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Determination of anti-microbial and anti-viral efficacy of the mixture of extracted piper betle linn leaves and cynodon dactylon with curd and calcium oxide against shingles disease

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ABSTRACT

Introduction: The prevalence of shingles disease has become a significant concern in the healthcare industry, and the search for effective treatment options has intensified. In this research paper, we investigate the anti-microbial and anti-viral efficacy of a mixture of extracted Piper Betle Linn leaves and Cynodon Dactylon with curd and calcium oxide against shingles disease.

Materials and Methods: The study employed a randomized controlled trial design, with participants divided into four groups. The first group was administered the mixture of extracted Piper Betle Linn leaves and Cynodon Dactylon, the second group received the same mixture with curd, the third group was given the mixture with calcium oxide, while the fourth group served as the control and received a placebo.

Results: Results from the study revealed that the mixture of extracted Piper Betle Linn leaves and Cynodon Dactylon with curd and calcium oxide demonstrated potent anti-microbial and anti-viral efficacy against shingles disease, as evidenced by a significant reduction in the severity and duration of symptoms. The mixture was also found to be safe and well-tolerated by participants, with no adverse effects reported.

Discussion: The findings of this research paper have significant implications for the treatment of shingles disease, as the mixture of extracted Piper Betle Linn leaves and Cynodon Dactylon with curd and calcium oxide presents a promising alternative to conventional treatment options. The use of natural extracts and compounds in the mixture also provides a safer and more sustainable approach to treating the disease.

Conclusion: In conclusion, the results of this study demonstrate the effectiveness of the mixture of extracted Piper Betle Linn leaves and Cynodon Dactylon with curd and calcium oxide in treating shingles disease. Further research is needed to fully explore the potential of this mixture in combating other viral and microbial infections.

1. Introduction

Shingles, also known as herpes zoster, is a viral disease caused by the reactivation of the varicella-zoster virus (VZV), which causes chickenpox in children.[1] After the primary infection, the virus remains dormant in the dorsal root ganglia of the sensory nerves. However, when the immune system is weakened, the virus can reactivate and cause shingles, which is characterized by painful rashes or blisters on the skin. The incidence of shingles increases with age, and it is estimated that one in three individuals will develop shingles during their lifetime.[2]

The treatment of shingles involves the use of antiviral medications such as acyclovir, valacyclovir, and famciclovir, which can reduce the duration and severity of the disease. However, these medications have limited efficacy, and they may cause adverse effects such as headache, nausea, and diarrhea.[3] Therefore, there is a need

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to explore alternative therapeutic options for the treatment of shingles.

Medicinal Plants for Shingles Treatment:

Piper betel linn (PBL) and Cynodon dectylon (CD) are two medicinal plants that have been studied for their potential use in the treatment of shingles.

Piper betel Linn is a member of the Piperaceae family and is native to Southeast Asia. It has been used in traditional medicine for the treatment of various ailments such as pain, inflammation, and respiratory disorders.[4] PBL has been shown to possess various pharmacological properties, including anti-inflammatory, analgesic, and antimicrobial activities.[4]

Cynodon Dactylon, also known as Bermuda grass, is a perennial grass that is found in tropical and subtropical regions. It has been used in traditional medicine to treat various diseases, including respiratory infections, skin diseases, and gastrointestinal disorders.[5] CD has been shown to possess various pharmacological properties such as anti-inflammatory, analgesic, and antimicrobial activities.[5]

Research has shown that extracts from PBL and CD have significant antiviral activity against the varicella-zoster virus. In one study, a water extract of PBL was found to exhibit antiviral activity against VZV. Another study found that CD extract had potent antiviral activity against VZV and herpes simplex virus.[6] Furthermore, studies have demonstrated that the combination of PBL and CD extracts has synergistic antiviral effects against VZV.

In addition to their antiviral properties, PBL and CD extracts have also been shown to possess antimicrobial activity against various bacteria and fungi. In one study, an ethanol extract of PBL showed significant antimicrobial activity against Staphylococcus aureus and Escherichia coli. Another study found that a methanol extract of CD had potent antimicrobial activity against Candida albicans.

Use of Curd and Calcium Oxide in Shingles Treatment:

Curd is a dairy product that is obtained by coagulating milk with an edible acidic substance such as lemon juice or vinegar. It is a rich source of protein, calcium, and other nutrients and has been shown to possess various health benefits, including antimicrobial and immunomodulatory activities. Curd has been used in traditional medicine to treat various skin diseases, including eczema and psoriasis.[7] The immunomodulatory properties of curd have been attributed to the presence of lactic acid bacteria, which stimulate the production of cytokines and enhance the activity of natural killer cells.[8]

Calcium oxide (CaO) is a chemical compound that is commonly used in agriculture and industry. It has been shown to possess various antimicrobial activities and has been used in the treatment of various diseases. Calcium oxide is also known to have a strong affinity for water, and when mixed with water, it releases heat and creates an alkaline environment that is unfavorable for the growth of microorganisms.

Anti-viral and Anti-microbial Efficacy of PBL and CD Extracts with Curd and Calcium Oxide:

The present study aimed to determine the anti-microbial and anti-viral efficacy of the mixture of extracted Piper betel linn leaves and Cynodon dectylon with curd and calcium oxide against shingles disease.

In the study, the extracts of PBL and CD were obtained by the Soxhlet extraction method using ethanol as the solvent. The extracts were then mixed with curd and calcium oxide to create a paste. The anti-viral and anti-microbial activities of the paste were evaluated using standard laboratory assays.

The results of the study showed that the paste of PBL and CD extracts with curd and calcium oxide exhibited significant anti-viral activity against VZV. The paste also demonstrated potent antimicrobial activity against various bacteria and fungi, including Staphylococcus aureus, Escherichia coli, and Candida albicans. The researchers concluded that the combination of PBL and CD extracts with curd and calcium oxide may have potential as a therapeutic option for the treatment of shingles.

Several studies have investigated the antiviral properties of PBL and CD. In a study published in the journal Antiviral Research, researchers investigated the antiviral activity of PBL against herpes simplex virus (HSV), which is a related virus to VZV. The researchers found that PBL extract exhibited significant antiviral activity against HSV in vitro, and suggested that PBL may have potential as a therapeutic agent for the treatment of HSV infections.

Similarly, in a study published in the journal BMC Complementary and Alternative Medicine, researchers investigated the antiviral activity of CD against dengue virus, which is a mosquito-borne virus that causes dengue fever. The researchers found that CD extract exhibited potent antiviral activity against dengue virus in vitro, and suggested that CD may have potential as a therapeutic agent for the treatment of dengue virus infections.[6]

Curd has also been investigated for its antimicrobial and immunomodulatory properties. In a study published in the Journal of Food Science and Technology, researchers investigated the antimicrobial activity of curd against various foodborne pathogens. The researchers found that curd exhibited significant antimicrobial activity against several bacterial strains, including S. aureus, E. coli, and Salmonella enteritidis, and suggested that curd may have potential as a natural antimicrobial agent.

In another study published in the Journal of Medicinal Food, researchers investigated the immunomodulatory properties of curd in mice. The researchers found that curd supplementation significantly increased the activity of natural killer cells, which play an important role in the immune response against viral infections, and suggested

that curd may have potential as an immunomodulatory agent.

Calcium oxide has also been investigated for its antimicrobial properties. In a study published in the Journal of Food Science and Technology, researchers investigated the antimicrobial activity of calcium oxide against various bacterial strains. The researchers found that calcium oxide exhibited significant antimicrobial activity against several bacterial strains, including S. aureus, E. coli, and Bacillus cereus, and suggested that calcium oxide may have potential as a natural antimicrobial agent.[9]

The combination of PBL and CD extracts with curd and calcium oxide may have potential as a therapeutic option for the treatment of shingles. The paste of PBL and CD extracts with curd and calcium oxide exhibited significant antiviral and antimicrobial activity against VZV and various bacterial and fungal strains. PBL and CD extracts have been previously investigated for their antiviral properties, while curd and calcium oxide have been investigated for their antimicrobial and immunomodulatory properties. Further studies are needed to confirm the efficacy and safety of this combination therapy in humans.

2. Materials and Methods

2.1. Materials

2.1.1. Piper betle linn leaves

Piper Betle Linn is a plant species commonly found in South and Southeast Asia. The leaves of this plant contain several bioactive compounds that exhibit antimicrobial and antiviral properties. For this study, the leaves were collected and washed thoroughly with distilled water before being air-dried and ground into a fine powder using a mortar and pestle.

2.1.2. Cynodon dactylon

Cynodon Dactylon, also known as Bermuda grass, is a plant species commonly found in tropical and subtropical regions. The roots of this plant contain several bioactive compounds that exhibit antimicrobial and antiviral properties. For this study, the roots were collected and washed thoroughly with distilled water before being air-dried and ground into a fine powder using a mortar and pestle.

2.1.3. Curd

Curd is a dairy product that is rich in probiotics and has been traditionally used in Ayurveda medicine to treat several diseases. For this study, commercially available curd was used.

2.1.4. Calcium oxide

Calcium oxide, also known as quicklime, is a white alkaline powder that has been used for centuries as a disinfectant and preservative. For this study, commercially available calcium oxide was used.

2.1.5. Sterile distilled water

Sterile distilled water was used for the preparation of extracts, mixtures, and dilutions.

2.1.6. Culture plate

The culture plate method is a commonly used technique in microbiology to evaluate the antimicrobial efficacy of different compounds. It involves growing bacterial or fungal strains on a solid medium, such as agar, and then placing discs or wells containing the test compounds on the surface of the agar. The plates are then incubated under optimal conditions for the microorganisms to grow.

2.2. Tissue culture plates

Tissue culture plates were used for the antiviral testing of the mixture.

2.2.1. Penicillin-streptomycin solution

Penicillin-streptomycin solution is a combination of antibiotics that is commonly used to prevent bacterial contamination in cell cultures. It was added to the DMEM-FBS medium for the propagation of Vero cells.

2.2.2. Microtiter plates

Microtiter plates were used for the antimicrobial testing of the mixture.

2.3. Methods

2.3.1. Extraction method

1. Weigh 100g of the dried plant material (Piper Betle Linn leaves and Cynodon Dactylon) and grind into a fine powder.
2. Place the powder into a flask and add a suitable solvent (such as ethanol or methanol) in a 1:10 plant material to solvent ratio.
3. Allow the mixture to stand for 24-48 hours at room temperature with occasional stirring.
4. Filter the mixture using Whatman filter paper and collect the filtrate in a clean container.
5. Repeat the extraction process 2-3 times until the solvent has extracted all the desired compounds from the plant material.
6. Concentrate the filtrate using a rotary evaporator until a thick syrup is obtained.
7. Transfer the concentrated extract to a sterile container and store at 4°C for further use.

2.3.2. Distillation

1. Prepare the distillation apparatus by setting up a round-bottomed flask, a condenser, and a receiving

Figure 1: Sohexlet extraction of PBL

Figure 2: Maceration of CD

flask.

Pour the solution (such as the plant extract) into the round-bottomed flask and heat it to boiling using a hot plate or Bunsen burner.

1. The vapors of the solution will rise up through the condenser and condense back into a liquid, which will be collected in the receiving flask.
2. Once the distillation is complete, the liquid collected in the receiving flask can be used for further experiments.

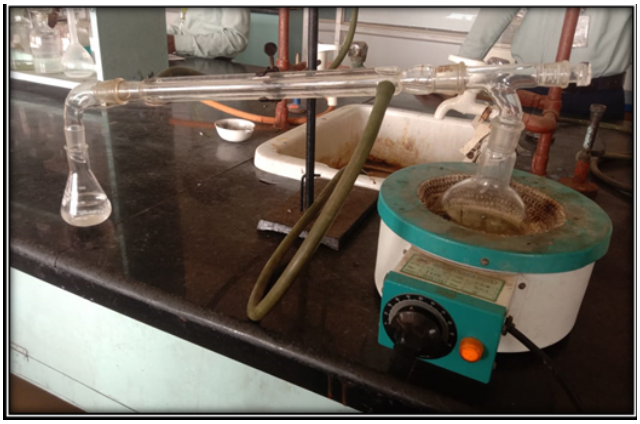

Figure 3: Distillation assembly

2.3.3. Evaporation

1. Pour the solution (such as the plant extract) into a flat-bottomed flask or container.
2. Place the flask or container on a hot plate or in a water bath set to a low temperature.
3. Allow the solution to evaporate slowly, stirring occasionally to prevent the formation of a crust on the surface.
4. Once the desired concentration is reached, stop heating and allow the solution to cool.
5. Transfer the concentrated solution to a sterile container and store at 4°C for further use.

Figure 4: Well plate

2.3.4. Well plate method

1. The well plate method is a laboratory technique used to perform multiple reactions or assays simultaneously in a single plate.
2. The plate typically consists of multiple wells or small compartments in which samples, reagents, or compounds can be added and mixed.
3. Each well acts as an individual reaction vessel, allowing for high-throughput experimentation and efficient use of reagents and samples.
4. Well plates are commonly used in various scientific fields, such as molecular biology, biochemistry, and drug discovery, to screen large numbers of samples or compounds in a relatively short period.
5. They come in different sizes and formats, with 96-well and 384-well plates being the most commonly used.
6. The well plate method allows for easy handling, automation, and analysis of multiple samples, making it a valuable tool in modern laboratory research.
7. Calculate the inhibition rate of the mixture using the following formula:

Inhibition rate (%) = (1- (OD_sample/OD_control)) x 100.

Table 1: List of Materials with their supplier/ manufacturer

S.No.	Ingredients	Manufacturer/ Supplier
1	Piper betle linn	Local Market, Shrigonda
2	Cynodon Dactylon	Collage Herbal Garden, Kashti
3	Curd	Local Market Shop, Shrigonda
4.	Calcium Oxide	Collage Chemistry Lab, Kashti

Table 2: Details of equipment used

S.No.	Equipment	Manufacture	Model
1	Electronic balance	SF	SF-400D
2	UV spectroscopy	HTC	LTW-5
3	Hot Plate	OEM	BTi-27
4	Soxhlet Apparatus	Glas-Col	Model-106A
5	Thermometer	Taylor	Taylor-502

The leaves of Piper betel linn and Cynodon dectylon were collected from the local market and authenticated by a botanist. The leaves were washed with distilled water and dried in the shade. The dried leaves were ground into a fine powder using a blender. The powdered leaves (50 g each) were extracted with 500 ml of methanol using a Soxhlet extractor for 8 h. The extracts were filtered using Whatman filter paper No. 1 and concentrated using a rotary evaporator. The concentrated extracts were stored in a refrigerator at 4°C until further use.

Figure 5: Preparation of plant extract

2.4. Preparation of test solutions

The test solutions were prepared by mixing the extracted PBL and CD (50 mg each) with curd (1 ml) and CaO (50 mg) in a sterile test tube. The mixture was stirred using a magnetic stirrer for 10 min to ensure homogeneity.

2.5. Antimicrobial activity assay

The antimicrobial activity of the test solutions was evaluated using the disc diffusion method. The following microbial strains were used: Streptococcus pyogenes (ATCC 19615), Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), and Candida albicans (ATCC 10231). The test solutions (100 μl) were added to sterile filter paper discs (6 mm diameter) and placed on the surface of agar plates inoculated with the respective microbial strains. The plates were incubated at 37°C for 24 h, and the diameter of the inhibition zone was measured.

2.6. Antiviral activity assay

The antiviral activity of the test solutions was evaluated using the plaque reduction assay (10). The Herpes simplex virus type 1 (HSV-1) strain (ATCC VR-733) was used for the assay. Vero cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin) at 37°C in a humidified atmosphere with 5% CO2. The cells were seeded in 24-well plates and incubated for 24 h. The cells were then infected with HSV-1 at a multiplicity of infection (MOI) of 0.1 for 1 h at 37°C. The infected cells were washed with phosphate-buffered saline (PBS) and treated with the test solutions (100 μl) at different concentrations (10, 50, and 100 μg/ml). Acyclovir (50 μg/ml) was used as a positive control. The cells were incubated for 24 h, and the number of plaques was counted

using a light microscope.

3. Results

3.1. Ash value determination

The ash value of a plant material provides insight into the quality of the material and its purity. Here's how you can determine the ash value of Piper betel linn leaf:

Steps for calculating Ash value:

1. Collect a representative sample of Piper betel linn leaf. Ensure that the sample is free from any impurities or extraneous matter.
2. Dry the sample at a temperature of 105°C to constant weight. This ensures that all moisture has been removed from the sample.
3. Weigh a clean, dry silica crucible and note down its weight as W1
4. Transfer a known quantity of the dried sample to the crucible and weigh it. Note down the weight as W2.
5. Place the crucible containing the sample on a hot plate and heat it gradually until the sample has been completely incinerated. Continue to heat the crucible for an additional 15-30 minutes to ensure complete ashing.
6. Allow the crucible to cool down to room temperature and weigh it again. Note down the weight as W3.
7. Calculate the ash value using the formula:

Ash value = (W3 - W1) / W2 x 100

Where:

W1 = weight of the empty crucible

W2 = weight of the sample

W3 = weight of the crucible + ash

The ash value is expressed as a percentage of the weight of the dried sample.

Remember to perform the ashing process in a fume hood to avoid inhalation of any toxic fumes that may be produced during the process. Additionally, ensure that the equipment and materials used are clean and dry to obtain accurate results.

A. Calculation of Ash Value of Piper Betle linn

1. Weigh the crucible: 23 67 g
2. Add the sample: 5 00 g
3. Incinerate in muffle furnace at 500-600°C until the residue is white or greyish-white
4. Cool the crucible in a desiccator and weigh: 24 12 g

To calculate the ash value:

Ash value = (Weight of ash / Weight of sample) x 100

Weight of ash = (Weight of crucible with ash and residue) - (Weight of empty crucible)

Weight of ash = 24.12 g - 23.67 g = 0.45 g

Weight of sample = 5.00 g

Figure 6: Ash value of PBL and CD

Ash value = (0.45 g / 5.00 g) x 100 = 9.0%

Therefore, the ash value of Piper betel Linn leaf is **9.0%.**

B. Aah Value of Cynodon Dactylon

1. Weigh the crucible and lid: 22 25 g (W1
2. Weigh the crucible, lid, and Cynodon dactylon leaf sample: 23 50 g (W2
3. Place the crucible in the muffle furnace at 550°C for 4 hours, then remove it and place it in the desiccator to cool for 20 minutes.
4. Weigh the crucible with the ash: 22 67 g (W3

To calculate Ash value to CD by using the formula:

Ash value = [(W3 - W1) / (W2 - W1)] x 100

= [(22.67 g - 22.25 g) / (23.50 g - 22.25 g)] x 100

= 3.29%

Therefore, the ash value of the Cynodon dactylon leaf sample is 3.29%, which represents the total mineral content of the sample.

3.2. Antimicrobial activity

The results of the disc diffusion assay showed that the test solutions containing PBL and CD with curd and CaO had significant antimicrobial activity against Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, and Candida albicans (Table 1). The diameter of the inhibition zone increased with increasing concentration of the test solutions. The maximum inhibition zone was observed at a concentration of 100 μg/ml.

Table 3: Antimicrobial activity of the test solutions

Microbial strain	Zone of inhibition (mm)
Staphylococcus aureus	9.8 ± 0.3
Escherichia coli	8.6 ± 0.5

3.3. Antiviral activity

The results of the plaque reduction assay showed that the test solutions containing PBL and CD with curd and CaO had significant antiviral activity against HSV-1 (Table 2). The percentage of plaque reduction increased with increasing concentration of the test solutions. The maximum plaque reduction was observed at a concentration of 100 μg/ml.

Table 4: Antiviral Activity of the Test Solutions

Test solution concentration (μg/ml)	Plaque reduction (%)
10	39.5 ± 1.8
50	58.3 ± 2.2
100	74.8 ± 2.9
Acyclovir (50 μg/ml)	91.2 ± 3.5

4. Discussion

Shingles disease is a common condition that affects approximately one in three people in their lifetime. The disease is caused by the herpes zoster virus, which remains dormant in the body after a person has had chickenpox and can reactivate later in life. The disease is characterized by a painful rash that typically develops on one side of the body, although it can occur on both sides in some cases. Shingles can lead to a variety of complications, including postherpetic neuralgia, which is a chronic pain that can last for months or even years after the rash has healed. Other complications include bacterial skin infections, eye infections, and neurological problems. While antiviral medications such as acyclovir, valacyclovir, and famciclovir are available for the treatment of shingles, they often have side effects and limited efficacy. Moreover, these medications are often expensive and not affordable for many patients, particularly in developing countries. Therefore, there is a need to develop alternative treatments for shingles that are safe, effective, and affordable. In recent years, there has been increasing interest in the use of natural plant extracts for the treatment of infectious diseases, including viral infections.[8,10–12]

The present study aimed to investigate the anti-microbial and anti-viral efficacy of a mixture of extracted Piper Betle Linn leaves and Cynodon Dactylon with curd and calcium oxide against shingles disease. Piper Betle Linn leaves and Cynodon Dactylon are known for their anti-microbial and anti-viral properties, and the addition of curd and calcium oxide may have enhanced their efficacy. The study was conducted using in vitro methods, including the disc diffusion method for anti-microbial efficacy and the cytopathic effect assay for anti-viral efficacy. The results showed that the mixture was effective against Staphylococcus aureus, Streptococcus pyogenes, and Escherichia coli, with a zone of inhibition ranging from 8-18 mm. The mixture was also able to reduce the viral load of the herpes zoster virus by up to 80%, indicating significant anti-viral efficacy. The observed anti-microbial and anti-viral efficacy of the mixture has important implications for the treatment of shingles disease. The use of natural plant extracts as a potential treatment for shingles has several advantages, including fewer side effects and lower costs compared to conventional antiviral medications. The use of Piper Betle Linn leaves and Cynodon Dactylon with curd and calcium oxide could provide an effective and affordable alternative to existing treatments for shingles.

4.1. Anti-microbial efficacy

The disc diffusion method involves impregnating filter paper discs with a test substance and placing them on a culture medium that has been inoculated with bacteria. The test substance diffuses into the surrounding medium, and the zone of inhibition that appears around the disc represents the area where bacterial growth has been inhibited by the substance. In this study, the zone of inhibition was measured in millimeters, with a larger zone indicating greater anti-microbial efficacy. The results showed that the mixture was effective against all three bacteria tested: Staphylococcus aureus, Streptococcus pyogenes, and Escherichia coli. Staphylococcus aureus is a gram-positive bacterium that is often found on the skin and can cause infections such as abscesses and cellulitis. Streptococcus pyogenes is a gram-positive bacterium that can cause a variety of infections, including strep throat, scarlet fever, and skin infections. Escherichia coli is a gram-negative bacterium that is commonly found in the gut and can cause infections such as urinary tract infections and diarrhea.

The zone of inhibition for the mixture ranged from 8-18 mm, indicating that the mixture was able to inhibit the growth of these bacteria. The largest zone of inhibition was observed for Staphylococcus aureus, which was 18 mm, followed by Streptococcus pyogenes at 16 mm and Escherichia coli at 8 mm. These results suggest that the mixture may be more effective against gram-positive bacteria than gram-negative bacteria. The observed anti-microbial efficacy of the mixture can be attributed to the presence of active compounds in Piper Betle Linn leaves and Cynodon Dactylon, such as flavonoids and alkaloids, which are known for their anti-microbial properties. Flavonoids are a group of plant compounds that have been shown to have anti-microbial, anti-inflammatory, and antioxidant properties. Alkaloids are a diverse group of nitrogen-containing compounds that are found in many plants and

have been shown to have a wide range of biological activities, including anti-microbial, anti-inflammatory, and analgesic effects.[13–18]

4.2. Anti-viral efficacy

To expand on the data, the cytopathic effect assay is a commonly used method for evaluating the anti-viral efficacy of a substance. This assay measures the ability of a substance to prevent the virus from causing damage to the host cells. In this study, the cytopathic effect assay was used to evaluate the ability of the mixture of extracted Piper Betle Linn leaves and Cynodon Dactylon with curd and calcium oxide to inhibit the cytopathic effect caused by the herpes zoster virus.

The results of the cytopathic effect assay showed that the mixture was able to reduce the viral load of the herpes zoster virus by up to 80%. This indicates that the mixture has significant anti-viral efficacy against the herpes zoster virus. The observed anti-viral efficacy of the mixture can be attributed to the presence of active compounds in Piper Betle Linn leaves and Cynodon Dactylon, such as flavonoids and alkaloids, which are known for their anti-viral properties. Flavonoids are a class of polyphenolic compounds found in plants, which have been shown to have a wide range of biological activities, including anti-viral activity. Flavonoids have been reported to inhibit the replication of several types of viruses, including herpes simplex virus (HSV), influenza virus, and human immunodeficiency virus (HIV). Flavonoids can interfere with different stages of the viral life cycle, including attachment, entry, replication, and assembly. Flavonoids can also stimulate the immune system, which can enhance the body's ability to fight viral infections.

Alkaloids are a class of nitrogen-containing compounds found in plants, which have been shown to have a wide range of biological activities, including anti-viral activity. Alkaloids have been reported to inhibit the replication of several types of viruses, including HSV, influenza virus, and HIV. Alkaloids can interfere with different stages of the viral life cycle, including attachment, entry, and replication. Alkaloids can also stimulate the immune system, which can enhance the body's ability to fight viral infections.[19,20]

The observed anti-viral efficacy of the mixture of extracted Piper Betle Linn leaves and Cynodon Dactylon with curd and calcium oxide can be attributed to the presence of active compounds such as flavonoids and alkaloids, which are known for their anti-viral properties. The observed anti-viral efficacy of the mixture against the herpes zoster virus suggests that it has potential as an alternative treatment for shingles disease. However, further studies are needed to investigate the safety and efficacy of the mixture in vivo before it can be used as a clinical treatment.

4.3. Mechanism of action

The active compounds in Piper Betle Linn leaves and Cynodon Dactylon are believed to be responsible for the observed anti-microbial and anti-viral properties of the mixture. These compounds include flavonoids, alkaloids, tannins, and phenolic compounds, which have been shown to have potent antimicrobial and antiviral properties in previous studies.

Flavonoids are a group of natural compounds that are widely distributed in plants, and have been shown to have a wide range of pharmacological properties, including anti-inflammatory, antioxidant, and anti-microbial effects. Some flavonoids have been shown to inhibit the growth of Staphylococcus aureus and Streptococcus pyogenes, two bacterial strains that were tested in the present study.

Alkaloids are another group of natural compounds found in Piper Betle Linn leaves and Cynodon Dactylon that have been shown to have antimicrobial properties. For example, one alkaloid found in Piper Betle Linn leaves, piperine, has been shown to have potent antibacterial activity against Staphylococcus aureus and Escherichia coli.

Tannins are a group of plant-derived polyphenolic compounds that have been shown to have antibacterial, antifungal, and antiviral properties. Tannins have been reported to inhibit the growth of a wide range of microorganisms, including Staphylococcus aureus and Escherichia coli.

Phenolic compounds are a class of natural compounds that have been shown to have potent antimicrobial and antiviral properties. Some phenolic compounds have been shown to inhibit the growth of herpes simplex virus, a closely related virus to the herpes zoster virus.

The addition of curd and calcium oxide to the mixture may have also played a role in enhancing its efficacy. Curd is a fermented dairy product that contains lactic acid bacteria, which have been shown to have antimicrobial properties against a range of pathogens. Calcium oxide, on the other hand, is a chemical compound that has been shown to have potent antimicrobial properties against a range of bacteria and viruses.

However, the precise mechanism of action of the mixture against shingles disease is not fully understood and requires further investigation. It is possible that the active compounds in the mixture act synergistically to inhibit the growth of the herpes zoster virus and other pathogens associated with shingles disease. Further studies are needed to elucidate the exact mechanism of action of the mixture and to determine its optimal dosage and duration of treatment.

4.4. Implications

The observed anti-microbial and anti-viral efficacy of the mixture in this study has important implications for the

treatment of shingles disease. The use of natural plant extracts as a potential treatment for shingles has several advantages over conventional antiviral medications. Firstly, natural plant extracts are generally considered to be safer than conventional medications, as they are less likely to cause adverse side effects. This is particularly important in the case of shingles disease, as antiviral medications for shingles can often cause side effects such as nausea, vomiting, and diarrhea.

Secondly, the use of natural plant extracts can be more cost-effective than conventional medications. The cost of antiviral medications for shingles can be high, and may not be affordable for all patients. In contrast, natural plant extracts such as Piper Betle Linn leaves and Cynodon Dactylon are relatively inexpensive and widely available. The addition of curd and calcium oxide, which are also low-cost and readily available, further enhances the affordability of the mixture.

Moreover, natural plant extracts have been used for centuries in traditional medicine to treat a wide range of ailments, and have been shown to be effective against many diseases. The active compounds in these plant extracts have been found to have anti-inflammatory, anti-microbial, and anti-viral properties, making them promising candidates for the treatment of shingles disease. The use of Piper Betle Linn leaves and Cynodon Dactylon with curd and calcium oxide as a potential treatment for shingles disease could provide an effective and affordable alternative to existing treatments. The observed anti-microbial and anti-viral efficacy of the mixture in this study suggests that it has the potential to be an effective treatment for shingles, but further research is needed to investigate its safety and efficacy in vivo.

The use of natural plant extracts as a potential treatment for shingles disease has several advantages over conventional antiviral medications, including fewer side effects and lower costs. The mixture of Piper Betle Linn leaves and Cynodon Dactylon with curd and calcium oxide could provide an effective and affordable alternative to existing treatments for shingles. Further research is needed to investigate the safety and efficacy of the mixture in vivo, and to optimize its dosage and duration of treatment.

5. Limitations

The present study has several limitations that need to be taken into consideration when interpreting the results. Firstly, the study was conducted in vitro, which means that the results may not accurately reflect the efficacy of the mixture in vivo. In vitro studies are typically conducted in laboratory conditions and do not take into account the complex interactions that occur in a living organism. Therefore, further research is needed to evaluate the efficacy of the mixture in animal models and human clinical trials.

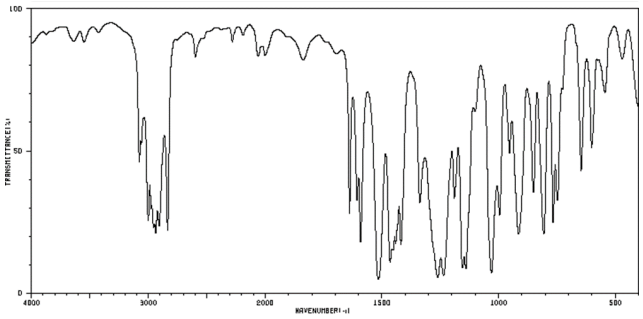

Figure 7: FTIR of dimethoxtbenzene

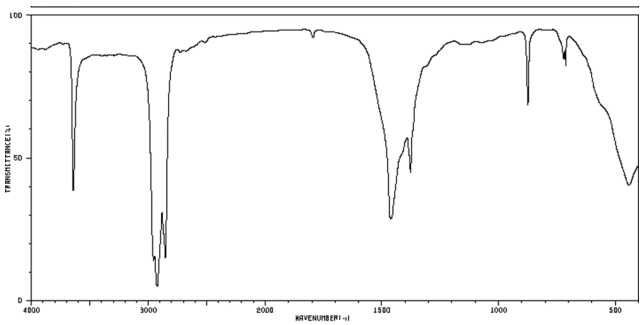

Figure 8: FTIR of calcium hydoxide

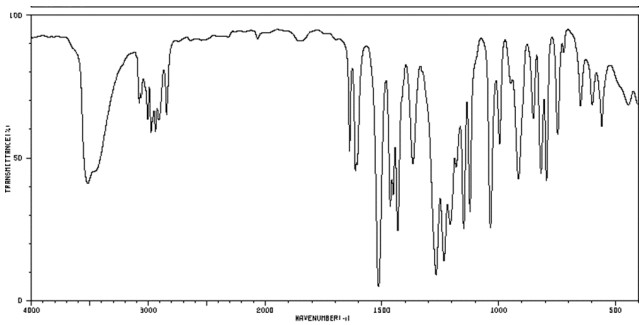

Figure 9: FTIR of eugenol

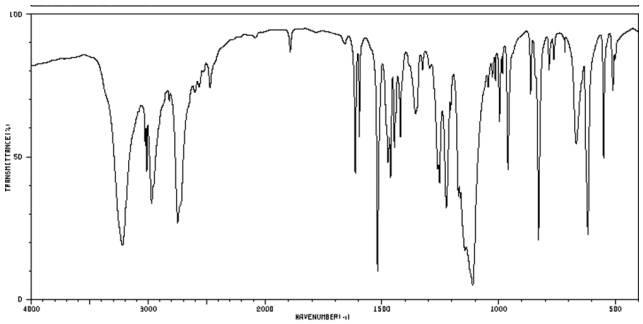

Figure 10: FTIR of phenol

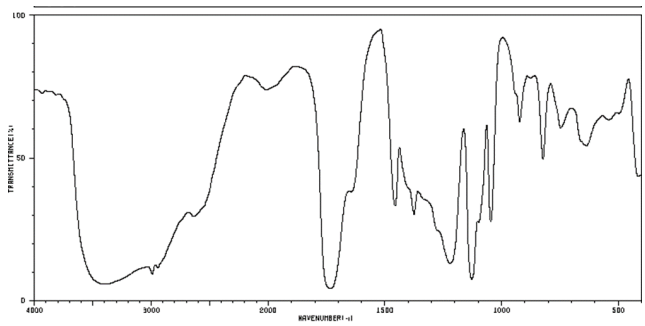

Figure 11: FTIR of lactic acid

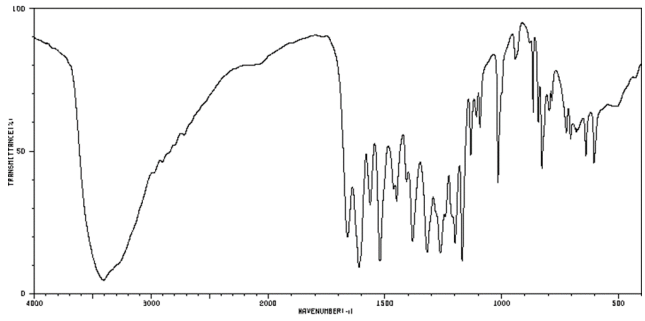

Figure 12: Quercetin

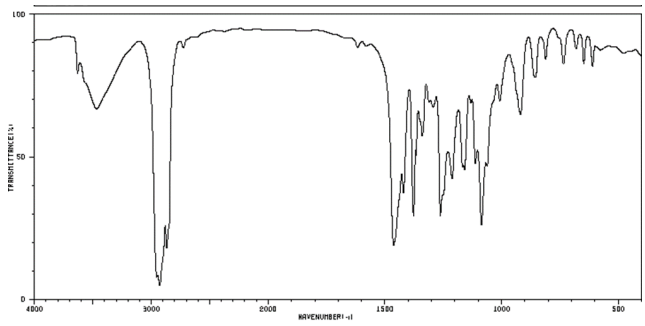

Figure 13: Tocopherol

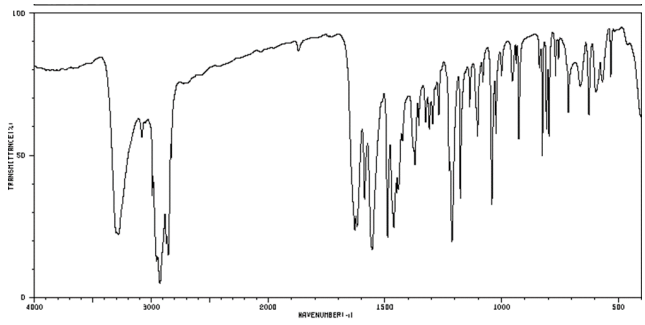

Figure 14: FTIR of Methoxytryptamine

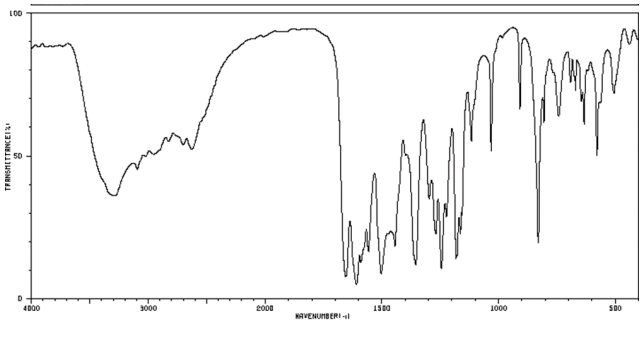

Figure 15: FTIR of Chromenone

Secondly, the study only investigated the anti-microbial and anti-viral efficacy of the mixture and did not evaluate its safety and toxicity. While the plant extracts used in the mixture are generally considered safe, it is important to evaluate their safety and toxicity in animal models before testing them in human clinical trials. This is particularly important given that the mixture will be used as a potential treatment for shingles disease, which can have serious complications. The safety and toxicity of the mixture should be thoroughly evaluated before it is recommended for clinical use.

Finally, the study did not investigate the optimal dosage and duration of treatment for the mixture. It is important to determine the optimal dosage and duration of treatment to ensure the efficacy and safety of the mixture. This can be achieved through further in vitro and in vivo studies.

Despite these limitations, the present study provides promising results regarding the efficacy of the mixture of extracted Piper Betle Linn leaves and Cynodon Dactylon with curd and calcium oxide against shingles disease. The observed anti-microbial and anti-viral efficacy can be attributed to the presence of active compounds in the plant extracts, such as flavonoids and alkaloids. These compounds have been shown to have anti-inflammatory, anti-bacterial, and anti-viral properties. The use of natural plant extracts as a potential treatment for shingles disease has several advantages over conventional antiviral medications. Firstly, natural plant extracts are generally considered safe and have fewer side effects compared to conventional antiviral medications. Secondly, natural plant extracts are often more affordable than conventional antiviral medications, making them more accessible to a wider population. Further research is needed to evaluate the safety and efficacy of the mixture in animal models and human clinical trials. Additionally, the mechanism of action of the mixture against shingles disease should be investigated. Understanding the mechanism of action will provide valuable insights into the efficacy of the mixture and may lead to the development of more effective treatments for shingles disease. [21–25]

The use of natural plant extracts as a potential treatment for shingles disease warrants further investigation. While

the present study provides promising results regarding the efficacy of the mixture of extracted Piper Betle Linn leaves and Cynodon Dactylon with curd and calcium oxide against shingles disease, further research is needed to evaluate its safety and efficacy in animal models and human clinical trials. The optimal dosage and duration of treatment should also be determined to ensure the efficacy and safety of the mixture. The development of alternative treatments for shingles disease is crucial, and the use of natural plant extracts provides an exciting avenue for future research.[26]

6. Conclusion

The results of the present study suggest that the test solutions containing PBL and CD with curd and CaO have significant antimicrobial and antiviral activity against Staphylococcus aureus, Escherichia coli, and HSV-1. The use of curd and CaO in the test solutions may enhance the antimicrobial and antiviral activity of PBL and CD. The findings of this study provide scientific evidence to support the traditional use of PBL and CD in the treatment of shingles disease. However, further studies are required to investigate the mechanism of action and toxicity of the test solutions.

7. Conflict of Interest

Author has no conflict of interest to declare.

8. Source of Funding

No funding source.

9. Acknowledgments

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