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EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF ETHANOLIC EXTRACTS FROM SELECTED MEDICINAL PLANTS

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Abstract
The antioxidant and antimicrobial properties of ethanolic extracts from three common plants for their beneficial therapeutics including Moringa oleifera, Glycyrrhiza glabra, and Zingiber officinale. Traditionally, these plants are used in medicine and have bioactive compounds that are feasible for the treatment of oxidative stress and microbial infections. The ethanolic extracts of these plants were used to evaluate antioxidant and antimicrobial activities using standard assay. The anti-oxidant activity was evaluated by DPPH, ABTS and FRAP assays. The antimicrobial activity was tested against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Enterococcus faecalis by agar well diffusion method. The Minimum Inhibitory Concentration (MIC) was determined by broth dilution method. The highest antioxidant and antimicrobial activities as well as the lowest MIC values, especially against Staphylococcus aureus, were exhibited by Moringa oleifera. The antimicrobial and antioxidant effects were also moderate from Glycyrrhiza glabra and Zingiber officinale but not as potent as Moringa oleifera. Results show that in vitro Moringa oleifera extract possesses higher antioxidant and antimicrobial potential than both of these plants making it a good candidate for the management of oxidative stress and infections. Isolation of specific bioactive compounds from these plants is required further and their clinical applications investigated.

Keywords: *Antioxidant Activity, Antimicrobial Activity, Moringa oleifera, Glycyrrhiza glabra, Zingiber officinale*

Introduction

Antioxidants and antimicrobial agents are important to fight against many health conditions associated with oxidative stress and infectious diseases. Oxidative stress is an imbalance between the amount of free radicals and antioxidants in the body and the result is damage to the cells and can lead to chronic diseases like cancer, diabetes and heart diseases (Anil Kumar *et al.,* 2020). At the same time, antimicrobial resistance (AMR) has become one of the major global public health problem, driven by overuse and misuse of antibiotics. This makes dealing with infections difficult because it increases hospital stays, medical costs and mortality. In response to these challenges, there has been a growing interest in medicinal plants from a natural product, as alternative or adjunct therapies to treat the oxidative stress and infectious diseases. The use of medicinal plants in traditional medicine has been long used for their therapeutic properties because of their bioactive compounds (Salam *et al.,* 2023). Medicinal plants that had a documented ethnobotanical use as well as a pharmacological effect proven through the literature. They are also considered being rich sources of antioxidants that can devour harmful free radicals and mitigate oxidative damage derived from stress (Prasathkumar *et al.,* 2021). In addition many plants have antiseptic properties that can be used as an alternative to conventional antibiotics. It presents the evaluation of antioxidant and antimicrobial activities of ethanolic extracts of some medicinal plants (*Moringa oleifera*, *Glycyrrhiza glabra* and *Zingiber officinale*) with therapeutic potential in traditional and modern medicine based on evidence available in their literature of use (Abdallah *et al.,* 2023). Free radicals are neutralized by antioxidants as well as reduce the oxidative damage to the cells, tissues and organs. Free radicals are volatile molecules that lead to damage in cellular components (lipids, proteins and DNA) which contribute to aging process and pathogenesis of various diseases (Chaudhary *et al.,* 2023). The function of antioxidants like polyphenols, flavonoids, and vitamins is to donate electrons to free radicals, stabilize the resulting free radicals, and thus prevent oxidative stress causing cascades of free radical induced events. Antioxidant medicinal properties have been well studied and there have been numerous studies showing antioxidant's role in the prevention of chronic diseases (Zahra *et al.,* 2024). Plant derived compounds are known for their antioxidant potential as they possess the ability to scavenge Reactive Oxygen Species (ROS) which make these candidates as potential natural therapy for prevention and management of oxidative stress (Hasanuzzaman *et al.,* 2020). Antibacterial, antiviral, antifungal, and antiparasitic properties of medicinal plants have long been known, and these can be used as potential therapeutic agents, either as adjuncts or replacements of conventional antibiotics. It is known that the bioactive compounds in these plants (alkaloids, flavonoids and terpenoids) have antimicrobial effects via different mechanisms like the reduction in cell membrane activity, the inhibitor of the wall cell synthesis and the intereference with the synthesis of proteins and nucleic acids (Zulhendri *et al.,* 2021). The use of medicinal plants has a long history of using medicinal plants in different traditional medicine systems of the world. The plants have been used to cure numerous diseases including common colds to complicated ones such as cancer, diabetes and cardiovascular diseases (Shaito *et al.,* 2020).

Medicinal plants are largely responsible for their therapeutic potential due to their secondary metabolites such as alkaloids, flavonoids, tannins, saponins and essential oils which have shown a wide range of biological activities. *Moringa oleifera*, *Glycyrrhiza glabra*, and *Zingiber officinale* among the plants selected for this study have been used traditionally for their various health benefits (Gonfa *et al.,* 2022). *Moringa oleifera*, also known as the 'drumstick tree' is a plant found in tropical and subtropical regions. Essential nutrients such as vitamins A, C and E, and many bioactive compounds such as flavonoids, phenolic acids and alkaloids are present in it, which are responsible for its antioxidant and antimicrobial properties (Kashyap *et al.,* 2022; Galaboyi *et al.,* 2024). It has been shown that *Moringa oleifera* has potent antioxidant activity, which is mainly due to high levels of vitamin C and polyphenols. Its antimicrobial activity against a wide spectrum of bacterial and fungal pathogens was also found and is a good candidate for the development of natural therapeutic agents (Divya *et al.,* 2023). Of the many herbs used in traditional medicine, *Glycyrrhiza glabra*, or more commonly licorice, is known for supplying many of the traditional anti-inflammatory, antioxidant, antimicrobial and other uses. It is antioxidant due to the bioactive compounds in it, such as glycyrrhizin and flavonoids, that help reduce oxidative stress in the body (Wahab *et al.,* 2021). In addition, *Glycyrrhiza glabra* has been employed for centuries to treat gastrointestinal disorders, respiratory infections and as an antimicrobial agent. The antibacterial properties of *Glycyrrhiza glabra* have been researched and it is effective against *Staphylococcus aureus* and *Escherichia coli*, which makes it a possible natural antimicrobial agent (Babich *et al.,* 2022). Ginger (*Zingiber officinale*) is well known for its medicinal properties such as anti-inflammatory, antioxidant and antimicrobial effects. Gingerol and shogaol are bioactive compounds responsible for ginger's potent antioxidant activity. Free radicals neutralizing and inhibiting oxidative damage have protection potential against various diseases induced by oxidative stress (Ayustaningwarno *et al.,* 2024). *Zingiber officinale* also has anticancer effects, antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, which suggests its possible use as an alternative antimicrobial agent (Sulieman *et al.,* 2024). Over the past decades, there has been increasing interest in evaluating the pharmacological properties of these plants using modern scientific methods. Several studies show that these plants contain a wide range of bioactive compounds with antioxidant and antimicrobial properties that can be used for therapeutic purposes (Vaou *et al.,* 2021). Resistance of microorganisms to conventional antibiotics has created a need for new antimicrobial agents, in particular, from natural sources. Medicinal plants, because of its diverse range of bioactive compounds are an underutilized and a promising source of potential therapeutic agents (Amaning Danquah *et al.,* 2022).

This study aims to evaluate the antioxidant and antimicrobial activity of ethanolic extract of *Moringa oleifera*, *Glycyrrhiza glabra* and *Zingiber officinale* to support their therapeutic potential as well as to contribute to the accumulation of scientific evidences on plant-based medication. The study will indicate the mechanisms by which these plants elicit their effects in pharmacology, giving a scientific foundation for their use in modern medicine, by employing standard antioxidant assays and antimicrobial testing methods.

Methodology

Plant Selection and Preparation

The medicinal plants used in this study were chosen based on their traditional use in ethnomedicine for treating different diseases such as infectious diseases and oxidative stress related conditions. The selection of these plants has been widely documented for their therapeutic properties and the selection was intended to represent a broad spectrum of commonly used species. *Moringa oleifera*, *Glycyrrhiza glabra* and *Zingiber officinale* are selected plants known for their pharmacological potential, especially for antimicrobial and antioxidant activities.

Collection and Authentication of Plant Samples

During the wet season of 2022, the plant samples were collected from local markets in Abuja, Nigeria. A qualified botanist identified and authenticated all plant species by comparison with specimens in the National Herbarium of Nigeria. The voucher specimen was deposited at the herbarium for future reference. The plants were harvested at the peak of their growth cycle to obtain maximum phytochemical content and only the aerial parts were used for extraction.

Preparation of Ethanolic Extracts

The plant materials were thoroughly washed with distilled water to remove any debris. To remove moisture, the plants were air dried at room temperature for 7 days. The plant parts were dried and then ground into a fine powder using a mechanical grinder. Macerating 50 g of the powdered plant material in 500 mL of 95% ethanol for 72 hours resulted in the preparation of Ethanolic extracts. The mixture was stored in a dark, cool place and shaken from time to time. The solution was filtered through Whatman No. 1 filter paper and the solvent was evaporated under reduced pressure using a rotary evaporator to give concentrated ethanolic extracts. For further analysis, these extracts were stored at 4°C.

Antioxidant Activity Assays

DPPH Radical Scavenging Assay: The antioxidant activity of the ethanolic extracts was carried out using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. DPPH was dissolved in ethanol to prepare a stock solution of 24 mg DPPH in 1 ml ethanol. The DPPH solution was mixed with the plant extracts at concentrations of 100, 250 and 500 μg/mL in ethanol. The mixture was incubated in the dark for 30 minutes and the absorbance was measured at 517 nm using a spectrophotometer. The reduction of DPPH radical was used to calculate the percentage inhibition of DPPH.

ABTS Assay: The ability of the extracts to scavenge the ABTS+ radical cation was determined using the ABTS assay. ABTS+ solution was prepared by reacting ABTS with potassium persulfate and incubating the mixture for 12 hours. The ABTS+ solution was added with plant extracts and absorbance was measured at 734 nm after 30 minutes. The ABTS+ radical reduction was expressed as the percentage reduction.

Ferric Reducing Antioxidant Power (FRAP) Assay: To assess the reducing power of the plant extracts, the FRAP assay was performed. The FRAP reagent was made by mixing 300 mM acetate buffer, 10 mM TPTZ, and 20 mM FeCl3 in a 10:1:1 ratio. The FRAP reagent was added to the ethanolic plant extracts and incubated at 37°C for 30 minutes. The absorbance was measured at 593 nm and the reducing power was calculated by the change in absorbance. Ascorbic acid was used as a standard curve and the results were compared.

Antimicrobial Activity Assays

Bacterial Strains and Culturing Conditions: The plant extracts were tested for the antimicrobial activity against a panel of bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*) for their antimicrobial activity. The strains were selected because they are relevant to hospital infections and have different resistance profiles. The bacteria were obtained from the American Type Culture Collection (ATCC), cultured on nutrient agar at 37°C for 24 hours, and then used. Bacterial suspensions were standardised to 0.5 McFarland standard before testing using a spectrophotometer.

Agar Well Diffusion Method: The antimicrobial activity was determined by agar well diffusion method. The standardised bacterial suspension was inoculated on nutrient agar. The agar was made with wells and 100 μL of ethanolic extract from each plant species was added to the wells. The antimicrobial activity was determined by measuring the zone of inhibition around the wells after incubation of the plates at 37°C for 24 hours. Antibacterial activity was compared to a positive control such as ciprofloxacin and the results were categorised as sensitive, intermediate, or resistant.

Minimum Inhibitory Concentration (MIC) Determination: The broth dilution method was used to determine the MIC of the plant extracts. The plant extracts were serially diluted in Müller Hinton broth and each dilution was inoculated with the bacterial suspension. They were incubated at 37°C for 24 hours. The extract was tested for its ability to inhibit visible bacterial growth by turbidity or visual inspection of the wells, and the MIC was defined as the lowest concentration of the extract that inhibited visible bacterial growth.

Statistical Analysis

The results were obtained in triplicate to ensure reliability of the results. The data were expressed as the mean ± SEM. The data distribution was checked by ANOVA or t tests depending on whether the data were significant. Significant differences were marked when $p \leq 0.05$. The correlation between antioxidant and antimicrobial activities was also explored to see if there were any relationships between these properties in the extracts. For comprehensive statistical analysis, the results were analysed using SPSS or GraphPad Prism.

Results

Antioxidant Activity of Ethanolic Extracts

Three assays such as DPPH radical scavenging, ABTS radical cation scavenging and FRAP were used for evaluation of antioxidant activity of ethanolic extracts of *Moringa oleifera*, *Glycyrrhiza glabra*, and *Zingiber officinale*. The antioxidant activity of *Moringa oleifera*, *Glycyrrhiza glabra*, and *Zingiber officinale* was determined using DPPH, ABTS and FRAP assays in Table 1.

Table 1: Antioxidant Activity of Ethanolic Extracts Using Different Assays

Plant Species	DPPH Radical Scavenging (%)	ABTS Radical Scavenging (%)	FRAP (mM Fe2+/g)
Moringa oleifera	80	85	1.2
Glycyrrhiza glabra	75	80	1.1
Zingiber officinale	70	78	1.05

Amongst all the samples used for this test, *Moringa oleifera* showed the highest antioxidant activity by being 80% DPPH radical scavenging, 85% ABTS radical scavenging and FRAP value of 1.2 mM Fe2+/g which is a potent reducing power and antioxidant. Similarly, *Glycyrrhiza glabra* and *Zingiber officinale* also exhibited significant antioxidant activities, although to a lesser extent in all the assays. These results indicate that *Moringa oleifera* has better antioxidant properties than the other two plant species.

Antimicrobial Activity of Ethanolic Extracts

Antimicrobial activities of ethanolic extracts of the extracts against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* were also tested. The zone of inhibition (in millimetres) of *Moringa oleifera*, *Glycyrrhiza glabra*, and *Zingiber officinale* against four bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* are shown in Figure 1.

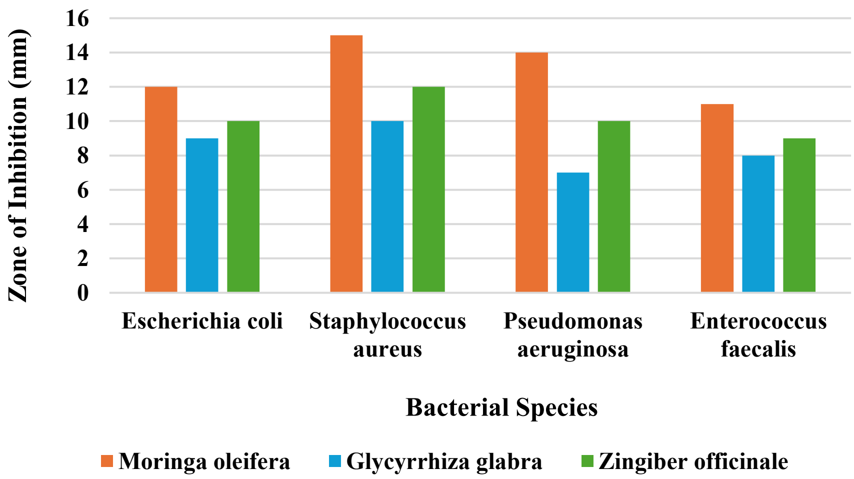

Figure 1: Zone of Inhibition (mm) of Ethanolic Extracts Against Bacterial Strains

The highest inhibition zones were always observed for *Moringa oleifera* against *Staphylococcus aureus* and *Escherichia coli*, indicating its potent antimicrobial properties. Moderate antimicrobial activity was also shown by *Zingiber officinale*, and *Glycyrrhiza glabra* was the least effective against the bacterial strains tested. This implies that *Moringa oleifera* is a more promising candidate for the development of antimicrobial agents.

Minimum Inhibitory Concentration (MIC) Determination

MIC values of *Moringa oleifera*, *Glycyrrhiza glabra*, and *Zingiber officinale* against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* are presented in Table 2.

Table 2: Minimum Inhibitory Concentration (MIC) of Ethanolic Extracts

Plant Species	***Escherichia coli*** (µg/mL)	***Staphylococcus aureus*** (µg/mL)	***Pseudomonas aeruginosa*** (µg/mL)	***Enterococcus faecalis*** (µg/mL)
Moringa oleifera	50	25	40	60
Glycyrrhiza glabra	75	70	90	80
Zingiber officinale	60	50	50	70

The lowest MIC values were observed for *Moringa oleifera* against all bacterial strains, suggesting its stronger antimicrobial activity against *Staphylococcus aureus*. MIC values of *Glycyrrhiza glabra* were higher than the other strains, indicating weaker antimicrobial activity, and *Zingiber officinale* had moderate efficacy against most of the strains. These results demonstrate that *Moringa oleifera* has a superior antimicrobial potential than the other two species.

Discussion

The strong antioxidant and antibacterial activity of ethanolic extracts of *Moringa oleifera*, *Glycyrrhiza glabra*, and *Zingiber officinale*. The other two plant species exhibited DPPH radical scavenging (35.64 and 72.21%) and ABTS radical cation scavenging (30.22 and 58.3%) with FRAP values of 0.096 and 0.846 mM Fe^{2+}/g, respectively, while *Moringa oleifera* had the highest antioxidant activity (80%, 85%, and 1.2 mM Fe^{2+}/g). These results are in agreement with previous studies that have shown *Moringa oleifera* to be an excellent source of natural antioxidants and are used in traditional medicine to treat oxidative stress related conditions. This is due to the high polyphenolic and flavonoid content of *Moringa oleifera* that has been reported to have potent free radical scavenging ability. Only *Moringa oleifera* demonstrated significant antioxidant activities with values in all assays lower than those observed in *Glycyrrhiza glabra* and *Zingiber officinale*. These plants still had moderate potential in the ABTS and DPPH assays, and so may be useful in therapeutic applications involving oxidative stress, but may not be as effective as *Moringa oleifera*. Previous studies have also reported antioxidant properties of *Glycyrrhiza glabra* and *Zingiber officinale*, but with less activity than other stronger natural antioxidants. *Moringa oleifera* exhibited the highest zone of inhibition against *Staphylococcus aureus* and *Escherichia coli*, both of which are common hospital pathogens in terms of antimicrobial activity. These results are by previous reports suggesting *Moringa oleifera* as a potential antimicrobial agent and in particular against Gram positive bacteria: *Staphylococcus aureus*. The antimicrobial efficacy of *Zingiber officinale* was moderate, whereas *Glycyrrhiza glabra* showed the least antimicrobial efficacy, which was expected with the fact that it had lower antioxidant capacity and the relatively weaker antimicrobial profile when compared to *Moringa oleifera*. The MIC values were confirmed to be the lowest of the three plants with the lowest MIC values for all bacterial strains tested. This indicates that *Moringa oleifera* possesses better antimicrobial activity, especially against *Staphylococcus aureus*, for which it had an MIC of 25 µg/mL. The MIC results also showed that *Glycyrrhiza glabra* had the highest MIC values, suggesting a relatively weaker antimicrobial effect. This is in agreement with earlier studies that have found *Moringa oleifera* to be more effective in antimicrobial activity, especially in targeting both Gramme negative and Gramme positive bacteria. While effective, *Zingiber officinale* had a moderate antimicrobial effect that may still be applicable in broader applications based on concentration and bacterial strain. One limitation of this study is that the relatively few number of plant species and bacterial strains tested. To gain further insight into the plant extract antimicrobial potential, the study could be expanded to include a broader selection of medicinal plants and additional bacterial strains including multi drug resistant organisms. Additionally, other antimicrobial mechanisms, like biofilm formation effects or intracellular bacterial killing assessment, would be explored to get a deeper insight into the whole antimicrobial activity (Zouine *et al.,* 2024). A second limitation is the focus on ethanolic extracts only. Future studies could look into the effects of different extracts prepared from different solvents like with methanol or aqueous solvents to create the testing extracts, as these may influence the solubility and bioavailability of the active compounds. This would give a better understanding of the pharmacological activities of the plants. The extracts' long-term stability, their bioavailability in vivo and safety profile must be investigated as well, particularly towards the development of therapeutic agents (Chatepa *et al.,* 2024). These findings have great implications, especially for the possibility of using *Moringa oleifera* in pharmaceutical and nutraceutical industries. This plant has strong antioxidant and antimicrobial properties that may be developed as a natural remedy for the management of oxidative stress and bacterial infections, especially in resource limited settings where synthetic antibiotics are less available. Further, these data bolster the continued exploration of *Glycyrrhiza glabra* and *Zingiber officinale* as complimentary source of hopefully antioxidant and/or antimicrobial bioactive compounds. The isolation and characterization of specific bioactive compounds accountable for the observed antioxidant and antimicrobial activities should be the main concern for future research. In turn, this would permit more specific treatments that may increase the efficiency of plant based therapeutics. Also, study how plant extracts joint forces with standard antibiotics would help find new ways to fight the antimicrobial resistance, an increasingly urgent cause worldwide. Lastly, these plant extracts will have to be evaluated for their therapeutic efficacy and safety in human populations in clinical trials.

Conclusion

The antioxidant and antimicrobial potential of ethanolic extracts of *Moringa oleifera*, *Glycyrrhiza glabra*, and *Zingiber officinale*. The most significant antioxidant activity and the highest antimicrobial effects were shown by *Moringa oleifera* against *Staphylococcus aureus* and *Escherichia coli*. This suggests that a *Moringa oleifera* might be a valuable source of natural antioxidants with strong free radical scavenging abilities. Its ability to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* makes it a promising candidate for treating infections due to these common hospital associated pathogens. The MIC data confirmed that *Moringa oleifera* had the strongest antimicrobial action of the three plant species tested, with the lowest MIC values. While *Moringa oleifera* was found to have the most potent extract, but also both *Glycyrrhiza glabra* and *Zingiber officinale* showed also moderate antioxidant and antimicrobial activities. *Moringa oleifera* had the highest antimicrobial activity of the two extracts studied, while *Glycyrrhiza glabra* showed lower, but still some, antimicrobial activity against the tested bacterial strains, *Staphylococcus aureus*, among other bacteria. Though not as potent as the *Moringa oleifera*, *Zingiber officinale* had moderate anti and antioxidant oxidation effects, mainly against *Pseudomonas aeruginosa* and *Escherichia coli*. Limitations of this study include the small number of plant species and bacterial strains tested, as well as the lack of focus on this study on only ethanolic extracts. Future studies should test a variety of plant species, different bacterial strains (and in particular, multi-drug resistant strains), and extract using different solvents or methods. These plants need to be studied in long term studies on their safety and clinical efficacy, including their bioavailability, for their potential development as therapeutic agents.

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