African Journal of Pharmaceutical Research and Development
Available online at https://ajopred.com
Vol. 17 No.1; pp. 27-37 (2025)

Original Research Article

ANTIMICROBIAL ACTIVITIES, PHYTOCHEMICAL AND PROXIMATE COMPOSITION OF NON-FERMENTED AND FERMENTED BULB OF *ALLIUM SATIVUM*

MANSURAT BOLANLE FALANA[1,*], MUHAMMED ROBIU ASINMI[1], MUHAMMAD ALI DIKWA[1], QUADRI OLAIDE NURUDEEN[1]

1. Department of Biological Sciences, Al-Hikmah University, Ilorin, Nigeria.

ABSTRACT

Allium sativum, also known as garlic, has been eaten as food and used as medicine against numerous ailments for thousands of years. This study investigated the pH, In-vitro antimicrobial assays: Minimum Inhibitory Concentration (MIC)/ Minimum Fungicidal Concentration (MFC), and disc diffusion assay of non-fermented and fermented samples of *Allium sativum* against *Staphylococcus aureus, Escherichia coli*, and *Candida albicans*. The phytochemical and proximate compositions of the samples were also screened. Constituents of the fermented samples were further analyzed using high-performance liquid chromatography (HPLC). Fermentation of the samples for 14 days influenced the results of this study. The pH decreased from 6.8 to 4.5, and MIC/ MFC against all the test organisms ranged between 12.5 - 100 mg/mL and 12.5 – 25 mg/mL for the non-fermented and fermented samples. Concentration-dependent ranges of activities (2-8 mm for the non-fermented and 10-16 mm for the fermented samples) were recorded. Varying quantities of moisture, ash, fat, carbohydrate, fiber, and protein were obtained for the samples. However, these values were higher for the fermented than the non-fermented samples. In addition to alkaloids, flavonoids, glycosides, saponins, and terpenoids detected in the non-fermented samples, anthraquinone and tannin were also detected in the fermented samples. In order of peak height, allicin, allin, coumaric acid, ajoene, beta phellendrene, hydroxycinnamate, and dithin were presented in the fermented *A. sativum*. The increase in acidity of *A. sativum* due to fermentation signifies preservation, the presence of organic acids, and other compounds. This may correlate to the increased phytochemical components in the fermented samples. Consequently, the constituents identified by HPLC in the fermented sample may be linked to better biological properties than the non-fermented sample, making it a better source of nutritional and therapeutic agents.

ARTICLE INFO

Received 13 November, 2024
Accepted 09 June, 2025
Published 30 June, 2025

KEYWORDS

Allium sativum,
E. coli,
Fermentation,
Phytochemicals,
Antimicrobial activities,
Proximate composition

INTRODUCTION

The bulb of *Allium sativum* (Garlic), recurrently known as garlic, of the family Alliaceae, is a known beneficial spice commonly consumed in its raw, dried, or processed (oil or extract) form for numerous nutritional and biological properties [1]. The functional and beneficial effects of a food substance must be based on the available scientific evidence on the food [2]. Garlic is packed with high concentrations of sulfur-containing compounds such as allicin, contributing significantly to its therapeutic properties. Other beneficial components of garlic include steroids,

***Corresponding author:** bolman4ever@yahoo.com, +234 806 041 1288

https://doi.org/10.59493/ajopred/2025.1.4 ISSN: 0794-800X (print); 1596-2431 (online)

terpenoids, flavonoids, saponins, sugars, proteins, fats, and vitamins [1, 3]. Due to its active composition, garlic is notable for its therapeutic potential against numerous health conditions (including high blood pressure and cancer) and diseases (caused by bacteria and fungi) [1, 4]. Efforts to unfold more potentials of *A. sativum* are rapidly ongoing among scientists, especially its potential for discovering new drugs.

The potential of microorganisms to develop resistance to the same drug has become a crucial public health challenge [5]. Therefore, researchers continue to assess strategies to enhance the effectiveness of agents with known antimicrobial potentials and search for newer sources of antimicrobial compounds.

Fermentation is a traditional technique that improves the biological properties of herbs by decomposing their complex substrate into simpler ones, thereby increasing their assimilability, digestibility, bioactivity, and reducing their adverse effects [6,7]. Samtiya *et al*. [7] reported that fermentation produces enzymes such as phytase that break down phytic acid, an antinutritional compound of plant-based foods. The influence of fermentation in enhancing the phenolic constituents and the biological activities of products such as agricultural products, tea, and cereal foods is gaining relevance [8]. During fermentation, microorganisms produce many enzymes that disintegrate components, such as phenolic substituents, to release their phenolic components [9].

Despite the numerous scientific papers reporting the efficacy and compounds in garlic extracts, there is a need to explore techniques to improve the constituents and properties of *A. sativum*. Also, the European Food Safety Authority (EFSA) states that the scientific proof of the beneficial effect of a food determines its functionality [1]. Therefore, this study focuses on the bioprocessing of *A. sativum* to explore the impact of fermentation on the phytochemical, proximate constituents and its *in-vitro* antimicrobial activities on selected pathogens.

MATERIALS AND METHODS

Preparation and fermentation of the plant material

Bulbs of *Allium sativum* were obtained from vendors in Mandate market in Kwara State, Nigeria. The bulbs were authenticated, and a referenced specimen (number UILH/1209) was dropped at the Herbarium of the University of Ilorin, Ilorin, Nigeria. Healthy bulbs were selected, oven-dried at 30^{o}C for 72 hours, and squashed to powder using a China-made Blender (Master Chef, Mode MC-BL 1980).

An adapted method reported by Kim *et al.* [10] was observed to ferment 100g of the *A. sativum* powder in a sterile mason jar (2-liter capacity heat-tempered glass) holding 1000 mL of the brine (2% of NaCl w/v), resulting in a powder/brine ratio of 1:10 w/v. This gave an initial concentration of 100mg/ml. The tightly covered mason jar was submerged in a water bath (Lab Tech product; Model LWB-111D) at 72 ˚ C for 15 minutes to discourage microbial growth and cooled to room temperature (25 ° C). Subsequently, the cap of the jar was carefully slacked to release trapped air, then closed tightly back at room temperature for 14 days. After that, the sample was filtered with a membrane mesh sized 0.4 mm. The filtrate was kept refrigerated at -18 ˚ C before analysis. The pH reading of the sample was done on days 7 and 14 of the fermentation time using a digital pH meter (Multifunction quality tester; Model Number-EZ-9908).

Preparation of different concentrations of the samples

Three concentrations of the sample were prepared in sterile test tubes. The pure sample representing a 100 % concentration was used to prepare other concentrations. The 50 % concentration was prepared by transferring 10 mL from the 100% concentration into another tube holding 10 mL of sterile distilled water, whirled to mix, and the 25 % concentration was prepared by transferring 10 mL from the mixture in the second test tube into the third tube containing 10 mL of distilled water. All the tubes were centrifuged for 20 mins at 25^{o}C at 3000 rpm to separate the supernatants through filtration with Whatman no. 1 filter paper and stored.

Procurement and standardization of inoculum

The cultural, physiological, and biochemical characteristics of the isolates used in this study were done before storage at 4^{o}C for use in the study. The isolates used, *Candida albicans* (yeast), *Staphylococcus aureus, and Escherichia coli* (bacteria), were procured from the Microbiology Department, University of Ilorin Teaching Hospital, Ilorin, Nigeria, on Potato Dextrose Agar (PDA) and Nutrient agar slants, respectively.

An aseptic laboratory technique was adopted throughout the study. Using a sterile rod, a few (4-5) discrete five colonies were taken from a primary agar plate and introduced into the sterile tube containing 5 mL sterile saline, Vortexed using a Genie Mixer and the arising suspension was noted for cloudiness using a spectrophotometer that was previously modified at 530 nm to 1.5 x 10^{8} CFU/mL (for the bacteria) and 1.5 X 10^{4} conidia/mL (for the yeast isolate), respectively [11]. This was equivalent to 0.5 McFarland standard.

Determination of MIC of the sample

The MIC was determined by adopting the microdilution techniques described by Wayne [12], and different concentrations (100 mg/mL, 50 mg/mL, and 25 mg/mL) of the samples were prepared. A two-fold serial dilution was done in a plate (microtiter) of Mueller Hinton broth to get different concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, and 3.25 mg/mL). Into each tube containing the different concentrations of the sample, 0.1 mL of an 18-hour broth culture of the test organism that had been adapted to 0.5 McFarland standard was introduced. Cotton wool was placed over the test tubes and incubated at 37 ° C for 24 hours. After this, clear test tubes with the

lowest concentration were selected after 24 hours and recorded as the MIC.

Phytochemical analysis of non-fermented and fermented bulb of *A. sativum*

Phytochemical screening was done following the modified methods of Ezeonu and Ejikeme [13] as well as Shaikh *et al*. [14] to determine the presence of Alkaloids, anthraquinones, flavonoids, glycosides, phlobatannins, saponins, steroids, tannins, and terpenoids in the samples.

Estimation of proximate compounds in the non-fermented and fermented bulb of *A. sativum*

The method described by Magu *et al*. [15] was followed to quantify the Moisture, ash, fat, fiber, protein, and carbohydrate present in the samples.

HPLC analysis of the fermented bulb of *Allium sativum*

The fermented sample was investigated for the active constituents that may be responsible for its biological activities based on the HPLC method described by Mernissi *et al*. [16]. The modular chromatographic system Shimadzu (Nexeramx), comprising a Workstation (LC-10), an interface (CBM-10A), an LC-10AD pump, a column oven (CTO-10A), and a UV-DAD detector (SPD-10A). The sample (1.0 g) was suspended in acetonitrile/water (1:1 v/v), centrifuged (for 10 minutes at 3000 rpm), and filtered. The constituents were further analyzed on a 250 mm x 4.6 mm ID x 5 mm at 30°C using the mobile phase acetonitrile with water (40:60 v/v) at a 1 mL /min flow rate. The evaluation period involved a separation of the constituents of the sample in the isocratic mode at a mobile phase of constant composition.

Susceptibility Testing of Non-fermented and Fermented *A. sativum*

Testing of the non-fermented and fermented samples for activity against the test pathogens was done by the Kirby-Bauer disc diffusion technique as described by the CLSI [17]. In this method, paper discs (6 mm in diameter) were produced from Whatman filter paper (Grade No. 1) using an office hole paper perforator, arranged in Petri dishes, left 5 meters apart, sterilized in an oven (hot air) at 160°C lasting one hour [18], and allowed to cool. A fixed amount of 0.01mL (10 µl) of the samples at different concentrations was carefully loaded on the paper discs using a micropipette, which came in slight contact with the paper; the sample was allowed to diffuse into the discs and dried at room temperature. Following this, solidified Mueller-Hinton (Oxoid, UK) agar of 4.0 mm depth in sterile plates was introduced (by streaking) with 0.5 mL of an 18-hour liquid culture of the standardized pathogen. The loaded paper discs were immediately arranged superficially on the agar plate and gently pressed down to make good contact with the inoculated agar top. The plates were turned upside down and incubated (for 18–20 hours at 35 ± 1°C), after which the diameter of the inhibition was measured as zones with clarity around the disc. An average of the triplicate experiment was taken. Standard antibiotics discs (erythromycin and Gentamicin at 30 µg each) were used as control [19].

Statistical Analysis

The zones of inhibition were expressed as arithmetic means ± standard error of mean (SEM). The means were compared using One-way analysis of variance (ANOVA).

RESULTS

pH value of *Allium sativum* during the fermentation period

A reduction in value was observed on days 5, 10, and 14 of the fermentation periods with pH values of 6.8, 6.3, and 6.0, respectively (Figure 1).

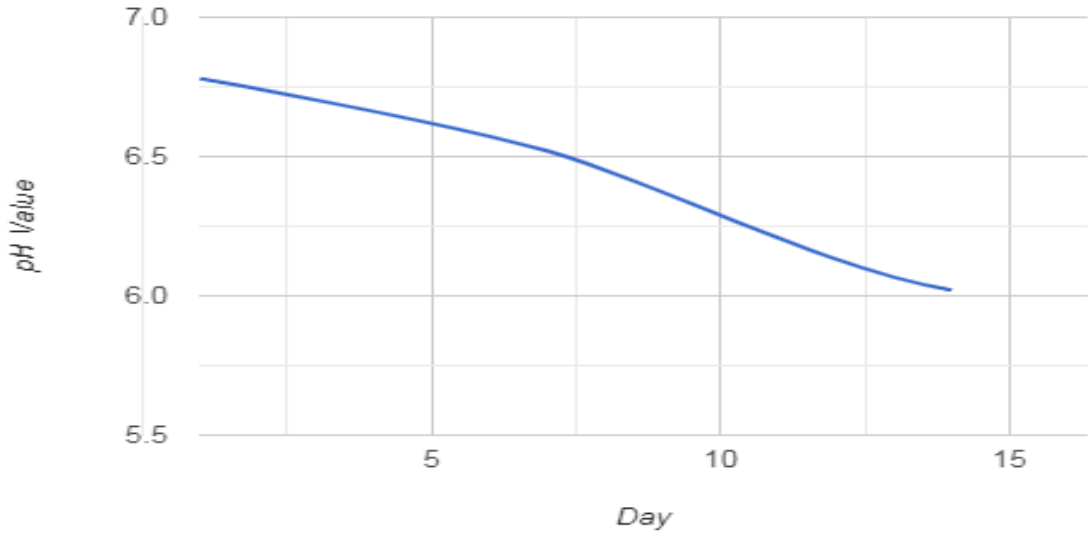


Figure 1: pH value of a sample of *A. sativum* during the fermentation period

Minimum Inhibitory Concentration (MIC) of Fermented and Non-fermented samples of *Allium sativum*

The Minimum Inhibitory Concentration/minimal fungicidal concentration of the non-fermented and fermented *A. sativum* showed a range of inhibition of 25-100 µg/mL and 12.5-50 µg/mL, respectively, against the selected pathogens (Table 1).

Table 1: Minimum Inhibitory Concentration (µg/mL) of non-fermented and fermented *Allium sativum* against some pathogens

	Minimum Inhibitory Concentration (µg/mL)		
Sample type	***S. aureus***	***E. coli***	***C. albicans***
Non-fermented	100	100	25
Fermented	12.5	50	25

Diameter of zones of inhibition (mm) of the non-fermented and fermented *A. sativum* against the test pathogens

In addition to the effect of concentration of the tested samples, fermentation influenced the zones of inhibition of *A. sativum* against the tested pathogens as diameters of 2mm was obtained against both *E. coli* and *S. aureus*; 6mm against *C. albicans* were obtained at the lowest (25 mg/mL) and highest (100 mg/mL) concentrations of the non-fermented sample. However, diameters of 8mm (against *E. coli*) and; 16mm (against *S. aureus*) were obtained at the lowest (25 mg/mL) and highest (100 mg/mL) concentrations of the fermented sample (Figure 2).

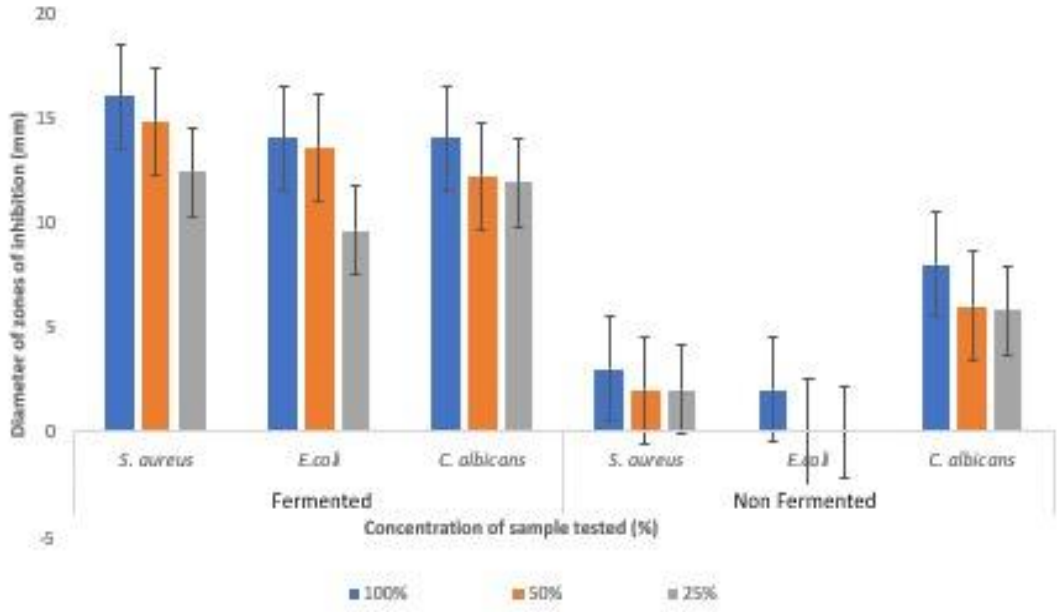


Figure 2: Diameter of zones of inhibition (mm) of the non-fermented and fermented *A. sativum* against the test pathogens

Comparative Nutritional Composition of the Fermented and Non-fermented sample of *Allium sativum*

Comparative Nutritional Composition of the fermented and non-fermented samples of *Allium sativum*. moisture content, ash, fat, carbohydrates, fiber, and protein were explored in the fermented and non-fermented samples of *Allium sativum* with only differences in their nutritional values (Table 2).

Table 2: Comparative proximate composition of the non-fermented and fermented *Allium sativum*

Proximate composition	Proximate values (%)	
	Non-fermented	Fermented
Moisture content	68.01 ± 0.12[a]	74.12 ± 0.34[b]
Ash	3.48 ± 0.62[a]	3.21 ± 0.032[a]
Fat	0.28 ± 0.02[a]	0.18 ± 0.18[a]
Carbohydrate	25.83 ± 0.12[a]	19.35 ± 0.14[b]
Fiber	1.04 ± 0.32[a]	1.28 ± 0.21[b]
Protein	1.64 ± 0.23[a]	2.04 ± 0.18[b]

Phytochemical constituents of the Non-fermented and Fermented *Allium sativum*

Anthraquinones, glycosides, saponin, steroids, and tannin were present in the non-fermented sample. Alkaloids, anthraquinones, flavonoids, glycosides, saponin, steroids, and tannins were present in the fermented sample of *Allium sativum* (Table 3).

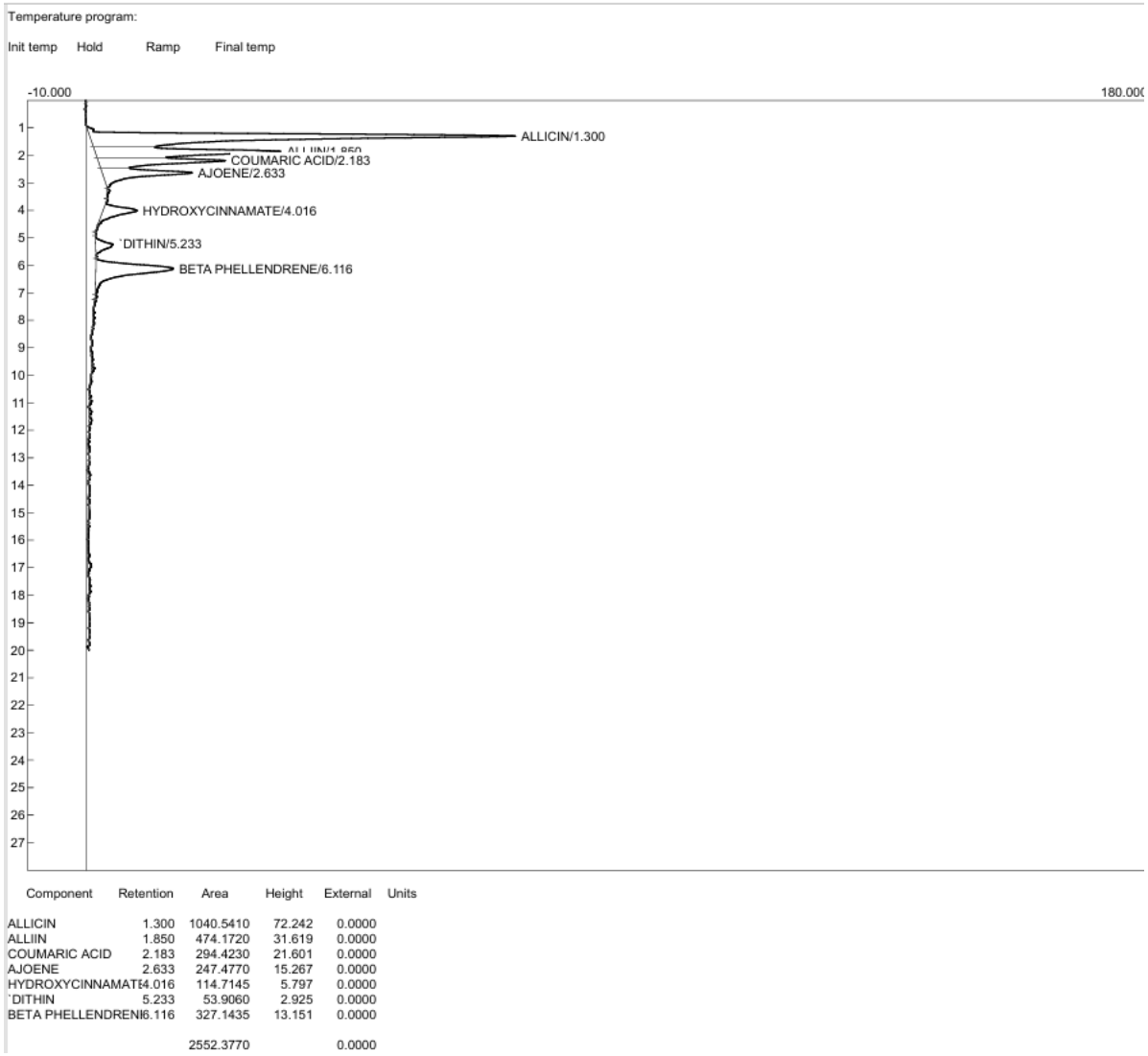
Table 3: Phytochemical components of the non-fermented and the fermented *Allium sativum*

Phytochemical constituents	Non-fermented *Allium sativum*	Fermented *Allium sativum*
Alkaloids	-	+
Anthraquinones	+	+
Flavonoids	-	+
Glycoside	+	+
Phlobatannin	-	-
Saponin	+	+
Steroids	+	+
Tannins	+	+
Terpenoid	-	-

Key: + Present; – Absent

HPLC Chromatogram of the constituents of fermented bulb of *Allium sativum*

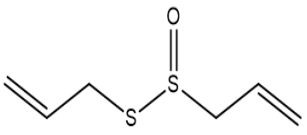
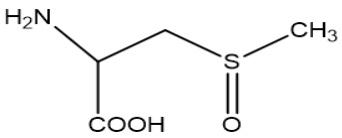
The HPLC chromatogram result of the fermented bulb of *A. sativum* shows different constituents at various retention times. Allicin, with the highest peak, represented the main constituent while dithin was the least constituent present in the fermented bulb of *A. sativum* leaves. (Figure 3). The functional activities of the compounds separated in the fermented A. sativum by HPLC are given in Table 4.



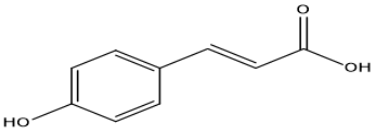
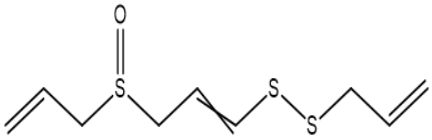
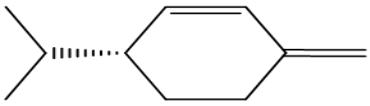
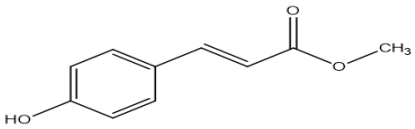
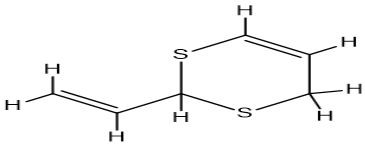
Component	Retention	Area	Height	External	Units
ALLICIN	1.300	1040.5410	72.242	0.0000	
ALLIIN	1.850	474.1720	31.619	0.0000	
COUMARIC ACID	2.183	294.4230	21.601	0.0000	
AJOENE	2.633	247.4770	15.267	0.0000	
HYDROXYCINNAMATE	4.016	114.7145	5.797	0.0000	
`DITHIN	5.233	53.9060	2.925	0.0000	
BETA PHELLENDRENE	6.116	327.1435	13.151	0.0000	
		2552.3770		0.0000	

Figure 3: HPLC chromatogram of the constituents of fermented bulb of *Allium sativum*

Table 4: Some of the functional activities attributed to the compounds separated in the fermented *A. sativum* by HPLC

Separated compound	Biological activity	References	Structure
Allicin	Antimicrobial, Antioxidant, Anti-inflammatory	Ankri and Mirelman [20]	
Allin	Antifungal, Anticancer, antiplatelet	Ankri and Mirelman	

[20]

Coumaric acid	Antioxidant, Antimicrobial Anticancer,	Boz [21] Kaschula *et al.* [22]	
Ajoene	Anti-leukaemia	Hassan [23]	
Beta phellandrene	Glucose Uptake and Adipogenesis	Seethalakshmi *et al*. [24]	
Hydroxycinnamate	Antioxidant Glycosidase inhibition	Simmermaker [25]	
Dithin	Anticancer	Talib *et al*. [26]	

DISCUSSION

The consistent decrease in the pH value of *A. sativum* during the days of fermentation is similar to the phenomenon described by Choi *et al.* [27], where the reduction in pH value was linked to increasing acidity. Scientific papers prove that fermented products are sources of nutraceuticals due to their functional, health-maintaining, and disease-prevention properties [28]. The increased acidity observed in this study may mean a decrease in undesirable strains of microorganisms in the fermented sample, and this corroborates the report of Masulli [29] who stated that the acidic pH of fermented samples acts as a preservative against undesirable microbial strains.

The lower MIC obtained for the fermented sample against all the test pathogens signifies the need for a lower concentration of the fermented sample to impede the growth of pathogens than the non-fermented sample. This directly translated to the higher diameter of the zone of inhibition obtained for the fermented samples than the non-fermented sample against all the tested pathogens. The fermented and non-fermented samples of *A. sativum* revealed varying activities against the test pathogens. Our findings agree with some scientific literature that reported the antimicrobial activities of organic and aqueous extracts of garlic and black garlic extracts [30 - 34].

A higher diameter of the zone of inhibition obtained for the Gram-positive pathogen than other pathogens concur with the statements of Moulia *et al.* [35] and Airaodion *et al*. [36], who individually reported a better activity of garlic against Gram-positive bacteria compared to Gram-negative bacteria. The higher diameter of inhibition zones of the fermented sample may be linked to the biotransformation process of fermentation that decomposed organic substances by fermenting organisms to yield active constituents with reduced toxicity and improved pharmacological effects. The influence of the concentration of the sample on the diameters of zones of inhibition obtained against the test pathogens conforms with Nweze *et al.* [37], who also reported a concentration-dependent activity of medicinal plants. This may invariably mean that the concentration of the sample is a function of its bioactive constituents. There was no remarkable difference between the ash and fat content of the

fermented and non-fermented *Allium sativum.* However, differences occurred in their moisture, carbohydrate, protein, and fiber content. The higher nutritional composition elucidated by the fermented sample in the present study may be attributed to better biological activity. Fiber helps in bowel movement, carbohydrate plays a crucial role in immunity, clotting of blood, and body development, proteins play a significant role in building body tissues, and fat contributes to the synthesis of membranes and tissue [38, 39].

More constituents being detected in the fermented sample than the non-fermented counterpart may translate to factors such as the increased acidity of the fermented sample and the associated compounds in the biotransformation process (fermentation) disintegrating more constituents. Leopoldini *et al*. [40] and Ghosh *et al*. [41] variously linked the influence of thermodynamics of hydrogen ion transfer (acidity) to the phytochemical composition of fermenting samples.

Different scientific studies have identified numerous bioactive components of *A. sativum* [42-45]. Organic sulfides, saponins, phenolic compounds, and polysaccharides are among the bioactive compounds that may be responsible for diverse biological properties such as antimicrobial, anti-inflammatory, antioxidant, anticancer, immunomodulatory, anti-diabetic, anti-obesity, and cardiovascular protective properties [46 - 48]. Bioactives are functional compounds, such as phytochemicals produced during fermentation, and are implicated in numerous health benefits [49]. Therefore, the fermented sample of *A. sativum* would be a better alternative to the non-fermented sample. Alkaloids and flavonoids in the fermented sample may be attributed to its improved antimicrobial and antioxidant activity [50]. The presence of tannin in this study may be linked to the tannin-hydrolyzing enzyme content of the sample, which may mean that the sample may support gut health and reduce diabetic and cardiovascular conditions [51].

Allicin and ajoene are among the predominantly mentioned active components in garlic with health-promoting potentials [52]. According to Bhatwalkar et al. [53], the antimicrobial effect of allicin was linked to the inhibition of DNA synthesis. Several scientific studies have reported that ajoene and allicin from garlic function in lipid profile alteration, vasodilation enhancement, and cholesterol biosynthesis inhibition [54, 55]. The improved properties of the fermented *A. sativum* observed in this study may be attributed to its organosulfur compounds, including allicin, adenosine, ajoene, flavonoid, and saponin, which provide numerous benefits, such as anticancer, antimicrobial, antioxidant, and anti-inflammatory properties [56].

CONCLUSION

The increased acidity of the fermented sample observed in this study may be linked to increased shelf life and the presence of acids and other organic compounds in fermented *A. sativum*. This may be attributed to the improved antimicrobial activities and increased phytoconstituents detected in the sample. The HPLC result on the constituents (Allicin, allin, coumaric acid. ajoene, beta phellandrene, hydroxycinnamate, and dithin) identified in the fermented *A. sativum* by HPLC corroborate its biological properties and its potential as a good source of nutritional and therapeutic agent. Hence, this study enriches the available scientific reports on the benefits of *A. sativum*, its active composition, and its health benefits. However, a more comprehensive exploration of the compounds associated with fermented *A. sativum* will promote antimicrobial studies and enhance pharmaceutical intervention and disease prevention.

ACKNOWLEDGMENT

The authors acknowledge the Microbiology Laboratory, Department of Biological Science, Al-Hikmah University, Ilorin, Nigeria, for using their facility.

AUTHORS' CONTRIBUTION

MBF and QON conceptualized and searched the literature for the project. The first draft of the manuscript, the collection of plant material, and the antimicrobial studies were done by MBF. MBF, QON, and MRA carried out the phytochemical and HPLC assay. QON elucidated chemical structures. All authors edited and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

The authors received no funding.

REFERENCES

1. Singh R, Singh K. Garlic: A spice with wide medicinal actions. *Journal of Pharmacognosy and Phytochemistry*, 2019, 8(1):1349–1355.
2. EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A. Food manufacturing processes and technical data used in the exposure assessment of food enzymes. *EFSA Journal*, 2023, 21(7):e08094.
3. Torres-Palazzolo C, Ramirez D, Locatelli D, Manucha W, Castro C, Camargo A. Bioaccessibility and permeability of bioactive compounds in raw and cooked garlic. *Journal of Food Composition and Analysis,* 2018,70:49-53.
4. Alare K, Alare T, Luviano N. Medicinal importance of garlic and onions on autonomic nervous system. *Clinical Pharmacology and Biopharmaceutics*, 2020, 9(4), 204-206.
5. Sartorius B, Gray AP, Weaver ND, Aguilar GR, Swetschinski LR, Ikuta KS, Mestrovic T, Chung E, Wool EE, Han C, Hayoon AG. The burden of bacterial antimicrobial resistance in the WHO African

region in 2019: A cross-country systematic analysis. *The Lancet Global Health*, 2024,12(2):e201-16.
6. Samtiya M, Aluko RE, Puniya AK, Dhewa T. Enhancing micronutrients bioavailability through fermentation of plant-based foods: A concise review. *Fermentation,* 2021, 20: 7(2):63.
7. Hur SJ, Lee SY, Kim YC, Choi I, Kim GB. Effect of fermentation on the antioxidant activity in plant-based foods. Food chemistry, 2014,160:346-356.
8. Bei Q, Wu Z, Chen G. Dynamic changes in the phenolic composition and antioxidant activity of oats during simultaneous hydrolysis and fermentation. *Food chemistry*, 2020, 305:125269.
9. Dulf FV, Vodnar DC, Socaciu C. Effects of solid-state fermentation with two filamentous fungi on the total phenolic contents, flavonoids, antioxidant activities and lipid fractions of plum fruit (Prunus domestica L.) by-products. *Food chemistry*, 2016, 209:27-36.
10. Kim H, Kim J, Shin SG, Hwang S, Lee C. Continuous fermentation of food waste leachate for the production of volatile fatty acids and potential as a denitrification carbon source. *Bioresource Technology*, 2016, 207:440-445.
11. Onuoha CC, Muhsin MU, Udofia EV, Ojo OH, Asibe GA, Adekplorvi G, Sani AH, Ahmad BM, Surajo AA, Wankan B, Jasseh M. Prevalence and Antimicrobial Susceptibility of Enteric Bacteria from Poultry Farms in Kano State, Nigeria. *UMYU Journal of Microbiology Research*, 2023, 8(2):92-98.
12. Samtiya M, Aluko RE, Dhewa T. Plant food anti-nutritional factors and their reduction strategies: an overview, *Food Production, Processing and Nutrition*, 2020, 2:1-4.
13. Ezeonu CS, Ejikeme CM. Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian softwoods. *New Journal of Science*, 2016, (1):5601327.
14. Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, 2020, 8(2):603-8.
15. Magu TO, Louis H, Nzeata-Ibe N, Sunday EA, Udowo VM, Ugor JA, Oyo IE. Proximate Analysis and Mineral Composition of Jatropha curcas seeds obtained from Pankshin local government area of Plateau state of Nigeria. *Journal of Physical Chemistry and Biophysics*, 2018, 8(265):2161-0398.
16. Mernissi T, Bodeau S, André C, Zahr N, Mary A, Dupont H, Kontar L, Lemaire-Hurtel AS, Bennis Y. An HPLC assay for the therapeutic drug monitoring of cefiderocol in critically ill patients. *Journal of Antimicrobial Chemotherapy*. 2021, 76(6):1643-1646.
17. Clinical and Laboratory Standards Institute; CLSI. 2011. Performance standards for antimicrobial susceptibility testing. 20th Informational Supplement. CLSI document.
18. Riaz K, Rizvi ZF, Hyder S, Rashed R. Antibacterial, antitumor activity and phytochemical studies of methanolic extract of (*Catharnthus roseus*)(Linn.) G. Don. *Pakistan Journal of Botany*, 2021, 53:2259-2266.
19. Shobowale EO, Solarin AU, Elikwu CJ, Onyedibe KI, Akinola IJ, Faniran AA. Neonatal sepsis in a Nigerian private tertiary hospital: bacterial isolates, risk factors, and antibiotic susceptibility patterns. *Annals of African medicine*, 2017, 16(2):52-58.
20. Ankri S, Mirelman D. Antimicrobial properties of allicin from garlic. *Microbes and infection*, 1999, 1(2):125-129.
21. Boz H. p-Coumaric acid in cereals: presence, antioxidant and antimicrobial effects. *International Journal of Food Science & Technology*, 2015, 50(11):2323-2328.
22. Kaschula CH, Hunter R, Parker MI. Garlic-derived anticancer agents: Structure and biological activity of ajoene. *Biofactors*, 2010, 36(1):78-85.
23. Hassan HT. Ajoene (natural garlic compound): a new anti-leukaemia agent for AML therapy. *Leukemia Research*, 2004, 28(7):667-671.
24. Seethalakshmi S, Sarumathi R, Sankaranarayanan C. Effect of Alpha-Phellandrene on Glucose Uptake and Adipogenesis in Insulin Resistant 3T3-L1 Adipocytes: an in vitro and in silico Approach. *Asian Journal of Biological and Life Sciences*, 2023, 12:603.
25. Simmermaker C. *Synthesis of Hydroxycinnamate Derivatives and Characterization of Bioactivity As Radical Scavengers and α-Glucosidase Inhibitors*. 2023, Doctoral dissertation, University of the Pacific.
26. Talib WH, Atawneh S, Shakhatreh AN, Hamed RA, Al-Yasari IH. Anticancer potential of garlic bioactive constituents: Allicin, Z-ajoene, and organosulfur compounds. *Pharmacia*, 2024, 71:1-23.
27. Choi IS, Cha HS, Lee YS. Physicochemical and antioxidant properties of black garlic. *Molecules*, 2014, 19(10):16811-16823.
28. Sachdeva V, Roy A, Bharadvaja N. Current prospects of nutraceuticals: a review. *Current Pharmaceutical Biotechnology*, 2020, 21:884–896.
29. Masulli D. Measuring pH of yoghurt. *Food Quality and Safety*, 2016,1-4.
30. Jain I, Jain P, Bisht D, Sharma A, Srivastava B, Gupta N. Comparative evaluation of antibacterial efficacy of six Indian plant extracts against *Streptococcus mutans*. *Journal of Clinical and Diagnostic Research*, 2015, 9(2): ZC50.
31. Vlachojannis C, Chrubasik-Hausmann S, Hellwig E, Vach K, Al-Ahmad A. Activity of preparations from *Spilanthes oleracea*, propolis, *Nigella sativa*, and black garlic on different microorganisms involved in

oral diseases and on total human salivary bacteria: a pilot study. *Phytotherapy Research*, 2018, 32(10):1992-2001.
32. Chidinma O, Timothy OC, Samuel S, Isaac E, Hauwa S. Therapeutic effects of garlic: A review. *Journal of Biology and Life Sciences*, 2019;1(1):1-5.
33. Ahmed T, Wang CK. Black garlic and its bioactive compounds on human health diseases: A review. *Molecules*, 2021, 26(16):5028.
34. Setiyoningrum F, Herlina N, Afiati F, Priadi G. Antibacterial activities of Solo garlic. *IOP Conf Ser Materials Science and Engineering*, 2021, 1011(1): 012067.
35. Moulia MN. Antimikroba ekstrak bawang putih. Jurnal Pangan, 2018, 27(1):55-66.
36. Airaodion AI, Ngwogu AC, Ngwogu KO, Ekenjoku JA, Megwas AU. Pharmacotherapeutic activity of *Allium sativum* (garlic) bulb against Gram-positive and Gram-negative bacteria. *Asian Journal of Research in Infectious Diseases*, 2020, 3(3):22-27.
37. Nweze EI, Okafor JI, Njoku O. Antimicrobial activities of methanolic extracts of *Trema guineensis* (Schumm and Thorn) and *Morinda lucida* benth used in Nigerian. *Bio-research*, 2004, 2(1):39-46.
38. Williams MJ, Sutherland WH, McCormick MP, De Jong SA, Walker RJ, Wilkins GT. Impaired endothelial function following a meal rich in used cooking fat. *Journal of the American College of Cardiology*, 1999, 33(4):1050-1055.
39. Uboh FE, Ebong PE, Mbi E. Cultural discrimination in the Consumption of black snail (*Archachatina marginata*) and white snail (*Achatina achatina*); any scientific justification. *International Research Journal of Microbiology*, 2010, 1(1):013-017.
40. Leopoldino WM, Lana RP, Borges AC, Mantovani HC, Teixeira RM, Oliveira JS, Jaremtchuk AR, Eifert EC, Martins RG. In vitro effect of pH on resistance of ruminal bacteria to intracellular potassium depletion, and effect of pH and ionophores on ammonia and microbial protein production. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 2005, 57:778-783.
41. Ghosh S, Chakraborty R, Raychaudhuri U. Determination of pH-dependent antioxidant activity of palm (Borassus flabellifer) polyphenol compounds by photoluminol and DPPH methods: a comparison of redox reaction sensitivity. *Biotech*. 2015, 5:633-640.
42. Izol E, Temel H, Yilmaz MA, Yener I, Olmez OT, Kaplaner E, Fırat M, Hasimi N, Ozturk M, Ertas A. A detailed chemical and biological investigation of twelve Allium species from Eastern Anatolia with chemometric studies. *Chemistry & Biodiversity*, 2021,18(1):e2000560.
43. Diretto G, Rubio-Moraga A, Argandoña J, Castillo P, Gómez-Gómez L, Ahrazem O. Tissue-specific accumulation of sulfur compounds and saponins in different parts of garlic cloves from purple and white ecotypes. *Molecules*, 2017, 22(8):1359.
44. Liu J, Ji F, Chen F, Guo W, Yang M, Huang S, Zhang F, Liu Y. Determination of garlic phenolic compounds using supercritical fluid extraction coupled to supercritical fluid chromatography/tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, 2018, 159:513-523.
45. Shang A, Cao SY, Xu XY, Gan RY, Tang GY, Corke H, Mavumengwana V, Li HB. Bioactive compounds and biological functions of garlic (*Allium sativum* L.). *Foods,* 2019, 5: 8(7): 246.
46. Szychowski KA, Rybczynska-Tkaczyk K, Gawel-Beben K, Swieca M, Karas M, Jakuczyk A, Matysiak M, Binduga UE, Gminski J. Characterization of active compounds of different garlic (*Allium sativum* L.) cultivars. *Polish Journal of Food and Nutrition Sciences*, 2018, 68(1).
47. Yun HM, Ban JO, Park KR, Lee CK, Jeong HS, Han SB, Hong JT. Potential therapeutic effects of functionally active compounds isolated from garlic. *Pharmacology & therapeutics*, 2014, 142(2):183-195.
48. Boonpeng S, Siripongvutikorn S, Sae-wong C, Sutthirak P. The antioxidant and anti-cadmium toxicity properties of garlic extracts. *Food science and Nutrition*, 2014, 2(6):792-801.
49. Guo Q, Chen P, Chen X. Bioactive peptides derived from fermented foods: Preparation and biological activities. *Journal of functional foods,* 2023, 101:105422.
50. Dhalaria R, Verma R, Kumar D, Puri S, Tapwal A, Kumar V, Nepovimova E, Kuca K. Bioactive compounds of edible fruits with their anti-aging properties: A comprehensive review to prolong human life. *Antioxidants*, 2020, 9(11):1123.
51. Kitabatake M, Matsumura Y, Ouji-Sageshima N, Nishioka T, Hara A, Kayano SI, Ito T. Persimmon-derived tannin ameliorates the pathogenesis of ulcerative colitis in a murine model through inhibition of the inflammatory response and alteration of microbiota. *Scientific reports,* 2021, 11(1):7286.
52. Guizellini GM, Torres EA, Freitas RA, Saldanha T, Sawaya AC, Gamallo OD, Soares MJ, de Oliveira VS, Sampaio GR. The anticholesterol oxidation effects of garlic (*Allium sativum* L.) and leek (*Allium ampeloprasum* L.) in frozen fish burgers submitted to grilling. *Journal of Food Science,* 2020, 85(8):2416-2426.
53. Bhatwalkar SB, Mondal R, Krishna SBN, Adam JK, Govender P, Anupam R. Antibacterial Properties of Organosulfur Compounds of Garlic (*Allium sativum*), *Frontiers in Microbiology,* 2021, 12:613077.

54. Walag AM, Ahmed O, Jeevanandam J, Akram M, Ephraim-Emmanuel BC, Egbuna C, Semwal P, Iqbal M, Hassan S, Uba JO. Health benefits of organosulfur compounds. *Functional foods and nutraceuticals: bioactive components, formulations and innovations,* 2020:445-72.
55. Ribeiro M, Alvarenga L, Cardozo LF, Chermut TR, Sequeira J, Moreira LD, Teixeira KT, Shiels PG, Stenvinkel P, Mafra D. From the distinctive smell to therapeutic effects: Garlic for cardiovascular, hepatic, gut, diabetes and chronic kidney disease. *Clinical Nutrition,* 2021, 40(7):4807-4819.
56. Wibisono Y, Izza NM, Savitri D, Dewi SR, Putranto AW. Ekstraksi senyawa fenolik dari bawang putih (*Allium sativum* L.) untuk agen anti-biofouling pada membran. Jurnal Ilmiah Rekayasa Pertanian dan Biosistem, 2020, 8(1):100-109.