
Phytochemical Screening and Antimicrobial Properties of *Allium sativum* Against *Lactobacillus*

Mr. Stanley Kiprof^{a*}, Dr. Mutemi Muthangya^b

^aPG Student, Biomedical Science, University of Eastern Africa Baraton, P.O.BOX. 2500-30100 Eldoret, Kenya

^bChair, Department of Biochemistry and Molecular Biology, South Eastern Kenya University, P.O.BOX 170-90200 Kitui, Kenya

^aEmail: skiprop32@gmail.com

^bEmail: mmuthangya@seku.ac.ke

Abstract

The objectives of this study were to extract phytochemical components of *Allium sativum* and screen the phytochemical composition of allium extracts for bioactivity against *Lactobacillus*. The methanol extract of *Allium sativum* was obtained from a dried sample of garlic, was screened for phytochemical composition and tested for antimicrobial properties against probiotic bacteria lactobacillus. Antimicrobial analysis was done using agar well diffusion method where different concentration of garlic extract were tested against lactobacillus. The experiment was arranged in 3 replicates according to 4 treatments of different extract concentrations and in the control experiment the bacterial were grown without extract. The result of the phytochemical screening revealed the presence of alkaloids, saponins, cardiac glycosides, steroids, and flavonoids in garlic, but tannins were absent. The antibacterial activity of the extracts against the test lactobacillus showed inhibitory effect where different concentrations showed different inhibitory activities. This review goes over some relevant research that has already been done in this area where garlic has been tested for antimicrobial activities against numerous human pathogens. It therefore lays a ground for new research in testing allium varieties for antimicrobial activities against human resident microbes like lactobacillus that may be subject to susceptibility on these antimicrobial natural products.

Keywords: Spices; Probiotic; Growth Inhibitory Effect; Natural Products.

* Corresponding author.

1. Introduction

Spices are products from plants seeds, fruits, flowers, roots, leaves or bark that are added to food to improve flavor, taste, color, or act to minimize the rate of rancidity and as preservatives that suppress microbial activities [1]. Each spice has a unique aroma, flavor and antimicrobial activity which is derived from its phytochemicals [2]. Concisely Spices are strongly flavored parts of plants usually rich in essential oils used in fresh or dry forms [3]. Some spices are reported to have microbicidal or microbistatic activities [4]. The inhibitory effects of these spices are mostly due to the volatile oils present in their composition [5]. More recently, the interest in the use of spices has grown not only because of their seasoning and flavoring properties, but their ability to protect the body from many potential disease causing pathogens [6]. Thus the Consumption of spices have been implicated in the prevention of many disease [7]. Garlic (*Allium sativum*), as indicated by research has a broad range of antimicrobial activity due to presence of allicin, an organosulfur in it, *Allium sativum* has been shown to act as a growth inhibitor for both Gram-positive and Gram-negative bacteria including *E. coli*, *Salmonella*, *Streptococcus*, *Staphylococcus*, *Klebsiella*, *Proteus*, *Campylobacter jejuni*, *Aeromonas hydrophila*.and *Helicobacter pylori* [8]. This antibacterial activity of garlic could be due to the action of allicin, diallyl thiosulfonic acid, or diallyl disulfide [9]. The healthy human body ecology is made up of a friendly micro flora (good bacteria) that resides in our intestines and keeps us healthy and strong. Lactobacillus is one bacteria that resides in the body of human beings. It is very acid and bile tolerant and plays a beneficial roles in the digestive tract. It helps digestion of food, produces vitamins, and antagonizes other disease causing microbes like *E.coli* and increase immune response. It is therefore possible that with the use of natural products and spices that have antimicrobial property either with view of adding flavor to food or as a remedy against disease causing pathogens the activity of these natural products might have effect on resident microbes which are beneficial to the body. This research was aimed at evaluating the phytochemicals present in garlic, its antibacterial bioactivity against lactobacillus, with a view to educating the public on the need for food safety consciousness.

2. Materials and Methods

a) Preparation of Aqueous Extract of *Allium sativum*

The raw *Allium sativum* were sliced, crushed, dried in air for 3 weeks and then pulverized to powder. Extraction was performed by soaking 50g of the pulverized garlic in 300ml of methanol for 24 hours, the residue and the filtrate were obtained using what man No. 1 filter paper, evaporated to concentrate the phytochemicals and then stored at 4°C for use later use.

b) Qualitative Analysis of Phytochemicals

Foam test: 5ml of the test sample solution were placed in a test tube and then shaken gently for 5 min, presence of saponins was confirmed by formation of a foam. Alkaline reagent test: a few drops of 5% NaOH were added to 1 ml of test sample solution followed by addition of 2M HCl. Formation of an intense yellow color which turns colorless upon addition of HCl indicates presence of flavonoid. Mayer's test: 1 ml of test sample solution were placed in a test tube. 2 drops of Mayer's reagent (potassium mercuric acid) were added. Presence of

alkaloids was be confirmed by formation of cream precipitate. Ferric chloride test: test sample solution was added to 5% FeCl₃ reagent the formation of brown green precipitate or dark brown precipitate confirms presence on phenols. Ferric chloride test: a few drops of FeCl₃ solution were added to 2 ml of the extracts. Purple coloration indicates presence of tannins. Fehling's reagent were be added to 5 ml of test sample extracts and then boiled for 2 minutes. A brick red coloration indicates the presence of glycosides.

c) Antimicrobial Screening

Filter-paper disc-agar diffusion procedure, known as the Kirby-Bauer method (Yousufi, 2012) was used to determine the garlic spice susceptibility on *lactobacillus*. These bacterial microbes used in this experiment were obtained from Daija plain yoghurt. Which was bought from a supermarket at Kitui town. The yoghurt fortified with bacteria was kept in a fridge for 3 days and tested for viability by inoculation on a blood agar plate following an overnight incubation. These were retained as stock cultures.

d) Media Preparation

MacConkey agar was prepared according to manufacturer's instruction where 25.7g were dissolved in 500ml distilled water. The solution was then be warmed on a hot plate to mix homogeneously followed by autoclaving at 121°C for 15 minutes and left to cool down. It was then dispensed in sterile petri dishes and allowed to solidify. Following incubation at 37°C for 24 hours.

e) Preparation of Extract Concentrations

The stock extract was prepared by dissolving 3mg of crude extract in 2ml of distilled water to make a stock concentration of 1000mg/ml this was then labelled as T1. From the stock, serial dilutions of 10⁻¹ and 10⁻² were made and labelled as T2 and T3 respectively.

f) Preparation of McFarland Standard

Barium sulphate (190w/v) standard suspension was used as turbidity standard. It was prepared by adding 1ml of concentrate H₂SO₄ in 99ml of water. 1g of barium chloride was dissolved in 100ml H₂SO₄ acid solution to yield 1.0% w/v barium sulphate solution. The turbid solution that was formed was then transferred into a test tube as the standard for comparison (Saeed, 2005). This match with the 0.5 McFarland's standard turbidity to prepare using the test bacteria culture. Exactly 0.5 McFarland gives equivalent approximate density of bacteria 1.5x10⁸ cfu (Saeed, 2005).

g) Standardization of Inoculum

The previously prepared overnight nutrient broth culture of *lactobacillus* were used as innocula by diluting with sterile saline solution. The sterile normal saline was prepared by weighing 0.5g of NaCl and dissolved into 100cm³ of sterile distilled water. 10ml of the solution was transferred to cool, and 0.1ml viable colonies of an overnight broth culture of *lactobacillus* was dispensed into the separate test tubes containing the sterile normal

saline. The suspension was adjusted to match the 0.5 McFarland's standard which has a similar appearance of an overnight broth culture. This served as the standard inocula which was used for the antibacterial activity testing and for different concentration of the extract.

h) Test for Bioactivity

12 agar plates will be arranged in triplicates and labelled as T0, T1, T2 and T3 respectively. The prepared media will then be placed in the petridishes and left to solidify following inoculation with 0.01 ml of lactobacillus bacteria using inoculation loop. 2 drops of the extracts will then be spread on the surface of the media-where T1 will receive the extract prepared by dissolving 3mg of extract in distilled water. T2 will receive dilution one of extract, T3 dilution 2 and T0 will receive no extract and hence used as a control. All plates will then be incubated at 37 degrees Celsius for 48 hours after which the observations will be made on the growth of bacterial colonies.

i) Statistical Data Analysis

All assays were performed in triplicate in three independent experiments where tables and bar graphs were used to analyze data obtained from the experiments. Data analysis results were expressed as means \pm S.E. (Standard Error) and differences between means analyzed statistically using R-console statistical tool. And the differences will be considered significant if $p \leq 0.05$.

3. Results

The phytochemical screening of the methanol crude extract of *Allium sativum* (garlic) is shown in Table 1. The result revealed the presence of alkaloids, saponins, cardiac glycosides, steroids and flavonoids in both plants while tannins were absent.

Table 1: Qualitative Analysis for Phytochemical Constituents of Methanol Extract of Garlic

Phytoconstituent	Presence(+)/Absence(-)
Tannins	-
Flavonoids	+
Saponins	+
Cardiac glycosides	+
Phenols	+
Alkaloids	+

The antibacterial activity of the methanol extract of *Allium sativum* against lactobacillus is shown in Table 2. The result shows the number of bacterial colonies growing at different concentrations of garlic extracts. A, B and C represents the replicates of the same treatment.

Table 2: Number of Lactobacillus Colonies against the Concentration of Garlic Extract Treatment

Treatment		No. of colonies	Mean
T0(control)no extract	A	51	59
	B	59	
	C	67	
T1(1g of extract in 2ml distilled water)	A	9	6
	B	4	
	C	5	
T2(1 ml of t1 dissolved in 9 ml distilled water)	A	24	21
	B	15	
	C	22	
T3(1 ml of t2 dissolved in 9 ml distilled water)	A	34	32
	B	30	
	C	31	

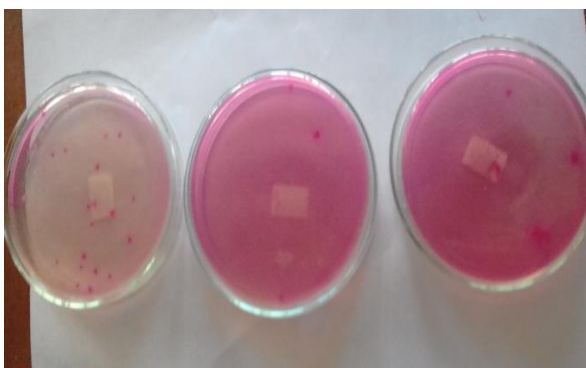


Figure 1: T1 treated with concentrated extract

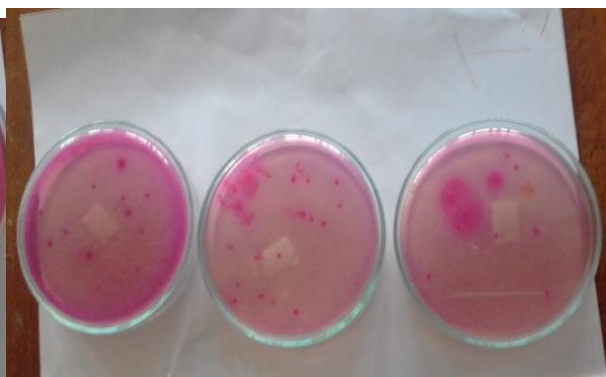


Figure 2: T2 treated dilution 1 of extract

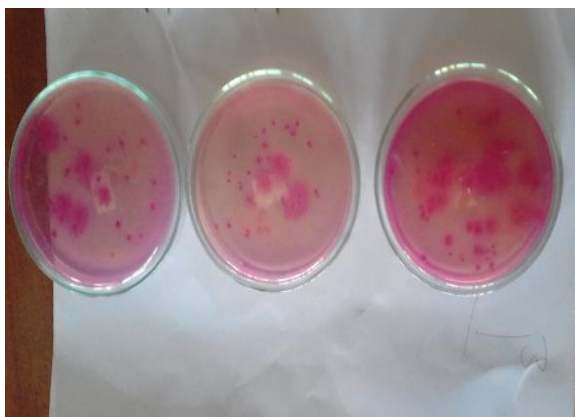


Figure 3: T3 treated with dilution 2 of extract

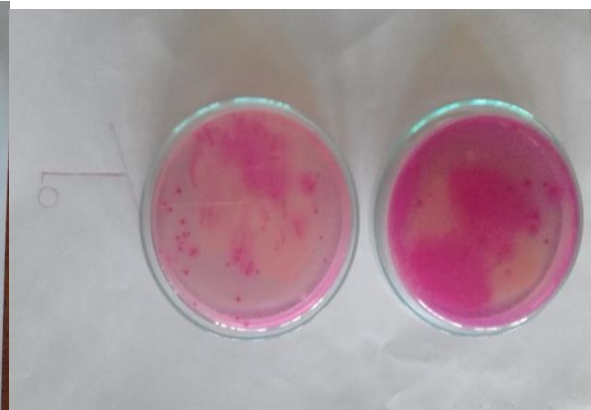


Figure 4: T0 (control) bacteria without extract

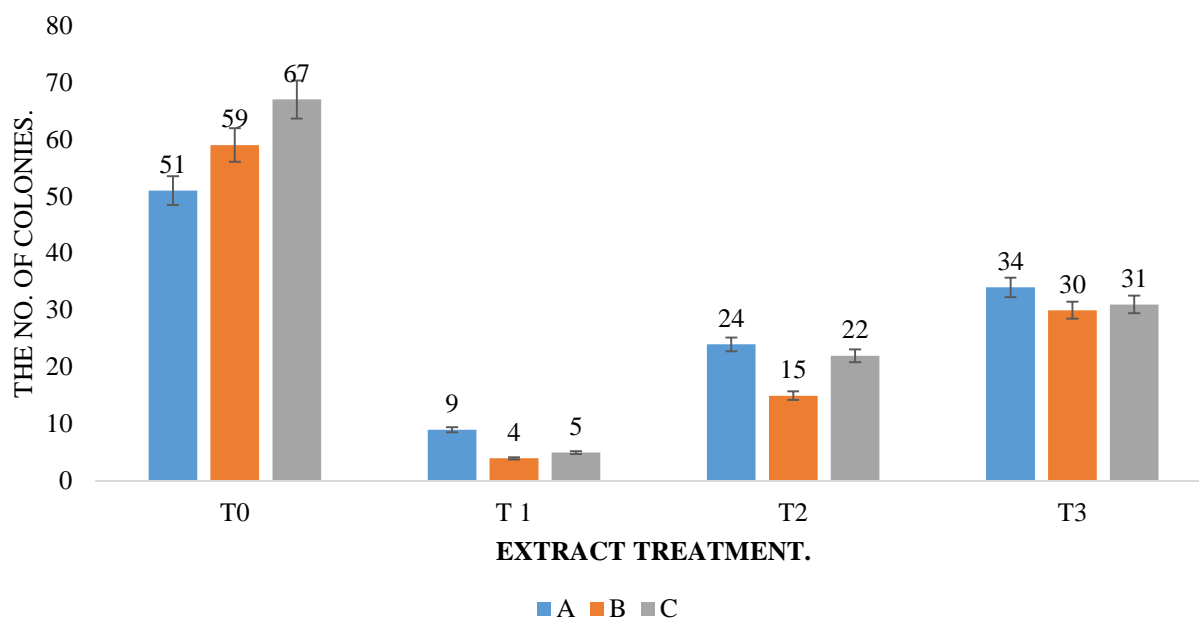


Figure 5: Number of Colonies against Different Extract Treatment

Table 2: Comparisons between Treatments

Treatments compared	T	Df	P-value	Remarks
t0 and t1	10.895	2.4323	0.004015	P value <0.05
T0 and t2	7.2079	3.2443	0.004216	P value <0.05
T0 and t3	5.7271	2.748	0.02167	P value <0.05
T1 and t2	-4.5838	3.1416	0.01762	P value <0.05
T1 and t3	13.205	3.7902	0.0002621	P value <0.05
T2 and t3	-3.8013	2.748	0.03722	P value <0.05

The comparison between control and t1 shows that there is a significant difference in bacterial response to the two treatments given that: p-value 0.004015 is less than 0.05. The comparison between control and t2 shows a significant difference in bacterial response to the two treatments given that: p-value 0.004216 is less than 0.05. The comparison between the control and t3 shows there is a significant difference in bacterial response with respect to the two treatments; p-value 0.02167 is less than 0.05. The comparison between t1 and t2 shows a significant difference bacterial response with respect to the two treatments i.e. p-value 0.01762 is less than 0.05. The comparison between t2 and t3 shows a significant difference bacterial response with respect to the two treatments given that: p-value 0.03722 is less than 0.05. The comparison between t1 and t3 shows a significant difference bacterial response with respect to the two treatments given that: p-value 0.0002621 is less than 0.05.

4. Discussion

The result of the phytochemical screening revealed the presence of alkaloids, saponins, cardiac glycosides, steroids, and flavonoids in garlic, but tannins was absent in garlic. The findings of this study agrees with the findings of other studies which identified that alkaloids, saponins, cardiac glycosides, steroids, and flavonoids are present in *Allium sativum* extract [10,11,12,13]. Therefore, these findings are confirmation that the presence

of alkaloids, saponins, cardiac glycosides, steroids, and flavonoids are responsible for the medicinal properties of the extracts. They looked at the ability of these garlic-derived compounds to kill bacteria [10,13,11]. Their results indicated that extract of *Allium sativum* showed excellent antibacterial activity against *Escherichia coli*, *Salmonella* and *Aeromonas hydrophila* [13], extract of *Allium sativum* showed increasing growth inhibition with concentration [11] and extract of *Allium sativum* can be effectively used to treat periodontal and dental caries infections [13]. This was not different in the current study which also showed that alkaloids, saponins, cardiac glycosides, steroids, and flavonoids possess antimicrobial activities by showing growth inhibitory activities. However, the antibacterial activity of the extracts against the test lactobacillus shows that the extracts of garlic shows different inhibitory activities against the test bacteria. This difference in inhibitory activities may be due to the differences in the bioactive compositions on different concentrations of the extract. The highest inhibitory activity of *Allium sativum* against lactobacillus was observed in t1 with a mean growth bacterial colony of 6 and the inhibition activity was decreasing with decreasing in concentration of extract through t2 and least in t3 with a mean bacteria colony 31. Therefore, the higher the concentration of the extract, the higher the inhibitory activity of *Allium sativum* and the lower the concentration, the lower the inhibitory activity of *Allium sativum* as confirmed by the highest number of bacterial colony observed in t0 (control) which had no extract. Although the mechanism of action of these extracts was not studied, the phytochemical compounds such as tannins are known to coagulate the wall proteins, while saponins facilitate the entry of toxic material or leakage of vital constituents from the cell [5]. Flavonoids inhibit the activity of enzymes by forming complexes with bacterial cell walls, extracellular and soluble proteins, more lipophilic flavonoids disrupt cell wall integrity or microbial membranes at low concentrations [14]. This then demonstrates that the confirmed presence of the above discussed phytochemicals in garlic are responsible for the growth inhibition activity on lactobacillus as noted.

5. Conclusion and Recommendation

In this experiment, crude extracts of garlic was used; no separation of garlic components was done. Garlic showed growth inhibitory activity against lactobacillus that is dependent on the concentration and it was significantly varied among the different concentrations ($P < 0.05$). T3 had a lesser growth inhibitory effect on lactobacillus compared to other treatments in an increasing extract concentration i.e. t2 and t1 respectively. Therefore, the study can confidently conclude that garlic has effect on the growth of lactobacillus and thus the alternative hypothesis is accepted. This indicate that the activity of this bioactive phytochemicals against lactobacillus is dependent on their concentrations thus there is need to regulate their dietary composition. This is to avoid depletion of the micro floral microbe lactobacillus which are beneficial to the health of human body. The studies examined herein were done in vitro. However, more tests need to be conducted to determine inhibitory effects of garlic on lactobacillus in vivo, especially because large number of people consume garlic as their dietary spice. This could be a potential means through which the effects associated with absence or deficiency of microfloral lactobacillus in the gastrointestinal tract are caused. This research opens the doors to greater research on lactobacillus and the likely risk factor that may be associated with depletion of these microbes. The data already collected and methods of testing offers new directions for future experiments. To obtain more conclusive data, tests should be done to establish if growth inhibition was as a result of killing the bacteria or inactivation.

6. Study Limitations

This study was limited to extraction of phytochemical components of *Allium sativum* and screening the phytochemicals for bioactivity against *Lactobacillus*. Therefore, the study did not investigate the mechanism of action of these phytochemical components.

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