5 | 10 | 5 | 2.5 | 0.0039 |
| | 40 | 5 | 10 | 10 | >10 | 20 | 10 | 20 | 10 | 5 | >0.0156 |
| *S. aureus* | 10 | 2.5 | 2.5 | 2.5 | 2.5 | 5 | 2.5 | 10 | 5 | 2.5 | 0.0039 |
| | >40 | 10 | >10 | >10 | >10 | >20 | >20 | >40 | >20 | >10 | >0.0156 |
| *S. typhimurium* | >10 | 2.5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 2.5 | 0.0039 |
| | 40 | 10 | 10 | 20 | 10 | 20 | 20 | 10 | 10 | 5 | >0.0156 |
| *B. cereus* | >10 | 2.5 | 2.5 | 2.5 | 5 | 5 | 2.5 | 5 | 2.5 | 2.5 | 0.0039 |
| | 40 | 10 | 5 | 10 | 10 | 20 | 10 | 10 | 10 | 10 | >0.0156 |
| DTRP$_2$0[a] | 10 | 5 | 5 | 5 | 5 | 5 | 5 | 10 | 5 | 2.5 | 0.25 |
| | >40 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 10 | 0.50 |
| NCKT0$_7$[a] | 10 | 5 | 5 | 5 | 5 | 5 | 5 | 10 | 5 | 2.5 | 0.25 |
| | 40 | 10 | 10 | 20 | >20 | 20 | 20 | 40 | 20 | 10 | 0.50 |
| CMC-13[a] | >10 | 10 | 10 | 10 | 10 | >10 | 10 | 10 | 5 | 2.5 | 0.031 |
| | 25 | 40 | 20 | 20 | 40 | 40 | 40 | 20 | 10 | 5 | 0.125 |
| CMC-16[a] | >10 | 10 | 10 | 10 | 10 | 10 | 10 | >10 | 10 | 5 | 0.25 |
| | 40 | 40 | 20 | 20 | 40 | 40 | 20 | 25 | 20 | 10 | 0.50 |
| CMC-19[a] | >10 | 5 | 5 | 5 | 10 | 10 | 5 | 10 | 5 | 2.5 | 0.015 |
| | 30 | 10 | 10 | 10 | 20 | 40 | 20 | 20 | 10 | 5 | 0.50 |
| Pm$_1$[b] | >10 | 2.5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 1.25 | 0.0039 |
| | 30 | 5 | 10 | 20 | 10 | 10 | 10 | 20 | 20 | 5 | >0.0156 |
| Pm$_3$[b] | >10 | 2.5 | 5 | 5 | 5 | 5 | 5 | 10 | 5 | 1.25 | 0.0039 |
| | 30 | 5 | 10 | 10 | 10 | 20 | 10 | 20 | 10 | 5 | >0.0156 |
| Pm$_4$[b] | 10 | 1.25 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 5 | 5 | 1.25 | 0.0039 |
| | 40 | 5 | 10 | 10 | 10 | 10 | 10 | 20 | 10 | >5 | >0.0156 |
| Pm$_5$[b] | 5 | 1.25 | 2.5 | 2.5 | 2.5 | 2.5 | 1.25 | 5 | 5 | 1.25 | 0.0039 |
| | 20 | 5 | 10 | 10 | 10 | 5 | 5 | 20 | 10 | >5 | >0.0156 |
| Pm$_6$[b] | 5 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 | 5 | 2.5 | 5 | 0.0039 |
| | 20 | 5 | 5 | 5 | >5 | 5 | 5 | 10 | 5 | 20 | >0.0156 |

MIC-Minimum Inhibitory Concentration, MBC-Minimum Bactericidal Concentration.
MBC-Up to 4*MIC value of each garlic powder was tested.
Control-Ciprofloxacin.

[a] is expressed as multidrug-resistant *E. coli* isolates.

[b] is expressed as multidrug-resistant *Proteus* spp isolates.

*Bacillus cereus, Escherichia coli, Salmonella typhi* at lower MIC values (Ross, O'Gara, Hill, Sleightholme, & Maslin, 2001). Moreover, it showed strong antibacterial action against the tested multi-drug resistant *Proteus* spp. To our knowledge, the antimicrobial activity of garlic against this resistant organism is very limited.

Many studies propose that garlic could be consumed to prevent food poisoning due to its strong antimicrobial activity (Bhatwalkar, Mondal, Krishna, Adam, Govender, & Anupam, 2021). Nejad, Shabani, Bayat, and Hosseini (2014) tested the inhibitory effect of aqueous garlic extracts against Staphylococcus aureus in hamburgers and observed that their growth was retarded (Nejad, Shabani, Bayat, & Hosseini, 2014). Similarly, garlic exhibited high performance as a potential antimicrobial in Brazilian low-sodium frankfurters in a study conducted by others (Horita et al, 2016). These findings demonstrate that local garlic, which inhibits the growth of the foodborne bacteria examined in the current study, may also be evaluated for use as a natural food preservative. It may have the potential to extend the shelf life of food products by itself or in combination with other herbs and spices. However, it's crucial to separate and identify the bioactive compound of this garlic that shows a strong antibacterial effect. Although the most popular technique for the separation and analysis of biological samples is Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC) coupled to Mass Spectrometry (MS) and Diode Array Detector (DAD), the complex nature of garlic suggests the use of hyphenated IC-FLD/UV technique to identify its bioactive components (Muhammad et al., 2018; Muhammad et al., 2021).

The antioxidant properties of examined garlic varieties have been also ascertained by DPPH Radical Scavenging assay and are shown in Table 2. In the present study, we analyzed the methanolic extracts of our garlic for antioxidant activity. The antioxidant value ($EC_{50}$) differed among garlic varieties ranging from 11.59 to 18.7 mg/mL, where the highest level of antioxidant activity was obtained from BARI-3 ($EC_{50}$-11.59), followed by Chinese ($EC_{50}$-11.6 mg/mL) and BARI-1 garlic ($EC_{50}$-11.61 mg/mL). The activity of these garlic varieties was higher than the G4 (16.90 mg/mL), G6 (13.06 mg/mL), G7 (17.22 mg/mL), G8 (20.09 mg/mL) genotypes of Greece garlic (Petropoulos et al, 2018), but lower than the aqueous extracts of Korean garlic (95.04–166.92 μg/mL) as reported in a previous study (Nagella, Thiruvengadam, Ahmad, Yoon, & Chung, 2014). The antioxidant activity of BARI-3 garlic was also higher than that of other's garlic. Škrováncková, Mlček, Snopek, andPlanetová (2018) evaluated the antioxidant capacity of five types of garlic (Greek, Spanish, Czech Harnas, Czech Bjetin, Chinese) and two commercial dry garlic products (Škrovánková, Mlček, Snopek, & Planetová, 2018). Their $EC_{50}$values were 261.7–550.2 and 163.4–177.5 mg/mL, which was much higher than the $EC_{50}$value of BARI-3. The evaluation of antioxidant activity by the other two assays (reducing power, β-Carotene bleaching inhibition) could further support the findings as different assays display varying activity due to the multiple antioxidant mechanisms involved. The lowest antioxidant activity ($EC_{50}$ – 19.44) was found from BAU-1 variety. There was also dissimilarity within all the BARI varieties (11.59 to 15.66) and BAU varieties (12.41–19.44), although some had (BARI-3, BARI-1) nearly similar $EC_{50}$ values.

**Table 2**
Antioxidant properties and Total Phenolic Content (TPC) of garlic powder.

| Garlic Varieties | $EC_{50}$ value (mg/mL) by DPPH Assay | TPC as mg of GAE/g dry weight |
|---|---|---|
| BARI-1 | 11.61 ± 0.38 | 2.77 ± 0.24 |
| BARI-2 | 13.86 ± 0.39 | 2.42 ± 0.14 |
| **BARI-3** | **11.59 ± 0.48a** | **5.08 ± 0.05a** |
| BARI-4 | 15.66 ± 0.02 | 2.44 ± 0.05 |
| BAU-1 | 19.44 ± 1.64 | 2.12 ± 0.22 |
| BAU-2 | 12.41 ± 0.26 | 2.7 ± 0.02 |
| BAU-5 | 13.4 ± 0.67 | 2.38 ± 0.02 |
| Chinese | 11.6 ± 0.30 | 2.36 ± 0.22 |
| Indian | 14.54 ± 0.29 | 2.08 ± 0.11 |
| Local | 18.7 ± 0.60 | 1.64 ± 0.28 |

GAE-gallic acid equivalent. The antioxidant activity is expressed in $EC_{50}$ values. The lower the $EC_{50}$ value, the higher the antioxidant activity. $EC_{50}$: Concentration of extract representing 50 % of antioxidant activity. Lowercase letter a indicates the significant difference at $P < 0.05$.

The total phenolic compounds content (TPC) of the investigated garlic varieties was also measured using Folin-Ciocalteucolourimetric method. Regarding the content of total phenolic compounds, they were also different from each other (Table 2). This variation was evident as they were cultivated at different places with various microclimatic conditions and agricultural practices, although some were grown at identical places with similar cultivation conditions. Furthermore, the results were consistent with the report of Hirata, Abdelrahman, Yamauchi, and Shigyo, (2016) where the TPC content varied in a wide variety of garlic. Significantly, the highest value of TPC content was observed for the BARI-3 variety (5.08 mg GAE/g dry extract), while the values were between 1.64 and 2.77 mg GAE/g dry extract for the other varieties. There were no noticeable differences in TPC within the other BARI varieties (2.42–2.77 mg GAE/g dry extract), apart from the BARI-3 variety. Likewise, the differences among BAU varietieswere almost negligible (2.12–2.7 mg GAE/g dry extract). Unexpectedly, the local garlic was the lowest in total phenolic content (1.64 mg GAE/g dry extract) in comparison to that of others. These results recommend that the BARI-3 variety may be a superior source of phenolic compounds than the other tested garlic varieties. It might have a significant amount of Phenolic acids, Flavonoids in comparison with the other varieties. The TPC content of this variety is also much higher than the TPC observed in Australia-grown garlic (Phan, Netzel, Chhim, Netzel, & Sultanbawa, 2019), Kenyan garlic (Alide, Wangila, & Kiprop, 2020) as well as other garlic cultivars including Chinese Spring, Spanish Roja and California White (Toledano Medina, Pérez-Aparicio, Moreno-Ortega, & Moreno-Rojas, 2019), but lower than the extract of fresh Brazilian garlic bulb (6.99–8.70 mg GAE/g dry extract) (Queiroz, Ishimoto, Bastos, Sampaio, & Torres, 2009). The TPC content in this garlic was also greater than the TPC value (1.55–4.73 mg GAE/g dry weight) of fifteen commercial-grade garlic bulbs of various country origins (Wongsa, Spreer, Sruamsiri, & Müller, 2014) produced from conventional and organic conditions. The differences in total phenolic content between this BARI-3 garlic and garlic from various country origins may be due to a variety of factors, including genetic, environmental, and agronomic factors (Waterer & Schmitz, 1994) that have been proven in earlier investigations. However, the relatively higher amount of total phenolic content in BARI-3 in regard to other BARI varieties from the same location and almost identical growth conditions might represent the major role of genotype rather than the other conditions.

There was no distinct positive or negative correlation between antioxidant activity and total phenolic content for methanolic extracts of garlic varieties (Fig. 2). Only a positive correlation was seen in the case of the BARI-3 variety which was in agreement with Chen et al. (2013) who reported that out of 43 garlic cultivars, the cultivar "74-x" had the highest phenolic content and the maximum antioxidant activity. However, the variations in antioxidant activity and total phenolic content within BARI and BAU local landraces of two distinct regions (Gazipur, Mymensingh) indicate that genotype, rather than the microclimate conditions and cultivation methods used to develop the landraces, is the main determinant of their bioactive content. This is because the varieties from the two groups were grown under the group-specific same environmental and growing conditions in the particular two locations.

The organosulfur compounds content of Garlic varieties were analyzed by GC–MS techniques and are shown in Table 3. The identified major compounds were Diallyl Disulfide; Tri-sulfide,di-2-propenyl; Trisulfide,methyl-2-propenyl; Tetrasulfide,di-2-propenyl; 3-vinyl-1,2-dithiacyclohex-4-ene; 3-vinyl-1,2-dithiacyclohex-5-ene; 2-vinyl-4H-1,3-dithiine. Other compounds were also present in some garlic but in a lower amount representing <5 % of total organosulfur compounds (data was not shown). These types of compounds were also detected in Iraqi Garlic, Spanish Garlic of White, Purple, and Chinese Origin (Al-

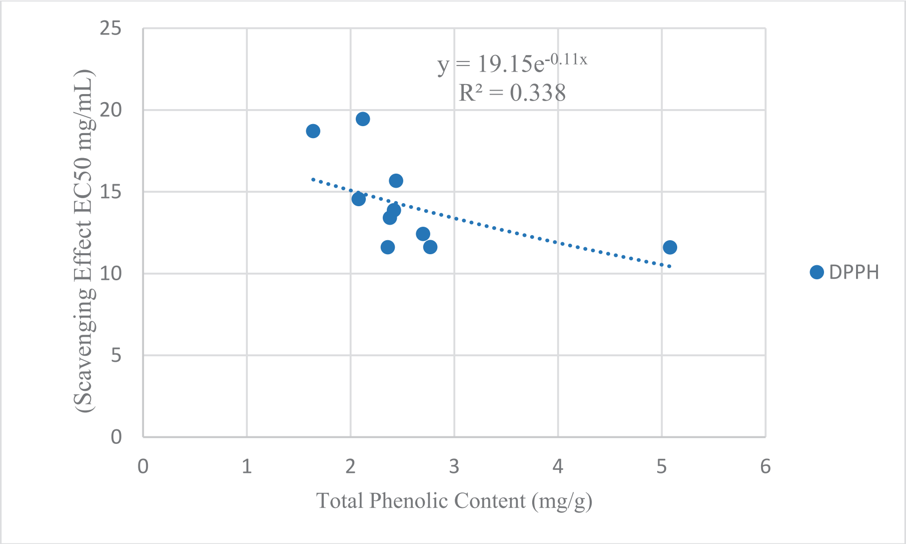

**Fig. 2.** Correlation between Total Phenolic Content and Antioxidant Activity of garlic varieties.

**Table 3**
Organosulfur Compound profiling of tested garlic varieties.

| Sl. No | Major Organosulfur Compounds (Conc.) | Garlic Varieties | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BARI-1 | BARI-2 | BARI-3 | BARI-4 | BAU-1 | BAU-2 | BAU-5 | Local | Indian | Chinese |
| 1 | Diallyl disulfide | 7.97 % | 6.11 % | 4.20 % | 6.99 % | 11.21 % | 9.28 % | 15.14 % | 10.36 % | 40.48 % | 13.67 % |
| 2 | Trisulfide, di-2-propenyl | 47.35 % | 46.91 % | 5.25 % | 7.91 % | 43.92 % | 4.03 % | 23.61 % | 2.79 % | 1.76 % | 0.98 % |
| 3 | Trisulfide, methyl 2-propenyl | 18.96 % | 8.76 % | 1.41 % | 2.19 % | 6.11 % | 2.32 % | 7.97 % | 1.71 % | ND | ND |
| 4 | Tetrasulfide, di-2-propenyl | 0.41 % | 1.78 % | ND | ND | ND | ND | ND | 9.04 % | 13.63 % | 6.27 % |
| 5 | 3-Vinyl-1,2-dithiacyclohex-4-ene | 0.84 % | 2.16 % | 10.19 % | 8.26 % | 5.49 % | 8.24 % | 2.51 % | 13.19 % | ND | ND |
| 6 | 3-Vinyl-1,2-dithiacyclohex-5-ene | ND | ND | ND | ND | ND | ND | ND | ND | 28.59 % | 50.28 % |
| 7 | 2-Vinyl-4H-1,3-dithiin | 5.61 % | 13.08 % | 78.15 % | 73.45 % | 30.87 % | 74.57 % | 13.24 % | 56.82 % | ND | ND |

ND: Not Detected.

Taai, Al-Fekaiki, & Jamail, 2019; Molina-Calle, Priego-Capote, & de Castro, 2016). Diallyl disulfide and Trisulfide, di-2-propenyl were derived from each garlic variety in different relative concentrations where diallyl disulfide was the most abundant in Indian garlic ((40.48 %)) and Trisulfide, di-2-propenyl was in BARI-1 (47.35 %). Trisulfide, methyl-2-propenyl was common in almost all garlic except Indian and Chinese garlic. On the other hand, tetrasulfide,di-2-propenyl was only detected in BARI-1, BARI-2, and Local, Indian and Chinese garlic. Apart from Indian, and Chinese garlic, 3-vinyl-1,2-dithiacyclohex-4-ene was present in all garlic varieties. 3-vinyl-1,2-dithiacyclohex-5-ene was only derived from Indian and Chinese garlic. Surprisingly, 2-vinyl-4H-1,3-dithiine was found at the highest concentration in the BARI-3 variety with a relative amount of 78.15 %, followed by BAU-2 (74.57 %), BARI-4 (73.45 %). The elevated amount of this compound in BARI-3 compared to other analyzed garlic could be linked to the key effect of genotype, because the content did vary not only among the tested five distinguished groups but also among BARI group varieties where the growing areas and cultivation conditions were identical. The other studies on several garlic varieties from four locations in Spain and the Italian province of Foggia also revealed that genotype, rather than other factors, had a certain influence on the level of specific organosulfur compounds (Beato, Orgaz, Mansilla, & Montaño, 2011; Bonasia et al., 2020). Further molecular analysis is also required for final confirmation. In addition, it was also the only local landrace that was identified with the highest level of 2-vinyl-4H-1,3-dithiine compared to other garlic assessed in different parts of the world to the best of our knowledge. This result agreed with that of earlier studies where the compounds variation in different garlics might be occurred due to the impact of garlic cultivar, ecotype, and geographical distribution (Beato, Orgaz, Mansilla, & Montaño, 2011; Hirata, Abdelrahman, Yamauchi, & Shigyo, 2016).

The vinyldithiins of garlic have significant implications in the regulation of cardiovascular diseases. An in vivo study on hypertensive rats reported that intake of 2-vinyl-4H-1,3-dithiine could provide significant protective effects against arterial remodelling in hypertension (Torres-Palazzolo, de Paola, Quesada, Camargo, & Castro, 2020). In our current study, the level of 2-vinyl-4H-1,3-dithiine was very high in the BARI-3 variety which could be an excellent source of this promising compound for protection against hypertension in humans.

There was a pronounced difference in organosulfur compounds between the local garlic, two imported garlic, and the newly developed garlic varieties. Principal Component Analysis graph discriminates them into six clusters based on the distribution of their organosulfur compounds where the principal components explain above 80 % of the variance of data (Fig. 3). BAU-2, BARI-3, and BARI-4 are clustered quite on the right side of the PC1 for the higher concentration of 2-vinyl-4H-1,3-dithiine, 3-vinyl-1,2-dithiacyclohex-4-ene. On the other hand, Indian garlic is clustered separately on the left side of PC1 for the level of Diallyl disulfide, 3-vinyl-1,2-dithiacyclohex-5-ene and Tetrasulfide-di-2-propenyl. BARI-1 garlic is placed on the upper side of PC2 and characterized by the concentration of Trisulfide-di-2-propenyl, Trisulfide-methyl-2-propenyl. Other garlic varieties are set in a distinct position on the quadrant relatively with no visible distribution of compounds. It could be again mentioned that some of the examined garlic varieties were from different locations and cultivated with various growth and agricultural conditions, while some (BARI, BAU varieties) were from respective similar regions with identical cultivation conditions. Hence, the observed variations confirm the effect of many factors including genotypic, agricultural, and environmental factors (Liu et al., 2020) on

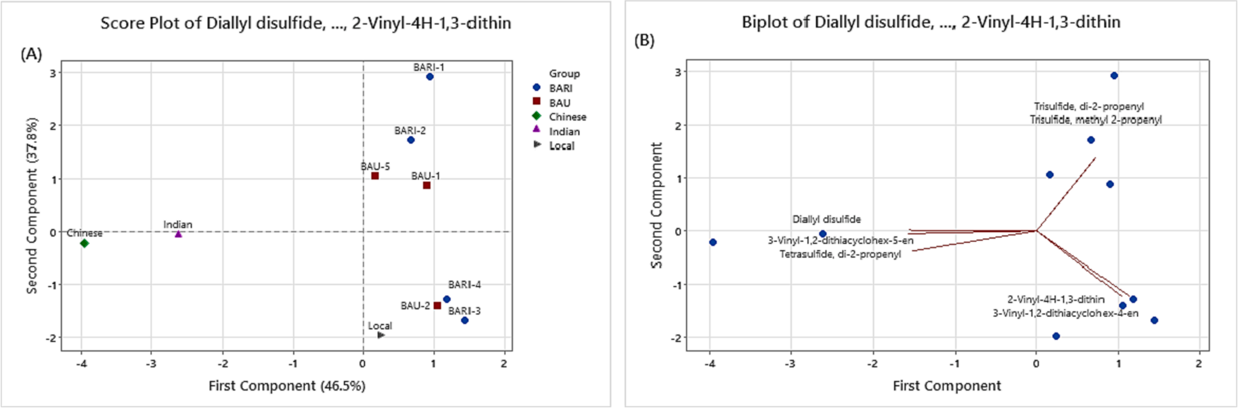

**Fig. 3.** Principal Component Analysis graph demonstrates the spatial distribution of the major organosulfur compounds in different garlic varieties. Graph A shows their respective clusters whereas graph B indicates the concentration of compounds in each clusters. The major organosulfur compounds were Diallyl Disulfide; Trisulfide,di-2-propenyl; Trisulfide,methyl-2-propenyl; Tetrasulfide,di-2-propenyl; 3-vinyl-1,2-dithiacyclohex-4-ene; 3-vinyl-1,2-dithiacyclohex-5-ene; 2-vinyl-4H-1,3-dithiine.

the quality of these kinds of garlic. Genotype could be the major contributing factor, otherwise, the organosulfur compounds would not differ within the garlic varieties of the same group. However, the wide differences in the level of volatile organosulfur compounds between the imported two garlic and the collected local cultivars, new varieties could be associated with different pre-harvest and post-harvest factors. Besides, certain factors such as sulfur fertilization, storage time, and temperature possibly may have an impact on the accumulation of different levels of organosulfur compounds in the tested garlic varieties as widely proved in several studies (Ludlow et al., 2021).

## Conclusion

In the present study, some key differences regarding the bioactive properties and organosulfur compounds content were observed between the new varieties and the other varieties available in local commercial markets of Bangladesh. The new variety, BARI-3 was found with the highest antioxidant activity as well as total phenolic compounds content in comparison to other tested garlic. Additionally, 2-vinyl-4H-1,3-dithiine, an organosulfur molecule, was detected in it at the highest amount (78.15 %) ever recorded in any garlic evaluated worldwide. On the other hand, the local garlic showed potent antimicrobial activity against a wide range of tested pathogens than the other garlic including the imported garlic. Therefore, these two garlic varieties need more consideration in consumption and breeding programs for their further improvement. The comparison of whole genome of BARI-3 garlic with our native and foreign varieties might be useful to determine the reason for its increased bioactive properties and 2-vinyl-4H-1,3-dithiine content than other varieties. The isolation and purification of an active antibacterial component from local garlic for use as a natural food preservative could be equally important.

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## CRediT authorship contribution statement

**Md. saddam Hossain:** Conceptualization, Methodology, Writing – original draft. **Md. Shahiduzzaman:** Resources. **Mohammad Abdur Rahim:** Resources. **Methun Paul:** Investigation. **Rajib Sarkar:** Writing – review & editing. **Farjana Showline Chaity:** Writing – review & editing. **Md. Najem Uddin:** Resources. **G.M. Masud Rana:** Investigation, Visualization. **Mst. Sarmina Yeasmin:** Validation. **Amena Kibria:** Investigation. **Saiful Islam:** Supervision, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability

Data will be made available on request.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100577.

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