Eco. Env. & Cons. 31 (4) : 2025; pp. (1740-1743)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2025.v31i04.061
Website: www.envirobiotechjournals.com

Assessment of Mask-Associated Microorganisms and the Antimicrobial Effectiveness of Traditional Herbal Extracts

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(Received 18 August, 2025; Accepted 12 October, 2025)

ABSTRACT

Health care workers were the most likely to be exposed to COVID-19 because they were in close contact with suspected, probable, or confirmed cases. The present study investigated risk factors for health care workers using surgical face masks. Microorganisms were isolated from the used masks, and four pathogenic strains were identified. These microorganisms were found to be predominant, and the antimicrobial activity of Kabasura Kudineer and Nilavembu (*Andrographis paniculata*), traditionally used to treat fever, was evaluated against these organisms. Gentamycin was employed as the positive control. Different concentrations of Kabasura Kudineer extracts were prepared and tested for their antimicrobial activity. The extracts inhibited the growth of all tested microbes at all concentrations, with inhibition increasing as concentration increased. It was concluded that the plant constituents of Kabasura Kudineer and Nilavembu were effective against these pathogenic organisms and could potentially be incorporated into masks, thereby protecting health care workers from infections.

Key words *: Mask-associated microorganisms, Herbal extracts, Antimicrobial*

Introduction

During the COVID-19 pandemic, face masks were widely recommended by international, national, and local authorities as a key source control measure to limit the spread of respiratory droplets from both symptomatic and pre-symptomatic individuals. Masks have been shown to reduce the transmission of respiratory viruses and improve clinical outcomes; however, their effectiveness can be influenced by factors such as improper usage, prolonged wear, contamination, and the presence of fine aerosols. Health care workers, who are frequently in close contact with infected patients, remain at high risk of exposure despite mask usage. Prolonged use of N95 masks, for example, can cause discomfort, headaches, and reduced compliance, while masks themselves can become contaminated and potentially increase the risk of infection if not handled properly.

In this context, herbal formulations with antimicrobial and immunomodulatory properties have gained attention as adjunctive protective measures. Traditional herbal remedies such as Kabasura Kudineer and Nilavembu (*Andrographis paniculata***)** have been used for centuries to manage fevers and

respiratory illnesses. Recent studies indicate that these plant extracts exhibit significant antimicrobial activity against pathogens, including those associated with COVID-19. Incorporating such herbal extracts into masks or protective materials may enhance their efficacy by reducing microbial contamination and providing additional protection for health care workers. This approach combines conventional protective measures with natural therapeutics, offering a potential strategy to reduce infection risk while supporting overall health and immunity.

Materials and Methods

Collection of Masks

Used surgical masks were collected from various health care workers at SMS Hospital to serve as a source of microbial contamination.

Isolation and Identification of Pathogens

Microorganisms were isolated from the masks by streaking onto **nutrient agar** and **potato dextrose agar (PDA).** Pure cultures were obtained using standard sub-culturing techniques, and isolates were identified by morphological and biochemical methods.

Preparation of Plant Extracts

Two medicinal plants were selected for antimicrobial testing: **Nilavembu (*Andrographis/paniculata*)** and **Kabasura Kudineer**. Leaves were collected, thoroughly washed, shade-dried, and ground into a fine powder. The powdered material was extracted by dissolving in **dimethyl sulfoxide (DMSO)** to prepare stock extracts (Kumar *et al*., 2021)

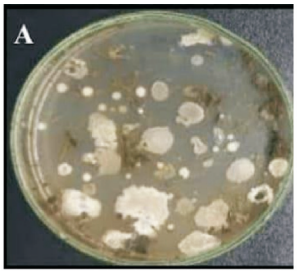
Antimicrobial Testing

The antimicrobial activity of the plant extracts was evaluated against the isolated pathogens using the **agar well diffusion method**, following protocols similar to those described in previous studies (e.g., the antimicrobial testing of *Andrographis paniculata* extracts). Briefly, wells were punched into agar plates seeded with the test organism, and aliquots of each extract were loaded into the wells. Plates were incubated (bacterial cultures at ~37 °C; fungal cultures at room temperature) for an appropriate period, after which zones of inhibition were measured to assess activity. (Positive controls (e.g., standard antibiotic) and negative controls (DMSO) were included to validate the assay, as has been done in similar herbal antimicrobial studies (Aruna *et al*., 2024).

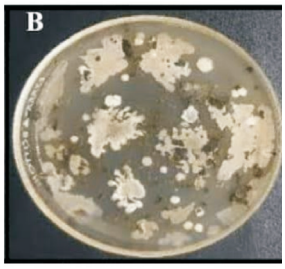
Results and Discussion

Isolation of Microorganisms

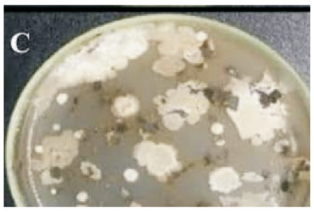
The isolation of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger* from used face masks of healthcare workers reflects the high potential for microbial accumulation on personal protective equipment. The detection of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger* from used masks of healthcare workers indicates significant microbial accumulation on PPE. Similar studies have shown that prolonged mask use and repeated handling promote contamination with both bacterial and fungal pathogens (Chughtai *et al*., 2020). The presence of common hospital-associated microbes aligns with previous reports that masks can act as reservoir for opportunistic and nosocomial organisms (Ronksley *et al*., 2021). Successful culturing and identification using nutrient agar,



A. Staphylococcus aureus

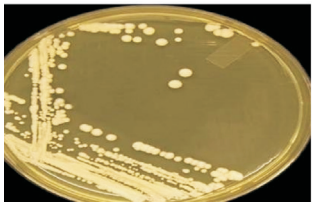


B. *Pseudomonas aeruginosa*

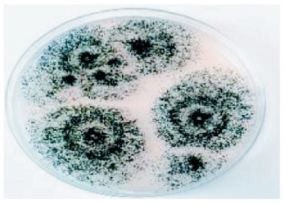


C. *Escherichia coli*

D. *Bacillus subtilis*



E. *Candida albicans*



F. *Apergillus niger*

PDA, Gram staining, and biochemical tests confirm the effectiveness of standard laboratory methods for mask-associated microbial detection. These findings highlight the need for proper mask hygiene and timely replacement in clinical environments.

Antibacterial Effect of KabasuraKudineer

Table 1 summarizes the antibacterial activity of KabasuraKudineer extract. Concentrations ranging from 25 µL to 100 µL of the DMSO-based extract were evaluated. Among these, the 100 µL concentration demonstrated the highest antibacterial efficacy, producing the largest zones of inhibition against all tested bacterial strains: *Staphylococcus aureus* – 40 mm, *Pseudomonas aeruginosa* – 40 mm, *Escherichia coli* – 36 mm, *Bacillus subtilis* – 35 mm.These findings indicate that the selected bacterial isolates were highly

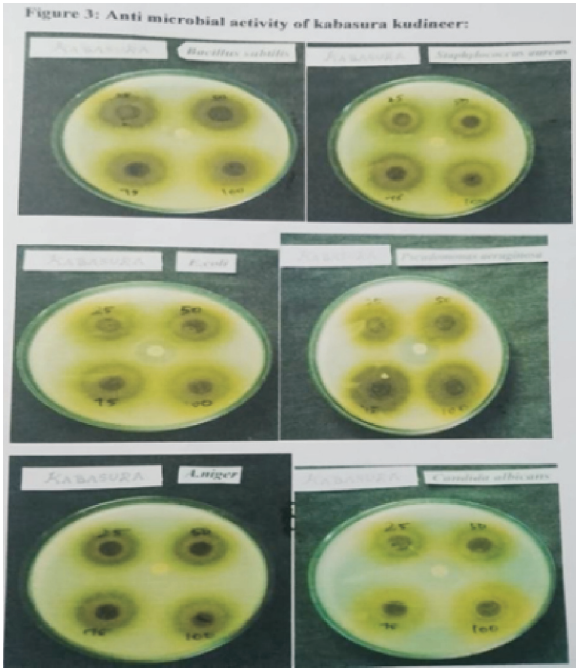


Fig. 2. Anti-microbial effect of Kabasura Kudineer extract against different microorganisms

The antifungal activity observed against *Candida albicans* and *Aspergillusniger* was moderate compared with the antibacterial effects. This difference may be attributed to the structural complexity and rigidity of fungal cell walls, which contain chitin, glucans, and mannoproteinsreducing the permeability of many plant-derived antimicrobial compounds (Gow *et al.,* 2017). Despite this, the increasing zone of inhibition with higher extract concentrations indicates that Nilavembu contains phytochemicals capable of exerting antifungal, effects when present at sufficient levels. Recent investigations have likewise reported that andrographolide and related constituents of *Andrographis paniculata* exhibit inhibitory activity against pathogenic *Candida* and *Aspergillus* species (Almalki *et al.*, 2024). Further studies involving phytochemical isolation, mechanistic exploration, and in vivo validation are warranted to identify the most active compounds and enhance their drug-development potential (Pandey and Tripathi, 2024).

Conclusion

This study demonstrates that KabasuraKudineer and Nilavembu (*Andrographis paniculata*) extracts possess significant antimicrobial activity against the bacterial and fungal species isolated from the used masks of healthcare workers during the COVID-19 pandemic, including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillusniger*. Both herbal formulations exhibited concentration-dependent inhibition, with KabasuraKudineer showing strong antibacterial and antifungal effects and Nilavembu displaying broad-spectrum efficacy, particularly against Gram-negative pathogens. The ability of these extracts to inhibit microbes commonly associated with mask contamination suggests their potential use as supportive herbal agents for reducing secondary microbial

Table 1. Anti-microbial effect of Kabasura Kudineer extract against different microorganisms

Sample	Zoneofinhibition (mm)				
Bacillus subtilis	25 µl	50 µl	75 µl	100 µl	Control
Staphylococcus aureus	25	29	32	35	20
Escherichia coli	30	34	38	40	20
Pseudomonasaeruginosa	28	30	33	36	20
Candida albicans	25	30	35	40	22
Aspergillus niger	22	25	28	32	20
	22	25	27	30	20

infections in high-risk environments such as hospitals. Their activity against both bacteria and fungi highlights their relevance during pandemics, where prolonged mask use and environmental exposure increase the risk of microbial colonization.Overall, the findings support the traditional use of these herbal formulations and emphasize their promise as natural antimicrobial candidates. Further research on their active phytochemicals, mechanisms of action, and possible integration into preventive healthcare products is recommended to enhance their effectiveness in real-world applications.

Conflict of Interest- None

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