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Phytochemical Profiling and Multifunctional Bioactivity of High-Altitude Kodaikanal Hill Garlic (*Allium sativum*): Antidiabetic, Antioxidant, Antimicrobial, and Anticancer Evaluation

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Abstract

Diabetes mellitus and oxidative stress-related disorders represent major global health challenges, necessitating the exploration of effective natural therapeutics. This study investigates the phytochemical profile and multifunctional biological activities of high-altitude Kodaikanal Hill garlic (*Allium sativum*) with a focus on its antidiabetic, antioxidant, antimicrobial, and anticancer potential. Garlic juice extract was characterized using FTIR and GC–MS analyses, revealing the presence of key functional groups and bioactive organosulfur compounds, including trisulfides. The extract exhibited strong antioxidant activity, with a maximum DPPH radical scavenging of 82%, alongside significant ABTS and hydrogen peroxide scavenging effects. In vitro antidiabetic assays demonstrated dose-dependent inhibition of carbohydrate-hydrolyzing enzymes, with IC_{50} values of 561.15 µg/mL for α-amylase and 686.70 µg/mL for α-glucosidase. The antimicrobial activity showed potent inhibition against pathogenic strains, including Escherichia coli and Staphylococcus aureus, with low minimum inhibitory concentrations. Furthermore, cytotoxic evaluation against MCF-7 breast cancer cells revealed a significant antiproliferative effect with an IC_{50} of 129 µg/mL, likely mediated through reactive oxygen species (ROS)-induced apoptosis. Overall, the enhanced biological activities observed in Kodaikanal Hill garlic may be attributed to its rich composition of organosulfur and phenolic compounds, potentially influenced by high-altitude agro-climatic conditions. These findings highlight its promise as a natural source for the development of functional foods and therapeutic agents targeting diabetes, oxidative stress, microbial infections, and cancer.

Keywords: Garlic, antioxidant, antidiabetic, antimicrobial, organosulfur compounds

1. Introduction

Garlic (*Allium sativum*) has acquired a reputation in different traditions as a prophylactic as well as therapeutic medicinal plant. Garlic (*Allium sativum*) is an aromatic herbaceous plant that is consumed worldwide as food and traditional remedy for various diseases. It has been reported to possess several biological properties including anticarcinogenic, antioxidant, antidiabetic, renoprotective, anti-atherosclerotic, antibacterial, antifungal, and antihypertensive activities in traditional medicines. *A.sativum* is rich in several sulfur-containing phytoconstituents such as alliin, allicin, ajoenes, vinyldithiins, and flavonoids such as Quercetin[1].

The rapid rise of antimicrobial resistance has been recognized as a threat to human, there is need to develop potential antibacterial agents. Garlic organosulphur compounds exhibit antibacterial activity. A recent increase in the popularity of alternative medicine and natural products has renewed interest in garlic and their derivatives as potential natural remedies [2]. Efforts have been made to understand the molecular mechanism of cancer reduction, a specific approach to reduce breast cancer has not yet been identified. The garlic extracts able to inhibit the growth of several cancer cell types [3]. Though, research has focused on the use of fresh garlic to prevent or treat breast cancer was less. Although it is shown that garlic may have a significant clinical potential either in their own right or as adjuvant therapy in different disorders, however, due to some issues, such as methodological inadequacies, small sample sizes, standard experiments there needed to confirm the beneficial effect of garlic in various diseases especially its anti-diabetic property. Thus, the present study focuses on exploring the anti-diabetic property and antibreast cancer of fresh garlic juice and also its antioxidant and antibacterial properties.

Materials and Methods

Sample collection and Extraction of Fresh Garlic juice

Garlic from the Kodaikanal Hill is recognised for its pungency and is considered one of the aromatic spices growing in this area. The Singapore Red variety is also known as Malai Poondu or Kodaikanal Hill garlic. Garlic was purchased at a local market. The peeled bulbs were immediately chopped into small pieces and placed in a clean mortar and pestle. To make a fine paste, it was carefully ground. To extract the fresh garlic juice, this was filtered again using a Whatman No1 filter paper and transferred to sterile screw cap tubes and refrigerated at 4°C.

FT -IR and GC-MS analysis

To make fine powder, fresh garlic juice was evaporated at 37°C. For the FTIR analysis, the powdered material was employed. The sample carrier was potassium bromide (KBr). Because of its optical transparency for light in the IR spectrum area, it is mostly utilised for analysis. In the region of 4000 to 400 cm-1, the transmittance of KBr is 100 percent, which is the range in which the FTIR analysis is performed. Garlic juice powder was crushed with KBr and pressed under pressure to produce a fine, clear pellet for analysis. In the PerkinElmer, the scan range was set to 4000 to 400 cm^{-1}. Analysis of garlic samples extract was performed under the conditions with GC. Then filtered the mixture after 5 min shaking in path. Then injected 1µl from sample into the GC-MS. The retention indices of the components were used to identify them, and the mass spectrum was interpreted using the National Institute of Standards and Technology's database.

Antibacterial activity

Two-fold serial dilution procedures were used to determine the minimal inhibitory concentration (MIC). The test organisms were *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Vibrio cholerae* and 1 ml of the inoculum was taken in an aliquot of test tubes. The samples were dissolved in increasing concentrations and then incubated overnight at 37°C. Visual inspection and spectrophotometer measurements of the optical density (OD) at 630 nm were used to monitor growth. Following the visual reading, the OD was measured[4].

DPPH free radicals scavenging assay

To determine the DPPH free radical scavenging capacity of *A. sativum* extract, different concentrations (2, 4, 6, 8 and 10 µg) were taken. To this 2 mL of 0.1 mM DPPH solution in methanol was added and the mixture was incubated in dark for 30 min. The absorbance of the samples was measured at 517 nm[5]. Different concentrations of Ascorbic acid were used as the control and the percentage of free radical scavenging was calculated.

ABTS$^{\cdot+}$ free radicals scavenging assay

To evaluate the ABTS·+ free radical scavenging ability of *A. sativum*, 7 mM of ABTS·+ was dissolved in double distilled water to which 140 mM of potassium persulfate solution was added. The mixture was incubated in dark for 14 h and before the experiment they were diluted to give an absorbance of 0.700 ± 0.02 at 734 nm. Various concentrations (2, 4, 6, 8 and 10 µg/ mL) of *A. sativum* extract were mixed with the diluted ABTS·+ solution and incubated for 10 min. The measurements were taken at 734 nm and Ascorbic acid was used as the control [6]. The free radical scavenging ability was calculated.

Hydrogen peroxide radicals scavenging assay

To investigate the hydrogen peroxide scavenging activity different concentrations of (2, 4, 6, 8 and 10 µg/ mL) of *A. sativum* extract were taken. To this 0.6 mL of 40 mM hydrogen peroxide solution was added and mixed well. After 10 min the absorbance of the reaction mixture was measured at 230 nm and Ascorbic acid was used as the standard [7].The hydrogen peroxide radical scavenging ability of *A. sativum* extract was calculated.

Antidiabetic activity

α- Amylase inhibition assay

Different concentrations of the Sample and 500 µl of 0.02 M sodium phosphate buffer (pH 6.9) containing porcine pancreatic α-amylase enzyme (0.5 mg/ml) were incubated at 25℃ for 10 min. After the incubation, 500µl of 1% starch solution in 0.02 M sodium phosphate buffer was added to the reaction mixture. Subsequently, the reaction mixture was incubated at 25℃ for 10 min, followed by addition of 1.0 ml of dinitrosalicylic acid (DNSA). Finally the reaction was stopped by incubation in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 10 ml distilled water, and the absorbance was measured at 540 nm. The mixture of all other reagents and the enzyme except the sample was used as a control. The α-amylase inhibitory activity was expressed as percentage inhibition [8]. The IC_{50} value was defined as the concentration of the sample extract to inhibit 50% of α-amylase activity under assay condition.

α- Glucosidase inhibition assay

Various amounts of garlic extracts (50-250µg/ml) and 100µl of α-glucosidase (0.5 mg/ml) in 0.1 M phosphate buffer (pH 6.9) solution were incubated at 25℃ for 10 min. Then, 50 µl of 5M p-nitrophenyl-α-D-glucopyranoside in 0.1M phosphate buffer (pH6.9) solution was added. Reaction mixtures were incubated at 25°C for 5 min and the absorbance was taken at 405 nm. The mixture of all other reagents and the enzyme except the sample was used as a control and the results of α-glucosidase inhibition activity were expressed in terms of inhibition percentage [9]. The IC_{50} value was defined as the concentration of the sample extract to inhibit 50% of α- glucosidase activity under assay condition.

MTT assay

3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15µl of MTT (5mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader.

Total Reactive Oxygen species (ROS) assay

The garlic sample was tested for ROS using MCF 7 cells (Human breast carcinoma cells). Briefly, the cultured MCF 7 cells were harvested by trypsinization, pooled in a 15 ml tube. Then, the cells were plated at a density of 1×106 cells/ml into 24-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24 hour at 37°C. The wells were washed and 1 ml of ROS assay buffer was added followed by 100 µl of 1X ROS assay stain solution was added to the wells and mixed gently. Then the plate was incubated for 60 minutes in a 37°C incubator with 5% CO_2. After the incubation period, the cells were treated with 129 µl of drug and the production of ROS was evaluated immediately by fluorescence microscope using a fluorescent filter at 520 nm.

Results and Discussion

FT-IR and GCMS analysis

The functional groups in garlic juice were identified via FT-IR analysis. In this study, FTIR bands indicating the functional groups of bioactive substances in garlic juice were obtained at various wave numbers (Table 1). Functional groups such as hydroxyl, carbonyl, carboxylic, and organosulfur compounds were detected in the FTIR of the garlic extract (Fig.1). The O-H stretching of a hydroxyl group causes the broad peak at 3265 cm^{-1}, indicating the presence of polyhydroxy chemicals such as flavonoids, non-flavonoids, and saponins respectively. The garlic juice was subjected to a GC-MS analysis in order to identify the bioactive compounds. Table 2 showed the lists the compound present in garlic juice such as 3-Pentanone, Trisulfide,di-2-propenyl, (2,4-hexadienylthio)dimethyl malonate, Thymine, 7-hexamethyl-5(-4methylphenyl),N,N-Dimethylhydroxylamine respectively. The rich sulphur-containing compounds from garlic, which might be responsible for antidiabetic, antioxidant and antibacterial activity [10].

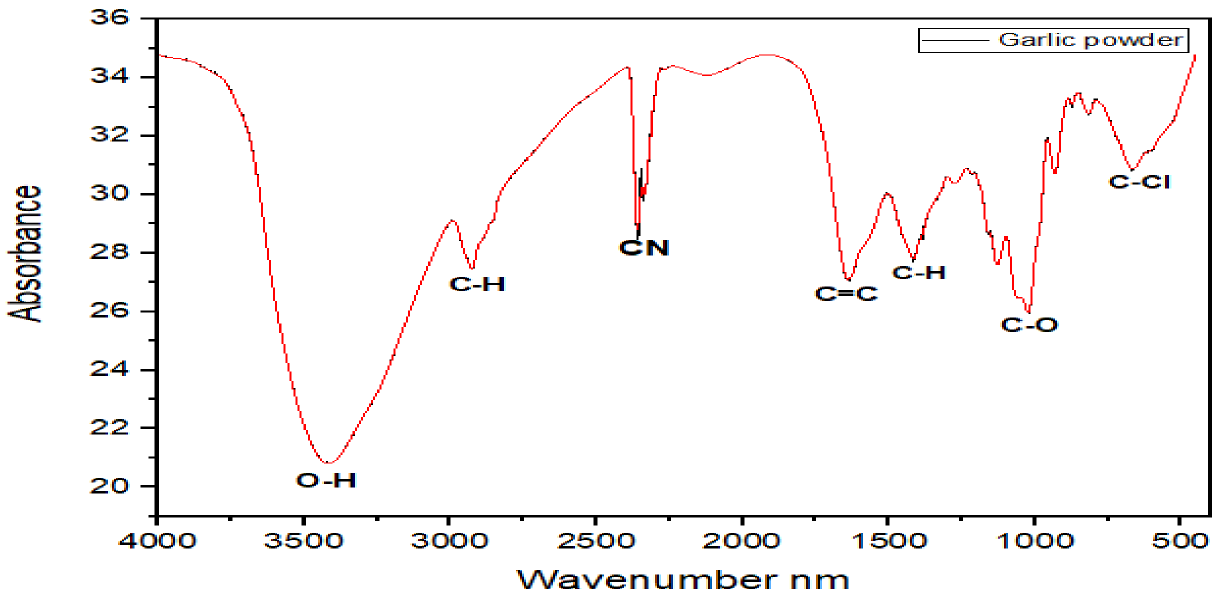

Figure 1: FTIR analysis of *A. sativum*

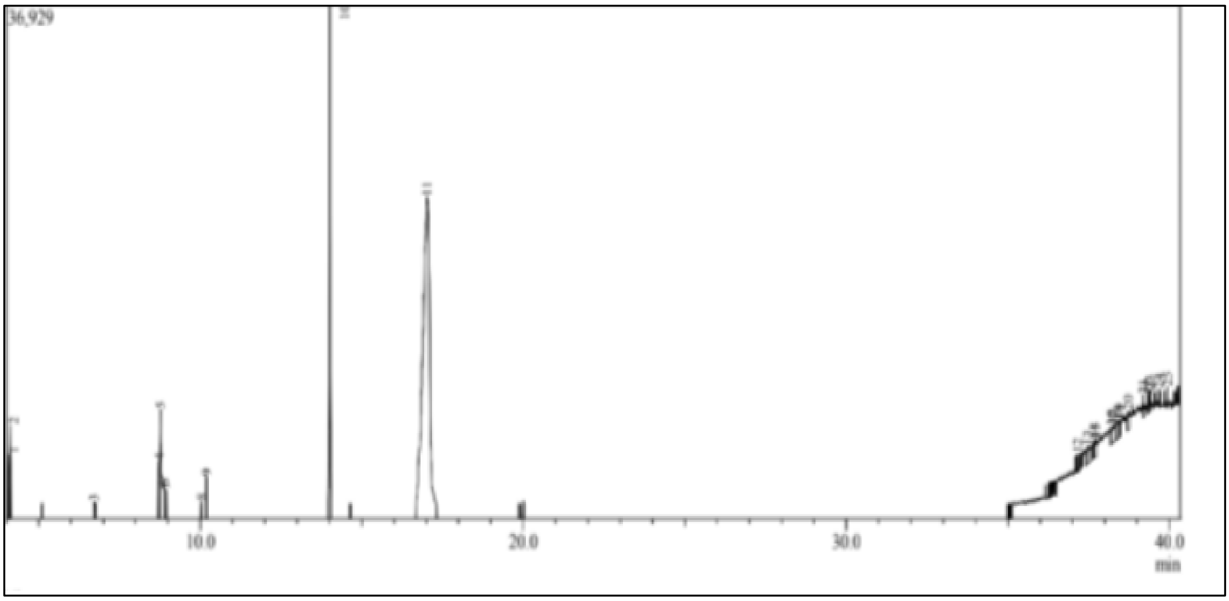

Figure 2: GC-MS spectrum of *A. sativum*

Table1: GCMS analysis of Garlic bulb

S.No	**Compound**	**Area %**	**Biological Function**
1	3-Pentanone	63.92	Antimicrobal, Aroma and Flavour
2	Trisulfide,di-2-propenyl	13.06	Antimicrobial, Anticancer, Antioxidant, and Cardioprotective activities
3	(2,4-hexadienylthio)dimethyl malonate	5.68	Antimicrobial,Antioxidant
4	2-(trimethyl siloxy)ethynyl)hepata methyl trisilane	1.98	Antibacterial, Drug development
5	Thymine	1.72	DNA Repair
6	3,6-dioxa-2,7disilaoctane4-(4-chlorophenyl)-2,2,4,5,7,7-hexamethyl-5(-4methylphenyl)	1.45	Pharmaceutical property
7	N,N-Dimethylhydroxylamine	1.49	Antioxidant

Antibacterial activity

The MIC of garlic juice was measured in this investigation using the broth dilution method. Various concentrations ranging from 31.25 to 1000 g were evaluated (Table 3). Plant extracts were refereed to have high antimicrobial activity if their MIC was less than 100.0 g/ml, moderate if their MIC was between 100.0 and 500.0 g/ml, and poor if their MIC was greater than 500.0 g/ml. The lowest concentration necessary to suppress the development of four distinct organisms ranged from 53.21 to 96.5 g/ml, according to the results of the MIC determination. This demonstrates that garlic juice has a high potential for preventing harmful bacterial development. Allicin, derived from amino acid allin gives the pungent odour of crushed garlic and is believed to be responsible for the pharmacologic activity of antimicrobial [11] .

Table 2: Percentage of Inhibition of Bacterial growth

Organisms	Percentage of inhibition					
	31.25 µg/ml	62.5µg/ml	125µg/ml	250µg/ml	500µg/ml	1000µg/ml
E. coli	41.2 ± 1.2	57 ± 0.4	62 ± 1.2	76 ± 0.4	89 ± 1.2	100
B. cereus	36.6 ± 0.4	47 ± 3.2	58 ± 3.4	63 ± 0.3	81 ± 1.4	94 ± 0.2
S. aureus	21.4 ± 0.3	45 ± 2.1	56 ± 0.7	60 ± 3.2	79 ± 0.4	87 ± 3.2
V.cholerae	12.1 ± 2.1	31 ± 1.2	51 ± 1.2	58 ± 2.1	64 ± 0.2	73 ± 2.1

Antioxidant activity

The order of radical scavenging activities by garlic extract was in the following order: DPPH (82%) > H_2O_2(55%)>ABTS (32%). The studies have suggested that aged garlic contains plentiful phenol, flavonoid, and various sulfur compounds such as *S*-ally-(L)-cysteine (SAC, hydrophilic) and disulfide (hydrophobic) compared to fresh garlic [12]. In addition, SAC has high radical scavenging activities. The number of phenolic compounds and flavonoids has positive correlation with DPPH and ABTS radical scavenging activities due to hydrogen and electron donation from hydroxyl groups of these compounds. In particular, ABTS method is regarded as potentially more efficient than DPPH method since ABTS can measure both hydrophilic and hydrophobic substances. Flavonoids have antioxidant activities promoting anti-cancer,antibacterial and antilung cancer anti-inflammatory [13].

Antidiabetic activity

The enzyme was inhibited at all of the concentrations tested. The lowest inhibitory effect was found at a concentration of 200 g/ml, while the largest inhibitory impact was found at a concentration of 1000 g/ml. The inhibitory concentration (IC_{50}) at which 50% of the enzyme was effectively inhibited was 561.15 g/ml (Table 4). Studies consistently showed that S-allyl cysteine sulphoxide, (allicin), a sulphur-containing amino acid in garlic had a potential to reduce the diabetic condition. The α-Glucosidase inhibition assay was performed to determine the ability of the garlic juice to inhibit this exoenzyme in different concentration. The results showed that garlic juice was able to inhibit the enzyme even at a concentration of 200 µg/ml at the highest inhibitory potential was observed at a concentration of 1000µg/ml. The concentration required to inhibit 50% of the enzyme was 686.70 µg/ml. Most of the herbal plants and its parts have the tendency to reduce the blood glucose level. Tannins and flavonoids have potential inhibitory effects on alpha amylase and alpha glucosidase [14].

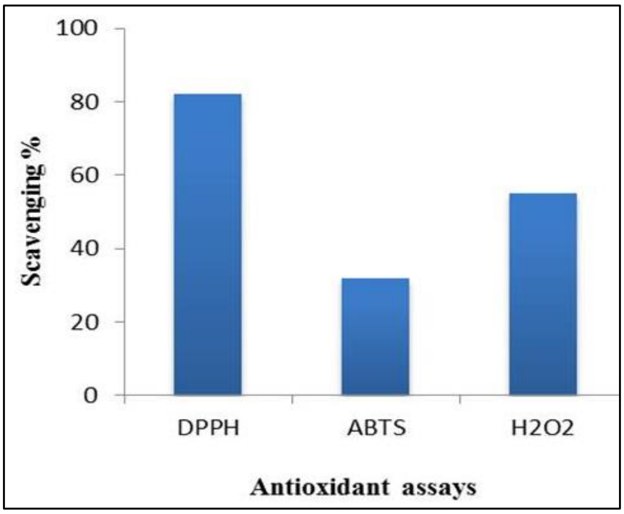

Figure-2 Antioxidant activity of *A. sativum*

Table 3: Alpha amylase and α- Glucosidase inhibition assay of *A. sativum*

Garlic juice (µg/ml)	% Inhibition Alpha amylase	% Inhibition α- Glucosidase
200	17.50 ± 0.45	15.29 ± 0.10
400	36.30 ± 0.50	33.48 ± 0.30
600	53.20 ± 0.20	45.20 ± 0.30
800	69.38 ± 0.30	57.28 ± 0.30
1000	84.20 ±0.20	73.39 ± 0.40
IC_{50} value	561.15	686.70

Cytotoxicity and Total reactive species assay in MCF-7 cells

The cytotoxic effect of *A.sativum* was tested on MCF-7 cells. The results demonstrated that the extract effectively inhibited the growth and proliferation of the cells at an IC_{50} of 129µg/ml. Morphological changes was visible in cells treated with the extract when compared to the normal cells. In the total reactive species assay it was witnessed that the extract induced apoptosis of cancer cells by triggering the generation of Reactive Oxygen Species. Production of ROS within the cells causes internal stress that leads to the apoptosis pathway in the cells. Studies have revealed that cancer cells showed an increased level of ROS, which is the consequence of high metabolic activity, mitochondrial dysfunction, peroxisome activity, increased cellular receptor signalling, oncogene activity, increased activity of oxidases, cyclooxygenases, lipoxygenases and thymidine phosphorylase. Similar studies was carried out using fresh extracts of garlic stopped the growth and changed MCF7 breast cancer cells morphology. Derestricted levels of E-cadherin, cytokeratin8/18, and β-catenin correlated with the altered phenotype early down-regulation of cyclin D1, reduced phosphorylation of ERK1, and increased phosphorylation of eIF2-α triggered the phenotypical changes[15].

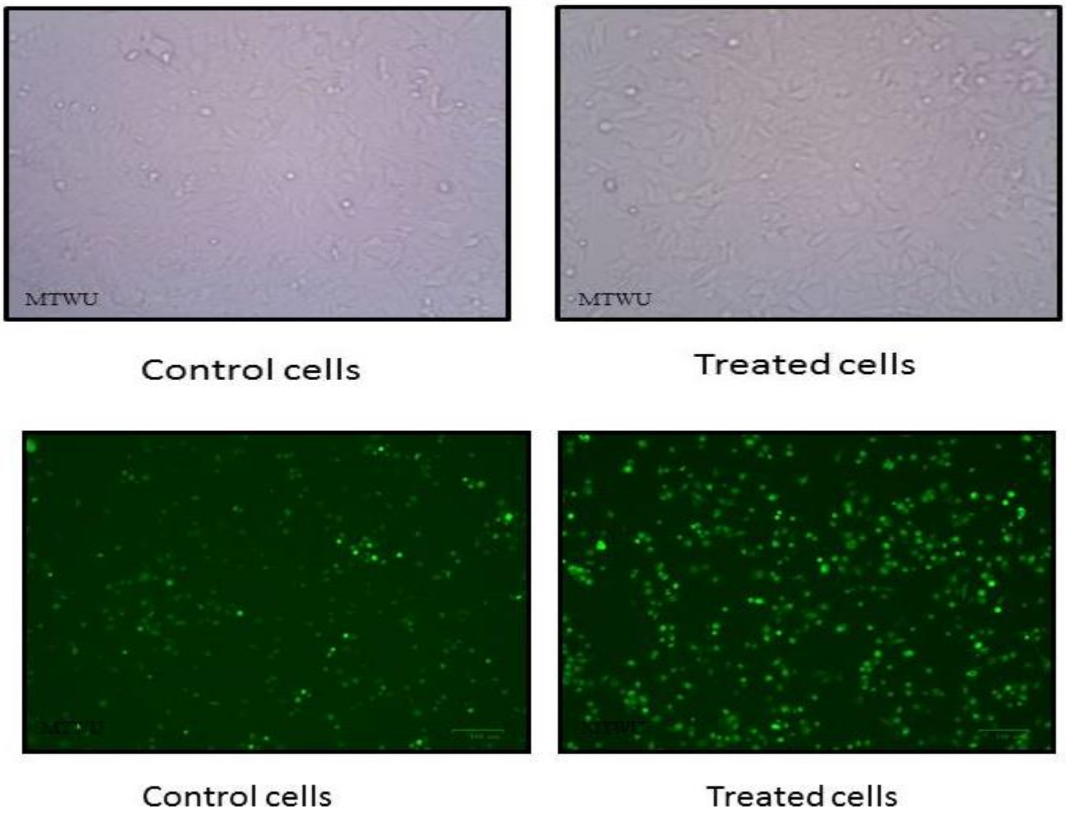

Figure-3: Cytotoxic activity in MCF-7 Cells: (a) Control cells; (b) *A.sativum* treated cells
c) Generation of ROS in MCF-7 Cells

The phytochemical analysis revealed a rich profile of bioactive compounds, primarily organosulfur constituents like trisulfides and 3-Pentanone. These findings align with the established reputation of *Allium sativum* as a therapeutic plant rich in sulfur-containing compounds such as alliin and allicin, which contribute to its multifaceted biological properties [1]. The presence of these functional groups reinforces garlic's role as a "phytoceutical powerhouse" with broad applications in alternative medicine [2].

In the evaluation of antioxidant capacity, the garlic juice demonstrated a high percentage of inhibition against DPPH radicals, outperforming several commercial varieties. This scavenging activity is consistent with standard protocols used to measure antioxidant properties in plant extracts, where hydroxyl groups from phenolics serve as primary electron donors[18] [4]. The additional scavenging of ABTS and H_2O_2 radicals mirrors the activity patterns observed in other potent natural antioxidants, such as green tea catechins, which are used as benchmarks for free radical neutralization.[5][6]

The antidiabetic results showed significant inhibition of α-amylase (IC_{50}: 561.15 μg/ml) and α-glucosidase (IC_{50}: 686.70 μg/ml). This inhibitory potential against key carbohydrate-hydrolyzing enzymes is a recognized trait of several *Allium* species, which have been explored for their ability to manage postprandial hyperglycemia [7]. The inhibitory profile observed in Kodaikanal garlic is comparable to other natural inhibitors like theaflavins, which effectively target these enzymes to reduce glucose absorption [22][8]. These effects are likely mediated by bioactive compounds revealed through GC-MS, which are responsible for the metabolic stability associated with garlic consumption [9].

Furthermore, the antimicrobial activity against pathogens like *E. coli* and *S. aureus* was robust, with MIC values indicating high potency. This is largely attributed to allicin, which is known to inhibit bacterial DNA gyrase activity[10]. The high phenolic and flavonoid content of high-altitude garlic contributes significantly to this inhibitory effect, similar to findings in other Egyptian *Allium* species [11][12].

The cytotoxic study on MCF-7 breast cancer cells (IC50: 129 μg/ml) demonstrated that the extract effectively induces apoptosis through ROS generation. This mechanism is supported by previous studies where garlic constituents, such as diallyl trisulfide, were shown to induce programmed cell death in MCF-7 cells [3]. The observed morphological changes and growth arrest are characteristic of the therapeutic action of sulphur compounds in *Allium sativum*, which trigger phenotypic changes in malignant cells [13][14].

Table 4. Comparative evaluation of biological activities of Kodaikanal Hill garlic (Allium sativum) with previously reported literature values, highlighting its enhanced antidiabetic, antioxidant, antimicrobial, and anticancer potential.

S. No	Biological Parameter	Present Study (Kodaikanal Hill Garlic)	Literature Value / Range	Reference
1.	α-Amylase Inhibition (IC_{50})	561.15 μg/mL	680.54 ± 0.58 μg/mL (Aqueous extract)	[15]
2.	α-Glucosidase Inhibition (IC_{50})	686.70 μg/mL	16.93 mg/mL (Garlic oil)	[16]
3.	MCF-7 Cytotoxicity (IC_{50})	129 μg/mL	~200 μg/mL (Crude extracts)	[17]
4.	MIC (*Staphylococcus aureus*)	79 μg/mL	400 μg/mL (Aqueous extract)	[18]
5.	MIC (Escherichia coli)	89 μg/mL	700 μg/mL (Aqueous extract)	[19]

6.	DPPH Scavenging (%)	82% (at 10 µg/mL)	11.7% – 15.2% (at fixed conc.)	[20]
7.	Total Phenolics (TPC)	Strongly present (FTIR)	566 – 612 mg/kg	[21]

The comparative analysis clearly demonstrates that Kodaikanal Hill garlic exhibits superior or comparable biological activities relative to previously reported garlic extracts. Notably, the α-amylase inhibitory activity (IC_{50}: 561.15 µg/mL) was more effective than earlier reports, indicating a stronger potential for regulating postprandial glucose levels. Similarly, the α-glucosidase inhibition (IC_{50}: 686.70 µg/mL) was significantly more potent than garlic oil-based studies, suggesting that fresh garlic juice retains higher bioactive efficacy, possibly due to the preservation of thermolabile organosulfur compounds.

The cytotoxic activity against MCF-7 cells (IC_{50}: 129 µg/mL) also surpassed previously reported crude extracts, highlighting its enhanced anticancer potential. Furthermore, the antimicrobial activity showed markedly lower MIC values against *Staphylococcus aureus* and *Escherichia coli*, confirming stronger antibacterial potency compared to aqueous extracts reported in literature. The antioxidant activity, particularly DPPH radical scavenging (82%), was substantially higher than reported ranges, indicating a rich presence of phenolic and sulfur-containing compounds contributing to redox potential. Overall, these findings suggest that the unique agro-climatic conditions of Kodaikanal hills may enhance the phytochemical composition of garlic, thereby improving its pharmacological efficacy. This comparative evidence strongly supports the potential of Kodaikanal Hill garlic as a potent natural therapeutic agent for managing oxidative stress, microbial infections, diabetes, and cancer.

Conclusion

The study was to determine the antioxidant, antibacterial, and antidiabetic potential of garlic juice extracted from high-altitude garlic bulbs in vitro. Diabetes has become one of the world's most serious health problems, and increased use of chemically generated medications has resulted in catastrophic adverse effects. As a result, garlic consumption is a natural strategy to avoid diabetes through a healthy diet. It has a great ability to block the alpha amylase and alpha glucosidase enzymes, which are important carbohydrate hydrolysing enzymes that play a key role in diabetes. Garlic and its bioactive compounds revealed through GC-MS analysis in this work can be created as effective diabetes control and prevention medicines. Apart from being a good antidiabetic agent, the antioxidant and antibacterial properties of garlic juice are additional reasons to include garlic in regular diet.

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