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Comparative Evaluation of Commercial Chemical and Traditional Natural Disinfectants by Phenol Coefficient Test and Antibacterial Assays

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Abstract:

Disinfectants play a crucial role in eliminating harmful microorganisms from various surfaces, ensuring hygiene and reducing infection risks. This study compares the effectiveness of chemical and natural disinfectants, specifically neem-based and citrus-based with neemleaf extract and citrus fruit juice with phenol coefficient test and antimicrobial assay. Efficacy of both commercial and natural disinfectants were tested with Klebsiella spp and Staphylococcus spp were isolated from the laboratory surfaces . The results indicates that neem and citrus-based commercial disinfectants show significantly higher antimicrobial efficacy than natural extracts. However, natural disinfectants present eco-friendly and non-toxic alternatives, despite lower effectiveness. The findings highlight the potential for developing improved herbal disinfectant formulations to reduce dependence on chemical-based solutions.

Keywords: Disinfectants, Neem, Lemon, Phenol Coefficient, Antibacterial

1. INTRODUCTION

Neem and Citrus are two important traditional disinfecting agents in India, Disinfectants are antimicrobial agents applied to non-living surfaces to destroy microorganisms, though they may be less effective against resistant forms such as bacterial spores. They differ from antibiotics, which act inside the body, germicides for living tissues, and biocides, which target all life forms. In healthcare, disinfection and sterilization are essential to prevent hospital-acquired infections, achieved through surface cleaning, immersion of instruments, or chemical treatment of infectious waste. Efficacy depends on factors such as temperature, contact time, pH, concentration, organic load, and water hardness.Chemical disinfectants, such as phenol and its derivatives, are valued for broad-spectrum antimicrobial activity but pose health and environmental risks, including skin irritation, respiratory issues, toxicity, and promotion of antimicrobial resistance (AMR). Phenol, historically the first surgical antiseptic, remains a reference standard via the phenol coefficient for comparing disinfectant potency. Growing concerns over synthetic chemicals have driven interest in eco-friendly herbal alternatives. Neem (Azadirachta indica) contains bioactive compounds—saponins, alkaloids, flavonoids, tannins, azadirachtin, nimbin, and nimbolide—

that disrupt microbial cell structures and inhibit a wide range of bacteria, fungi, and viruses. Citrus essential oils, rich in limonene, citral, and flavonoids, also exhibit significant antimicrobial effects. These plant-based agents offer sustainable options for replacing synthetic disinfectants in healthcare and household settings.The agar well diffusion method and phenol coefficient test are reliable methods for evaluating antimicrobial efficacy, measuring inhibition zones to determine potency. Effective disinfectants—whether chemical or herbal—must be broad-spectrum, cost-effective, safe to handle, and environmentally sustainable to meet sanitation needs across healthcare, industrial, and domestic applications.

MATERIALS AND METHODS

A. Screening and identification of of Klebsiella and Staphylococcus from Laboratory Surface

Bacteria from contaminated laboratory benches were isolated using sterile cotton swabs and cultured on Mannitol salt agar and MacConkey agar. Plates were incubated at 37°C for 24–48 hours, and colonies were observed, sub-cultured, and stored for analysis. Klebsiella spp. were identified from MacConkey agar and Staphylococcus spp. from Mannitol salt agar using colony morphology, Gram staining, motility, and biochemical tests.(Nandhini R & Dr. V. Judia Harriet Sumathy 2016)

B.Determination of Phenol Coefficient Test of Commercial Neem and Citrus-based Disinfectants:

The two commercially available herbal disinfectants used in our study was neem based and citrus-based disinfectants brought from the supermarket at K G Chavadi, Coimbatore, Tamilnadu. Test tubes with different phenol dilutions (1:80, 1:90, 1:100) and test chemical dilutions (1:400, 1:450, 1:500) were prepared. 5 ml of these dilutions were added into test tube. With sterile 1ml pipette one ml of overnight nutrient broth culture of Staphylococcus species and Klebsiella species was added to all the test tube. The time of inoculation of bacteria into the disinfectant were recorded. The content of the tubes was mixed aseptically . At intervals 5, 10, 15 minutes one loop-full from each test tube was transferred to appropriately labelled sterile nutrient broth tubes. One sterile test tube of nutrient broth was kept as control. All the inoculated nutrient broth were incubated at 37°C for 24-48 hours. After incubation the tubes were observed for the growth of organisms and results was tabulated and phenol coefficient was determined.

$$\text{Phenol coefficient} = \frac{\text{Reciprocal of test chemical dilution}}{\text{Reciprocal of phenol dilutions}}$$

C..Determination of Phenol Coefficient Test of Natural Disinfectants Neem leaf extract and Citrus Fruit juice

Fresh neem leaf extract and citrus fruit juice were prepared immediately before use. Phenol dilutions 1:10, 1:20, 1:30 and 1:10, 1:20, 1:30 dilutions of neem leaf extract and citrus fruit juice were prepared. 5ml of these dilutions are added into test tubes. It is placed in the test tube rack. With sterile 1ml pipette one ml of 24 hours Nutrient broth culture of Staphylococcus species and Klebsiella species as added into each of the test tube of the disinfectant.The time of inoculation of bacteria into the disinfectant were recorded. The content of the tubes was mixed aseptically at intervals of 5, 10, 15 minutes.One loop-full from each test tube was transferred to appropriately labelled nutrient broth tubes. One sterile test tube of nutrient broth was kept as control. All the inoculated nutrient broth were incubated at 37°C for 24-48 hours. After

incubation the tubes were observed for the presence of organism and results were tabulated and phenol coefficient were determined.(Shital Lakhe & Sneha Khadse 2020).

$$\text{Phenol coefficient} = \frac{\text{Reciprocal of test chemical dilution}}{\text{Reciprocal of phenol dilutions}}$$

D. Determination of antibacterial activity of commercial neem based and citrus based disinfectant and traditional natural disinfectant neem leaf extract and citrus fruits by well diffusion method

The antibacterial properties of commercial and natural disinfectants were evaluated using the agar well diffusion method. Nutrient broth cultures of isolated bacteria, Klebsiella and Staphylococcus were incubated at 37°C for 24 hours and standardized to 0.5 McFarland (OD 570 nm). Four sterile Mueller Hinton Agar (MHA) plates were inoculated with standardised inoculum of Klebsiella and four MHA plates were inoculated with standardised inoculum of Staphylococcus and spread with sterile swabs. Four wells were made in all MHA plates with sterile borer.In both the plates inoculated with Klebiella and Staphylococcus three wells added with commercial neem based and citrus based disinfectant of 1:400,1:450,1:500 dilution one well with phenol as control.The plates thus prepared were kept at incubator at 37°C for 24 hours. After incubation for 24 hrs at 37°C, the plates were observed. The zone of inhibition was measured and expressed in mm for both commercial neem based and citrus based disinfectant.In another two plates inoculated with Klebiella and Staphylococcus three wells added with neem Leaf extract and citrus juice of 1:10,1:20,1:30 dilution and one well with phenol as control.The plates thus prepared were kept at incubator at 37°C for 24 hours. After incubation for 24 hrs at 37°C, the plates were observed. The zone of inhibition was measured and expressed in mm for both commercial neem leaf extract and citrus juice.

E.Determination of Effectiveness Disinfectants studied:

Surfaces from laboratory benches and washing areas were swabbed before and after cleaning with the commercial neem based and citrus based disinfectant and traditional natural disinfectant neem extract and citrus fruit. extract. Swabs were plated on nutrient agar, incubated at 37°C for 24 h, and CFU counts compared to evaluate post-cleaning bacterial reduction.

RESULT

A.Isolated colonies present in mannitol salt agar with yellow colony and change of colour of medium from pink to yellow were observed and the colony morphology indicated the presence of Staphylococcus spp. Large mucoid pink colonies were observed on MacConkey agar indicated the presence of Klebsiella spp which were further confirmed by staining and biochemical Tests.

Fig :1 Colony of Klebsiella on Mac Conkey and Colony of Staphylococcus on Mannitol Salt Agar

B.Determination of Phenol Coefficient Test of Commercial Neem and Citrus-based Disinfectants: Neem and citrus-based commercial disinfectants exhibited significantly higher phenol coefficients ranging from 4.4 –5.0 with highest activity for neem based disinfectant against Staphylococcus compared to natural extracts ranging from 0.5–2.5 with highest activity for citrus against Staphylococcus indicating stronger bactericidal activity.The results suggest that chemical modifications and additional active ingredients in commercial disinfectants enhance their efficacy.

Sl.No	Disinfectant	Phenol coefficient of Klebsiella	Phenol coefficient of Staphylococcus
1	Neem based Commercial Disinfectant	4.4	5
2	Citrus based Commercial Disinfectant	4	4.4
3	Neem leaves extract	0.5	1
4	Citrus fruit Juice	2	2.5

Fig 2: Neem Leaves and Citrus Fruits

C. Determination of antibacterial activity of commercial neem based and citrus based disinfectant and traditional natural disinfectant neem leaf extract and citrus fruits by well diffusion methodUsing various dilutions of commercial neem based and citrus based disinfectant and traditional natural disinfectant neem leaf extract and citrus fruits antibacterial activity was performed against isolated Klebsiella and Staphylococcus. The result indicated neem based chemical disinfectant exhibited in highest zone of inhibition in 1:450 dilution for Staphylococcus and for natural disinfectant citrus fruit juice had highest activity in 1:1 dilution against Staphylococcus. Antimicrobial activity was present for Chemical disinfectant and Natural Disinfectant against Staphylococcous where as Klebsiella found to be resistant for Chemical disinfectant and Neem leaf extract but sensitive to Citrus juice.

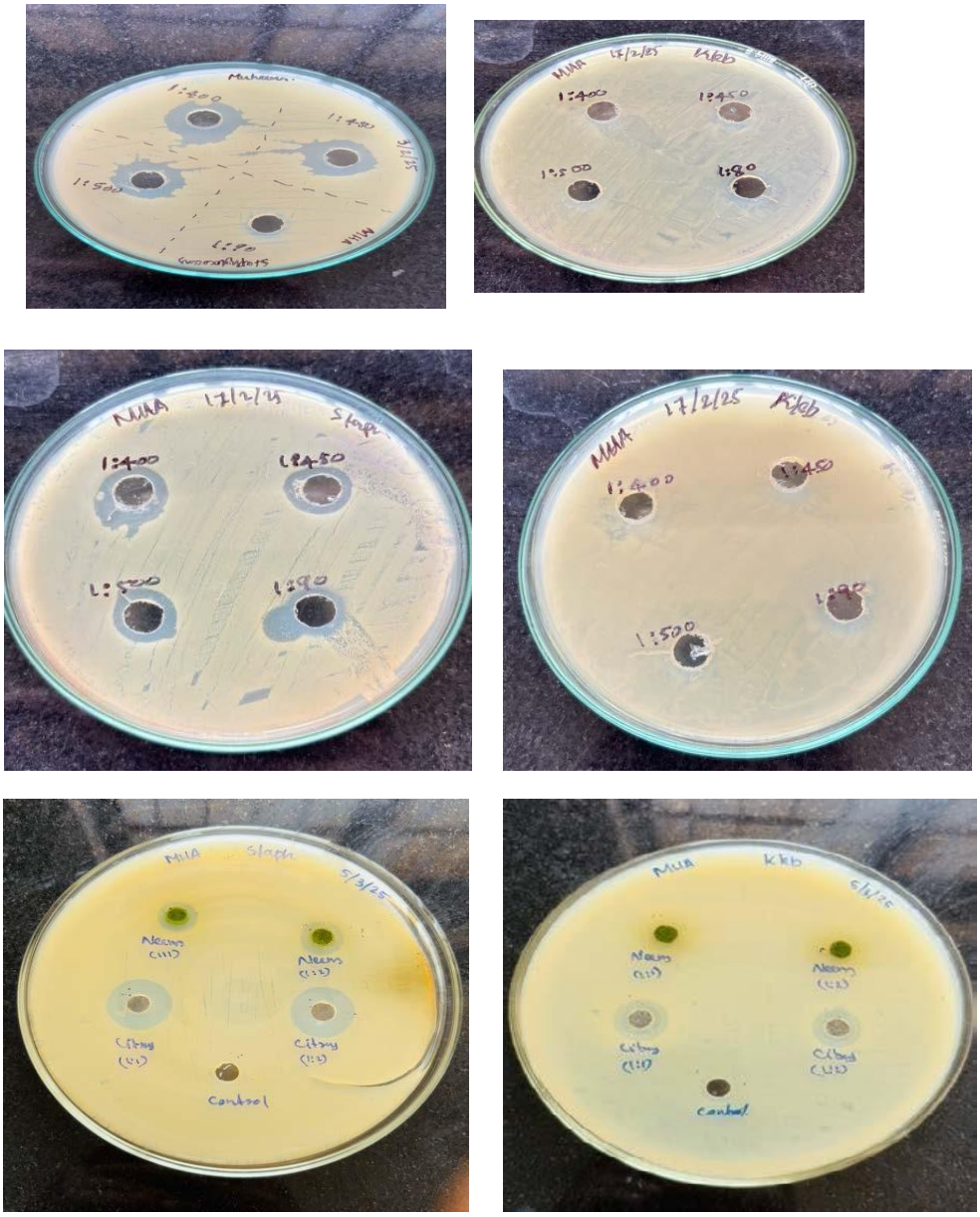

Fig 3: Antimicrobial efficacy of Commercial disinfectants and Natural Disinfectants

D.Determination of Effectiveness Disinfectants studied:

Swab samples were taken from benches before and after disinfection and inoculated on nutrient agar plates. Number of colonies were noted. It is found that neem based disinfectant is more effective than

citrus based disinfectant. In case of natural disinfectant, citrus fruit is more effective compared to neem extract.

Table 2 :Comparison of Effectiveness of commercial and traditional natural disinfectants.

Disinfectant	Number of colonies before disinfection	Number of colonies after disinfection	Percentage of reduction (%)
Neem based Disinfectant	156	2	97%
Citrus based disinfectant	148	10	86%
Neem leaves extract	106	30	68%
Citrus fruit juice	120	6	93%

DISCUSSION

The study assessed the antimicrobial properties of commercial and natural disinfectants using the phenol coefficient test and agar well diffusion method. Neem and citrus-based Lysol disinfectants exhibited .In conclusion, while commercial disinfectants provide superior antimicrobial protection, natural alternatives offer a safer, environmentally sustainable approach. Further research into formulation enhancement of herbal disinfectantscan contributes to the development of effective, non-toxic solutions for hygiene and infection control.

significantly higher phenol coefficients compared to natural extracts indicating stronger bactericidal activity.The results suggest that chemical modifications and additional active ingredients in commercial disinfectants enhance their efficacy. However, the presence of toxic compounds in these chemical disinfectants raises concerns over skin irritation, respiratory effects, and environmental hazards.Natural disinfectants, though less effective in the short term, offer biodegradable, non-toxic solutions. The antimicrobial properties of neem and citrus fruit extracts from their bioactive compounds such as flavonoids, alkaloids, and limonene, which interfere with bacterial cell walls.Despite the lower phenol coefficient, citrus extract demonstrated a 95% bacterial reduction, making it a promising alternative if further concentration adjustments and formulation optimizations are applied. Neem extract, though slightly less effective, remains a viable antibacterial agent

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