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Invitro Antioxidant and Antimicrobial Activities of Ethanolic Extract of *Musa paradisiaca* (Plantain) Pseudostem

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Abstract

The pseudostem of *M. paradisiaca* has been used traditionally in the treatment of wounds, infections, gastrointestinal disorders and inflammation in West Africa. This study reports the evaluation of the antioxidant and antimicrobial activities of the plant using standard in vitro assays. Antioxidant activity was assessed using DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) assays. Agar well diffusion method was used to determine antimicrobial activities. The extract exhibited a dose-dependent inhibition in both models. DPPH scavenging activity, which achieved 83.325 % inhibition at 80 mg/ml, compared to butylated hydroxytoluene (BHT) reaching 98.501 % at 80 mg/ml. Thus, the extract exhibited moderate inhibition of free radicals. The extract gave a ferric reducing power of 87.999% at 80 mg/ml compared to gallic acid with ferric reducing power of 96.19% at 80 mg/ml, indicating moderate ferric reducing power. Antimicrobial screening revealed bacterial zones of inhibition 10.00 – 14.00 mm against *S. aureus, E. coli,* and *P. vulgaris,* compared to 25 – 38 mm for ciprofloxacin, and fungal zones of inhibition 10.0 – 11.5 mm against *A. niger, C. albicans* and *R. stolonifer,* compared to 14.5 – 32.0 for Nystatin. MIC values ranged between 200–250 mg/mL, while MBC/MFC values

ranged from 250 to > 250 mg/mL, the MBC to MIC, and MFC to MIC ratio are 1.00, and 1.25 respectively, indicate bacteriocidal and fungicidal effects. These results justify its use in traditional medicine in the treatment of conditions related to oxidative stress and microbial infections. Further studies can lead to development of natural drugs from the plant.

Keywords: *Musa paradisiaca,* antimicrobial activity, antioxidant activity, therapeutic agent.

Introduction

Natural products are very important sources of biologically-active compounds which have varying applications in nutrition and therapy. Phytochemicals called secondary metabolites are responsible for the biological activities of natural products, and make them useful in the treatment and management of diseases. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are free radicals which are responsible for oxidative stress. Overexpression of ROS and RNS due to imbalance in the equilibrium between their production and neutralization are the major causes of many diseases such as, inflammation, neurodegenerative disorders, cardiovascular diseases, cancer, cataract, asthma, rheumatoid arthritis, diabetes,[1] ,[2]. Pathogenic microbes are responsible for large number of infections worldwide challenge the immune system in many ways, many bacteria make us sick, they kill cells and tissues, or produce toxins that can paralyze or destroy cells' metabolism, or express a massive toxic immune reactions .[3]

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Plantain *(Musa paradisiaca)* pseudo stem (Figure 2), is a neglected waste despite reports of its medicinal potential and wide pharmacological properties.[4] It is a monoherbacious plant belonging to the family *Musaceae,* distributed throughout the tropical and sub-tropical countries. From the literature, the juice from plantain stem (Figure 2) removes toxins from the body. The juice can dissolve kidney stones and it is diuretic. Obesity, urinary stones, u*lcers,* burning sensation, acidity, diabetes, bleeding disorders can be treated with the plantain stem.[5] *M. paradisiaca* have been identified to possess antioxidant effects against oxidative stress-related conditions such as diabetes and hypertension, and antimicrobial properties against E. coli and Staphylococcus aureus[6].

The antioxidant and antimicrobial properties of *M. paradisiaca* are due to the presence of secondary metabolites, mainly, phenolics and flavonoids [7] ,[8] . Phenolics and flavonoids neutralize free radicals, thereby preventing cellular damage which may lead to oxidative stress, they also inhibit or kill microorganisms by disrupting their cellular structures or functions.[9]

Many diseases have formed resistance to synthetic drugs, and some of the synthetic drugs are associated with harmful side effects and damage to body organs. The prohibitive cost of many conventional medicines keep them beyond reach, hence there is urgent need for research into natural medicines that are not only effective and harmless, but affordable.

Despite the use of plantain stem by traditional medicine practitioners in the effective treatment of different diseases, there is limited scientific evidence of its medicinal properties and the compounds responsible for those properties, knowledge of these can be harnessed to produce safer, more effective and affordable medicines to improve people's well-being.

The antioxidant and antimicrobial studies were carried out in the present study to confirm the pharmacological potentials of ethanolic extract of *M. paradisiaca*.

Fig. 1A *Musa paradisiaca* (plantain) tree Fig.1B *Musa paradisiaca* pseudostem

Materials and Methods

Plant Material and Extraction

Pseudostem of *Musa paradisiaca* was harvested from the botanical garden belonging to Alvan Ikoku Federal University of Education, Owerri Imo State, it was authenticated by Dr. Evans Kemka, an expert in Plant Taxonomy, at the department of Biology, Alvan Ikoku Federal University of Education. The pseudo stem was dried under laboratory condition. The sample (500

g) was soaked in 2.5 L of 95% ethanol. After 72 h, it was filtered, and concentrated using rotary evaporator under reduced pressure condition at 40 °C. The crude extract was collected in a flask.

Antioxidant Assays

DPPH Radical Scavenging Activity:

A total of 24 milligrams of DPPH were dissolved in 100 mL of methanol for making the stock solution. Filtration of DPPH stock solution using methanol yielded a usable mixture with an absorbance of around 0.973 at 517 nm. In a test tube, 3 mL DPPH workable solutions were combined with 100 µL of extract. Three milliliters of solution containing DPPH in 100 µL of methanol was as a standard. After that, the tubes were kept in complete darkness for 30 min. The absorbance was therefore determined at 517 nm. The following formula was used to compute the percentage of antioxidants or RSA .[10] The IC_{50} values were calculated using Graphpad.

$$\% \text{ of antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

where: Ac—Control reaction absorbance; As—Testing specimen absorbance.

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Ferric Reducing Antioxidant Power (FRAP):

FRAP assay was performed according to the methods of [11] with slight modification.[12] An amount of 200 µl extracted samples were mixed with 3 mL FRAP reagent in test tubes and undergoes vortex. Blank samples were prepared for both methanol and deionized water extracted samples. Both samples and blank were incubated in water bath for 30 minutes at 37°C and the absorbance of the samples was determined against blank at 593 nm. Series of stock solution at 200, 400, 800, 1200 and 1600 µM were prepared (r2= 0.9944) using aqueous solution of $FeSO_4.7H_2O$ as standard curve. The values obtained were expressed as µM of ferrous equivalent Fe (II) per gram of freeze dried sample as well as EC_{50} values in Trolox equivalents

Antimicrobial assays

Test organisms:

Gram-positive: *Staphylococcus aureus, Streptococcus mutans;* Gram-negative: *Escherichia coli, Pseudomonas aeruginosa; fungi: Candida albicans, Aspergillus niger.*

Determination of the Antimicrobial Activities of the Extracts

The agar well diffusion method as described by [13] was adopted in the determination of the antimicrobial activities of the extracts. A sterile Pasteur pipette was used to drop 0.2 ml standardized inoculums equivalent to 0.5 McFarland's turbidity standards on the surface of already prepared and dry Mueller-Hinton agar and Sabouraud dextrose agar. The inoculum was evenly spread using sterile Hockey stick shaped glass rod. Two wells were carefully bored into each agar plate after standing for about 5 minutes with heat sterilized 6 mm diameter cork borer and labeled. 0.1 ml of 125 mg/ml of the extracts were then poured into the wells and the plates were allowed to stand for about 30 minutes for proper diffusion of the solutions before being incubated at 37 ^{o}C for 24 hours for bacterial isolates and 27 ^{o}C for 48hours for fungal isolates. After incubation, antimicrobial activity was evaluated by measuring the diameter of the zones of inhibition produced by the extracts against the test organisms in millimeters.

Tests for Minimum Inhibitory Concentrations (MIC) of the Extracts

Tube dilution method as described by [14] was adopted in the determination of the minimum inhibitory concentration of the extracts. Two milliliters (1 ml) each of the extracts was added in to four milliliters (2 ml) of peptone water; this gives 500 mg/ml. Thereafter, two-fold serial dilutions was carried out from the 500 mg/ml concentration by transferring 1 ml of the 500 mg/ml concentration to 2 ml of peptone water contained in a test tube and homogenized properly. This procedure of transferring 1 ml of the tube to 2 ml of peptone water contained in the subsequent tube was continued until the last test tube. The following concentrations, 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.50 mg/ml and 31.25mg/ml were thereafter obtained. Having obtained the different concentrations and dilutions, three drops of overnight broth cultures of the test organisms were inoculated into the dilutions in each case of the test organisms. The tubes were then incubated at 37 ^{0}C for 24 hours for bacterial isolates and 27 ^{0}C for 48 hours for fungal isolates. The lowest concentration of the extracts that inhibited the growth of the test organisms were recorded as the MIC.

Test for Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC) of Extracts

Tubes showing no visible growth from the MIC test were subcultured onto sterile nutrient agar plates and incubated at 37 ^{0}C for 24 hours for bacterial isolates and 27 ^{0}C for 48 hours for fungal isolates. The lowest concentration of each the extracts yielding no growth was recorded as the minimum bactericidal/fungicidal concentrations.

Statistical Analysis

Determinations were carried out in triplicate, results are presented as mean ± standard deviation (SD). The IC_{50} (half maximal inhibitory concentration) for DPPH radical scavenging activity and EC_{50} (effective concentration) for ferric reducing antioxidant potential were calculated using GraphPad, based on non-linear regression analysis. Comparison of the extract and standard antioxidant results were carried out using one-way ANOVA (analysis of variance) at 95% confidence level, with statistical significance set at $p < 0.05$

Results and Discussion

Antioxidant activity by DPPH Method

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The DPPH radical scavenging activity of the extract and the standard antioxidant (BHT) is presented in Table 1. The extract exhibited considerable free radical scavenging activity across all tested concentrations (10–80 mg/mL).

At 10 mg/mL, the extract showed a mean scavenging activity of 76.50%, which increased progressively with concentration to 83.33% at 80 mg/mL. In comparison, BHT demonstrated higher scavenging activity at most concentrations, with values ranging from 84.61% at 20 mg/mL to 98.50% at 80 mg/mL.

Although the extract showed slightly lower activity than BHT, the increase in scavenging activity with concentration indicates a dose-dependent antioxidant effect.

Table 1: DPPH Radical Scavenging Activity of Plantain Pseudostem Vs BHT.

Concentration (mg/ml)	% scavenging activity of Extract			% scavenging activity of BHT		
	1st	2nd	Average	1st	2nd	Average
10	76.422	76.577	76.4995 ± 0.110	82.627	92.478	87.5525 ± 4.9255
20	82.161	82.265	82.213 ± 0.0735	85.109	84.102	84.6055 ± 0.5035
40	82.627	83.040	82.8335 ± 0.292	89.100	88.489	88.7945 ± 0.3055
80	83.299	83.351	83.325 ± 0.0368	98.501	98.501	98.501 ± 0.5352

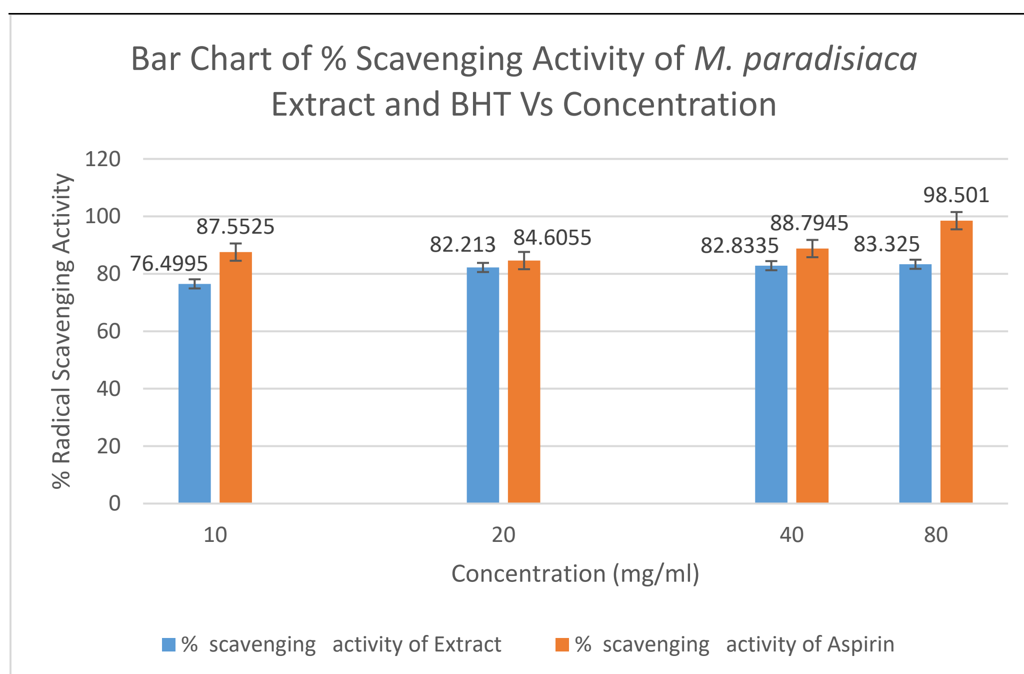

Figure 2: Percentage Scavenging Activity of *M. paradisiaca* Extract and BHT Vs Concentration

Similarly, as shown in figure 4, the ferric reducing potential of the plant extract is slightly lower at all concentrations measured than gallic acid, except at 10 mg/ml, where % FRAP has an average value of 77.478 mg/ml, compared with 76.950 mg/ml for gallic acid.

Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing antioxidant capacity of the extract and gallic acid is shown in Table 2 . The extract demonstrated moderate reducing power, with average values ranging from 69.62% to 87.99%.

At lower concentrations (10 mg/mL), the extract exhibited a relatively high reducing power (77.48%), which decreased slightly at intermediate concentrations (20–40 mg/mL) before increasing significantly at 80 mg/mL (87.99%).

In contrast, gallic acid exhibited consistently higher reducing power across all concentrations, with values ranging from 76.95% to 96.19%. The highest activity for gallic acid was observed at 80 mg/mL.

Table 2. Percentage Ferric Reducing Potential of Extract vs Gallic acid

Conc. (mg/ml)	% ferric reduction of extract				% ferric reduction of gallic acid			
	1st	2nd	3rd	Average $\pm$ SD	1st	2nd	3rd	Average $\pm$ SD
10	76.426	77.523	78.475	77.478 ± 1.0254	76.219	75.995	78.636	76.95 ± 0.2760
20	74.748	74.251	71.435	73.478 ± 1.7867	93.847	93.311	93.693	93.617 ± 0.2760
40	70.421	68.998	69.453	69.624 ± 0.7267	95.314	94.654	92.659	94.209 ± 1.3823
80	86.57	86.912	90.515	87.999 ± 2.1856	97.001	96.871	94.695	96.189 ± 1.2955

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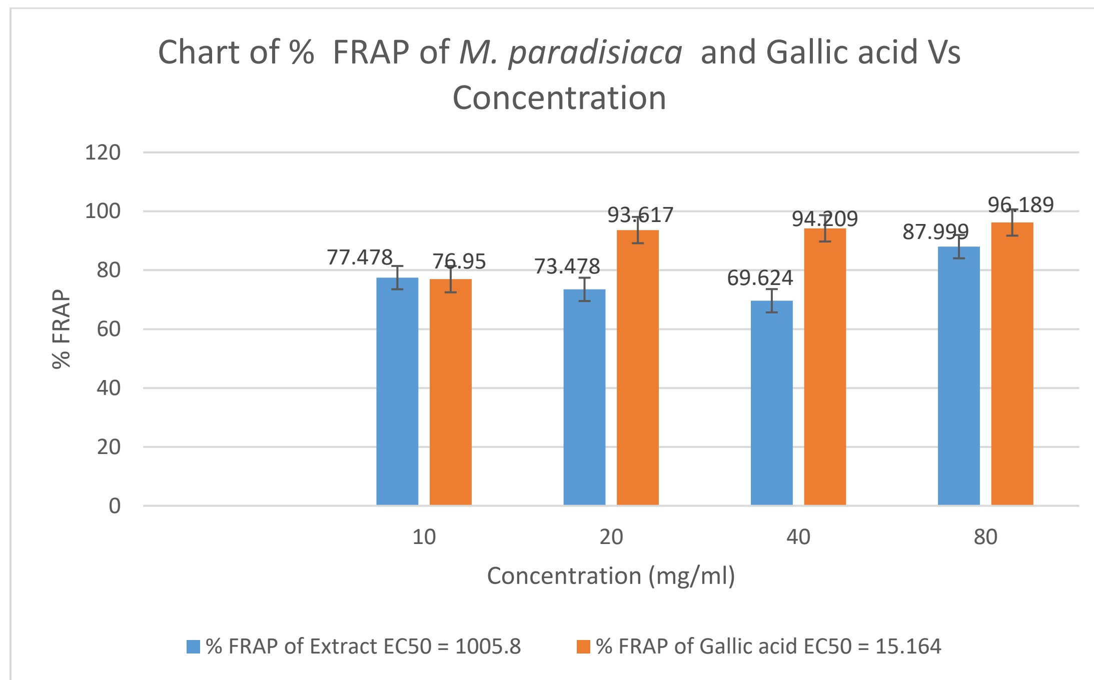

Figure 3. Percentage FRAP of *M. paradisiaca* and gallic acid.

Antimicrobial activity

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The extract produced bacterial zones of inhibition ranging from 10.00 – 14.00 mm against *S. aureus, E. coli,* and *P. vulgaris,* compared to 25 – 38 mm for ciprofloxacin; 10.0 – 11.5 mm against *A. niger, C. albicans* and *R. stolonifer,* compared to 14.5 – 32.0 for Nystatin (table 3). Bacterial MIC values (table 4) is 200 mg/ml, while fungal MIC ranged between 200–250 mg/mL, while MBC and MFC (table 5) values ranged from 250 to > 250 mg/mL, for both bacteria and fungi. With MBC/MIC, and MFC/MIC ratio, being 1 and approximately 1. *Bacillus species, V. cholera, A. flavus* and *M. indicus* were not susceptible.

Table 3: Antimicrobial susceptibility test of the extract

Test organisms	Mean zone of inhibition (mm)	Control (mm)
Staphylococcus aureus	10.00	26.5
Escherichia coli	10.5	38.0
Bacillus species	-	31.5
Vibrio cholera	-	32.5

Proteus vulgaris	14.0	25.0
Candida albicans	11.0	32.0
Aspergillus niger	10.0	26.0
Aspergillus flavus	-	14.5
Rhizopus stolonifera	11.5	26.0
Mucor indicus	-	26.0

Key: Control = Chloramphenicol for the bacterial test organisms and Nystatin for the fungal test organisms

Table 4: Minimum Inhibitory Concentration of the extracts

Test organisms	Concentration of extracts (mg/ml)						MIC (mg/ml)
	250	200	100	50	25	12.5	
Staphylococcus aureus	-	+	+	+	+	+	250
Escherichia coli	-	+	+	+	+	+	250
Bacillus species	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Vibrio cholera	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Proteus vulgaris	-	+	+	+	+	+	250
Candida albicans	-	-	+	+	+	+	200
Aspergillus niger	-	+	+	+	+	+	250

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Aspergillus flavus	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Rhizopus stolonifera	-	+	+	+	+	+	200
Mucor indicus	N.D	N.D	N.D	N.D	N.D	N.D	N.D

Key: mg/ ml = milligram per mililitre

N.D = Not done because the raw extract did not show any zone of inhibition against the test organisms. + = growth, - = no growth

Table 5: Minimum Bactericidal/ Fungicidal Concentration of the extracts (mg/ml)

Test organisms	Concentration of extracts (mg/ml)						MIC (mg/ml)
	250	200	100	50	25	12.5	
Staphylococcus aureus	-	+	+	+	+	+	250
Escherichia coli	-	+	+	+	+	+	250
Bacillus species	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Vibrio cholera	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Proteus vulgaris	+	+	+	+	+	+	>250
Candida albicans	+	-	+	+	+	+	>250
Aspergillus niger	-	+	+	+	+	+	250
Aspergillus flavus	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Rhizopus stolonifera	+	+	+	+	+	+	>250

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Mucor indicus	N.D	N.D	N.D	N.D	N.D	N.D	N.D

Key : mg/ ml = milligram per mililitre

N.D = Not done because the raw extract did not show any zone of inhibition against the test organisms.

+ = growth., - = no growth

Statistical Analysis: one way ANOVA showed that there was significant difference ($P < 0.05$) between the extract and standard in the two antioxidant assays. The 95% confidence intervals showed that the plant extract exhibited significant antioxidant properties, although less potent than BHT.

Discussion of Results

The combined results from both assays confirm that the ethanolic extract of plantain pseudo stem has strong dose-dependent antioxidant activity, though slightly lower than standard antioxidants (BHT and gallic acid). These results support the use of the plant extract as a natural antioxidant.

The DPPH assay evaluates the ability of compounds to donate hydrogen atoms or electrons to neutralize free radicals. The observed increase in scavenging activity with concentration suggests that the extract contains bioactive compounds capable of stabilizing free radicals.

Although the extract showed slightly lower activity compared to BHT, its high percentage inhibition (above 76% even at the lowest concentration) indicates strong antioxidant potential. This activity may be attributed to the presence of phytochemicals such as flavonoids, phenolics, and tannins, which are known for their radical scavenging properties.

The near plateau observed between 40 mg/mL and 80 mg/mL suggests that the active constituents may reach saturation at higher concentrations, limiting further increases in activity.

The FRAP assay measures the electron-donating capacity of antioxidants, reflecting their reducing power. The extract demonstrated appreciable ferric reducing ability, particularly at higher concentrations.

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The initial decrease in reducing power at 20 and 40 mg/mL may suggest possible interaction effects among phytoconstituents or experimental variability. However, the marked increase at 80 mg/mL indicates enhanced electron-donating capacity at higher concentrations.

Phenolic compounds, including flavonoids, tannins and vitamin C, which have been identified in plantain and banana pseudostems may contribute to the antioxidant activities of the plant extract.[15] These findings corroborate with [16] and [17], who reported that various parts of banana and plantain exhibit strong radical scavenging activities due to their phenolic content.

The extract exhibited moderate antimicrobial activity against selected bacteria and fungi, with zones of inhibition ranging from 10–14 mm, while no activity was observed against some organisms, indicating selective effectiveness. Compared to the standard control, the extract showed much lower potency.

MIC values (200–250 mg/mL) suggest that high concentrations are required to inhibit microbial growth, indicating relatively weak activity in crude form. The extract showed better activity against Candida albicans and Rhizopus stolonifer (MIC = 200 mg/mL) compared to other organisms.

The antimicrobial activity shows that extracts from *Musa paradisiaca* inhibit pathogenic bacteria and fungi, the results support the finding by [18]. The ratio of the MBC/MIC or MFC/MIC values indicate that the extract is bacteriocidal and fungicidal respectively.[(19,20)] Overall, the extract shows limited but promising antimicrobial potential.

These findings are significant because natural antioxidants and antimicrobials are increasingly preferred over synthetic ones due to safety concerns. The extract could therefore serve as a potential source of bioactive compounds for pharmaceutical or nutraceutical applications. The phytochemicals in the plant extract could be isolated to serve as lead candidates for future drug development.

Conclusion

The present study has identified that the ethanolic extract of *Musa paradisiaca* pseudostem possesses significant antioxidant and antimicrobial properties. These findings highlight the medicinal potential of plantain pseudostem, and align with the growing interest in the search for plant-based medicines for oxidative stress and infectious diseases. There is need for further work

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to be carried out to isolate the compounds responsible for the observed antioxidant and antimicrobial activities.

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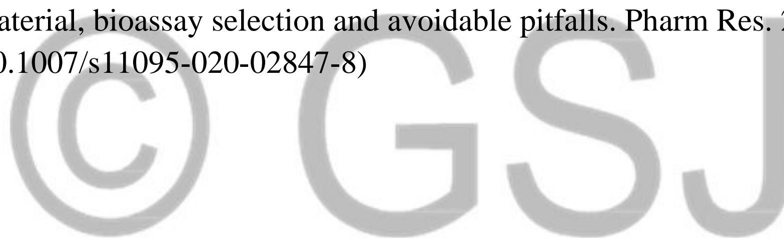
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