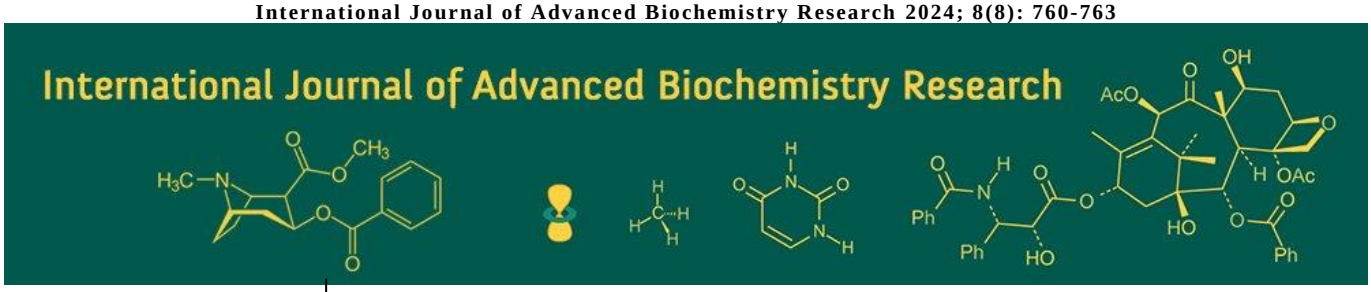

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Shuddi and its variants: natural alternatives to chemical floor cleaners and disinfectants

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Abstract
This study assesses the effectiveness of Shuddi Multipurpose Spray and Multipurpose Liquid, which are eco-friendly enzyme-based cleaning and disinfecting products created by Multiplex Group of Companies. These products use cell-free extracts of beneficial microbes, specifically *Bacillus* spp. and *Aspergillus* spp., obtained through fermentation. They also contain antimicrobial enzymes such as amylase, cellulase, pectinase, lipase, and protease. A quantitative analysis of the enzymes showed that the commercial product had the highest total enzyme concentration (1.511 mg/ml), followed by Shuddi Multipurpose Spray (1.102 mg/ml) and Shuddi Multipurpose Liquid (1.000 mg/ml). The cleaning efficacy, tested according to ASTM D4488-95-A5 standards, revealed that the commercial product had the highest efficacy (98.3%), with Shuddi Multipurpose Spray at 92.8%, and Shuddi Multipurpose Liquid at 73.6% when diluted (1:100 at 10 °C). Regarding disinfectant efficacy, assessed using a suspension time-kill procedure, all products achieved >99.9% kill against *S. aureus*, *P. aeruginosa*, *E. coli*, *C. albicans*, and *A. niger* within 30 seconds. These findings underline the impact of enzyme concentration, dilution, and temperature on the cleaning performance of enzyme-based products. Shuddi products, which are free from harsh chemicals, artificial fragrances, and synthetic dyes, provide a safer and environmentally friendly alternative to conventional chemical cleaners. The results support the potential of enzyme-based cleaners and disinfectants as effective and sustainable solutions for maintaining hygiene in various conditions.

Keywords: Cleaning agents, disinfecting agents, Enzymes based products, Shuddi and environmentally friendly

1. Introduction
Hygiene plays a crucial role in reducing the burden of infections, both in developing countries and among specialized populations. Chlorinated phenols found in toilet bowl cleaners are toxic to the respiratory and circulatory systems. Tile cleansers, usually liquid in nature, help remove dirt, dust, stains, foul smells, and clutter from surfaces. However, many of these contain strong chemicals, and continuous exposure to such chemicals may lead to various acute and chronic ailments, ranging from allergic colds and skin irritation to serious diseases like bronchial asthma and cancer (IARC, 2010) [8].

Conventional chemical cleaners, which often contain harsh substances like ammonia, chlorine, and various acids, are widely used for their powerful cleaning and disinfecting properties. However, these cleaners come with significant downsides. They can cause respiratory issues, skin irritation, and other health problems due to their toxic ingredients (AIHA, 2020) [1]. Additionally, the strong fumes and residues left by these chemicals can be harmful, especially in households with children and pets (ASPCA, 2023) [2]. Environmental concerns are also paramount, as chemical runoff can contaminate water sources and harm aquatic life. Homes, hospitals, and other healthcare settings extensively use antiseptics and disinfectants to control microbial growth on living tissues and inanimate objects (Saha *et al*., 2009) [12]. The term "disinfectant" specifically refers to chemical agents used to disinfect inanimate/animate objects. These agents aim to reduce or eliminate pathogenic microbes on materials, rendering them non-hazardous.

Various classes of chemical disinfectants exist, including acids and their esters, alcohols, aldehydes, biguanides, halogens, heavy metals, oxidizing compounds, phenols and phenolic compounds, surface-active agents, quinoline and isoquinoline derivatives, and dyes.

However, the continuous use of these chemical disinfectants has raised concerns due to environmental damage and the development of resistance among pathogens. Some clinical isolates have shown resistance to disinfectant formulations, even at manufacturer-prescribed dilutions (EPA, 2021) [5].

In recent years, there has been a growing interest in natural and eco-friendly cleaning solutions. In response to these concerns, the Multiplex Group of Companies has developed a bio-based solution for cleaning and disinfecting purposes. This eco-friendly approach aims to reduce reliance on chemical disinfectants, promoting a safer and greener environment. The Indian patent for the procedure viz., Compositions and methods for augmenting the capabilities of the population of biological agents thereby facilitating growth survivability and functionalities thereof. Which relates the method of treating waste using proprietary product is selected from proprietary product 1 for water purification, proprietary product 2 for decomposition of biodegradable waste, proprietary product 3 for toilet& floor cleaning, proprietary product 4 for house fly repellent, proprietary product 5 for mosquito repellent, proprietary product 6 for rat repellent or proprietary product 7 as plant growth promoter. The Indian patent was received on 13 July 2021 from the National Biodiversity Authority bearing patent number 201841041691.

Shuddi and its variants offer effective, natural alternatives to traditional chemical floor cleaners and disinfectants. Made from the fermentation of beneficial microbes, Shuddi formulations include antimicrobial extracts and are free from harsh chemicals, artificial fragrances, and synthetic dyes. This makes them safer for humans, pets, and the environment.

Our study focuses on Shuddi Multipurpose spray and Multipurpose liquid, which are highly suitable for a wide range of surfaces, removing dirt, grime, and stains while leaving a pleasant natural scent. They can be used as floor cleaners, which are designed for deep cleaning floors without residue, gentle on hardwood, tile, and laminate. As disinfectants, they can target high-touch areas, killing germs and bacteria without harsh chemicals. In addition to this, they have other benefits of creating a healthier living environment and reducing the risks of respiratory issues and skin irritation. They are eco-friendly because of their biodegradable properties, directly reducing environmental impact. Their non-toxic formula ensures safety for pets and children.

Shuddi's natural composition, effectiveness, and environmental benefits make it an excellent choice for those seeking a cleaner, safer, and more sustainable home. As awareness of chemical cleaner hazards grows, natural solutions like Shuddi are becoming household staples worldwide. Our study aims to evaluate the cleaning and disinfecting ability of the commercial product (Shuddi Multipurpose spray and Multipurpose liquid).

2. Materials and Methods

Shuddi Multipurpose spray and Multipurpose liquid are innovative products that use the cell-free extract of beneficial microbes obtained through fermentation. The production process begins with the fermentation of specific strains of microorganisms (*Bacillus* spp. and *Aspergillus* spp.) in a controlled environment. These selected microbes are known for their ability to produce enzymes that effectively break down organic matter and eliminate pathogens. The active components responsible for the cleaning and disinfecting properties of the product are the enzymes found in the cell-free extract. These enzymes are capable of degrading various organic materials, such as fats, proteins, and carbohydrates, making them highly effective at removing dirt and grime. Additionally, certain enzymes possess antimicrobial properties, enabling them to kill or inhibit the growth of harmful bacteria, viruses, and fungi. This ground-breaking cleaner has secured an Indian patent, highlighting its uniqueness and potential impact.

2.2 Qualitative and quantitative analysis of enzymes

Cellulase activity was evaluated by incubating a medium consisting of 5 mL of Winogradsky saline solution and 10 g carboxymethylcellulose at 28 °C for 4 days. The development of hydrolysis halos was observed using Congo red and NaCl solutions, following. Chitinase activity was determined by treating colloidal chitin with phosphoric acid, keeping it at 4 °C for 24 hours, neutralizing it to pH, and sterilizing it at 121 °C, following the procedure outlined by (Romero-Cortes *et al*., 2016) [16]. Pectinase activity was tested using Hankin's medium, consisting of yeast extract, pectin, agar (Solution A), and various salts (Solution B), all sterilized at 121 °C as described by (Hankin *et al*., 1971) [17]. The Shuddi Multipurpose was inoculated and incubated at 28 °C for 3 days. Lugol's solution was used to detect pectinase activity.

2.3 Cleaning ability

To compare how well products clean resilient flooring and washable walls, we used the ASTM D4488-95-A5 (reapproved 2001) Standard Guide for Testing Cleaning Performance of Products Intended for Use on Resilient Flooring and Washable Walls: Particulate and Oily Soil/Vinyl tiles test method.

The cleaning efficiency can be calculated using the formula:

% Cleaning Efficiency = (Rc - Rs) / (Ro - Rs) x 100

Where Rc = cleaned reflectance; Ro = original reflectance; Rs = soiled reflectance.

2.4 Antibacterial activity and efficacy of test Substance:

The test sample was sent to Skanda Life Sciences Pvt. Ltd. in Bangalore, Karnataka. The antibacterial activity and efficacy of the test substance were assessed using a suspension time-kill procedure. *S. aureus*, *P. aeruginosa*, and *E. coli* cell suspensions were prepared individually using tryptone broth, and then incubated for 24 hours at 37±1 °C. Similarly, *C. albicans* and *A. niger* cell suspensions were prepared and grown in potato dextrose broth, then incubated for 24-48 hours at 27±2 °C. The suspension of the test microorganisms was standardized to a minimum concentration of 1-2 x 10^6 CFU/mL using diluents by spectrophotometry. The testing involved exposure times of 15 and 30 seconds with the test pathogens (CLSI, 1999) [4].

Calculation of results was based on the following formulas:

Percentage reduction = (B-A/B) x 100

Log 10 Reduction = Log (B/A)

Where:

B = Number of viable test microorganisms in the control substance immediately after inoculation

A = Number of viable test microorganisms in the test substance after the contact time.

3. Results

3.1 Quantitative analysis of enzymes from the products/test sample

The commercial product has the highest total enzyme concentration at 1.511 mg/mL, followed by Shuddi Multipurpose Spray at 1.102 mg/mL, and Shuddi Multipurpose Liquid at 1.000 mg/mL. Shuddi Multipurpose Liquid has the highest concentration of Pectinase at 0.980 mg/mL. The Commercial product exhibits the highest Amylase concentration at 0.732 mg/mL. The percentage of unknown concentration is highest in the commercial product at 6.04% and lowest in Shuddi Multipurpose Liquid at 4.00%. The qualitative analysis revealed that the enzymes Amylase, Cellulase, Pectinase, Lipase, and Protease are present in both of the Shuddi products.

Table 1: Qualitative and quantitative analysis of enzymes in the test products

Sl. No.	Testing sample	Amylase	Cellulase	Pectinase	Lipase	Protease	All enzymes concentration	% of unknown concentration
1	Shuddi Multipurpose Spray	0.206	0.174	0.100	0.331	0.291	1.102	4.408
2	Shuddi Multipurpose Liquid	0.200	0.168	0.980	0.330	0.250	1.000	4.00
3	Commercial product	0.732	0.137	0.172	0.208	0.261	1.511	6.04

3.2 Evaluation of the cleaning efficacy of the test products

The commercial product demonstrated the highest cleaning efficacy at 98.3% when used in its ready-to-use form, making it the most effective cleaner among the tested products. Shuddi Multipurpose Spray also used in its ready-to-use form, showed a cleaning efficacy of 92.8%, indicating strong performance. Shuddi Multipurpose, when diluted (1:100 at 10 °C in tap water), had the lowest cleaning efficacy at 73.6%, suggesting that dilution and temperature conditions can significantly impact the performance of enzyme-based cleaners.

Table 2: Cleaning efficacy of test samples

Product	Dilution	Avg. % C.E.
Shuddi Multipurpose Spray LC#21-T0825	RTU	92.8
Shuddi Multipurpose Liquid LC#21-T0829	1:100 @ 10 °C, in tap water	73.6
Commercial Product LC#21-T0827	RTU	98.3

3.3 Evaluation of the disinfectant efficacy of the test products

The results of the time-kill inhibition test for the sample against the test pathogens are shown in Table 3. The test sample has successfully met the antibacterial test criteria by demonstrating a >3 Log 10 reduction (>99.9% kill) against *S. aureus*, *P. aeruginosa*, and *E. coli* after 30 seconds of exposure. Similarly, the test sample has met the antifungal test criteria by demonstrating a >3 Log 10 reduction (>99.9% kill) against *C. albicans* and *A. niger* after 30 seconds of exposure.

Table 3: Disinfectant efficacy of test samples Shuddi Multipurpose Spray

Organism	*S. aureus*		*P. aeruginosa*		*E. coli*		*C. albicans*		*A. niger*	
Contact Time (sec.)	Log 10 reduction	% Reduction	Log 10 reduction	% Reduction	Log 10 reduction	% Reduction	Log 10 reduction	% Reduction	Log 10 reduction	% Reduction
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
15	1.195	93.610	1.324	95.257	1.549	97.173	1.865	98.636	1.646	97.739
30	3.291	99.949	3.517	99.970	3.818	99.985	3.736	99.982	3.717	99.981

4. Discussion

The analysis of enzyme concentrations and cleaning efficacy, along with the disinfectant efficacy of various test samples, provides valuable insights into the performance of these products. The data presented is crucial for evaluating the effectiveness of enzyme-based cleaners and disinfectants. The quantitative analysis of enzymes shows that the Shuddi Multipurpose Spray (sample 1) and Shuddi Multipurpose liquid (sample 2) contain varying concentrations of amylase, cellulase, pectinase, lipase, and protease. The commercial product (sample 3) has the highest overall concentration of enzymes (1.511), followed by Shuddi.

The commercial product shows a significantly higher amylase concentration (0.732) compared to the Shuddi products. This indicates that enzyme concentration could be a distinguishing factor in the effectiveness of the products. The results of the present study are consistent with the findings of Brown and Green (2023) [3]. The data on cleaning effectiveness shows that all products perform well, but there are variations in their performance. The Shuddi Multipurpose Spray (Ready to Use) achieved an average cleaning effectiveness of 92.8%, which is commendable but lower than the Commercial product (Ready to use), which achieved 98.3%. The Shuddi Multipurpose Liquid (diluted 1:100 in tap water at 10 °C) had the lowest effectiveness at 73.6%. This suggests that the cleaning performance of enzyme-based cleaners is significantly affected by dilution and temperature conditions.

These findings are in line with studies that demonstrate how enzyme activity can be affected by environmental conditions such as temperature and pH (Jones and Wang, 2021) [9]. Shuddi products suggest that enzyme concentration could be a differentiating factor in product effectiveness. The results of the current study align with those of Brown and Green (2023) [3]. The cleaning efficacy data shows that all products perform well, but there are differences in their performance. The Shuddi Multipurpose Spray (RTU) achieved an average cleaning efficacy of 92.8%, which is commendable but lower than the Commercial product

(RTU), which achieved 98.3%. The Shuddi Multipurpose Liquid (diluted 1:100 in tap water at 10 °C) had the lowest efficacy at 73.6%. This suggests that the dilution and temperature conditions significantly impact the cleaning performance of enzyme-based cleaners (NRDC, 2022) [11]. These findings are consistent with studies showing that enzyme activity can be influenced by environmental conditions such as temperature and pH (Jones and Wang, 2021) [9].

The results of the disinfectant efficacy tests indicate that all samples demonstrated strong antimicrobial activity against common pathogens such *as S. aureus*, *P. aeruginosa*, *E. coli*, *C. albicans*, and *A. niger*. The data shows a significant reduction in microbial counts within 15 seconds, which further improved after 30 seconds of exposure. Specifically, the test samples achieved more than a 3 Log 10 reduction (>99.9% kill) against the mentioned pathogens within 30 seconds, meeting the criteria for effective disinfectants. This high efficacy aligns with literature that notes enzyme-based disinfectants for their effectiveness in disrupting biofilms and enhancing microbial inactivation compared to traditional chemical disinfectants (Williams and Gracia, 2023) [15]. The combination of enzymes in these products likely contributes to their effectiveness by breaking down microbial cell walls and other structural components, facilitating the antimicrobial action of other active ingredients.

The results highlight the potential of using enzyme-based cleaners and disinfectants as an effective alternative to traditional products. Further research could investigate the stability and activity of these enzymes in different environmental conditions, as well as the possibility of synergistic effects with other cleaning agents. Furthermore, it's important to study the impact of enzyme formulations on various surfaces and materials, and to assess their long-term safety and environmental impact. This research will contribute to the development of more effective, sustainable, and user-friendly cleaning solutions.

5. Conclusion

The patented enzyme-based floor cleaner and disinfectant is a significant innovation in the cleaning and disinfecting industry. By harnessing the power of beneficial microbes and their enzymes, this product offers a natural, effective, and environmentally friendly alternative to traditional chemical cleaners. The Indian patent for the synthesis protocol highlights the uniqueness and potential of this technology, paving the way for broader adoption and further advancement in green cleaning solutions.

6. Acknowledgement

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