Antifungal Screening of Surveyed Plants of Gwadabawa/Illela Communities of Sokoto State-Northwest Nigeria

Mathias Sylvester Nefai¹*, Mshelia Halilu Emanuel², Giaze Tijani Rabiu³, Hussain Yahaya Ungo-Kore⁴

INTRODUCTION

The use of medicinal plants, especially infectious diseases, are increasing in recent years. It has been established that since bioactive compounds are from natural sources and have been structured within living systems, they are more biologically friendly than chemically synthetic molecules.¹ Today, several effective anti-fungal and anti-bacterial drugs are used to treat infectious agents; however, the genetic diversity caused by microbial pathogens, the emergence of resistant strains, and side effects of drugs, importantly necessitated replacing them with antimicrobial drugs of plant origin.² Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc. Hence, medicinal plants are widely exploited in traditional folk medicine, and their curative potentials are well documented.³ About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful, especially in the areas of infectious disease and cancer.⁴ The therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of Traditional Medicine.⁵ Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world.

The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies by 80% of the world’s population.⁶ It has been noted not quite recently that invasive fungal diseases are important causes of morbidity and mortality.⁷ Hence, these days, the incidence of invasive fungal infections (IFIs) has been increasing, mostly due to advances in medicine that may produce immunocompromised individuals.⁸ It has been shown that 10–20% of HIV/AIDS patients die directly from fungal infection.⁹ At the same time, additional antifungal agents have become available, but despite these advances, mortality rates of IFIs remain unacceptably high, especially among immunocompromised patients.

ABSTRACT

Background: An earlier ethnobotanical study afforded fifteen surveyed plants, which are used as antifungal and wound healing remedies. This informed their antifungal screening to validate folklore claims. Materials and Methods: Identified, procured, and authenticated medicinal plants were each, defatted in hexane and extracted with methanol before partitioning with n-butanol to obtain an n-butanol extract with solubilized antifungal compounds in the organic phase. The resulting concentrated n-butanol extracts were then screened by subjecting each plant extract to in vitro antifungal assay for the determination of the minimum inhibitory concentration (MIC) of the extract on Aspergillus niger in a 96-well flat bottom polystyrene microtiter plate using the broth microdilution method as outlined in the 2021 Clinical and Laboratory Standard Institute guideline. Results: The antifungal screening of the plants presented active plant extracts with variable antifungal properties of MIC values ranging from 3.9 to 250 (mg/ml). Only the extract of Balanites aegyptica showed no activity at all concentrations tested. The lowest extreme was recorded for Ficus platypylla, which gave a slight activity at the highest MIC of 125mg/ml, while the highest activity was recorded for Faidherbia albida at the lowest test concentration with a MIC of 3.90625 mg/ml. The other thirteen extracts exhibited antifungal activity in varying degree with the following MICs: Steculia setigera (7.8125 mg/ml), Annona senegalensis (7.8125 mg/ml), Urana picta (7.8125 mg/ml) Combretum collinum (15.65 mg/ml), Afromosia levilflora (15.65 mg/ml), Waitheria indica (15.65 mg/ml), Gueira senegalensis (15.65 mg/ml), Carica papaya (31.25 mg/ml), Regeria adenophylla (31.25 mg/ml), Pennisetum hordeoides (31.25 mg/ml), Acacia nilotica (31.25 mg/ml) and Sida ovata (62.5 mg/ml). Conclusion: The in vitro antifungal potential of these surveyed plants supports their traditional use, hence, information (data) furnished by this study can be exploited for their further evaluations as antimicrobial drug leads of nature. Keywords: Antifungal remedy, Surveyed plants, Gwadabawa/Illela, Plant extract, Faidherbia albida.
In this regard, the development of new bioactive compounds or new formulations of antifungals might be useful for a better therapeutic outcome.

Ethnobotanical or ethnopharmacological surveys are recognized as the most viable methods of identifying new medicinal plants leading to drug discovery of natural or semi-synthetic analogs. More so, Farnsworth and Fabricant highlighted the potential of ethnopharmacological approaches in the discovery of new therapeutic agents and the importance of confirming folklore use of herbal remedies by in vitro investigations. Hence, scientific investigations of medicinal plants have been initiated in many countries due to ethnomedicinal plants’ contributions to the fight against various diseases including fungal infections. Several ethnopharmacological surveys have been published during the last years on Traditional Medicine in several cultures in Africa to preserve their herbal remedy usage as well as find an evidence-based approach to their corresponding use. To provide data useful for the conservation of cultural traditions and biodiversity, and at the same time useful for community healthcare as well as drug discovery in the present and the future, the present study seeks to investigate by screening some earlier surveyed medicinal plants of Gwadabawa and Illela communities of Sokoto State-Nigeria, which were widely acclaimed and therapeutically cited by the local as being used as antifungal and wound healing remedies. Consequently, an in vitro antifungal screening of the plant extracts on which scientific knowledge is limited is thus performed to provide scientific evidence of their use.

**MATERIALS AND METHODS**

**Study Area**

The Gwadabawa/Illela people are predominantly Hausa-Fulani situated in Sokoto State-Nigeria. They are communities that lie within the coordinates 13°43’57” N (Latitude) and 5°18’1” E (Longitude) in DMS (Degrees Minutes Seconds) or 13.7325 and 5.30028 (in decimal degrees), North West of the Sahel Savannah region of Nigeria. The Hausa-Fulani people of this area are largely rural with a population of about 17,461 people who mostly pursue an agrarian trader/nomadic herder economy. See the map in Figure 1.

Retrieved from Mapmaker Interactive (https://mapmaker.nationalgeographic.org/); map of Nigeria (bottom right corner) showing especially, Gwadabawa/Illela Local Government Areas where the study was performed. The maps were adopted from our earlier published study of different areas in the same state with slight modifications; designed using the academic version of Map Maker 4 software obtainable from www.mapmaker.com.

**Ethnobotanical Survey**

An ethnobotanical survey carried out earlier for the Gwadabawa/Illela areas of Sokoto State from January to March 2021 has been reported elsewhere in our previous study.

**Plant Material**

**Collection and identification of plants**

The informants, through a focal TMP, guided us to the field where the cited medicinal plants were seen and the plant specimens in question were procured, most especially in cases where the plants were not found around their homes. Standard methods were used in plant material collection, drying, mounting, preparation, and preservation. Photograph images of the collected plant species were made to facilitate their identification processes. Final identification (both colloquially and scientifically) was made at the herbarium unit of the Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto-Nigeria, following compilations of Hausa plant names; vernacular names of Nigerian plants and other books of regional floras. The specimens were labeled with voucher specimen numbers and deposited (where it was not initially available) in the same department for reference.

**Plant extraction**

Air-dried plant samples were ground and defatted with hexane three times. The defatted samples were then extracted with methanol three times. The combined methanol extracts were suspended in water and the suspension was partitioned with n-butanol to solubilize antifungal compounds in the organic phase, leaving sugars, amino acids, and salty compounds in the water phase. The n-butanol extract was vacuum dried and the resultant residue was used for antifungal susceptibility tests.

**Determination of minimum inhibitory concentrations**

The minimum inhibitory concentration (MIC) of the extracts was determined in a 96-well flat bottom polystyrene microtiter plate using the broth microdilution method as outlined in the 2021 Clinical and Laboratory Standard Institute guideline (CLSI). Briefly, a stock concentration (500 mg/mL) of the extracts was prepared in 10% DMSO. Into 100 µL of double-strength Sabroud Dextrose broth in the wells of the polystyrene plate, an aliquot (100 µL) of the extract was dispensed into the first wells of the first rows and serially two-fold-diluted aseptically into 10 different concentrations (250 to 0.49 mg/mL). Thereafter, 10 µL of the standardized 0.5 McFarland turbidity suspension of the test fungi strain, *Aspergillus niger* obtained from the Department of Pharmaceutical Microbiology, Usmanu Danfodiyo University, Sokoto, diluted at 1:100 to obtain a final inoculum density of 1 × 10⁵ CFU/mL was inoculated into the wells. Negative (broth and DMSO) and growth (broth and fungi inoculum, without extract) controls were maintained for each test. The plates were thereafter covered and incubated aerobically at 35°C for 48hr. After incubation, the end-point was determined as the lowest concentration of the extracts in the wells that showed no visible growth.

**RESULTS**

**Ethnobotanical Survey**

A list of fifteen (15) out of the forty (40) ethnobotanical surveyed plants earlier reported and acclaimed by the informants of Gwadabawa/Illela communities was made at the herbarium unit of the Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto-Nigeria.
Illela communities as potent antifungal and wound healing medicinal plant remedies are shown in Table 1.

**In vitro Antifungal Assay**

The antifungal assay screened the fifteen (15) surveyed plants against *Aspergillus niger* using the 96 well microtiter plate-based method. The literature search also showed limited reports on most of the plants on this aspect. It was found that all the extracts showed activity at the highest test concentration (250mg/ml) except one. This is as presented in Table 2.

**DISCUSSION**

**Minimum Inhibitory Concentration (MIC)**

Antimicrobial susceptibility test is most often carried out to determine the effective potential antimicrobial agents from biological extracts of plants informatively sourced through ethnopharmacological research.

<table>
<thead>
<tr>
<th>Table 1. Ethnobotanical characteristics of 15 surveyed plants of Gwadabawa/Illela (Screened for antifungal properties)</th>
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</thead>
<tbody>
<tr>
<td>Family/Scientific name</td>
</tr>
<tr>
<td>[Fabaceae] Acacia nilotica L.</td>
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<tr>
<td>[Leguminosae] Afromosia lexiflora (Benth. ex Baker)</td>
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<tr>
<td>[Anonaceae] Annona senegalensis Pers.</td>
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<tr>
<td>[Zygophyllaceae] Balanites aegyptica Del</td>
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<tr>
<td>Carica papaya Linn.</td>
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<tr>
<td>[Combretaceae] Combretum collinum Fresen</td>
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<tr>
<td>[Fabaceae] Faidherbia albida (Delile) A.Chev.</td>
</tr>
<tr>
<td>[Uritricaceae] Ficus platyphylla De</td>
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<tr>
<td>[Malvaceae] Sida ovata Forssk</td>
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<tr>
<td>[Poaceae] Pennisetum hordeoides (Lam.) Steud.</td>
</tr>
<tr>
<td>[Pedaliaceae] Rogeria adenophylla J.Gay</td>
</tr>
<tr>
<td>[Malvaceae] Sterculia setigera Delile</td>
</tr>
<tr>
<td>[Combretaceae] Uraria picta (Jacq.) Desv. ex DC.</td>
</tr>
<tr>
<td>[Malvaceae] Waltheria indica L.</td>
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The extracts could be against different pathogenic microorganisms as portrayed by their determined MICs. Hence, our investigation of antifungal assay performed on the fifteen (15) surveyed plants against A. niger using the 96 well microtiter plate-based method indicated all the screened extracts as active at the highest test concentration (250mg/ml) except for one. Balanites aegyptica showed no activity at all test concentrations, while other fourteen (14) plant extracts showed activity at varying degrees. The lowest extreme showed that while Ficus Platyphylla gave slight activity at a MIC of 125mg/ml, the highest activity recorded was for Faidherbia albida at the lowest test concentration with a MIC of 3.90625 mg/ml. Thus, active extracts presented variable antifungal properties with MIC values ranging from 3.9 to 250 mg/ml (Table 2). The extract of Faidherbia albida had significant antifungal activity against the tested fungi (MIC: 3.90625 mg/mL). The high activity indicated for F. albida extract might be due to its high content in phenolic and flavonoid compounds, which are known to possess antimicrobial activity.

This might as well explain the success recorded traditionally in the use of this plant to effectively treat chronic and infected wounds, as seen in Table 1. Corroboration with our findings, more reports have also shown the inhibitory effect of the same plant on the growth of pathogenic bacterial and fungi species.

Similarly, Sterculia setigera had earlier been reported to show moderate antifungal activity in a typical evaluation study that agrees strongly with our findings. Total phenolics and flavonoid contents were the phytochemicals identified and attributed to the antifungal activity of S. setigera. The activity recorded for Annona senegalensis here, also support other reported works, where flavonoids and phlobatannins among other phytoconstituents have been assessed and attributed as responsible active antifungal agents in the plants. Similarly, the recent works of Mishra and Kumavat support our findings on Uraria picta leaves, where its extract was effectively used for the green synthesis of AgNPs. The new product formed from this experiment turned out to give a great antimicrobial and antifungal potential with high activity MIC, which goes to identify newer ways of understanding and developing new antimicrobial drug leads of U. picta and from other plants bioactive. In a recent study, the antimicrobial potency of Combretum collinum was demonstrated where its phytochemical and antimicrobial study revealed myricetin-3-O-rhamnoside and myricetin-3-O-glucoside as the main compounds in the hydrophilic extracts. Activities of hydroethanolic extract of the bark of Aframomum axilliflorum have also been evaluated showing it as a bioactive dermatophyte agent. Overall, results from this study agree strongly with those from reported literature and thereby support the antifungal potentials of active extracts recorded with significant MICs such as F. albida, S. setigera, S. setigera, U. picta C. collinum, and A. laxiflora plant species. The antifungal information (data) presented herein can be exploited for their further evaluations as antimicrobial drug leads of nature. Their use by the locals of Gwadabawa/Illa LGAs has thus, been validated of their folkloric therapeutic indications.

CONCLUSION
Fifteen sampled plant species from an earlier ethnobotanical survey study used in the treatment of fungal and wound ailments in the Gwadabawa/Illa communities of Sokoto State showed good antifungal potential when subjected to an in vitro antifungal assay. Hence, this supports the traditional use of the surveyed plants. Further studies should be considered for extract fractionation as well as the isolation of active compounds. In these ways, newer, effective, safe, and affordable phytomedicines can be developed.

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CONFICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

IFIs: Invasive fungal infection; TMP: Traditional medicine practitioner; MIC: Minimum inhibitory concentration.

REFERENCES

SUMMARY

Fifteen ethnobotanical surveyed plants were identified, procured, and authenticated, for which each was defatted in hexane, extracted with methanol, and partitioned with n-butanol. The concentrated n-butanol extracts for each plant were subjected to in vitro antifungal assay on *Aspergillus niger* in a 96-well microtiter plate using the broth microdilution method to determine their MICs. The results revealed active plant extracts with variable antifungal properties of MIC values ranging from 3.9 to 250 (mg/ml). The lowest extreme was recorded for *Ficus platyphylla*, with slight activity at a MIC of 125mg/ml, while the highest activity was recorded for *Faidherbia albida* at a MIC of 3.90625 mg/ml. Hence, all the extracts exhibited antifungal activity in varying degrees except that of *Balanites aegyptica*, which showed no activity at all concentrations tested. Consequently, the in vitro antifungal potential of the surveyed plants supports their traditional use, and thus, information obtained herein can be exploited for further targeted evaluations of the plant extracts’ phytomolecules as possible antimicrobial drug leads.