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Analysis of Photochemical and Antibacterial Activity of Gooseberry Leaf Extract (Phyllanthus acidus L. Skeels) for Formulation of Fortified Broiler Feed

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Abstract: The aim of the research was to assess the phytochemical composition, total flavonoid content and antibacterial activity of Phyllanthus acidus leaf extract (PALE). The study utilized the disc diffusion method to test the in vitro antibacterial properties of the extract against Escherichia coli and Lactic Acid Bacteria. The data collected were analyzed using analysis of variance and significant differences were further analyzed using Duncan's multiple range test. The results of the study showed that PALE contains bio-active compounds such as alkaloids, flavonoids, tannins and steroids. The total flavonoid content was 663.92 µg/ml QE. PALE exhibited statistically significant inhibition zone against both pathogenic and non-pathogenic bacterial species compared to the standard antibiotic zinc bacitracin. The extract at 2% concentration produced the highest inhibition zone compared to the 1.5% and 1% concentrations, but its effect was significantly lower than that of the control antibiotic zinc bacitracin. Based on these findings, it was concluded that PALE has the potential to be used as an antimicrobial feed additive in broilers. However, further research is necessary to investigate its efficacy in vivo and to determine its safety and potential side effects.

Key Words: phytochemical composition; flavonoid content; antibacterial activity; Phyllanthus acidus leaf extract

1. Introduction

The current research focuses on Phyllanthus acidus leaf extract as a potential candidate for broiler antimicrobial feed additive. Phyllanthus acidus, also known as gooseberry, is an underutilized botanical plant that contains bio-active compounds with various properties such as antimicrobial, antioxidant, immunomodulatory, and acidifying effects, making it a promising phytobiotic for improving broiler performance.

The research aims to identify the phytochemical composition of Phyllanthus acidus leaf extract, including the presence of secondary metabolites such as flavonoids. Flavonoids are known to be potent antimicrobial substances and are commonly found in plants, making them a potential alternative to antibiotics in poultry production. The total flavonoid content of Phyllanthus acidus leaf extract will also be quantified, which will provide valuable information on the concentration of flavonoids present in the extract. This information will help determine the potential efficacy of Phyllanthus acidus leaf extract as an antimicrobial feed additive for broilers.

In addition, the antibacterial activities of Phyllanthus acidus leaf extract will be evaluated. This will involve testing the extract against various bacterial strains to determine its effectiveness in inhibiting bacterial growth. Positive results in antibacterial activity would further support the potential use of Phyllanthus acidus leaf extract as a natural alternative to antibiotics in broiler production. Moreover, the research will also investigate other pharmacological effects of Phyllanthus acidus leaf extract, such as its anti-cholesterol properties. This information will provide additional insights into the potential benefits of Phyllanthus acidus leaf extract as a feed additive for broilers.

Overall, this research aims to explore the potential of Phyllanthus acidus leaf extract as a candidate for broiler antimicrobial feed additive, by identifying its phytochemical composition, quantifying total flavonoid content, evaluating

antibacterial activities, and investigating other pharmacological effects. The findings of this research could contribute to the development of natural alternatives to antibiotics in poultry production, addressing public health concerns related to antibiotic resistance and residue in food products.

2. Material and Methods

Materials:

A variety of materials were utilized in this study, including fresh Phyllanthus acidus leaves, a soxhlet extractor, a microwave, an incubator, an autoclave, a spectrophotometer, a vacuum drying oven, phytochemical reagents, MHA medium, and a bacterial isolate.

Extraction:

To extract the Phyllanthus acidus leaf, a previous method was followed, which involved macerating the leaf in 96% ethanol at a ratio of 1:4 for 48 hours. The extract was then subjected to microwave extraction for 10-15 minutes at a temperature of 40-50°C and filtered through muslin cloth before being stored at 4°C for further analysis.

Photochemical Screening:

Qualitative tests were conducted on the extract to screen for the presence of bio-active compounds, including alkaloids, terpenoids, saponins, flavonoids, tannins, and steroids. The presence of each compound was identified through characteristic color changes using standard procedures.

Alkaloids: To determine if alkaloids were present in the extract, Mayer and Wagner's tests were conducted. The formation of a yellow cream precipitate with Mayer's reagent or a brown/reddish brown precipitate with Wagner's test would indicate the presence of alkaloids.

Terpenoids: Salkowski's test was used to test for the presence of terpenoids in the extract.

Saponins: The methanol extract was boiled and filtered, and then 2.5 ml of the extract was mixed with 10 ml of distilled water in a test tube. The mixture was shaken for about 30 seconds and observed for frothing to determine the presence of saponins.

Flavonoids: To test for the presence of flavonoids, 1.5 ml of 50% methanol was added to 4 ml of the extract, and the mixture was warmed. Magnesium fillings were added, followed by a few drops of concentrated hydrochloric acid. A pink or red color would indicate the presence of flavonoids.

Tannins: A portion of the extract was diluted with distilled water at a 1:4 ratio, and a few drops of 10% ferric chloride solution were added. The presence of tannins would be indicated by a blue or green color.

Total Flavonoid:

Total flavonoid content (TFC) was also measured using an aluminum chloride colorimetric assay with quercetin (QE) as the calibration curve.

Antibacterial Activity:

Finally, the well diffusion test was used to assess the antibacterial activity of the extract on MHA medium. The medium was sterilized through autoclaving at 121°C for 15 minutes, cooled until 50-55°C, and then inoculated with a bacterial suspension. A sterile cotton swab was used to streak the extract on the surface of the MHA plates.

Methods:

Sample Collection and Preparation:

Fresh leaves of Phyllanthus acidus L. Skeels (gooseberry) should be collected, washed thoroughly, and air-dried. The dried leaves should then be ground into a fine powder and stored in an airtight container until further use.

Phytochemical Analysis:

The phytochemical analysis should be carried out to determine the presence of different bioactive compounds in the gooseberry leaf extract. The extract should be prepared by maceration or solvent extraction. The various bioactive compounds such as alkaloids, flavonoids, tannins, saponins, phenols, etc. should be analyzed using standard protocols.

Total Flavonoid Content:

The total flavonoid content of the gooseberry leaf extract should be determined using a standard colorimetric method. The results should be expressed in milligrams of quercetin equivalents per gram of dry weight.

Antibacterial Activity:

The antibacterial activity of the gooseberry leaf extract should be evaluated against some common bacterial pathogens such as Escherichia coli, Salmonella enteritidis, and Staphylococcus aureus. The agar well diffusion method should be used, and the results should be expressed as the diameter of inhibition zones in millimeters.

Broiler Study:

A total of 60-day-old broiler chickens should be randomly divided into three groups, with 20 chickens in each group. Group 1 (control) should be fed a basal diet without any additives, Group 2 should be fed a basal diet supplemented with 1% gooseberry leaf extract, and Group 3 should be fed a basal diet supplemented with 2% gooseberry leaf extract. The study should be conducted for 42 days, and the following parameters should be evaluated:

1. Body weight gain
2. Feed intake
3. Feed conversion ratio
4. Mortality rate

Subjects &Selection Method:

1. Plant Material: The selection of the plant material is critical since it would influence the quality and quantity of the phytochemicals present in the extract. The plants should be collected from a location where they are known to grow abundantly and should be free from any contaminants or diseases.

2. Extraction Method: The extraction method used to isolate the phytochemicals from the plant material should be standardized and optimized to ensure reproducibility and accuracy of the results.

3. Antibacterial Activity: The selection of bacterial strains for testing the antibacterial activity of the extract should be based on the intended use of the fortified boiler feed. The selected bacteria should be representative of the types of bacteria that are likely to be present in the feed and should be clinically relevant.

4. Study Design: The study design should be appropriate to answer the research question and objectives. For example, a randomized controlled trial could be used to evaluate the efficacy of the fortified boiler feed in reducing bacterial growth in the feed.

5. Sample Size: The sample size should be large enough to provide statistically significant results. The power analysis should be conducted to determine the appropriate sample size based on the expected effect size, level of significance, and statistical power.

Inclusion Criteria:

1. Source of Plant Material:

The plant material used for the extraction of the gooseberry leaf extract should be from a reliable source, and the identity of the plant should be confirmed through taxonomical identification.

2. Extraction Method:

The method used for the extraction of the gooseberry leaf extract should be described in detail, including the type of solvent used, extraction temperature, extraction time, and solvent-to-sample ratio.

3. Phytochemical Analysis:

The phytochemical constituents present in the gooseberry leaf extract should be identified and quantified using standard analytical methods, such as HPLC, GC-MS, or LC-MS.

4. Antibacterial Activity:

The antibacterial activity of the gooseberry leaf extract should be evaluated against relevant bacterial strains using standard methods, such as the disk diffusion assay or the broth microdilution assay.

5. Broiler Feed Formulation:

The formulation of the fortified broiler feed using the gooseberry leaf extract should be described in detail, including the amount of extract used, the nutrient composition of the feed, and the method of mixing the extract with the feed.

6. Statistical Analysis:

The data obtained from the phytochemical and antibacterial analyses should be subjected to appropriate statistical analysis to determine the significance of the results.

Exclusion Criteria

1. Sample Contamination:

Samples contaminated with other plant species or substances may yield inaccurate results. Therefore, samples that are not pure or contain impurities should be excluded from the analysis.

2. Sample Inconsistency:

Samples that vary in age, growth stage, or storage conditions may yield inconsistent results. Therefore, samples that are not uniform should be excluded from the analysis.

3. Sample Quality:

Samples that are damaged, wilted, or discolored may yield inaccurate results. Therefore, samples that are not fresh

and of high quality should be excluded from the analysis.

4. Antibacterial Resistance:

Bacterial strains that are resistant to commonly used antibiotics may not respond to the antibacterial activity of the gooseberry leaf extract. Therefore, bacterial strains that are known to be resistant to the extract should be excluded from the analysis.

5. Inadequate Data:

Samples that do not have enough data to draw meaningful conclusions should be excluded from the analysis. This may include samples with incomplete or missing data, or samples with unreliable or inconsistent data.

6. Poor Experimental Design:

Studies with poor experimental design or inadequate controls may yield unreliable or inconsistent results. Therefore, studies with poor design should be excluded from the analysis.

7. Lack of Relevance:

Studies that are not relevant to the research question or objectives may not provide useful information. Therefore, studies that are not relevant to the research should be excluded from the analysis.

Procedure Methodology:

1) Collection and Preparation of Plant Material:

Collect fresh and healthy Gooseberry leaves from a location where they are known to grow abundantly. The leaves should be thoroughly washed with water to remove any dirt or debris, and then air-dried or oven-dried at a low temperature to remove excess moisture.

2) Extraction of Phytochemicals:

Use a standardized and optimized extraction method to isolate the phytochemicals from the dried Gooseberry leaves. Commonly used methods include maceration, soxhlet extraction, and ultrasound-assisted extraction. The extracted phytochemicals should be concentrated and purified to remove any impurities.

3) Phytochemical Analysis:

Determine the total phenolic and flavonoid content of the extracted phytochemicals using colorimetric assays. High-performance liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS) can be used to identify and quantify specific phytochemical compounds.

4) Antibacterial Activity Testing:

Test the antibacterial activity of the Gooseberry leaf extract against relevant bacterial strains using standard microbiological techniques such as the disc diffusion method or the broth dilution method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) should be determined to evaluate the effectiveness of the extract against the bacteria.

5) Formulation of Fortified Boiler Feed:

Based on the results of the phytochemical and antibacterial activity analysis, formulate a fortified boiler feed containing an appropriate concentration of the Gooseberry leaf extract. The feed should be prepared according to standard protocols and sterilized to prevent contamination.

6) Evaluation of Feed Efficacy:

Conduct a randomized controlled trial to evaluate the efficacy of the fortified boiler feed in reducing bacterial growth in the feed. The bacterial load in the feed should be measured at regular intervals using standard microbiological techniques. Statistical analysis should be performed to determine the significance of the results.

7) Data Analysis and Interpretation:

Analyze the data collected from the phytochemical and antibacterial activity analysis and the feed efficacy trial. Interpret the results in the context of the research question and objectives, and draw conclusions about the effectiveness of the Gooseberry leaf extract in formulating a fortified boiler feed with antibacterial activity.

Statistical Analysis:

The phytochemical constituents and total flavonoid content of Phyllanthus acidus L. Skeels leaf were analyzed descriptively, which involved quantifying various bio-active compounds present in the leaf. The diameter of the zone of inhibition, which is a measure of antibacterial activity, was analyzed using one-way analysis of variance (ANOVA) to determine if there were significant differences among different treatments or groups. If significant differences were found, Duncan's Multiple Range Test (DMRT) was used to identify which groups or treatments differed significantly from each

other.

3. Result and Discussion

A. Qualitative Analysis of Phytochemical Constituents of Leaf Extracts of Phyllanthus Acidus

The result showed that the leaf extract of Phyllanthus acidus(PALE) contains alkaloids, tannins, flavonoids and steroids.These results were consistent with previous studies showing that P. acidus leaf extracts contain a number of important phytochemicalcomponent, i.e.,flavonoids, phenolic compounds, alkaloids, steroids and glycosides, who found flavonoids in P. acidus leaf extracts.

B. Quantitative Analysis of Flavonoid Constituents of Leaf Extracts of Phyllanthus Acidus

Flavonoids are a type of polyphenolic compound with low molecular weight that are often extracted using polar solvents. In this particular study, an ethanol extract was found to have a total flavonoid content of 663.92 µg/mL QE. Previous research has shown that PALE extraction with 70% ethanol and absolute ethanol resulted in total flavonoid contents of 0.50 mg/g QE and 0.51 mg/g QE, respectively. Another study found that the methanol extract had a total flavonoid content of 61.28 mg/g QE. The amount of total flavonoid content can vary due to factors such as the type of solvent, extraction method, and concentration used. The high total flavonoid content observed in the PALE ethanol extract in this study suggests that ethanol is an effective solvent for dissolving flavonoid compounds in ceremai leaves.

C. Evaluation Antibacterial Activity of Phyllanthus Acidus Leaf Extract

The antimicrobial effects of PALE and antibiotic zinc bacitracin were evaluated by measuring the diameter of inhibition zones on agar plates where the test microorganisms were grown. The results were presented in table 3 and showed that PALE had a significant effect on the inhibition zone diameter ($P<0.01$). Although the antibiotic had the largest zone of inhibition, the highest level of PALE showed a response that was closest to the antibiotic group.

Both the antibiotic and PALE showed narrow inhibition activity with average zone of inhibition ranges from 0.3-4.6 mm (table 3), indicating weak antimicrobial activity (<5mm). A previous study on the methanol extract of P. acidus showed average zone of inhibition ranging from 8-12 mm. The use of PALE at various concentrations resulted in lower zone of inhibition compared to the antibiotic (P0). The highest inhibitory activity was observed against the growth of E. coli with a zone of inhibition of 4.6 mm (P0).

PALE was found to be more effective in inhibiting the growth of Gram-positive bacteria compared to Gram-negative bacteria, possibly due to differences in the structure of the cell wall between the two groups of bacteria. The widest zone of inhibition was observed with the highest concentration of PALE (P3) compared to P2 and P1.Flavonoids are known to have antibacterial properties through three mechanisms: inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting energy metabolism.

4. Conclusion

Research has indicated that the leaves of Phyllanthus acidus L. Skeels contain bio-active compounds that have potential antibacterial properties. These properties suggest that the leaves may serve as a natural feed additive for broilers to improve their health and growth. However, it is important to note that further research and testing would be needed to confirm these findings and determine the optimal dosage and application of the leaf as a feed additive for broilers.

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