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Original Article

ANTIMICROBIAL ACTIVITY OF CHLOROPHYLL EXTRACT ISOLATED FROM COWPEA (VIGNA UNGUICULATA) LEAVES AGAINST SELECTED BACTERIAL AND FUNGAL PATHOGENS

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ABSTRACT

Background: The rapid emergence of antimicrobial resistance has intensified the search for novel bioactive compounds from natural sources. Chlorophyll, a naturally occurring pigment abundant in green plants, has attracted increasing attention due to its antioxidant and potential antimicrobial properties. However, limited information is available regarding the antimicrobial activity of chlorophyll extracted from cowpea leaves (Vigna unguiculata).

Objective: This study aimed to evaluate the antimicrobial activity of chlorophyll extracted from cowpea leaves against selected bacterial and fungal pathogens.

Methods: Fresh cowpea leaves (Vigna unguiculata) were used for chlorophyll extraction using an acetone–methanol solvent system. The antimicrobial activity of the chlorophyll extract was assessed by the disc diffusion method following National Committee for Clinical Laboratory Standards (NCCLS) guidelines. The extract was tested at a concentration of 100 mg/mL against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans. Antimicrobial efficacy was evaluated by measuring inhibition zone diameters around the treated discs.

Results: The chlorophyll extract demonstrated antimicrobial activity against all tested microorganisms. Strong inhibitory effects were observed against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Candida albicans, with inhibition zones indicating active to highly active antimicrobial performance. In contrast, Pseudomonas aeruginosa exhibited only limited susceptibility, producing a smaller inhibition zone (10 mm), indicative of partial activity. The findings suggest that chlorophyll extracted from cowpea leaves possesses broad-spectrum antimicrobial potential, particularly against Gram-positive bacteria and fungal pathogens.

Conclusion: Chlorophyll extracted from Vigna unguiculata leaves exhibited promising antimicrobial activity against a range of clinically relevant microorganisms, with the exception of Pseudomonas aeruginosa, which showed reduced sensitivity. These findings support the potential use of plant-derived chlorophyll as a natural antimicrobial agent and warrant further investigations to elucidate its mechanisms of action, optimize extraction procedures, and evaluate its therapeutic applications.

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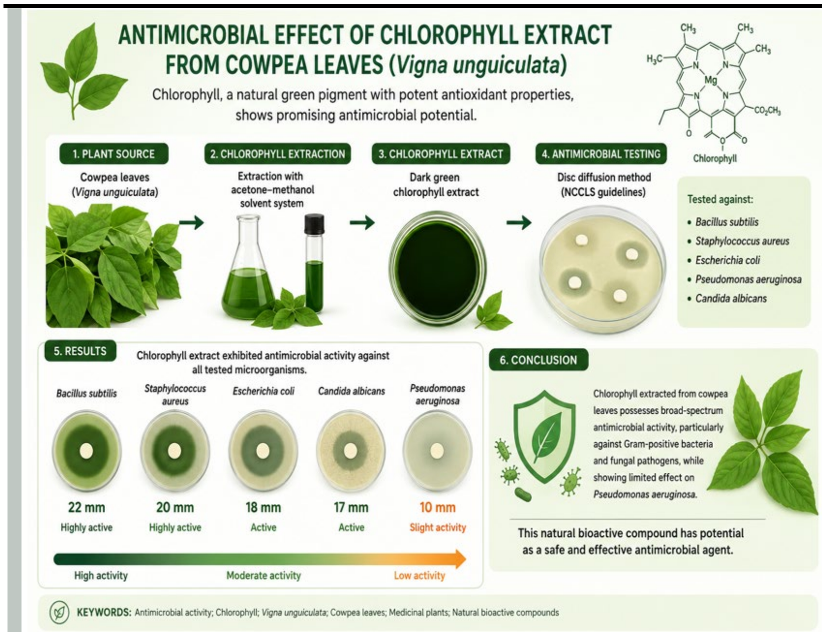

Keywords: Antimicrobial activity, Chlorophyll, Vigna unguiculata, Cowpea leaves, Medicinal plants, Natural antimicrobial agents

INTRODUCTION

Dark leafy greens are superfoods due to their high nutritional value. They are rich in antioxidants like vitamins C, E, and beta-carotene, which protect cells and may prevent cancer. These greens also provide vital nutrients such as folate, vitamins A and B for heart health, and vitamin K for bone health. Additionally, they are high in fiber and essential minerals like iron, magnesium, calcium, and potassium, while being low in cholesterol, sodium, and carbohydrates. Photosynthesis converts carbon dioxide, water, and energy into organic compounds, involving sunlight, pigments, and chlorophyll. Despite extensive research, the exact mechanisms of light absorption and energy conversion are still not fully understood. Key reviews on chlorophyll can be found in studies by Sanpietro and Black (1965), Vernon and Avron (1965), Whittingham (1965), and Clayton (1966). Early studies of photosynthesis initially credited green chlorophyll as the sole pigment in plants. It was later discovered to be a mix of yellow pigments, including chlorophyll alpha and beta, which were separated using column chromatography developed by Tswett (1906). Since then, various chlorophylls (a, b, c, d, e) and bacteriochlorophylls (a, b) have been extracted from plants, algae, and cyanobacteria (650 and 660) Strain (1958), Holt (1965),Allen (1966).

Medicinal plants play a crucial role in healing practices across various cultures worldwide and serve as a significant reservoir of therapeutic agents and healing solutions Elhussain et al. (2020). Numerous contemporary pharmaceuticals are indirectly derived from these plants, underscoring their ongoing significance in the process of drug development. Traditional medicinal systems, such as those in India and China, primarily depend on plants for their therapeutic benefits Rajalakshmi and Banu (2016). In Sudan, the use of medicinal plants is a vital aspect of cultural heritage, especially in rural regions, where they serve both nutritional and therapeutic roles. The Medicinal and Aromatic Plants Research Institute (MAPRI) in Sudan is crucial for researching these plants and assessing their healing properties Elhussain et al. (2020). Moreover, a review emphasizes the effectiveness of traditional herbal drinks in Indonesia for managing hypertension and the effects of herbal supplements on blood pressure. Several studies suggest that complementary therapies using herbal plants can significantly reduce blood pressure levels. Felis and Triyanto (2024). Likewise, Mimosa pudica, recognized for its medicinal benefits, is utilized in traditional medicine to address a variety of health issues, such as diarrhea, insomnia, and urogenital infections. For example, chlorophyllin derived from Mimosa pudica shows antimicrobial effects against various harmful bacteria and fungi Rajalakshmi and Banu (2016). Recent studies indicate that annually, 33,000 individuals in Europe die from infections caused by antibiotic-resistant bacteria, with 39% of these deaths linked to strains resistant to last-line antibiotics such as carbapenems and colistin. Antibiotic-resistant infections impose significant economic and healthcare-related costs. An analysis revealed that antibiotic usage increased by 65% between 2000 and 2015, with projections suggesting a rise of up to 200% by 2030. Polianciuc et al. (2020) Antibiotic-resistant pathogens, once confined to healthcare settings, are now common in the community, complicating containment and treatment. The rise of drug resistance in ESKAPE pathogens—Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species—is particularly concerning Boucher et al. (2009). Foodborne illnesses from pathogens like Escherichia coli and Salmonella also remain significant threats. Human antibiotic use has created selective pressure, enabling pathogens to acquire long-existing resistance elements Strahilevitz et al. (2009). With increasing drug resistance, new antibiotics are essential Gerard (2012). The introduction of antibiotics in the twentieth century marked a transformative era in modern medicine. Today, antibiotic-resistant bacteria are becoming increasingly common. The global dissemination of these resistant strains is mainly due to the misuse of antibiotics and a shortage of effective therapeutic options. Addressing this urgent issue necessitates international collaboration to develop new antimicrobial agents to combat resistant pathogens Browne et al. (2020). This study aims to identify the antibiotic effect of chlorophyll extract from cowpea leaves (Vigna unguiculata).

MATERIALS AND EXPERIMENTAL DESIGNS

PLANT COLLECTION

Vigna unguiculata leaf samples were taken from Omdurman's popular market in July 2021.

According to Medicinal & Aromatic Plants &Traditional Medicine Research Institute 2021 classification of Vigna unguiculata:

Botanical name: Lablab purpureous (L) Walp.

Vernacular name: Lubia Alaf or lubia warag (Ar).

Family: Fabaceae

Code number: Cultivated

CHLOROPHYLL EXTRACTION

Chlorophyll crude extract was prepared from sauropus androgynus leaves by extraction with acetone 80%: methanol 80% (7:3, v/v). 13 a given amount of leaves 200g was mixed with acetone: methanol (7:3, v/v) and magnetically stirred until the pigment was removed. Ascorbic acid was added to the solution to avoid degradation of the pigment. The extract was filtered to remove insoluble material. The filtrate obtained was then portioned with diethyl ether three times sequentially. The organic phase material results were added with Na2So4 anhydrate as far as the water contained was bound maximum. The solvent removal and drying process of chlorophyll used a rotary evaporator and N2 gas Zini et al. (2012)

The percentage yield was calculated as follows:

%Yield = $(wt.\ of\ extract\ /wt.\ of\ frish\ leaves)$ ×100

= (9/200) × 100% = 4.5%

METHODS

PREPARATION OF BACTERIAL SUSPENSIONS

One ml aliquots of a 24-hour broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37ºC for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 108 - 109 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique Miles and Misra (1938).

Serial dilutions of the stock suspension were made in sterile normal saline solution, and 0.02 ml volumes of the appropriate dilution were transferred by micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry, and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony-forming units per ml suspension.

The average number of viable organisms per ml of the stock suspension was determined through the surface viable counting technique Miles and Misra (1938). Serial dilutions of the stock suspension were made in sterile normal saline solution, and 0.02 ml volumes of the appropriate dilution were transferred by micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry, and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony-forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

PREPARATION OF FUNGAL SUSPENSION

The fungal cultures were maintained on Sabouraud dextrose agar and incubated at 25 °C for 4 days.

The fungal growth was harvested and washed with sterile normal saline, and finally, the suspension in 100 mL of sterile normal saline, and the suspensions were stored in the refrigerator until used.

TESTING OF CHLOROPHYLL ANTIMICROBIAL SUSCEPTIBILITY

Disc diffusion method: The paper disc diffusion method was used to screen the antimicrobial activity of plant extracts and performed by using Mueller-Hinton agar (MHA) and Sabouraud dextrose agar. The experiment was carried out according to the National Committee for Clinical Laboratory Standards (1999). Bacterial and fungal suspensions were diluted with a sterile physiological solution to 108cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial and fungal suspension were swabbed uniformly on the surface of MHA and SDA the inoculums were allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and SDA and soaked with 20 µl of a solution of each plant extract. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

Test Bacterial microorganism:

Bacillus subtilis NCTC 8236 (Gram + ve bacteria)

Staphylococcus aureus ATCC 25923(Gram +ve Bacteria)

Escherichia coli ATCC 25922(Gram -ve bacteria)

Pseudomonas aeruginosa ATCC 27853 (Gram -ve bacteria)

Test fungal microorganisms:

Candida albicans ATCC7596

National Collection of Type Culture (NCTC), Colindale, England.

American Type Culture Collection (ATCC), Rockville, Maryland, USA.

RESULTS

An antimicrobial test using dimethyl sulphoxide as a solvent at a concentration of 100 mg/ml demonstrated that chlorophyll extract from cowpea leaves (Vigna unguiculata) exhibited antimicrobial activity against all microorganisms tested in the experiment: Escherichia coli (E.c), Staphylococcus aureus (S.a), Bacillus subtilis (B.s), and Candida albicans (C.a) with 00-00 activity, except Pseudomonas aeruginosa (Ps.a), which displayed 00-10 activity. The antibacterial activity results were expressed in terms of the diameter of the zone of inhibition, and 18mm was considered very active. The results were represented by the diameter of the inhibition zone: < 9 mm indicates inactivity; 9-12 mm indicates partial activity; 13-18 mm indicates activity; and >18 mm indicates a very active status, as demonstrated in Figures 1,2,3.

Table 1

Table 1Comparative Sensitivity of Selected Microorganisms to Chlorophyll Extract from Vigna unguiculata Leaves

Plant name	Solvent	concent	E.c	Ps.a	S.a	B.s	C.a
Vigna unguiculata	Dimethyl sulphoxide	100mg\ ml	00-00	00-10	00-00	00-00	00-00

B.s = Bacillus subtilis; S.a = Staphylococcus aureus; C.a = Candida albicans:; E.c = Escherichia coli ; Ps.a= Pseudomonas aeruginosa

The chlorophyll extract obtained from cowpea (Vigna unguiculata) leaves demonstrated antimicrobial activity against the tested microorganisms. The observed inhibitory effect suggests that chlorophyll contains bioactive compounds capable of suppressing microbial growth. Among the tested organisms, Pseudomonas aeruginosa exhibited the lowest susceptibility, indicating a comparatively higher resistance to the extract than the other microorganisms.

Table 2

Table 2 Antimicrobial activity of chlorophyll extract from cowpea (Vigna unguiculata) leaves against selected microorganisms

Microorganism	Code	Response to Chlorophyll Extract	Activity Level
Escherichia coli	E.c	Visible inhibition zone observed	Active
Pseudomonas aeruginosa	Ps.a	Small inhibition zone observed	Partially active
Staphylococcus aureus	S.a	Clear inhibition zone observed	Active
Bacillus subtilis	B.s	Clear inhibition zone observed	Active
Candida albicans	C.a	Visible inhibition zone observed	Active

B.s = Bacillus subtilis; S.a = Staphylococcus aureus; C.a = Candida albicans:; E.c = Escherichia coli ; Ps.a= Pseudomonas aeruginosa

The chlorophyll extract obtained from cowpea (Vigna unguiculata) leaves exhibited antimicrobial activity against all tested microorganisms. Clear inhibition zones were observed against Staphylococcus aureus and Bacillus subtilis, while Escherichia coli and Candida albicans also showed visible growth inhibition. In contrast, Pseudomonas aeruginosa demonstrated only partial susceptibility, as evidenced by the relatively smaller inhibition zone. These findings suggest that chlorophyll extract possesses broad-spectrum antimicrobial activity against both bacterial and fungal pathogens, with varying degrees of effectiveness among the tested microorganisms.

Figure 1

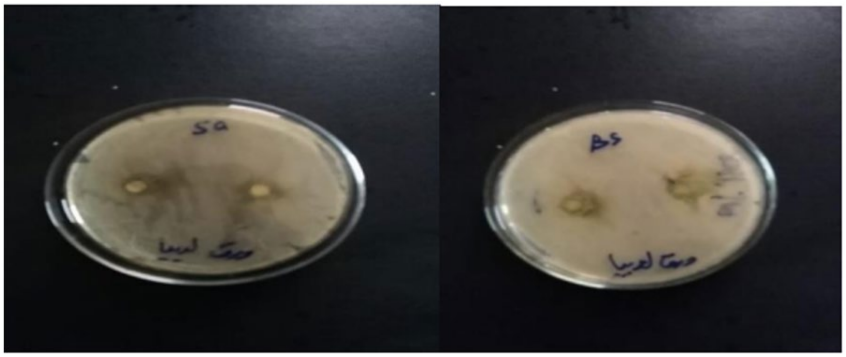

Figure 1 demonstrates the antimicrobial activity of chlorophyll extract against Staphylococcus aureus and Bacillus subtilis. The presence of visible inhibition zones surrounding the discs indicates that the extract effectively inhibited the growth of these Gram-positive bacteria. The findings suggest that chlorophyll extract possesses antibacterial properties and may be particularly effective against Gram-positive microorganisms.

Figure 2

Figure 2 illustrates the response of Pseudomonas aeruginosa to chlorophyll extract. A relatively small inhibition zone was observed, indicating limited susceptibility of this bacterium to the extract. This reduced sensitivity may be attributed to the intrinsic resistance mechanisms of P. aeruginosa, including its protective outer membrane and efflux pump systems.

Figure 3

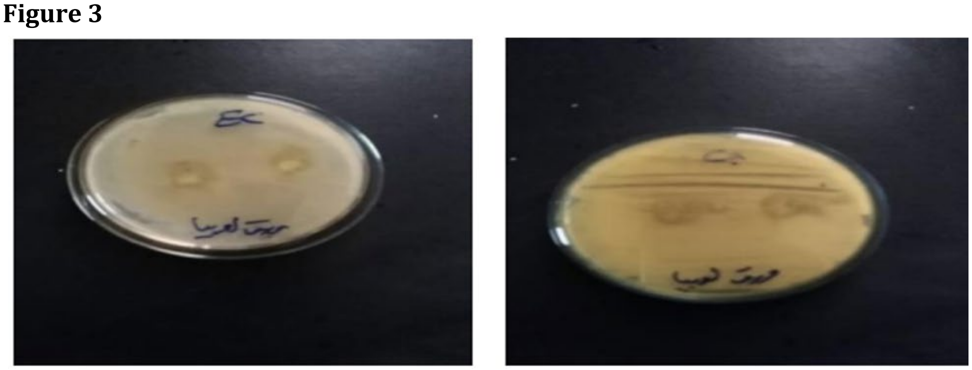

Figure 3 shows the antimicrobial activity of chlorophyll extract against Escherichia coli and Candida albicans. The visible inhibition zones observed around the discs indicate that the extract was capable of suppressing the growth of both the Gram-negative bacterium E. coli and the fungal pathogen C. albicans. These results suggest that chlorophyll extract exhibits broad-spectrum antimicrobial activity against diverse microbial groups. Collectively, the results indicate that chlorophyll extracted from cowpea (Vigna unguiculata) leaves possesses antimicrobial activity against both bacterial and fungal pathogens. The extract demonstrated stronger activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Candida albicans, while Pseudomonas aeruginosa showed comparatively lower susceptibility. These findings support the potential application of chlorophyll as a natural antimicrobial agent and warrant further studies to determine its active compounds and mechanisms of action.

DISCUSSION

Medicinal plants have long served as valuable sources of therapeutic compounds and continue to play an important role in traditional and modern healthcare systems. Numerous studies have documented the antibacterial, antifungal, antiviral, and anti-inflammatory properties of plant-derived bioactive compounds, highlighting their potential as alternative antimicrobial agents Samy and Ignacimuthu (2000), Stepanović et al. (2003). The present study evaluated the antimicrobial activity of chlorophyll extracted from cowpea (Vigna unguiculata) leaves against selected bacterial and fungal microorganisms. The findings demonstrated that chlorophyll extract exhibited antimicrobial activity against all tested microorganisms, including Escherichia coli, Staphylococcus aureus, Bacillus subtilis, and Candida albicans. Clear inhibition zones were observed against Staphylococcus aureus and Bacillus subtilis, indicating notable susceptibility of these Gram-positive bacteria to the extract. Visible inhibitory effects were also detected against Escherichia coli and Candida albicans, suggesting that chlorophyll possesses activity against both bacterial and fungal pathogens. In contrast, Pseudomonas aeruginosa exhibited only partial susceptibility, as evidenced by the relatively smaller inhibition zone. This reduced sensitivity may be attributed to the intrinsic resistance mechanisms of P. aeruginosa, including low outer membrane permeability, multidrug efflux systems, and biofilm-forming capacity, which are known to reduce the effectiveness of many antimicrobial agents. The antimicrobial activity observed in this study is consistent with previous reports describing the biological properties of chlorophyll and its derivatives. Tumolo and Lanfer-Marquez (2012) reported that chlorophyll possesses antimicrobial activity in addition to its recognized applications as a natural food colorant and wound-healing promoter. The ability of chlorophyll to inhibit microbial growth may be associated with its antioxidant properties and the presence of bioactive compounds capable of interfering with microbial cellular functions. The present findings are also in agreement with the work of Rajalakshmi and Banu (2016), who investigated the antimicrobial activity of chlorophyll extracted from Mimosa pudica and reported inhibitory effects against both Gram-positive and Gram-negative bacteria, as well as Candida albicans. Similar to the current study, their results demonstrated that chlorophyll-derived compounds possess broad-spectrum antimicrobial potential against diverse microbial groups. The comparable findings obtained from different plant sources suggest that chlorophyll itself may contribute significantly to the observed antimicrobial effects. The increasing prevalence of antimicrobial resistance has become a major global public health concern. Antimicrobial drugs have played a crucial role in reducing mortality and improving life expectancy; however, the widespread emergence of multidrug-resistant pathogens threatens their long-term effectiveness Suvorov et al. (2021). Consequently, there is growing interest in identifying natural products with antimicrobial properties that may complement existing therapeutic strategies. Plant-derived compounds such as chlorophyll represent promising candidates due to their natural origin, biological activity, and potential for development into alternative antimicrobial agents. The broad-spectrum activity observed in this study against Gram-positive bacteria, Gram-negative bacteria, and fungal organisms highlights the potential pharmaceutical value of chlorophyll extracted from cowpea leaves. Although the exact mechanism of action remains unclear, previous studies have suggested that chlorophyll and its derivatives may disrupt microbial cell membranes, interfere with essential metabolic pathways, and promote oxidative damage to microbial cells. These mechanisms may collectively contribute to the inhibition of microbial growth observed in the present investigation. To the best of our knowledge, this study is among the first to investigate the antimicrobial properties of chlorophyll extracted from cowpea (Vigna unguiculata) leaves. The findings provide preliminary evidence supporting the potential use of cowpea-derived chlorophyll as a natural antimicrobial agent. Nevertheless, further investigations are required to quantify antimicrobial activity using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays, identify the active constituents responsible for the observed effects, evaluate toxicity and safety profiles, and explore potential pharmaceutical and food-preservation applications. Such studies will contribute to a better understanding of the therapeutic potential of chlorophyll and its possible role in combating microbial infections and antimicrobial resistance.

CONCLUSION

The present study demonstrated that chlorophyll extract obtained from cowpea (Vigna unguiculata) leaves possesses antimicrobial activity against a range of bacterial and fungal microorganisms. The extract exhibited inhibitory effects against Escherichia coli, Staphylococcus aureus, Bacillus subtilis, and Candida albicans, while Pseudomonas aeruginosa showed comparatively lower susceptibility. These findings indicate that chlorophyll derived from cowpea leaves has the potential to function as a broad-spectrum natural antimicrobial agent. Given the increasing global challenge of antimicrobial resistance, plant-derived bioactive compounds such as chlorophyll represent promising alternatives for the development of novel antimicrobial products. The observed antimicrobial activity highlights the potential pharmaceutical, nutraceutical, and food-preservation applications of cowpea leaf chlorophyll. However, further studies are required to quantify its antimicrobial efficacy, identify the active constituents responsible for microbial inhibition, elucidate the underlying mechanisms of action, and evaluate its safety and effectiveness under in vivo conditions. Overall, this study provides valuable preliminary evidence supporting the antimicrobial potential of chlorophyll extracted from Vigna unguiculata leaves and contributes to the growing body of research exploring natural products as sustainable solutions for combating microbial infections and antimicrobial resistance.

AUTHORS' CONTRIBUTIONS

Israa Mohamed Fadla Alla Elhaj, Nuha Aboud Ahmed, and **Hiba Hassan Ibrahim** were responsible for conducting the laboratory experiments, sample collection, chlorophyll extraction, antimicrobial assays, and data acquisition. **Rabaa Musa Hamad** contributed to the study design, supervision of laboratory work, and interpretation of the results. **Azhari A. Mohammed Nour** conceived and supervised the study, contributed to data interpretation, manuscript preparation, critical revision of the manuscript, and overall project coordination. **Mohamed Awad Elkarim Mohamad Ibrahim** contributed to data analysis, interpretation of findings, manuscript review, and critical revision of the intellectual content.

RECOMMENDATIONS

1) Further studies should determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of chlorophyll extract from Vigna unguiculata leaves.
2) The bioactive compounds responsible for the antimicrobial activity should be isolated and characterized.
3) Additional in vivo studies are recommended to evaluate the safety and therapeutic efficacy of the extract.
4) The potential application of cowpea leaf chlorophyll as a natural antimicrobial agent in pharmaceutical and food-preservation products should be explored.

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