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Article

Effectiveness of Gambas (Luffa acutangula) Solution in Reducing Bacterial Counts on Tablespoons: A Quasi-Experimental Study

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Abstract
Microbiological contamination of tableware is a key aspect of food hygiene and sanitation, particularly when washing processes are unable to reduce the bacterial load sufficiently. Gambas (*Luffa acutangula*) contains various bioactive compounds and has been reported to exhibit antibacterial activity in laboratory tests; however, its application in reducing bacterial contamination on cutlery remains limited. This study aimed to analyse the effectiveness of various concentrations of gambas solution in reducing bacterial counts on tablespoons. The study employed an experimental design with 28 samples comprising a negative control group, a positive control group, and treatment groups using gambas solution at several concentrations. Bacterial counts on the surface of the spoons were measured after treatment and compared across groups using statistical analyses appropriate to the distribution and variance characteristics of the data. The results showed differences in bacterial counts between groups, with a tendency for bacterial counts to decrease as the concentration of the gambas solution increased. The highest concentration tested produced the greatest reduction in bacteria, although not all comparisons between concentrations showed statistically significant differences. These findings suggest that gambas solution has the potential to reduce the bacterial load on spoon surfaces under experimental conditions. However, further research is required to identify the microorganisms affected, determine the mechanism of antibacterial activity, evaluate the safety of residues, and test the consistency of efficacy under a wider range of usage conditions.

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Introduction

Food safety is a vital part of public health protection, as food contaminated with pathogenic microorganisms can serve as a vehicle for the transmission of various foodborne diseases. The World Health Organisation (WHO) estimates that in 2021, unsafe food was linked to around 866 million cases of illness and 1.52 million deaths globally, with the greatest burden falling on vulnerable groups, including children under five[1]. Contamination can occur at various stages of the food chain, including during food preparation and service; therefore, hygiene and sanitation practices in food service establishments are a key component of microbiological risk control.

Tableware is one type of surface that comes into direct contact with food and can play a role in the transfer of microorganisms if washing and sanitisation are not carried out adequately.

Microbiological assessments at food service establishments indicate that surfaces and equipment that come into contact with food can become contaminated and may potentially contribute to cross-contamination during the food service process[2]. Tablespoons are of particular importance because their surface comes into direct contact with food and the oral cavity, whilst washing them using only running water does not necessarily ensure an adequate level of microbiological cleanliness[2].

Control of microbiological contamination on food-contact surfaces can, in principle, be achieved through appropriate cleaning and sanitisation. The effectiveness of these processes is influenced by various factors, including the characteristics of the surface, the type and quantity of microorganisms, and the conditions under which sanitisation takes place[3]. The use of plant-based materials as a source of antimicrobial compounds is also an approach that continues to be researched. *Luffa acutangula* is a plant that has been reported to contain various bioactive compounds, including flavonoids, alkaloids, saponins, tannins and phenolic compounds, and to possess biological activities that include antimicrobial activity [4], [5]. Several experimental studies have also demonstrated the antibacterial activity of parts of the *L. acutangula* plant. Gambas fruit extract, for example, has been reported to inhibit the growth of *Clostridium perfringens* in tests using various concentrations of the extract [6], whilst another study reported the antimicrobial activity of an extract from the bark of *L. acutangula* against a number of test microorganisms [7].

Nevertheless, evidence regarding the use of gambas solution as a means of reducing bacterial contamination on cutlery remains limited and does not yet fully explain the response across different types of cutlery. Previous research on tableware has indicated that gambas solution can be used to reduce bacterial counts; however, those studies used items other than tablespoons and did not specifically evaluate the 25–45% concentration range under the washing conditions and contact times employed in this study. Furthermore, the available evidence of the antibacterial activity of *L. acutangula* stems partly from tests on extracts and *in vitro* systems; consequently, these results do not directly reflect the effectiveness of gambas solution on the surfaces of tableware used in food service environments [6], [7]. Consequently, research is required to compare different concentrations of gambas solution on the surface of a tablespoon using quantitative bacterial counts, so as to determine the pattern of bacterial reduction and the concentration that yields the greatest reduction under standardised experimental conditions.

In light of this gap, this study aims to analyse the effectiveness of various concentrations of gambas solution (*Luffa acutangula*), namely 25%, 30%, 35%, 40% and 45%, in reducing the bacterial count on tablespoons at Restaurant X in Baturraden. This study also compares the results of the treatments with those of the control group to determine whether there are differences in bacterial counts between treatments and to identify the concentration that shows the greatest reduction in bacteria. This approach is expected to provide experimental evidence regarding the potential of luffa solution as a plant-based agent for controlling microbiological contamination on cutlery, without generalising the results as a substitute for conventional disinfectants until further research has been conducted on safety, formulation consistency, antibacterial mechanisms, and effectiveness under broader conditions of use.

Materials and Method

This study employed a quasi-experimental design using a post-test only with control group approach. The study sample consisted of the tablespoons used at Restaurant X in Baturraden. The study comprised seven groups: one negative control group (washing spoons under running water), one positive control group (soap and running water), and five treatment groups with gambas solution concentrations of 25%, 30%, 35%, 40% and 45%. Each group was replicated four times, resulting in a total of 28 samples. Each sample was obtained from a combined swab of five tablespoons.

Data collection was carried out using a swab method on the surface of the tablespoon after treatment; the samples were then examined using the Total Plate Count (TPC) method and expressed in CFU/cm² at the Microbiology Laboratory of the Department of Environmental Health, Poltekkes Kemenkes Semarang. The independent variable in this study was the concentration of the gambas solution; the dependent variable was the bacterial count on the tablespoon; whilst the control variables included washing technique, type of water, spoon material, scrubbing tool, and contact time with the solution.

Data analysis was carried out using univariate analysis to determine the average bacterial count in each group, and bivariate analysis to examine differences between groups using the *One-way ANOVA* test if the data were normally distributed and homogeneous, or the Kruskal-Wallis test if these assumptions were not met. The effectiveness of the gambas solution in reducing the bacterial count on spoons was calculated using the formula:

The Effectiveness of Gambas Solution

$$\text{Effectiveness} = \frac{A-B}{A} \times 100\% \quad (1)$$

Results

Table 1 shows a consistent pattern of decline in bacterial count as the concentration of the gambas solution increases. The negative control group had the highest bacterial load, whilst the 45 % concentration resulted in the lowest bacterial count as well as the greatest absolute reduction compared with the negative control. Interestingly, the 25 % concentration already produced a greater reduction than the positive control, whilst increasing the concentration to 35 % and 40 % yielded even greater reductions. The most pronounced effect was observed at a concentration of 45 per cent, with a reduction efficiency of 93.25 per cent. However, the measurement variability at concentrations of 40 per cent and 45 per cent was relatively high, as reflected by the standard deviation and the wider confidence intervals. This indicates that the magnitude of the effect at high concentrations should be interpreted with caution and not based solely on the mean value.

Table 1. Bacterial counts, absolute reduction, and percentage reduction following treatment with different concentrations of gambas solution

Group	Mean bacterial count (CFU/cm²)	SD (CFU/cm²)	95% CI	Reduction vs. negative control (CFU/cm²)	Reduction (%)
Negative control	3.29×10^4	2.35×10^3	2.91×10^4–3.66×10^4	Reference	Reference
Positive control	1.87×10^4	1.63×10^3	1.61×10^4–2.13×10^4	1.42×10^4	43.12
Gambas 25%	1.46×10^4	6.20×10^2	1.36×10^4–1.56×10^4	1.83×10^4	55.49
Gambas 30%	1.36×10^4	4.17×10^3	6.92×10^3–2.02×10^4	1.93×10^4	58.76
Gambas 35%	9.35×10^3	2.43×10^3	5.48×10^3–1.32×10^4	2.36×10^4	71.56
Gambas 40%	7.24×10^3	6.72×10^3	-3.45×10^3–1.79×10^4	2.57×10^4	77.98

Group	Mean bacterial count (CFU/cm²)	SD (CFU/cm²)	95% CI	Reduction vs. negative control (CFU/cm²)	Reduction (%)
Gambas 45%	2.22×10^3	2.46×10^3	-1.70×10^3–6.14×10^3	3.07×10^4	93.25

Table 2. Overall and pairwise differences in bacterial counts among control and gambas solution groups

Analysis	Comparison	Mean difference (CFU/cm²)	p-value	Interpretation
One-way ANOVA	All groups	—	<0.001	Significant overall difference
Games–Howell	Negative control vs. positive control	1.42×10^4	0.001	Significant
	Negative control vs. 25%	1.82×10^4	0.002	Significant
	Negative control vs. 30%	1.93×10^4	0.005	Significant
	Negative control vs. 35%	2.35×10^4	<0.001	Significant
	Negative control vs. 40%	2.56×10^4	0.017	Significant
	Negative control vs. 45%	3.07×10^4	<0.001	Significant
	Positive control vs. 35%	9.35×10^3	0.010	Significant
	Positive control vs. 45%	1.65×10^4	0.001	Significant
	25% vs. 45%	1.24×10^4	0.008	Significant
	30% vs. 45%	1.13×10^4	0.044	Significant
	Other pairwise comparisons	—	>0.05	Not significant

Table 2 shows that there was a statistically significant overall difference in bacterial counts between the study groups, as determined by a one-way ANOVA ($p<0.001$). Further analysis using the Games–Howell test showed that all concentrations of the gambas solution differed significantly from the negative control; thus, the application of the gambas solution at all tested concentrations was associated with lower bacterial counts compared with washing using running water. The most pronounced difference was observed at the 45% concentration, which differed significantly from both the positive control and the 25% and 30% concentrations. Conversely, not all increases in concentration resulted in statistically significant differences. Some comparisons between closely related concentrations, such as 25% versus 30% and 35% versus 40%, did not show significant differences. These findings suggest a pattern of decreasing bacterial counts as concentration increases, but the statistical evidence does not support consistent differences with every increase in concentration..

Table 3. Effectiveness of gambas solution at different concentrations in reducing bacterial counts on tablespoons

Treatment	Mean reduction (CFU/cm²)	Effectiveness (%)
Positive control	1.42×10^4	43.12
Gambas 25%	1.83×10^4	55.49
Gambas 30%	1.93×10^4	58.76
Gambas 35%	2.36×10^4	71.56
Gambas 40%	2.57×10^4	77.98
Gambas 45%	3.07×10^4	93.25

Table 3 shows a trend towards increased efficacy as the concentration of the gambas solution increases. The positive control showed the lowest efficacy compared with all the gambas treatment groups, whilst the 45% concentration produced the highest efficacy. The increase in efficacy began to become more apparent at a concentration of 35% and continued to rise at 40%, before reaching its highest value at 45%. Overall, these results indicate that higher concentrations tend to result in a greater reduction in bacterial count under the experimental conditions used. However, as not all comparisons between concentrations showed statistically significant differences, these efficacy results are best viewed as an empirical pattern of increasing bacterial reduction, rather than as evidence that every increase in concentration always results in a significant increase in effect.

Discussion

The results of the study indicate that the application of a gambas solution (*Luffa acutangula*) using a tablespoon is associated with a reduction in bacterial count compared with washing using running water. This pattern of reduction was more pronounced at higher solution concentrations, although not all increases in concentration resulted in statistically significant differences. These findings suggest that the bacterial response to the gambas treatment was not entirely linear at every concentration level; therefore, a more accurate interpretation is that there was a tendency for the reduction in bacteria to increase with rising concentration, with the highest concentration tested yielding the strongest response under the study conditions. This pattern is significant because the research results not only demonstrate differences between groups but also show that the magnitude of the response can be influenced by the concentration of the substance used.

The reduction in bacterial counts on the surface of spoons can be understood in the context of the sanitisation of food-contact surfaces. Cutlery is a type of surface that can come into direct contact with food and may harbour microorganisms if the cleaning process fails to remove all contaminants. Research in restaurant environments has shown that various food-contact surfaces, including spoons and cutlery, can be subject to microbiological contamination even though they appear visually clean [8]. Surface contamination is also influenced by the presence of organic residues, surface conditions, humidity, and the characteristics of the microorganisms adhering to the surface [9]. Therefore, the reduction in bacterial count following treatment in this study can be regarded as an indication that additional treatment after the washing process is capable of reducing the microbiological load on the surfaces tested.

One possible explanation for this pattern is the presence of bioactive compounds in *L. acutangula*. Phytochemical studies have shown that this plant contains various groups of secondary metabolites, including flavonoids, phenolics, saponins, tannins, terpenoids and other groups of compounds that may contribute to its biological activity [10]. Research[11] also reported antimicrobial activity in the bark extract of *L. acutangula* and identified several groups of secondary metabolites in the extract. These findings provide a plausible biological basis for explaining why the gambas solution in this study may be associated with a reduction in bacterial counts; however, they cannot be used to confirm specific compounds as the primary cause, as this study did not undertake phytochemical characterisation or testing of the antibacterial mechanism.

The results of this study are consistent with previous experimental evidence regarding the antibacterial activity of *L. acutangula*. [12]reported that gambas fruit extract exhibited inhibitory activity against *Clostridium perfringens* in laboratory tests using various concentrations of the extract. The study [7][13]It also showed that the bark extract of *L. acutangula* exhibited antimicrobial activity against the test microorganisms, with variations in activity depending on the type of solvent used. Differences in extraction methods and the plant parts used are important when comparing these results with those of this study. Previous studies generally utilised extracts obtained through specific extraction processes, whereas this study employed a gambas solution as a treatment applied to the surface of spoons.

Consequently, the consistency of the results is best understood as supporting the possibility of antimicrobial activity in the gambas plant, rather than as evidence that the mechanism or potency of this activity is identical across all dosage forms.

The trend of increased bacterial inhibition at higher concentrations is also consistent with the general principle that the antimicrobial activity of plant-based materials can be influenced by the concentration of active compounds available in the test system. Phenolic compounds, flavonoids, terpenoids, alkaloids and saponins have been reported to possess various mechanisms of inhibition against microorganisms, including disruption of cell membranes, changes in membrane permeability, interactions with proteins, and disruption of cellular metabolic processes [13][14]. However, the relationship between the concentration of the substance and its antimicrobial activity is not always straightforward, as efficacy can be influenced by the type of compound, solubility, stability, pH, matrix composition, and the characteristics of the microorganisms being tested[15][16][15]. Therefore, the concentration patterns identified in this study should be understood as an empirical response to the experimental conditions employed, rather than as a universally applicable dose–response relationship.

The finding that some closely spaced concentrations did not show statistically significant differences also has scientific relevance. An increase in the concentration of an antimicrobial agent does not always result in a proportional increase in effect, as microbiological activity is influenced by interactions between the active ingredient, the microorganisms and surface conditions. On food-contact surfaces, the presence of organic residues and biofilm structures can reduce the access of antimicrobial agents to microbial cells, meaning that the response to treatment may differ from that observed in tests using bacterial suspensions or culture media [17][18]. Studies on the effectiveness of sanitisers against *Bacillus cereus* have also shown that the presence of biofilm can reduce the effectiveness of sanitising agents on various types of food-contact surfaces[19]. Thus, the fact that some of the comparisons between concentrations were not statistically significant does not contradict the overall downward trend in bacterial counts.

Another aspect to consider is that washing and sanitisation are two distinct stages with different functions. Washing is primarily intended to remove dirt and residues from surfaces, whilst sanitisation is aimed at reducing the number of microorganisms to an acceptable level in line with food safety objectives [20]. If organic residues remain on the surface, the effectiveness of the antimicrobial agent may be altered, as some of the active ingredients may interact with the organic matter before reaching the bacterial cells. This explains why test results on the surfaces of tableware cannot be directly equated with the results of testing plant extracts in culture media. Research on the sanitation of food-contact surfaces indicates that the type of surface, initial cleanliness, the presence of biofilm, the concentration of the agent, and contact time can all influence the success of the microorganism inactivation process [20][21].

From a food safety perspective, the findings of this study provide preliminary evidence that plant-based materials could be investigated further as part of a microbiological control approach for tableware. Research into plant-based antimicrobials indicates that various natural compounds exhibit activity against foodborne microorganisms; however, their application requires consideration of stability, effective concentration, matrix characteristics, safety and potential changes in sensory properties [15] [14]In the context of this research, this potential is more appropriately regarded as a basis for further development and testing, rather than as a basis for replacing the sanitising agents already in use in food hygiene practice. The use of natural substances on surfaces that come into contact with food also requires evidence that their residues are safe, do not affect the characteristics of the food, and do not cause any undesirable consequences for equipment or users.

The research findings should also be interpreted in light of the study's design characteristics. This study utilised a relatively small sample size and was conducted under experimental conditions with specific concentrations; consequently, the results cannot yet be generalised to all types of tableware, washing conditions, or food-service environments. Furthermore, whilst the study measured bacterial counts quantitatively, it did not identify the specific species or groups of bacteria that showed a reduction. Consequently, this study cannot yet explain whether the observed response stems primarily from specific bacteria or represents a change in the bacterial population as a whole. It is important to bear in mind similar limitations, as the microbial community on food-contact surfaces may consist of various groups of bacteria with differing survival capabilities, biofilm formation abilities, and sensitivities to antimicrobial agents[22].

The relatively high variability observed in some of the high-concentration treatments also indicates that the response to the beetroot solution was not entirely uniform across replicates. This variation may be related to heterogeneity in initial contamination, the distribution of the substance on the surface, the characteristics of the spoon's surface, or differences in microbiological conditions within each sample. Research on biofilms and food contact surfaces suggests that microorganisms may be distributed unevenly and that the physical characteristics of the surface can influence adhesion and the success of the cleaning process [23][24]. Therefore, studies with a larger number of replicates are needed to obtain more stable estimates of the effect and to test whether the patterns observed remain consistent under different conditions.

Future research should develop a more comprehensive approach by conducting a factorial design to test variations in concentration and contact time, so that the interaction between these two factors can be evaluated. The testing should also include the identification of dominant microorganisms, including relevant indicator bacteria and foodborne pathogens, as well as an assessment of the solution's ability to inhibit or remove biofilms. This approach is important because the effectiveness of antimicrobial agents against planktonic cells does not always reflect their effectiveness against bacteria attached to surfaces or present within a biofilm matrix. [25]. Furthermore, phytochemical characterisation and testing of the antibacterial mechanism are required to identify the compounds or groups of compounds that are likely to contribute to the observed activity.

Conclusions

This study shows that a gambas solution (*Luffa acutangula*) is associated with a reduction in bacterial counts on the surface of tablespoons, with a greater reduction observed at higher concentrations. Differences in bacterial counts between groups indicate that the treatments produced varying responses, although increasing the concentration did not always result in a significant difference at every concentration level. The highest concentration tested showed the greatest reduction in bacteria under the experimental conditions of this study. These findings provide preliminary evidence regarding the potential use of gambas as a plant-based material to help reduce the bacterial load on cutlery, but are not yet sufficient to conclude its effectiveness as a direct alternative to conventionally used sanitising agents.

Further research should evaluate a wider range of concentrations and contact times, identify the active compounds responsible for the antibacterial activity, and test their effectiveness against various types of microorganisms and under different surface conditions on tableware. Studies with a larger sample size and greater replication, as well as testing under real-world restaurant conditions, are also required to assess the consistency of the results and the feasibility of their application in food hygiene and sanitation practices.

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Conflicts of Interest

No aplicable

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