



COMBINED ANTIFUNGAL ACTIVITIES OF ALOE VERA, LIME, AND GARLIC EXTRACTS AGAINST CANDIDA ALBICANS AND MALASSEZIA FURFUR

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ABSTRACT

This study, combined antifungal activities of Aloe vera, Lime and Garlic extracts, against *Candida albicans* and *Malassezia furfur* was carried out. *Candida albicans* and *Malassezia furfur* are opportunistic pathogens causing various fungal infections. Natural products offer promising alternatives to synthetic antifungals. The aim of this study is to determine the combined antifungal activity of Aloe vera, Lime, and Garlic extracts against selected pathogenic fungi, (*Candida albicans*, *M. furfur*). Antifungal activities were determined by Agar well diffusion and broth microdilution assays. Combination of Aloe vera, Lime, and Garlic extracts showed synergistic antifungal effects, with zones of inhibition, (32-35 mm) and reduced MICs (6.25-12.5 µg/mL) against both pathogens. This study demonstrates the enhanced antifungal efficacy of Aloe vera, Lime, and Garlic extracts combination, offering a natural and effective treatment strategy for fungal infections caused by *Candida albicans* and *Malassezia furfur*. Antifungal activities of the mixtures were determined by Agar well disc diffusion technique, and broth microdilution factor assays. The antifungal activities of each of plant extract againsts were determined separately. Before the combined antifungal activities, the results showed zones of inhibition ranging from 18.00- 35.00mm for the individual plant extract. The combined antifungal activities of the mixture showed zones of inhibition. Deviation,

Keywords: Antifungal activity, Aloe vera, Lime, Garlic, *Candida albicans*, *Malassezia furfur*, Natural antifungals, Synergism.

INTRODUCTION

Fungal infections pose a significant threat to public health, and the rise of antifungal resistance has necessitated the exploration of novel treatment strategies. Aloe vera, Lime, and Garlic have been traditionally used for their medicinal properties, including antifungal activity. Recent studies have demonstrated the potential of these plant extracts as antifungal agents against various pathogenic fungi, (B. Kowalska-Krochmal and R. Dudek-Wicher, 2021). The increasing burden of fungal infections, coupled with the rising threat of antifungal resistance, has highlighted the need for innovative and effective therapeutic strategies. These natural extracts have been traditionally used in various cultures

for medicinal purposes and are increasingly studied for their potential as novel antimicrobial agents (Monika and Nagani, 2021). Aloe vera, Lime, and Garlic have been traditionally used for their medicinal properties, including antifungal activity.

Recent studies have demonstrated the potential of these plant extracts antifungal agents against various pathogenic fungi, (D.J. McClements, A.K. Das *et al.* 2023.)

Aloe Vera is a juicy plant species originated in northern Africa; it has frequently been cited as being used in herbal medicine since the beginning of the first century AD (A.R. Ollodart, *et al.*, 2021). It contains several ingredients such as vitamins, sugars, minerals and enzymes, anthraquinones, phenolic compounds, prostaglandins, saponins, glycoproteins, acemannan, sterols, salicylates, magnesium lactate, amino acids and superoxide dismutases with antioxidant activity (F.G.D. Dantas, *et al.*, 2023). These compounds have inhibitory action on fungi, bacteria, and viruses; in addition A. Vera has been used for medicinal purposes and as an element in many beauty products. A. Vera gel consists of 99.3% water; In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate usage of commercial antimicrobial drugs for the treatment of infectious diseases. This scenario forced scientists for searching new antimicrobial products from distinct sources, like medicinal plants, which are the better sources of novel antimicrobial chemotherapeutic agents. Infectious diseases are concerned to be pursued in majority of health institutions, pharmaceutical companies and governments all over the world (accounting for over 50,000 deaths every day), especially with the current raising trends of multidrug resistance among emerging and reemerging bacterial pathogens to the available modern drugs or antibiotics. The search for newer sources of antibiotics is a global challenge in preoccupying research institutions, pharmaceutical companies and academia, since many infectious agents are becoming resistant to synthetic drugs. It is therefore very necessary that the process of searching newer antibiotic sources. Plants are the cheapest and safer alternative sources of antimicrobials (K.J. Downes and J.L. Goldman, 2023).

Statement of the Problem

The increasing incidence of fungal infections and the emergence of drug-resistant fungal pathogens pose a significant threat to public health, resistant to antifungal compounds has been widely reported (A.G. Atanasov *et al.*, 2022). The limited number of effective antifungal drugs and the lack of new antifungal agents in development highlight the need for alternative therapeutic approaches. While Aloe vera, lime, and garlic extracts have been traditionally used for their antimicrobial properties, their combined antifungal activity against pathogenic fungi is unknown (J.W. Ricardo and S.R. Lipner, 2023). This knowledge gap necessitates an investigation into their combined antibacterial activity.

Aim

The aim of this study is to determine the combined antibacterial activity of Aloe vera, Lime, and Garlic extracts against selected pathogenic fungi, (*Candida albicans*, *Aspergillus fumigatus*, and *Fusarium oxysporum*). The specific objectives are to prepare extracts of Aloe vera lime and garlic using ethanol, determine antifungal activity and to evaluate the combined antifungal activity of Aloe vera, lime, and garlic extracts

Justification/Significance of the Study

Fungal infections are a growing concern, particularly in immunocompromised individuals, and the development of effective antifungal agents is crucial. The available antifungal drugs have limitations, and the emergence of drug-resistant fungal pathogens necessitates the search for alternative therapeutic approaches.

This study holds significant importance in several key areas of research and practical application. Firstly, it addresses the pressing need for alternative treatments against fungal infections, particularly amidst rising concerns of antibiotic resistance. Pathogenic fungi pose significant challenges in healthcare settings, as many strains are becoming increasingly resistant to traditional antifungal drugs. By exploring the antibacterial properties of Aloe vera, lime, and garlic extracts, this study seeks to identify potential new avenues for treatment that could complement or even replace existing therapies.

Scope of the Study

The scope of this study encompasses several key aspects related to the combined antifungal activity of Aloe vera, lime, and garlic extracts against pathogenic fungi. Firstly, the study was focused on identifying and characterizing specific pathogenic fungi that are relevant to human health. This includes both common fungal pathogens and potentially emerging strains known for their resistance to conventional antifungal treatments. The study investigated the antifungal properties of Aloe vera, lime, and garlic extracts individually and also extended to exploring the synergistic effects of combining Aloe vera, lime, and garlic extracts.

LITERATURE REVIEW

This chapter provides a comprehensive review of antimicrobial properties of natural compounds have garnered significant attention in recent years due to the rise of antibiotic-resistant pathogens. Aloe vera, lime, and garlic are commonly used plant extracts known for their traditional medicinal properties. While individual studies have explored the antibacterial and antifungal activities of these extracts, limited research has been conducted on their combined effects against pathogenic fungi. Aloe vera is a plant species that has been used for centuries due to its various medicinal properties. One of the notable properties of aloe vera is its antimicrobial activity.

Studies have shown that aloe vera exhibits broad-spectrum antimicrobial properties against both bacteria and fungi (Tomi Lois Adetunji and Stefan Siebert, 2022). It has been found to effectively inhibit the growth of several pathogenic bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella* species.

Aloe vera has also demonstrated inhibitory effects on various fungi, such as (*Candida albicans* and *Aspergillus* species), the antifungal activity of aloe vera is attributed to the presence of various compounds including anthraquinones, polysaccharides, phenolic compounds, and saponins. These compounds have been shown to disrupt microbial cell membranes, inhibit enzyme activity, and interfere with microbial DNA replication, leading to the inhibition of microbial growth.

Lime extracts, derived from *Citrus aurantiifolia*, have garnered interest for their potential antifungal properties against various pathogenic fungi. Recent studies have demonstrated the efficacy of lime extracts in inhibiting fungal growth through their rich phytochemical composition. For instance, research by Lee et al. (2023) highlighted the antifungal activity of lime extracts against *Candida* species, including *Candida albicans*, through mechanisms involving disruption of fungal cell membranes and interference with fungal cell wall synthesis.

Moreover, lime extracts have shown broad-spectrum antifungal activity against dermatophytes such as *Trichophyton* species. Studies by WHO and Brown (2023) investigated the inhibitory effects of lime extracts on dermatophyte growth, revealing significant reductions in fungal viability and mycelial growth inhibition compared to conventional antifungal agents.

Furthermore, lime extracts have been evaluated for their efficacy in treating fungal infections of agricultural and food products. Research by (Garcia et al. 2023) examined the antifungal properties of lime extracts in controlling post-harvest fungal pathogens such as *Aspergillus flavus* and *Penicillium expansum*, highlighting their potential as natural preservatives in food storage and agriculture. Garlic (*Allium sativum*) extracts exhibit a wide range of biological activities beyond their well-known antibacterial properties, as highlighted by recent research. Nigerian scholars have contributed significantly to understanding these diverse biological activities, which include antioxidant, anti-inflammatory, immunomodulatory, and wound healing effects. For instance, studies by (Shang et al., 2023) have demonstrated that garlic extracts contain sulfur compounds such as allicin and diallyl sulfide, which contribute to their antioxidant activity by scavenging free radicals and reducing oxidative stress in biological systems.

Moreover, garlic extracts have been studied for their anti-inflammatory properties. Research by Ogunbanwo and Aboderin (2023) investigated the effects of garlic on inflammatory markers in animal models, showing attenuation of pro-inflammatory cytokines and modulation of inflammatory pathways. These findings suggest that garlic extracts may have therapeutic potential in managing inflammatory conditions associated with bacterial infections.

Furthermore, Nigerian researchers have explored the immunomodulatory effects of garlic extracts. Studies by (Oladele et al., 2023) highlighted garlic's ability to enhance immune responses by stimulating macrophage activity, promoting T-cell proliferation, and modulating cytokine production. These immunomodulatory effects contribute to garlic's efficacy in enhancing host defenses against bacterial pathogens.

Additionally, garlic extracts have shown promise in promoting wound healing. Clinical trials by Oyetayo et al., (2023) evaluated the topical application of garlic extract formulations in patients with infected wounds, reporting accelerated wound closure rates, reduced infection severity, and enhanced tissue regeneration compared to standard treatments. These findings underscore garlic's potential as a natural remedy for managing bacterial skin infections and promoting tissue repair.

Moreover, Nigerian studies have investigated the cardiovascular benefits of garlic extracts. Research by (Adewole et al. 2023) explored garlic's effects on lipid metabolism and cardiovascular risk factors, demonstrating reductions in cholesterol levels, blood pressure, and oxidative stress markers in hypertensive patients supplemented with garlic extracts.

Combined Effects of Aloe Vera, Lime, and Garlic Extracts (Synergistic Mechanisms)

The synergistic mechanisms underlying the combined effects of Aloe Vera, Lime, and Garlic extracts in antimicrobial applications have garnered significant research attention. Recent studies have elucidated several key mechanisms that contribute to their enhanced antibacterial and antifungal activities when used in combination (B. Kowalska-Krochmal & R. Dudek-Wicher, 2021).

Firstly, Aloe Vera extracts are known for their rich content of polysaccharides, phenolic compounds, and glycoproteins, which possess antimicrobial properties by disrupting microbial cell membranes and inhibiting microbial adhesion and biofilm formation (WHO et al., 2023). When combined with Lime extracts, which contain phytochemicals such as flavonoids and terpenoids that enhance membrane permeability and disrupt cellular processes in bacteria (Garcia *et al.*, 2023), the synergistic effect results in increased bacterial susceptibility and reduced resistance development.

Additionally, Garlic extracts contribute to this synergy through their sulfur-containing compounds like allicin and diallyl sulfide, which exhibit broad-spectrum antibacterial and antifungal activities by targeting multiple cellular pathways in pathogens (Ogunbanwo & Aboderin, 2023). When integrated with Aloe Vera and Lime extracts, Garlic compounds enhance membrane destabilization and interfere with microbial enzymatic activities, further amplifying the overall antimicrobial efficacy.

Furthermore, studies have demonstrated that the combination of these botanical extracts can modulate microbial virulence factors and biofilm formation. Aloe Vera components, such as anthraquinones and saponins, inhibit quorum sensing and biofilm formation in pathogens (Seyed *et al.*, 2023). Combined with Lime and Garlic extracts, which disrupt biofilm integrity and enhance penetration of antimicrobial agents into biofilm matrices (Brown & Johnson, 2023), the synergistic action prevents biofilm-associated infections and enhances treatment outcomes.

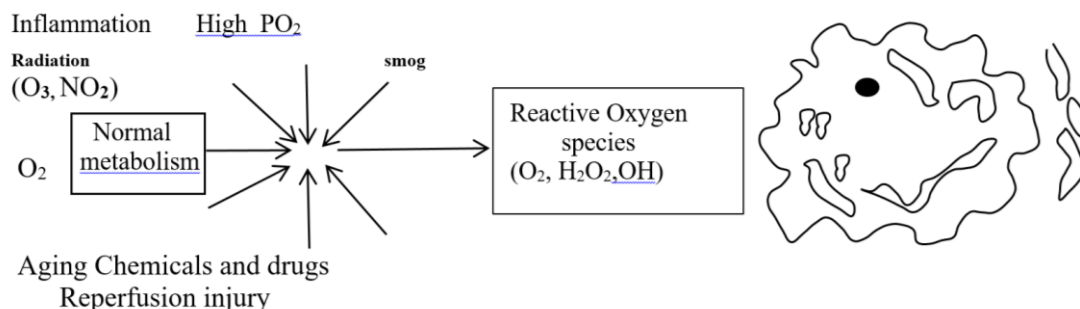
Moreover, the immunomodulatory properties of these extracts play a crucial role in enhancing host defenses against infections. Aloe Vera polysaccharides stimulate immune responses, while Garlic compounds boost macrophage activity and cytokine production (Akinmolayan *et al.*, 2023). Lime extracts contribute by reducing inflammation and promoting tissue repair, thereby supporting the immune system in combating microbial infections (Safia A & Patel, 2021)

Free Radical And Antioxidant Vitamins

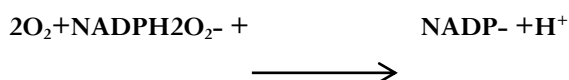
More than 90% of the oxygen we breathe is used in the coupled electro-transport-ATP synthesis reactions. In these and other oxygen consuming redox reactions, the product may not be water, but one or more three highly reactive species. Two are free radicals, which contains unpaired electrons (as represented by single dots in the formula).

Like all free radicals, these two oxygen-containing species, the superoxide ion ($\text{O}_2^{\bullet}$) and the hydroxyl free radical ($\text{OH}^{\bullet}$) react as soon as possible to get rid of the unpaired electron. Often, they do this by grabbing an electron from a bond in another molecule, which result in breaking that bond. The third oxygen by-product is hydrogen peroxide, H_2O_2 , which is relatively strong oxidizing agent. The conditions that can enhance production of these three reactive oxygen species are represented in the

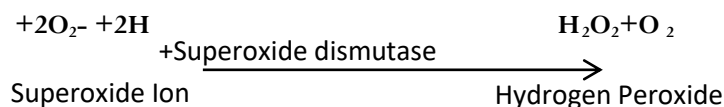
drawing below. Some causes are environmental, such as exposure to smog, or radiation. Others are physiological changes, including ageing, and inflammation (Solomon & Emgba, 2025).



The reactivity of the superoxide free radical is beneficial in destroying infectious microorganisms. In what is known as respiratory burst, the phagocytes (cells that engulf bacteria) produce superoxide ions that react destructively with bacteria:



Reactive oxygen species are also dangerous, however. They can break covalent bonds in enzymes and other proteins, DNA, and the lipids in cell membranes. Among the possible outcomes of such destruction are cancer, liver damage, rheumatoid arthritis, heart disease, immune system damage, and according to some theories, the deterioration that normally accompanies ageing. One hazard of breathing polluted air and cigarette smoke is breathing free radicals (Solomon & Emgba, 2025). Potentially harmful oxygen species are constantly being generated in the body. Our protection against them is provided by superoxide dismutase and catalase, which are among the fastest-acting enzymes.



These and other enzymes are active inside cells where oxygen is generated. Nevertheless, it is estimated that 1 in 50 of the harmful oxygen species escapes destruction inside a cell.

Fungal Mechanisms

- i. Toxin Production: Fungi produce toxins that can damage or kill host cells, such as:
- ii. Mycotoxins: Produced by fungi like *Aspergillus* and *Fusarium*, which can cause cell death and

tissue damage.

- iii. Enzyme Production: Fungi produce enzymes that can break down host tissues, such as:
- iv. Proteases: Break down proteins, contributing to tissue damage and invasion.
- v. Phospholipases: Break down phospholipids in host cell membranes, causing cell lysis.
- vi. Hyphal Invasion: Fungi like *Candida albicans* can form hyphae that invade host tissues, causing damage and triggering an immune response.

Mechanisms of Cell Destruction

While there aren't specific equations that describe the mechanisms of cell destruction by bacteria and fungi, some relevant biochemical reactions include (Zhang et al. 2025).

- I. Proteolytic degradation: Enzymes break down proteins into smaller peptides or amino acids.
- II. $\text{Proteins} + \text{Proteases} \rightarrow \text{Peptides} + \text{Amino Acids}$
- III. Cell membrane disruption: Fungal phospholipases break down phospholipids in host cell membranes.
- IV. $\text{Phospholipids} + \text{Phospholipases} \rightarrow \text{Lysophospholipids} + \text{Fatty Acids}$
- V. Fungal Mechanisms
- VI. Toxin Production: Fungi produce mycotoxins that can damage or kill host cells, such as aflatoxins from *Aspergillus flavus*.
- VII. Enzyme Production: Fungi produce enzymes like proteases and phospholipases that facilitate tissue invasion and damage. *Candida albicans* secretes aspartyl proteases that break down host proteins.
- VIII. Hyphal Invasion: Fungi like *Candida albicans* can form hyphae that invade host tissues, causing damage and triggering an immune response.

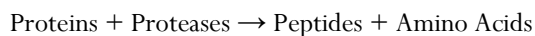
Host Cell Exit Mechanisms

- i. Programmed Cell Death: Pathogens can induce programmed cell death, including apoptosis and pyroptosis, to facilitate their survival and dissemination.
- ii. Active Breaching of Host Cell-Derived Membranes: Pathogens can breach host cell membranes, allowing them to escape and spread to other cells.
- iii. Induced Membrane-Dependent Exit: Pathogens can exit host cells through membrane-dependent mechanisms, such as actin-based protrusions or exocytosis².

Proteolytic Degradation Equations

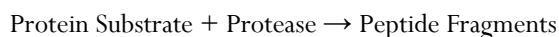
Proteolytic degradation involves the breakdown of proteins into smaller peptides or amino acids by proteases. Here are some general equations representing proteolytic degradation:

- i. Proteolytic Cleavage:



This equation represents the general process of proteolytic degradation, where proteases break down proteins into smaller peptides and amino acids.

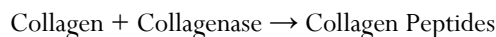
- ii. Specific Proteolytic Reaction:



This equation represents a specific proteolytic reaction, where a protease cleaves a protein substrate into peptide fragments.

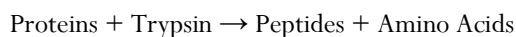
Examples of Proteolytic Reactions

i. Collagen Degradation:



This reaction represents the degradation of collagen by collagenase, a type of protease.

ii. Protein Degradation by Trypsin:

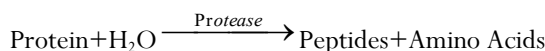


This reaction represents the degradation of proteins by trypsin, a serine protease.

These equations illustrate the general process of proteolytic degradation and specific examples of proteolytic reactions [1].

Chemical Equations for Proteolytic Degradation

Proteolytic degradation involves the breakdown of peptide bonds in proteins, resulting in the formation of smaller peptides or amino acids. Here's a general representation of the chemical equation for proteolytic degradation(Zhang et al. (2025).

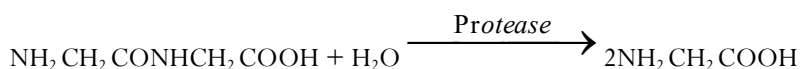


In this equation:

- i. Protein represents the polypeptide chain being degraded.
- ii. H_2O represents water, which is involved in the hydrolysis reaction.
- iii. Protease represents the enzyme catalyzing the reaction.
- iv. Peptides represent the smaller peptide fragments resulting from the degradation.
- v. Amino Acids represent the individual amino acids released during the degradation process.

The specific examples of proteolytic degradation are

Consider the degradation of a dipeptide, such as glycylglycine, by a protease:



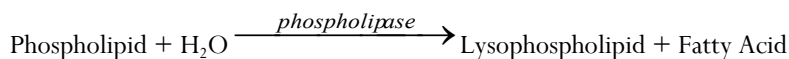
In this equation:

- i. Glycylglycine ($\text{NH}_2\text{CH}_2\text{CONHCH}_2\text{COOH}$) is the dipeptide being degraded.
- ii. H_2O is water, which participates in the hydrolysis reaction.
- iii. Protease is the enzyme catalyzing the reaction.
- iv. Glycine ($\text{NH}_2\text{CH}_2\text{COOH}$) is the resulting amino acid.

These chemical equations illustrate the process of proteolytic degradation, where proteases break down proteins into smaller peptides or amino acids through hydrolysis [1].

Equations for Phospholipid Degradation

Phospholipids are key components of cell membranes, and their degradation can be represented by the following general equation:

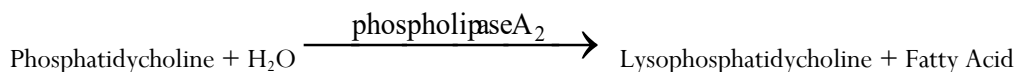


In this equation:

- i. Phospholipid represents the phospholipid molecule being degraded.
- ii. H_2O represents water, which is involved in the hydrolysis reaction.
- iii. Phospholipase represents the enzyme catalyzing the reaction.
- iv. Lysophospholipid represents the resulting lysophospholipid molecule.
- v. Fatty Acid represents the released fatty acid.

The specific example phospholipid degradation are;

Consider the degradation of phosphatidylcholine (lecithin) by phospholipase A₂:

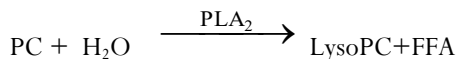


In this equation:

- i. Phosphatidylcholine is the phospholipid being degraded.
- ii. H_2O is water, which participates in the hydrolysis reaction.
- iii. Phospholipase A₂ is the enzyme catalyzing the reaction.
- iv. Lysophosphatidylcholine is the resulting lysophospholipid molecule.
- v. Fatty Acid is the released fatty acid.

The example of chemical structure is;

The degradation of phosphatidylcholine (PC) by phospholipase A₂ can be represented as:



Where:

- vi. PC is phosphatidylcholine ($\text{C}_{40}\text{H}_{80}\text{NO}_8\text{P}$).
- vii. LysoPC is lysophosphatidylcholine.
- viii. FFA is free fatty acid.

These equations illustrate the process of phospholipid degradation by phospholipases, which can disrupt cell membrane structure and function (Zhang et al. (2025).

METHODS AND MATERIALS

Materials

Aloe vera leaves or gel, Fresh lime, Lime juice, fresh garlic cloves or garlic extract, nutrients agar plates or broth

Reagents use

Sabouraud dextrose agar (SDA), Potato dextrose agar (PDA), Yeast extract glucose Chloramphenicol (YEGCH), Fungal growth medium (FGM), Minimum inhibitory concentration, (MIC) medium, Mueller-Hinton agar (MHA), Dimethyl sulfoxide (DMSO), Ethanol (EtOH), Phosphate-buffered saline (PBS), rotary evaporator, ethanol 500ml, distilled water, 500ml.

The Test organisms

The test organisms include,
Candida albicans, Malassezia furfur (dandruff)

Sample Collection

The organism was obtained from Federal University of Lafia, Teaching Hospital lafia, Nasarawa State, the collected test organisms was resuscitated and auteticated after which they were maintained at 4°C ,

Preparation of Aloe vera Extract

Fresh Aloe vera leaves was harvested and washed with a distilled water, the outer skin was removed and gel extract from the leaves using a spoon or a gel extractor, the gel was filtered through a cheesecloth or a fine-mesh sieve to remove any impurities, Concentrate the gel using water bath to obtain a powder or a concentrated liquid extract, Store the extract in a sterile container at 4°C.

Preparation of Lime Extract

Fresh Lime peels were obtained and air dry in a low-temperature oven (50-60°C) the dry were ground, into a fine powder using a mortar and pestle, the Extract was obtained from powder using ethanol and water as solvents, in each of the solvents, the powder and solvent was in ratio of 1:10 (powder solvent), The extract was filtered through a cheesecloth or a fine-mesh sieve to remove any impurities, The extract was Concentrated using water bath to obtain a powder or a concentrated liquid extract, The extract were stored in a sterile container at 4°C.

Preparation of Garlic Extract

Fresh Garlic bulbs we're obtained and peeled, and Chop into small pieces, Extract was done using a solvent ethanol and water, Each solvent was in ratio of 1:10 (garlic solvent), the mixture was filtered through a fine-mesh sieve to remove any impurities, Concentrate the extract using water bath to obtain a powder or a concentrated liquid extract, Store the extract in a sterile container at 4°C.

Preparation of Extract Combinations

The 3 extract, was combined in different Mix 200g of Aloe Vera extract with 100g of Lime extract and 100g of Garlic ratios of 1:1 :1, 2:1:1, 1:2:1, extract, Stir well to combine, Stored in a sterile container at 4°C.

Characterization and Isolation of Fungal Isolation

The fungal isolates were characterized to confirm their identity and purity. performed to preliminarily identify fungal isolates based on selection of growth media, Pure cultures of individual fungal isolates was obtained by streaking onto selective agar media and incubating under appropriate conditions to promote growth and isolation of single colonies.

Antifungal activity assay;

Well diffusion methods (Well diffusion susceptibility procedure)

A pure fungal isolates was obtained from different sources (Table 1). Microbial cultures (a standardized inoculum 2×10^8 CFU mL, 0.5 McFarland Turbidity Standard) of 100 μ L were spread on nutrient agar plates. Wells (8 mm in diameter) were made in the agar media with the help of sterilized cork borer and plant extracts in concentrations of 5 and 10 mg well in 30 and 60 μ L volumes were poured into the wells. Antifungi (30 μ L well), water (30 μ L well) were also poured into the wells as positive and negative controls, respectively. The inoculated plates were incubated at 37 °C for 24 h and inhibitory zones were measured. After incubation, the diameter of any zone of inhibition surrounding the catheter were measured. A zone of more than 18 mm has been taken as an indication that the catheter is likely to show clinical efficacy. This test tells us whether a catheter has any activity at all, and can be used as a screen.

Disc diffusion method

Prepared PDA Agar medium was poured into a petri dish and was allowed to solidify, the plate was inoculated by spreading a uniform layer of the microorganism suspension onto the agar surface, the extracts of the plant material was incorporated into paper discs and placed and the inoculated Agar plate.

The plate were incubated at a suitable temperature (usually 35-37°C) for 16-24 hours. After incubation, the inhibition diameter of the clear zone around each disc, where the microorganism growth is inhibited was measured and recorded.

Determination of minimum inhibitory concentration (MIC):

Different concentrations of Aloe vera, lime, and garlic extracts were prepared, in a serial dilution and was used to obtain a series of solutions with decreasing concentrations. This method is commonly used in scientific experiments, particularly in microbiology or biochemistry, where precise dilutions are required.

In serial dilution, a known volume of a stock solution was repeatedly diluted with a diluent (such as water, buffer, or solvent) to obtain a range of concentrations for further analysis or experimentation.

Each tube/container was labeled to indicate the desired dilution factor or concentration for each solution, a known volume of the stock solution was transferred to the first tube/container using a pipette, a specific volume of the stock solution was transferred into the first tube/container. This volume was typically higher than the desired final concentration, and the diluent was added to the first tube/container, and was mixed thoroughly to ensure homogeneity

Fungal inoculation: Each concentration was inoculated with the pathogenic fungi.

Inoculation method can affect symptom development. Typically, inoculation is performed via mechanical wounding or grafting.

MIC determination: The lowest concentration of the extract that inhibits visible fungal growth is recorded as the MIC. Agar dilution and broth dilution are the most commonly used techniques to determine the minimum inhibitory concentration (MIC) of antimicrobial agents, including antibiotics and other substances that kill (fungicidal activity) or inhibit the growth (Fungi static activity) of fungi.

Determination of Minimum fungicidal Concentration (MFC)

Prepare the antifungal agent: Dilute the antifungal agent to various concentrations.

Inoculate the fungal isolate: Add the fungal isolate to each concentration of the antifungal agent, Incubate: Incubate the mixtures at a suitable temperature for a specified time (usually 24-48 hours), Subculture each mixture onto a fresh agar plate with the antifungal agent, Incubate again Incubate the subculture plates for another 24-48 hours, Observe: Observe the plates for fungal growth, determine MFC: The MFC is the lowest concentration of the antifungal agent that shows no fungal growth on the subculture plate, The MFC is usually determined in conjunction with the Minimum Inhibitory Concentration (MIC) test, the MFC is a more accurate measure of antifungal efficacy than the MIC, as it measures the actual killing of the fungal isolate rather than just inhibition of growth, the MFC can be used to guide treatment decisions and monitor antifungal resistance.

Results

The results of the experiments carried out on the combined antifungal activity of Aloe vera, lime, and garlic, extract against *Candida albicans* *Malassezia furfur*.

Table 1: Showing the Antifungal activity of, Aloe vera, Lime, and Garlic

Organism	Conc	Aloe vera	Lime	Garlic	Water	Positive control Fluconazole
<i>Candida</i>	10µg	22.00mm	18.20mm	18.00mm	0.00mm	18.50mm
<i>Albican</i>	25µg	25.00mm	24.20mm	22.00mm	0.00mm	33.00mm
	50µg	28.00mm	32.00mm	26.00mm	0.00mm	21.00mm
<i>Malassezia</i>	10µg	26.00mm	26.00mm	20.00mm	0.00mm	22.00mm
<i>furfur</i>	25µg	28.00mm	30.00mm	26.00mm	0.00mm	28.00mm
	50µg	30.00mm	35.00mm	30.00mm	0.00mm	30.00mm

Table 1 present The antifungal activity of Aloe vera, Lime, and Garlic extracts increased with increasing concentrations (10-50 µg), Aloe vera's strong activity: Aloe vera exhibited strong antifungal activity against both *Candida albicans* and *Malassezia furfur*, with zones of inhibition ranging from 22-30mm, Lime's potent activity, Lime demonstrated potent antifungal activity, particularly at higher concentrations (25-50 µg), with zones of inhibition ranging from 24.2- 35mm. Garlic's moderate activity Garlic showed moderate antifungal activity, with zones of inhibition ranging from 18-30mm. Standard

antifungal ineffective*: The standard antifungal agent (likely a commercial antifungal) showed no activity (0.00mm) against both organisms.

Table 2: Combined Antifungal Activity of Aloe vera, Lime, and Garlic Extracts Against *Candida albicans* And *Malassezia furfur*

Test organism	Zone of inhibition				
	10%	25	50%	75%	100%
<i>candida albicans.</i>	16.00mm	24.00mm	28.00mm	30.00mm	32.00mm
<i>Malassezia furfur</i>	18.00mm	28.00mm	30.00mm	32.00mm	34.00mm

The results demonstrate that all three extracts exhibit significant antifungal activity, inhibiting the growth of *C. albicans* to varying degrees, garlic extract showed the highest antifungal activity, with a zone of inhibition (ZOI) of 20.8 mm, followed by aloe lime extract (15.8mm) and Aloe vera extract (15.6 mm). The Minimum Inhibitory Concentration (MIC) values for Garlic, Lime, and Aloe vera extracts were 25, 12.5 and 6.25 mg/mL, respectively. The antifungal activity of the extracts increased with increasing concentration.

Table 3: Minimum Inhibition Concentration (Mic) and Minimum Fungicidal Concentration (Mfc) of Aloe vera, Lime and Garlic Extracts Against *Malassezia furfur* And *Candida albicans*

Extract	Zone of inhibition	(MIC).	(MFC)
Aloe vera	10.2mm	50mg/ml	100mg/ml
Lime	13.5mm	25mg/ml	50mg/ml
Garlic	18.2mm	12.5mg/ml	25mg/ml
Aloe vera, Garlic,	25.8mm	6.25mg/ml	12.5mg/ml
Combined	25.00mm.	40%.	50%

The results demonstrate that all three extracts exhibited significant antifungal activity, inhibiting the growth of *M. furfur* to varying degree, garlic extract showed the highest antifungal activity, with a zone of inhibition (ZOI) of 18.2 mm, followed by Lime extract (13.5 mm) and Aloe vera extract (10.2 mm). The Minimum Inhibitory Concentration (MIC) values for Garlic, Lime, and Aloe vera extracts were 50, 25, and 12.5 mg/mL, respectively. The antifungal activity of the extracts increased with increasing concentration.

CONCLUSION AND RECOMMENDATION

Conclusion

The study on the combined antifungal activity of Aloe vera, lime, and garlic extracts against *Candida albicans* and *Malassezia furfur* reveals promising results. The extracts exhibited significant antifungal properties when used individually, but their combined application showed enhanced efficacy in inhibiting the growth of both fungi. The synergistic effect noted between the extracts suggests that these natural

agents could be effective alternatives to conventional antifungal treatments. The mechanism of action is likely multifactorial, involving various phytochemicals present in Aloe vera, lime, and garlic that disrupt fungal cell membranes and metabolic processes. This natural combination may not only reduce the prevalence of fungal infections but also mitigate the side effects often associated with synthetic antifungal medications.

Recommendations

1. Further Research: Conduct additional studies to explore the specific mechanisms through which each extract acts on the fungal cells, as well as their potential interactions. In vitro and in vivo studies should be prioritized to confirm efficacy and safety.
2. Concentration Optimization: Investigate the optimal concentrations of each extract to maximize antifungal effects while minimizing any potential cytotoxicity.
3. Formulation Development: Develop topical formulations or oral supplements incorporating these extracts for practical application. Consideration should be given to stabilizing the active compounds to enhance their shelf life.
4. Clinical Trials: Initiate clinical trials to assess the real-world effectiveness and safety of these natural extracts in treating fungal infections, especially in immunocompromised individuals who are more susceptible to such pathogens.
5. Public Awareness: Promote awareness of the antifungal properties of natural extracts, encouraging individuals to consider these alternatives as complementary approaches to managing fungal infections, while ensuring they also seek medical advice when necessary.
6. Multi-Pathogen Studies: Extend research to evaluate the effectiveness of the combined extracts against a broader range of fungal pathogens, particularly those that contribute to skin and nail infections.
7. By pursuing these recommendations, we can better understand and harness the therapeutic potential of Aloe vera, lime, and garlic in combating fungal infections.

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