

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

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History

- Submission Date: 15-07-2022;
- Review completed: 29-08-2022;
- Accepted Date: 19-09-2022.

DOI : 10.5530/pres.14.4.61

Article Available online

<https://www.phcogres.com/v14/i4>

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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 Platyphylla*, 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: *Stecullia setigera* (7.8125 mg/ml), *Annona senegalensis* (7.8125 mg/ml), *Uria picta* (7.8125 mg/ml) *Combretum collinum* (15.65 mg/ml), *Afromosia lexisflora* (15.65 mg/ml), *Waltheria indica* (15.65 mg/ml), *Guiera senegalensis* (15.65 mg/ml), *Carica papaya* (31.25 mg/ml), *Rogeria 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*.

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.

Cite this article: Mathias SN, Emanuel MH, Rabi GT, Ungo-Kore HY. Antifungal Screening of Surveyed Plants of Gwadabawa/Illela Communities of Sokoto State-Northwest Nigeria. Pharmacogn Res. 2022;14(4):417-22.

^[10] In this regard, the development of new bioactive compounds or new formulations of antifungals might be useful for a better therapeutic outcome.^[8]

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.^[11] More so, Farnsworth and Fabricant,^[12] 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.^[13] 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^[14] 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.^[15]

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.^[16] Photographs 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,

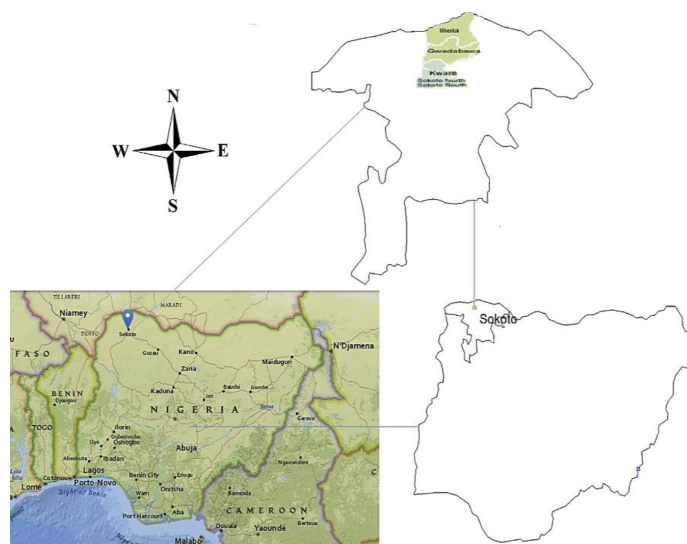


Figure 1: A map of Nigeria (bottom left corner) located along the Gulf of Guinea.

following compilations of Hausa plant names,^[17] vernacular names of Nigerian plants^[18] and other books of regional floras.^[19] 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).^[20] Briefly, a stock concentration (500 mg/ml) of the extracts was prepared in 10% DMSO. Into 100 μ L of double-strength Sabroud Dextrose broth in the wells of the polystyrene plate, an aliquot (100 μ L) 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 μ L 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 1×10^5 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,^[15] and acclaimed by the informants of Gwadabawa/

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 1. Ethnobotanical characteristics of 15 surveyed plants of Gwadabawa/Illela (Screened for antifungal properties)

Family/Scientific name	Source Community	Local name	Ailment uses	Plant part use	Habit	Mode of Preparation (Therapeutic indications)
[Fabaceae] <i>Acacia nilotica</i> L.	Illela town, Hura, Gaido, Chimmola Tungan-Kwangi	Bagaaruuwaa	Wounds, cuts	Stem bark	tree	Heal both fresh and old wounds, even with puss; wash and apply powdered drug; takes some 5 days to heal.
[Leguminosae] <i>Afromosia lexisflora</i> (Benth. ex Baker)	Gaido Illela	Makarfo	Wounds, bleeding	Stem bark	shrub	Powdered drug is sprayed on the wound; the wound heals; also stops bleeding.
[Anonaceae] <i>Annona senegalensis</i> Pers.	Gaido Illela Tungan-Kwangi Gatti, Galadi	Gwanda Daji	Bleeding wounds	Roots	shrub	Powdered drug is sprayed on the wound; can as well be soaked and drink; also stops bleeding.
[Zygophyllaceae] <i>Balanites aegyptica</i> Del	Gaido, Galadi	Aduwa	Wounds	Leaves	tree	Powdered drug is sprayed on the wound; takes some 3 days to heal; also stops bleeding.
Caricaceae <i>Carica papaya</i> Linn.	Bakin Dutsi, Galadi	Gwanda	Whitlow, wounds	leaves, seeds root bark	herb	Mixed plants powder is sprayed on fresh wound/whitlow to effect healing.
[Combretaceae] <i>Combretum collinum</i> Fresen	Gatti	Taramniyaa	Wounds, an infected wound, bleeding	Stem bark	tree	Powdered drug is mixed as a concoction with <i>W. indica</i> and alum, prayed on wounds due to knife, stabbing, old or infected. It stops bleeding and heals within a week.
[Fabaceae] <i>Faidherbia albida</i> (Delile) A.Chev.	Chimmola	Gawo	Wounds, boils	Stem bark	tree	spray powdered herb on the wound;
[Urticaceae] <i>Ficus platyphylla</i> De	Tudun Doki	Gamji	Wounds	Stem	tree	Wash the wound with cold water and apply the powdered drug
[Combretaceae] <i>Guiera senegalensis</i> J. F. Gmel.	Lakoda, Bakin Dutsi, Salame, Tungan-Kwangi	Saabaara	Eye injury Wounds Eczema	Leaves	shrub	Chew and apply leave sap on injurious eye
[Malvaceae] <i>Sida ovata</i> Forssk	Chimmola, Galadi	Miyar tsanya	Wounds, whitlow	Leaves	herb	Spray powder of drug on fresh or dried wound
[Poaceae] <i>Pennisetum hordeoides</i> (Lam.) Steud.	Chimmola	K'yasuwa	Wounds, bleeding	Leaves	grass	Squeeze and apply to the wound
[Pedaliaceae] <i>Rogeria adenophylla</i> J.Gay	Gaido	Loda	Wounds, bleeding	Roots	herb	Roots of the plant are powdered and applied on fresh wounds; stops bleeding as well.
[Malvaceae] <i>Sterculia setigera</i> Delile	Bakin Dutsi, Tudun doki	Kukkuukii	Wounds, bleeding	Stem bark	tree	Dried and powdered herb is sprayed on the wound, heals within a week; also stops bleeding.
[Combretaceae] <i>Uraria picta</i> (Jacq.) Desv. ex DC.	Gaido	Daakushee	Wounds, bleeding	Whole plant	herb	The powdered drug is sprayed on wounds and effect healing within thirty days; stops bleeding; can as well be infused and bath in for healing
[Malvaceae] <i>Waltheria indica</i> L.	Lakoda, Bakin Dutsi, Galadi	Gobir Hausa Yankufa	Wounds, bleeding	Flowers whole plant roots	herb	Powdered mixed herb is sprayed on the wound; stops bleeding, especially during circumcision; takes ten days to heal

Table 2: The minimum inhibitory concentration of the plant extracts against *Aspergillus niger*.

Conc (mg/ml)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	250	-	-	+	-	-	-	-	-	-	-	-	-	-	-
2	125	-	-	+	-	-	-	-	-	-	-	-	-	-	-
3	62.5	-	-	++	-	-	-	-	-	-	-	-	-	+	-
4	31.25	-	-	++	-	-	-	++	-	-	-	-	-	+	-
5	15.625	-	-	+++	-	+	-	++	++	+	-	+	-	++	-
6	7.8125	+	-	+++	+	+	-	+++	++	++	-	++	+	++	-
7	3.90625	++	+	+++	++	++	-	+++	+++	+++	++	++	++	+++	+
8	1.95313	++	+	+++	+++	++	+	+++	+++	+++	++	+++	++	+++	++
9	0.97656	+++	++	+++	+++	++	++	+++	+++	+++	+++	+++	+++	+++	+++
10	0.48828	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
1	<i>Combretum collinum</i> (Taramniya)	2	<i>Sterculia setigera</i> (Kukkuki)	3	<i>Balanites aegyptica</i> (Adua)	4	<i>Afromosia laxiflora</i> (Makarho)	5	<i>Rogeria adenophylla</i> (Loda)						
6	<i>Faidherbia albida</i> (Gawo)	7	<i>Sida acuta</i> (Miyar tsanya)	8	<i>Pennisetum hordeoides</i> (K'yasuwa)	9	<i>Acacia nilotica</i> (Bagaruwa)	10	<i>Annona senegalensis</i> (Gwanda daji)						
11	<i>Carica papaya</i> (Gwanda gida)	12	<i>Waltheria indica</i> (Yankufa)	13	<i>Guiera senegalensis</i> (Sabara)	14	<i>Ficus Platyphylla</i> (Gamji)	15	<i>Uraria picta</i> (Dakushee)						

- implies no growth observed; +implies little growth; ++implies more growth; +++implies highest growth.

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 seen 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.^[21] 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. Corroborative with our findings, more reports have also shown the inhibitory effect of the same plant on the growth of pathogenic bacterial and fungi species.^[22]

Similarly, *Sterculia setigera* had earlier been reported to show moderate antifungal activity in a typical evaluation study that agrees strongly with our findings.^[23] 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.^[24,25] Similarly, the recent works of Mishra and Kumavat,^[26] 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.^[27] Activities of hydroethanolic extract of the bark of *Afromosia laxiflora* have also been evaluated showing it as a bioactive dermatophyte agent.^[28] 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*, *A. senegalensis*, *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/Illela LGAs has thus, been validated of their folklore 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/Illela 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.

ACKNOWLEDGEMENT

We appreciate the support by the management of Usmanu Danfodiyo University, Sokoto (UDUS), for the release of funds from the TETFund as well as the technical assistance rendered by Abdulmalik B. S. of the Department of Veterinary Microbiology, Faculty of Veterinary Medicine, UDUS.

Funding

This research was funded by the tertiary education trust fund (TETFund), under the institutional-based research (IBR) scheme, Usmanu Danfodiyo

University, Sokoto (UDUS) with reference number, TETFUND/DR AND D/CE/UNIV/SOKOTO/RP/VOL.1.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

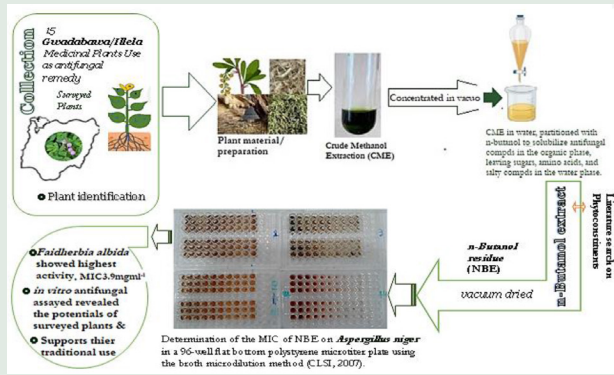
ABBREVIATIONS

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

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GRAPHICAL ABSTRACT



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.

Cite this article: Mathias SN, Emanuel MH, Rabiu GT, Ungo-Kore HY. Antifungal Screening of Surveyed Plants of Gwadabawa/Illela Communities of Sokoto State-Northwest Nigeria. Pharmacog Res. 2022;14(4):417-22.