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Antibacterial Potency of *Allium sativum*, *Psidium guajava*, *Zingiber officinale* Roots against the Food Poisoning Strains (*Escherichia coli* 0157:H7 and *Staphylococcus aureus*)

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Abstract

Escherichia coli 0157:H7 and *Staphylococcus aureus* are notorious food poisoning bacteria of great effect on human health. This study determined the phytochemical constituents of ethanolic, vacuum liquid chromatography (VLC) filtrates of *Allium sativum* (garlic), *Psidium guajava* (guava), *Zingiber officinale* (ginger) and their antibacterial activities on *E. coli* 0157:H7 and *S. aureus*. In a laboratory-based controlled experiment, the antibacterial and inhibitory activities of the VLC filtrates in doses of 0.01, 0.02, 0.04, and 0.08g/mL were investigated on *E. coli* 0157:H7 and *S. aureus* using the agar well diffusion method and compared with a standard antibiotic. The indices of the zone of inhibition on the agar plates were subjected to analysis of variance (p = 0.01). Phytochemical analysis revealed a high concentration of glycosides, steroids, tannins, terpenoids, and the absence of flavonoids in *A. sativum*. *P. guajava* root contained a high concentration of glycosides, steroids, tannins, and terpenoids. *Z. officinale* possessed a high concentration of alkaloids, glycosides, flavonoids, steroids, and terpenoids. Statistically, 0.02 g/mL of *Z. officinale* (12.0 mm) showed a significant result to 0.04g/mL of *A. sativum* (14mm) at p = 0.0049 and a higher inhibition index to 0.08g/mL of *P. guajava*. Inhibition by 0.02g/mL of *P. guajava* (8.8mm) was advanced than 0.04g/mL of *A. sativum* (6.3 mm) on *S. aureus*. Recommendable inhibition of 29.3 mm and 24.0 mm on *E. coli* 0157:H7 and *S. aureus*, respectively, were expressed by 0.08 g/mL *Z. officinale*, which was significant to the standard 0.04 g/mL of Neomycin (Positive control) at p = 0.0067. This study indicated a reliable antibacterial activity by Z. officinale than *A. sativum* and *P. guajava*, which might have been due to the high concentration of alkaloids and flavonoids. Nevertheless, *A. sativum*, *P. guajava*, and *Z. officinale* are normally used as fruit and food additives in the human diet, thus can be combined to prevent food poisoning by *E. coli* 0157:H7 and *S. aureus* and their exotoxins.

Keywords: *Allium sativum*, Antibacterial activity, *Escherichia coli* 0157:H7, *Psidium guajava*, *Staphylococcus aureus*, *Zingiber officinale*

Introduction

Food poisoning and infections caused by enteric pathogens are important causes of morbidity and mortality worldwide and have a major impact on public health (CDC 2018). The causes of food poisoning are microorganisms such as bacteria, viruses, fungi, and several other parasites, which can infect humans through contaminated food or drink (Nurjayadi et al. 2019). Common bacteria's that are associated with food poisoning are *Escherichia coli* and *Staphylococcus aureus* (Asime et al. 2020). Based on the Centre for Disease Control and Prevention (CDC), it is estimated that every year, there are two million cases of foodborne

diseases in Africa caused by *S. aureus* and *E. coli* (CDC 2018). *E. coli* is a large and diverse group of bacteria and normally lives in the intestines of human and animals at an optimum temperature of 35 to 40°C. Although most serotypes of *E. coli* are harmless, strains like *E. coli* 0157:H7 produce toxins that are associated with food poisoning, which has a significant impact on public health (Odo et al. 2020). The Gram-positive bacterium, non-spore-forming, non-motile, and facultatively anaerobic *S. aureus* is one of the important foodborne pathogens well known to cause foodborne intoxication. *S. aureus* is associated with toxin-mediated gastroenteritis in humans (Sahu et al. 2021).

S. aureus and *E. coli* 0157:H7 in food are important reservoirs of antimicrobial resistance genes, virulence factors, and exotoxins (Mourenza et al. 2021). This is now considered as a significant health problem because it increases the incidence of foodborne disease (Rasooly et al. 2021). Traditionally, the treatments against *E.coli* and *S. aureus* food poisoning are focused on either controlling exotoxins or the control of the transmission of the bacteria (Yang et al. 2017). The use of artificially made antibiotics to control *E.coli*, *S. aureus* and their toxins are not recommended owed that they lead to the release of toxins after bacterial cell death thus leading to septic shock (Mourenza et al. 2021).

Generally, there is a great interest in developing antibiotics from natural products that are daily consumed as supplements by a human in the prevention of *S. aureus* and *E. coli* 0157:H7 to reduce the incidence of gastro-intoxication (Yang et al. 2017). Plants and plant products consumed by man have been used as medicines since the start of history (Mauti et al. 2019a). These plants contain natural compounds inform of alkaloids, flavonoids, saponins, tannins and terpenoids that directly inhibit bacterial growth or replication. For example, the steroidal alkaloid; tomatidine, found in different solanaceous plants is a well-known antibacterial compound with demonstrated activity against *S. aureus* (Mauti 2021).

The plant species selected for this study have a historical report on their medicinal properties. *Allium sativum* L., commonly known as garlic, is a species in the onion family Alliaceae. It has a proven record of antibacterial activity against *Klebsiella pneumonia* and *Salmonella typhi* (Saravanan et al. 2010). *Psidium guajava* L., also known as guava is used for the prevention of stomach aches, gastroenteritis and in the treatment of diarrhoea, leukorrhea, cholera, external ulcers and skin diseases (Abdelrahim et al. 2002). *P. guajava* has antibacterial activity on *Pseudomonas spp*., *Klebsiella spp.*, *Proteus spp*., *Citrobacter spp*., *Alcaligenes fecalis* and *Salmonella typhi* (Madubuonu et al. 2020). *Zingiber officinale* R., of the family Zingiberaceae, is also known as ginger has been found to possess antibacterial activity on *Salmonella typhi*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Bacillus subtilis* (Lucky 2017). This study aims to determine the chemical compounds found in the roots of *Allium sativum, Psidium guajava,* and *Zingiber officinale.* Furthermore, to perform *in-vitro* antibacterial activity on *E. coli* 0157:H7 and *S. aureus* food poisoning bacteria.

Methods

Collection of plant material

The fresh roots of *Allium sativum, Psidium guajava,* and *Zingiber officinale* were collected from Kinole farm at Morogoro Rural District, Morogoro, Tanzania (06°54'S, 037°54'E) and transported to the Microbiology Laboratory at Kampala International University in Tanzania. Morphological identification was performed at the herbarium of the Pharmacy Department of the same institution, where the vouchers with the reference number DKK 3412, 3413, and 3424 for *Allium sativum, Psidium guajava* and *Zingiber officinale,* respectively were deposited. Neomycin (0.04 g/mL) was purchased from Gem Pharmaceuticals, Dar es Salaam, Tanzania for use as the positive control in the bioassay.

Collection of the test organisms

The test organisms used in this research consisted of *E. coli* 0157:H7 and *S. aureus*, which are Gram-negative and Gram-positive bacterial strains, respectively. The isolates were obtained from the Department of Microbiology, Kampala International University in Tanzania, for use in assessing the antimicrobial properties of the plant extract. The test organisms were cultured on nutrient agar, then subcultures were made in nutrient agar slants at two-week intervals before being stored under refrigeration at 4°C awaiting the bioassay.

Preparation of the plant extract

The plant materials were dried, grounded into powder, and fractionated through Vacuum Liquid Chromatography (VLC). The process involved measuring 20 g of each fine grounded powder of *A. sativum, P. guajava,* and *Z. officinale* on an electronic weighing balance. The measured fine powders were dispensed into sterilized beakers containing 80 mL of distilled water and soaked for 72 hours. The solution was carefully filtered, percolated through Whatman No. 1 filter paper into a sterilized conical flask of 100 mL, and the filtrate obtained was air-dried. The air-dried filtrates were subjected to VLC extraction using 95% ethanol (EtOH). The VLC filtrates were collected, dried using a rotary evaporator at 40 °C and then stored in the refrigerator at a temperature of 4 °C until required.

Phytochemical screening of plant extract

The VLC extracts of ethanol were subjected to phytochemical profiling to determine the presence of alkaloids, flavonoids, glycosides, steroids, tannins and terpenoids using standard methods. 0.5 g of VLC filtrate of *Allium sativum, Psidium guajava,* and *Zingiber officinale* and 0.5 mL honey was stirred with 0.5 mL of 1% aqueous hydrochloric acid on a steam bath at 60°C for 5 minutes. The sample was filtered with a Whatman no. 1 filter paper. 0.5 mL of the filtrate was treated with few drops of Draggendoff's reagent. Blue-black turbidity serves as preliminary evidence of alkaloids (Akintobi et al. 2013).

Flavonoids were analyzed by adding 0.5 mL of dilute ammonia solution to the aqueous filtrate of the VLC filtrate, followed by the addition of 0.2 mL concentrated sulphuric acid. A yellow colouration indicates the presence of flavonoids (Harborne and Williams 2000). Glycosides were analysed by adding 0.2 mL of glacial acetic acid containing a drop of ferric chloride solution to 0.5 g of the VLC filtrate. To this mixture, 1.0 mL of concentrated tetra-oxosulphate (VI) acid was added to give a brown ring formation at the interface (Brain and Turner 1975).

Steroids were analyzed by mixing 0.5 g of the VLC filtrate with 0.2 mL of acetic anhydride and 0.1 mL of chloroform. To the mixture, 0.1 mL of concentrated sulphuric acid was added. The formation of a brownish ring at the interface of the liquids and the appearance of violet colour in the supernatant layer indicated the presence of steroids (Younus et al. 2020). The presence of tannins was determined by stirring 0.5 g VLC filtrate, 0.5 mL of honey, and 10.0 mL distilled water and filtered. 0.1 mL of ferric chloride reagent was added to the filtrate. A blue-black or blue-green precipitate was an indication of the presence of tannins (Trease and Evans 2002). Terpenoids were analysed by mixing 0.5 g of the VLC filtrate with 0.2 mL of chloroform. To the mixture, 0.2 mL of concentrated tetra-oxosulphate (VI) acid was added to form a layer. A reddish-brown colouration of the interface indicated the presence of terpenoids (Younus et al. 2020).

Antibacterial test on VLC filtrate

A total of 24 sterilized Petri dishes were used for the agar diffusion method as described by Balouiri *et al.* to determine the antibacterial activity (Balouiri et al. 2016). In this study, the assay involved analysis of 0.01, 0.02, 0.04, and 0.08 g/mL of the *A. sativum, P. guajava* and *Z. officinale* VLC filtrates on *E. coli* 0157:H7 and *S. aureus.*

A quantitative amount of 0.5 mL of *E. coli* 0157:H7 was taken from 48 hr old cultures and inoculated into 12 sterile Petri

dishes. About 25mL of sterile nutrient media was aseptically poured into each dish and gently rocked for proper mixture with the test organism before solidification. Afterwards, five wells of 8.0 mm diameter were dug in each plate with the aid of a sterilized cork borer. One drop of the molten nutrient agar was used to seal the bottom of the bored well, to prevent the VLC filtrate from sipping beneath the agar gel. In a replicate of three Petri dishes, 0.01 g/mL of the *A. sativum, P. guajava,* and *Z. officinale* VLC filtrates were added to the first, second and third wells, respectively.

A volume of 0.04 g/mL of the positive control (Neomycin) and negative control (distilled water) was added to the fourth and fifth wells, respectively. The above method was carried out on 0.02, 0.04, and 0.08 g/mL of the *A. sativum, P. guajava* and *Z. officinale* VLC filtrates on *E. coli* 0157:H7 and *S. aureus*. After insertion of the test materials as described above, the plates were allowed to stand for one hour for proper diffusion and then incubated at 37°C for 24 hours. The sensitivity of the test organisms to the VLC filtrates and the positive control was indicated by a clear zone of inhibition around the wells. The diameter of the clear zone (zone of inhibition) was measured to the nearest millimetre using a transparent ruler. This was recorded as the degree of inhibition by the VLC filtrate against the test organisms.

Statistical analysis

Analysis of Variance (ANOVA) was used to test if there were significant differences between the antibacterial activities of the different VLC filtrates using SPSS software version 20. The analysis included comparing the means of the zones of inhibition at a 99% level of confidence utilizing the Tukey HSD test at $p = 0.01$. The independent variable in this model was the applied dose of the VLC filtrate, while the dependent variable was the diameter of the zone of inhibition.

Results

Phytochemical screening

The results of phytochemical analysis of *Allium sativum, Psidium guajava,* and *Zingiber officinale* VLC filtrate are reported in Table 1. *Allium sativum* extract showed a high concentration of glycosides, steroids, tannins and terpenoids. Also, there was a moderate concentration of alkaloids while flavonoids were absent. *Psidium guajava* root extract was found to contain a high concentration of glycosides, steroids, tannins, and terpenoids, while alkaloids and flavonoids were present in moderate concentrations. *Zingiber officinale* extract was found to possess a high concentration of alkaloids, glycosides, flavonoids, steroids, and terpenoids while tannins were found to be of moderate concentration.

Table 1: Phytochemical screening of the ethanolic extracts of *Allium sativum*, *Psidium guajava* and *Zingiber officinale*

Phytochemical	***Allium sativum***	***Psidium guajava***	***Zingiber officinale***
Alkaloids	++	++	+++
Flavonoids	-	++	+++
Glycosides	+++	+++	+++
Steroids	+++	+++	+++
Tannins	+++	+++	++
Terpenoids	+++	+++	+++

(+++) High concentration, (++) Moderate concentration, (+) Low concentration, (-) Absent.

Antibacterial activity

The antibacterial analysis was determined by the zone of inhibition on the agar plates as presented in Figures 1 and 2. At the lowest dose of 0.01 g/mL, *A. sativum* showed a significant inhibition activity against *E. coli* 0157:H7 compared to a similar dose by *P. guajava* and *Z. officinale.* At $p = 1$, the inhibition of 5.5 mm was visualized around the wells applied with 0.02 g/mL of *A.*

sativum and *P. guajava* at $p = 1$. Statistically, 0.02 g/mL of *Z. officinale* (12 mm) showed a significant result to 0.04 g/mL of *A. sativum* (14 mm) at $p = 0.0049$ and a higher inhibition index to 0.08 g/mL of *P. guajava.* The highest inhibition on *E. coli* 0157:H7 was expressed by 0.08 g/mL *Z. officinale*, which was significant to the standard 0.04 g/mL of Neomycin (Positive control) at $p = 0.0067$.

Graphically, inhibition on *S. aureus* was displayed by all doses of *A. sativum*, *P. guajava*, and *Z. officinale* (Figure 2). Statistically, 0.01 g/mL of *Z. officinale* recorded high inhibition compared to 0.01 g/mL of *P. guajava and* 0.02 g/mL of *A. sativum.* A comparable similarity in inhibition was recorded by 0.02 g/mL of *P. guajava* and *Z. officinale* ($p = 0.0071$). Inhibition by 0.02 g/mL of *P. guajava* (8.8mm) was higher than 0.04 g/mL of *A. sativum* (6.3 mm). The highest inhibition of 24mm was recorded by 0.08 g/mL of *Z. officinale* (Figure 2).

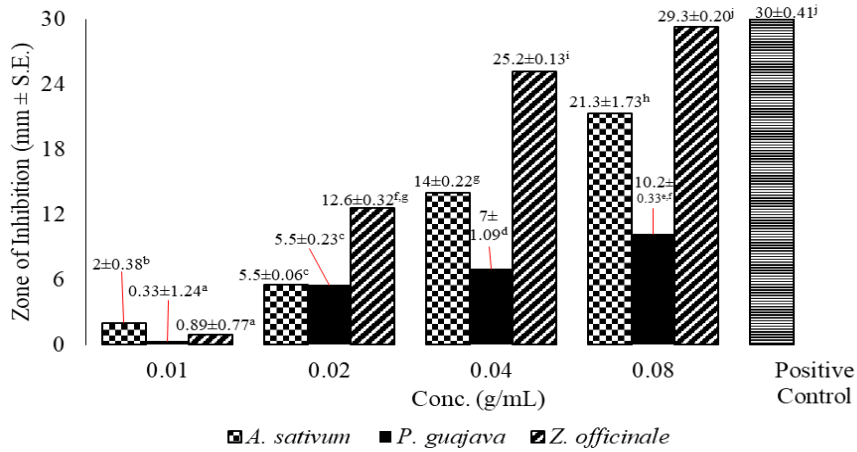

Figure 1: Antibacterial activity *Allium sativum*, *Psidium guajava* and *Zingiber officinale* on *E. coli* 0157:H7. Means that do not share a letter are significantly different (N = 60; $p < 0.01$).

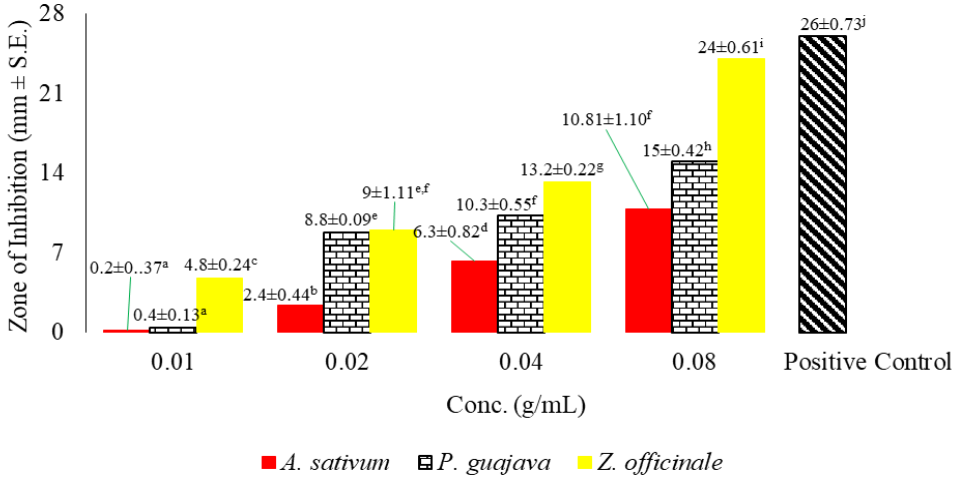

Figure 2: Antibacterial activity *Allium sativum*, *Psidium guajava* and *Zingiber officinale* on *S. Aureus*. Means that do not share a letter are significantly different (N = 60; $p < 0.01$).

Discussion

Antibacterial properties of plants and their relevant compounds are under investigation as a desirable tool in the control of undesirable microorganisms, especially in the treatment of infections and food spoilage (Mostafa et al. 2018). Vega et al. (2017) noted that successful extraction of botanical compounds from a plant material largely depends on the type of solvent used in the extraction procedure. Furthermore, research by Rafińska et al. (2019) revealed the active compounds from *A. sativum* and *Z. officinale* had poor solubility in water. The results from the present study exposed that the compounds present in *A. sativum, P. guajava,* and *Z. officinale* extracted by ethanol had antibacterial activity. These findings demonstrated the good solubility of the active compounds in ethanol as the organic solvent.

Findings of this study showed that a moderate concentration of alkaloids was found in *A. sativum*, similar results were recorded by Najeebullah et al. (2021). Research by Olusanmi and Amadi (2009) showed a low concentration of flavonoids, this was contrary to this present study where flavonoid is absent in the ethanolic extract of *A. sativum.* The present study showed the presence of a high concentration of glycoside and a moderate concentration of steroids in *Z. officinale*. Phytochemicals results obtained by Akintobi et al. (2013) on *Z. officinale* reported a high concentration of glycoside, and low concentration of steroid, as Chand (2013) reported the absence of glycosides, and high concentration of steroids. Furthermore, This study showed a high concentration of steroids, and terpenoids in *P. guajava*, on the other hand a low concentration of steroids and terpenoids was recorded in the ethanolic extract of *P. guajava* (Abdelrahim et al. 2002).

In this study, *A. sativum, P. guajava* and *Z. officinale* demonstrated antibacterial properties against life-threatening *E. coli* 0157:H7 and *S. aureus*. An inhibitory of 21.3 mm and 10.8 mm was recorded for the ethanolic extract of *A. sativum* on the *E. coli* 0157:H7 and *S. aureus* plates, this inhibitory activity was recommendable as to the results recorded by Saravanan et al. (2010) whereby 0.08 g/mL of methanol and water extracts of *A. sativum* had 3.0 mm and 6.0 mm, respectively on *E. coli*. Furthermore, an impressive inhibition of 30.0 mm was visualized on both *E. coli* and *S. aureus* plates by 0.1 g/mL of ethanolic extract of *A. sativum* (Mikaili et al. 2013, Najeebullah et al. 2021).

Research by Abdelrahim *et al.* (2002) recorded an inhibitory of 22.0 mm and 20.0 mm by 0.12 g/mL methanol and water extracts of *P. guajava*, respectively, on *E. coli*, these were promising compared to this current study where 0.08 g/mL of ethanolic extract of *P. guajava* attained an inhibitory of 10.2 mm on *E. coli* 0157:H7 plate. According to various researchers the antibacterial activity was due to the high concentration of steroids and terpenoids (Abdelrahim et al. 2002, Saravanan et al. 2010, Olusanmi and Amadi 2009). From these findings, it could be plausible that derivatives of the mevalonic acid pathway from these plants species could hold potential application as antibiotic scaffolds.

Inhibition of 19 mm and 17.3 mm was exhibited by 0.1 g/mL of ethanolic extract of *Z. officinale* against *E. coli* and *S. aureus*, respectively (Mikaili et al. 2013). Another report showed that inhibition of 15.0 mm and 17.5 mm were exhibited by 0.1 g/mL of methanolic extract of *Z. officinale* to *E. coli* and *S. aureus*, respectively (Njobdi et al. 2018). There was no inhibition on *E. coli* and inhibition of 2.2 mm was recorded on *S. aureus* by 1.3 g/mL of the ethanolic extract of *Z. officinale* (Akintobi et al. 2013). Results lower than what was observed in this study were further reported where 25.2 mm was inhibited by 0.04 g/mL of *Z. officinale* on *E. coli* 0157:H7and 24.0 mm by 0.04 g/mL of *Z. officinale* on *S. aureus* (Njobdi et al. 2018, Chand 2013). In this study, inhibition on *E. coli* 0157:H7 and *S. aureus* was highly expressed by *Z. officinale.* The presence of high concentration alkaloids and flavonoids have demonstrated an increase in inhibitory activities over glycosides, steroids, and terpenoids against most Gram-negative and Gram-positive bacteria's including *E. coli*

0157:H7 and *S. aureus* (Akintobi et al. 2013, Chand 2013, El-Saber et al. 2020, Patel 2021).

Conclusion

The incidences of antibacterial resistance by food poisoning bacteria's are increasing globally and the progress of new antibacterial therapies are slowing down on attainment and transmission of food poisoning bacterial resistance. *E. coli* 0157:H7 and *S. aureus* are important human pathogens, and they are quickly acquiring resistance to antibiotic drugs used in clinical medicine. Moreover, *E. coli* 0157:H7 and *S. aureus* exotoxins have a high impact on food contamination.

Many other plant materials with antibacterial activity against *E. coli* 0157:H7 and *S. aureus* have only been tested in preclinical trials due to their low absorption, dispersal, digestion or excretion features. Nevertheless, this study has shown *A. sativum*, *P. guajava*, and *Z. officinale* have antibacterial potency against *E. coli* and *S. aureus* and they are normally used as fruit and food additives in the human diet, thus can be combined to prevent food poisoning. However, more research is required to test the dosage and stability of the phytochemical compounds with the best antibacterial profiles.

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Declaration of interest

The author declares no potential conflict of interest.

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