Performance of two plant-based disinfectants for *Escherichia coli* inactivation

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Highlights:

- *H. esculentus* showed higher performance than *L. cylindrica*, achieving a maximum *E. coli* inactivation of 1.12 ± 0.16 log.
- Adding plant-based disinfectants into water may inadvertently increase the *E. coli* instead of decreasing due to its organic load.
- Among the parameters studied, dose exhibited the most significant influence on *H. esculentus*' disinfection efficacy.
- The performance of *L. cylindrica* was influenced by interactions among dose, contact time, and pH levels.

Keywords: disinfection; *Luffa cylindrica*; *Hibiscus esculentus*

INTRODUCTION

The utilization of conventional disinfectants poses drawbacks such as the generation of harmful by-products and potential risks during handling. These counterpoints motivate researchers to focus the studies on alternative options, like the plant-based disinfectants.

Luffa cylindrica, commonly known as vegetable sponge, contains an important antimicrobial group, Ribosome-Inactivating Proteins (RIPs), distributed across various parts of the plant, including roots, stems, leaves, flowers, fruits, and seeds (Stirpe, 2004). These RIPs are responsible for enzymatic damage to ribosomes, leading to allergic, cytotoxic, and antiviral effects against a broad spectrum of microorganisms (Shaheed et al, 2009).

Hibiscus esculentus, known colloquially as okra and often utilized as a dietary staple, exhibits attributes that hinder bacterial adhesion to gastrointestinal linings (Lengsfeld et al., 2004; Lengsfeld et al, 2007). These findings spurred a further investigation into the plant's antimicrobial properties, as exemplified by Carvalho et al (2001) in disinfection assays with different dosages of okra extract.

Nevertheless, there remain gaps in understanding the performance of *L. cylindrica* and *H. esculentus* disinfectants prepared through simplified techniques, particularly concerning their applicability in isolated communities. Based on this, our objective was to assess the potential of *L. cylindrica* leaf

powder and *H. esculentus* fruit extract as disinfectants for inactivating *E. coli* through batch factorial tests.

METHODOLOGY

The study utilized groundwater inoculated with 10^3 CFU/100mL of *E. coli* culture in 250 mL volumes. The efficacy of two plant-based disinfectants derived from *H. esculentus* fruit extract and powdered *L. cylindrica* leaves was assessed for *E. coli* inactivation. Various combinations of dosage, contact time (CT), and pH levels were employed in each disinfection trial.

A 3^3 factorial design was implemented to examine the impact of dose, CT, and pH on *E. coli* inactivation. The performance of *H. esculentus* extract was analyzed at doses of 1, 25 and 50 g/L over CT durations of 5, 30 and 60 min, across low, neutral, and high pH ranges. Similarly, *L. cylindrica* powder was evaluated under the same CT and pH conditions but with lower doses (0.1, 0.25, and 1 g/L).

E. coli quantification was by membrane filter technique following APHA et al. (2012) recommendations. The number of *E. coli* before and after the disinfection trials provided insight into the performance of each condition. Factorial analysis was carried out using Minitab software.

RESULTS AND CONCLUSIONS

Extract of *H. esculentus* in water favored more the growth than the reduction of *E. coli* in almost all conditions tested (Table 1), particularly at highest doses (25 and 50 g/L), attributable to the organic load introduced into the water. However, tests using the lowest dose (1.0 g/L) across neutral pH exhibited *E. coli* inactivation after 5 min. Factorial analysis revealed that only dosage influenced inactivation significantly ($p = 0.003$), despite pH values also seeming to demonstrate some influence.

Table 1 - Performance of two plant-based disinfectants for *E. coli* inactivation.

***Hibiscus esculentus* fruits extract**										
D (g L^{-1})		1.0			25			50		
CT (min)		5	30	60	5	30	60	5	30	60
pH	4.86	- 0.39 ± 0.08	- 0.47 ± 0.12	- 0.57 ± 0.10	- 0.69 ± 0.11	- 0.64 ± 0.06	- 0.64 ± 0.06	- 0.96 ± 0.15	- 0.96 ± 0.11	- 1.03 ± 0.12
	6.78	**1.06 ± 0.12**	**1.12 ± 0.16**	**0.94 ± 0.10**	- 0.37 ± 0.08	- 0.43 ± 0.11	- 0.42 ± 0.07	- 0.46 ± 0.01	- 0.64 ± 0.10	- 0.66 ± 0.11
	9.94	- 0.24 ± 0.19	- 0.25 ± 0.32	- 0.21 ± 0.17	- 0.66 ± 0.11	- 0.64 ± 0.10	- 0.66 ± 0.11	- 0.42 ± 0.29	- 0.61 ± 0.05	- 0.76 ± 0.06
***Luffa cylindrica* leaves powdered**										
D (g L^{-1})		0.1			0.25			1.0		
CT (min)		5	30	60	5	30	60	5	30	60
pH	2.73	**0.15 ± 0.12**	- 0.02 ± 0.12	- 0.14 ± 0.10	**0.03 ± 0.06**	**0.05 ± 0.15**	**0.29 ± 0.18**	- 0.11 ± 0.15	**0.24 ± 0.29**	**0.72 ± 0.33**
	7.33	**0.20 ± 0.04**	- 0.03 ± 0.12	- 0.25 ± 0.06	**0.00 ± 0.23**	**0.02 ± 0.07**	**0.16 ± 0.17**	- 0.01 ± 0.07	- 0.12 ± 0.12	- 0.32 ± 0.04
	9.71	- 0.02 ± 0.05	- 0.19 ± 0.05	- 0.22 ± 0.04	**0.34 ± 0.12**	**0.18 ± 0.14**	**0.03 ± 0.18**	**0.22 ± 0.37**	**0.18 ± 0.34**	- 0.35 ± 0.10

Notes: D: dose, CT: contact time, values in bold indicate positive inactivation

The optimal disinfection condition using *H. esculentus* extract (1 g/L, 30 min CT, pH 6.78) achieved a 1.12 ± 0.16 log *E. coli* inactivation, surpassing the 0.37 log reported by Carvalho et al. (2011) (97.7 g/L, 30 min CT). Differences in water quality, counting techniques, and trial conditions between studies may have contributed to these varied performance values. It is important to note that Carvalho et al. (2011) applied a dose almost 100 times higher, which, if applied to our study, would probably negatively affect its performance. Moreover, such high doses could potentially pose food safety concerns, given the primary culinary use of okra.

In all conditions using *L. cylindrica*, log values were predominantly close to zero, consequently, the influence of each parameter on *L. cylindrica*'s performance is less visually discernible than that of *H. esculentus*. The interaction CT*pH exerted the most significant influence on performance ($p = 0.001$), followed by the interaction among all three parameters ($p = 0.005$). pH was the sole isolated parameter with a significant effect on E. coli inactivation ($p = 0.038$).

None of the conditions employing *L. cylindrica* reached 1.0 log, with the highest being 0.72 ± 0.33 log (1.0 g/L, 60 min CT, 2.73 pH). Given the infrequency of natural water with low pH, this maximum performance may not be readily achievable. Nonetheless, this result falls within the range previously reported by Shaheed et al. (2009) using fruit extract (0.23 log) and seed extract (0.88 log). Despite the superior performance of seeds, their limited quantity per plant might render them less viable for isolated communities, as this restricts the amount of produced disinfectant, thereby limiting the volume that can be treated.

The availability and simple preparation of these plant-based disinfectants may prove attractive for home systems in isolated communities. However, further exploratory studies are warranted to optimize and enhance disinfection performance.

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