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ANTIFUNGAL ACTIVITIES OF HONEY AND ACACIA NILOTICA EXTRACTS

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ABSTRACT
The increasing threat of fungal resistant to current antifungal agents underscores the need for an alternative source of antifungal agents. This study was designed to investigate the antifungal activity of honey and Acacia nilotica extracts against four important fungal pathogens (Candida albican, Aspergillus niger, Trychophyton rubrum and Trychophyton mentagrophyte). The in vitro antifungal activity of honey, ethanolic leaves and stem-bark extracts from Acacia nilotica L was studied using agar well diffusion and micro broth dilution techniques. The honey and ethanolic crude leaf extract exhibited considerable fungistatic activity against the four fungal isolates. The maximum diameter zones of inhibition of 18.50 mm was observed in Candida albican with ethanolic leaves extracts while a minimum of 16.00 mm diameter zones of inhibition was found on Trychophyton rubrum and Trychophyton mentagrophyte. There was no activity against all the fungal isolates when ethanolic stem-bark extract was used. A maximum diameter zones of inhibition of 20.00 mm diameter was observed in Trychophyton rubrum with 50 %v/v of honey concentration, while a minimum 13.50 mm diameter zone of inhibition was found in Trychophyton mentagrophyte. The MIC and MFC of the extract against the fungal isolates ranged from 15.63 - 250.00 mg/ml and 31.25 - 250.00mg/ml for ethanolic leaves extract, 6.25-50% and 12.5-50% for honey, 32-128.00µg/ml and 32 - 128.00 µg/ml for fluconazole respectively. The in vitro antifungal activity revealed a considerable antifungal activity of honey and ethanolic leaves extracts of A. nilotica L. than stem-bark extract.

Keywords: *Acacia nilotica*, Honey, Antifungal activity, ethanolic leaves

INTRODUCTION

A medicinal plants is any plant which, in one or more of its organs, contains substance that can be used for therapeutics purposes or which are precursors for the synthesis of useful drugs (Jain *et al*., 2018). They are thought by some to have medicinal properties, but few plants have been proven by rigorous science or approved by regulatory agencies such as United State Food and Drug Administration or European Food Safety Authority to have medicinal effect (Awuchi, 2019).

Acacia nilotica known as ''Bagaruwa'' in Hausa language of Northern Nigerian..It is a proverbial, medium sized tree and broadly scattered in tropical and subtropical countries (Tripathi and Singh, 2020). It has an inspiring range of phytochemical, among which are alkaloid volatile essential oils, phenols and phenolic glycoside, resins, olesins, steroid, tannins (Prakash *et al*., 2018). Different parts of this plant such as leaves, root, seeds, bark, fruits, flowers, gum and immature pods act as anti-cancer, anti-mutagenic, spasmogenic, vasoconstriction, antibacterial, anti-hypertensive activities, and are also engaged for the treatment of different ailment in the indigenous system of medicine (Tripathi and Singh, 2020).

Homey has a valued place in the human diet due to its unique taste, nutritional value and health promoting properties. Consequently, honey has become one of the major therapeutic agents of traditional medicine (Mandal and Mandal, 2011)

A fungus is an organism (eukaryotic) that digests food externally and absorbs nutrients directly through its cell wall (White Jr *et al*., 2018). They are heterotrophs and like animal, obtain their carbon and energy from other organisms. There are four main groups (phyla) of true fungi: *Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota* (Tilston *et al*., 2020). Although formally considered to be plant, they are now generally assigned their own kingdom mycota (Cavalier-Smith, 2018)

The incidence and prevalence of invasive fungal infection have increased since the 1980s, especially in the large population of immune-compromised patients and or those hospitalized with serious underlying disease (Moro and David, 2021).

Fungal infection is increasing in both the community and hospital environment with several causative agent like yeast with *Candida* spp, which is among the leading organism and filamentous fungi such Aspergillus spp (Richardson and Lass-Flörl, 2008). The infection often becomes difficult to manage with the emergence of multi-drug resistance strains especially those observed in Trichophyton spp (Khurana *et al*., 2019). Candida infections also constitute the most common fungal infections in AIDS patients(Gnat *et al*., 2019). This study aimed to investigate the antifungal activity of honey and *Acacia nilotica* extracts against some pathogenic fungal isolates.

MATERIAL AND METHODS

Collection and Identification of Plant Materials

The Leaves and the stem bark of *Acacia nilotica* were collected in Sokoto state, North west-Nigeria in May, 2018 during dry season. The leaves and the stem bark were authenticated at the herbarium garden of the Department of Pharmacognosy and Ethno-pharmacy, Faculty of Pharmaceutical Science, Usmanu Danfodiyo University, Sokoto with a voucher number

PG9/UDUS/legn/0008. The leaves and the stem bark were sized reduced with mortar and pestle, shade-dried, powdered, labeled and stored in an air tight container prior to extraction.

Preparation of plant material

The powdered stem bark (100g) and powdered leaf (50g) of A*cacia nilotica* were weighed using weighing balance and transferred into 500ml empty beaker. Three (300)ml and 250ml of ethanol was transferred to the beaker containing powdered material and extracted by maceration for 24hour. The extracts were filtered and the filtrates were evaporated on an electric mantle. Both extracts were stored in a cool dry place until ready for use.

Fractionation of the Crude Extracts

The crude ethanolic stem-bark and leaves extracts of *Acacia nilotica* were partitioned using different solvent systems depending on their relative polarity, the solvents employed were: n-hexane, ethyl acetate, and ethanol. The fractionation was done using separating funnel for liquid-liquid partition method.

Crude leaves extract (15g) was suspended in a sufficient quantity of distilled water and (2.22g) of crude bark extract also in a separate 50ml beaker respectively. The solution of the both extracts were transferred into a different separating funnel and successively extracted with the organic solvents from increasing polarity (500ml each for leaves and bark) beginning with n-hexane (the least polar solvent), ethyl acetate, and ethanol, yielding n-hexane(nH_1), ethyl acetate (AE), and ethanol (ET) soluble fractions respectively. The solvents was removed in-vacuum using rotary evaporator and kept in a cool place for subsequent used.

Clinical isolates

The fungal isolates (*Candida albican, Aspergillus niger, Trychophyton rubrum* and *Trychophyton mentagrophyte*) were obtained from the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Science, Usman Danfodiyo University, Sokoto.

Antimicrobial screening of *A. nilotica* extracts, fractions and Honey.

Antifungal screening of *A. nilotica* crude ethanol extract of stem-bark and leaf, n- hexane, ethyl acetate and ethanolic soluble fraction of bark, leaf and 100% and 50% honey was carried out using the agar diffusion method. Sabouraud dextrose agar was prepared according to the manufacturer instructions and sterilize at 121^{o}c for 15 minutes. The sterilized medium was then poured into a sterile petri dish and the plate were covered and allowed to cool and solidify.

The suspension of the test organisms suspended in sterile saline were adjusted to an optical density of 0.1 at 530 nm (equivalent to 0.5 McFarland standard turbidity) was inoculated in Sabouraud dextrose agar plate. Then, 0.1 ml of the crude extract (1000 mg/ml and 125 mg/ml), its fractions and honey were added into wells bored with a standard sterile 6 mm cork borer. The plates were incubated aerobically at 30^{o}C for 72 hours and were observed. The diameter of zones of inhibitions were measured with a transparent ruler and recorded. This was done in triplicates.

Micro-Broth Dilution

Broth dilution is a method used to test the susceptibility of microorganism to antimicrobial agent. Using the multipipettor, 100 µl of double strength of Sabouraud dextrose broth was dispensed into all the 96 wells of a microtitre plate. Also, 100µl of the test agent was pipetted into the wells in column 1, multipipettor set at 100µl was used to mix the solution into the wells in column 1 by sucking up and down 6-8 times. Another 100 µl was withdrawn from column 1 and added to column 2. This make column 2 a two folded dilution of column 1. The solution was mixed up and 100µl was transferred to column 3, respectively. Finally, 100µl was discarded from column 10 rather than putting it in column 11. The fungi cell suspension was dispensed into wells in column 11to 1in that other with smaller multipipettor set to 5µl.The plate was incubated aerobically at 30^{o}C for 48-72 hours. The column 12 serves as the sterility control consisting the broth and fungal cell suspension without the extract (1000mg/ml to 0.49mg/ml), and the blank control containing only the medium were maintained for each test. MIC was taken as the lowest concentration of the agent that reduces, by more than 50% or 90% for MIC50 or MIC90 respectively using spectrophotometer. This same procedure was repeated for honey (100 – 3.91 %v/v) and fluconazole (1000mg/ml to 0.49mg/ml). The lowest concentration of the agent that inhibited the visible growth of the test organisms was taken as the MIC. All experiments were performed in duplicate and the wells without growth were reported as MIC (CLSI, 2002)

Determination of minimum fungicidal concentration (MFC)

Ten (10) µl of the culture from the wells with no visible growth were sub-cultured into a freshly prepared Sabouraud dextrose agar and incubated at 30 °C for 48-72 h (CLSI, 2002).

RESULTS

The antifungal screening of *A. nilotica* crude stem-bark extract revealed that, there was no activity against all the test fungal isolates at the concentration of 1000mg/ml and 125mg/ml while the result revealed the activity with crude ethanolic leaf extract at the same concentration with the stem-bark and the honey (50%) against all the five (5) test fungal isolates as shown in Table 1 and 2 below.

The antifungal screening of *A. nilotica* stem-bark extract revealed that, there was no activity against all the test fungal isolates when ethanolic, n-hexane and ethyl acetate stem-bark fraction was used, while there was activity with ethanolic, n-hexane and ethyl acetate leaf fraction as presented in the table 3 and 4 below. The antifungal efficacy of honey and ethanolic leaf and stem-bark extract of *Acacia nilotica* was evaluated by MIC and MFC assays as showed in Table 5 and 6 below. The result revealed that the leaf extract and honey represented effective fungistatic activity against all test fungal isolates while there was no activity against all the test fungal isolates when ethanolic stem-bark was used. The ethanolic leaf extract showed the highest MIC and MFC of 250mg/ml to *Trychophyton mentagrophyte* compared to fluconazole with highest MFC of 128µg/ml**.**

Table 1 Susceptibility of the test fungi to *A. nilotica* of crude Stem bark extract, Fluconazole and Honey

Organisms'	Diameter zone of inhibition (mm)			
	Stem-bark extract			
	A (1000mg/ml)	B (125mg/ml)	50% Honey	FLUC (125µg/ml)
TM	NA	NA	13.5 ± 0.50	26.67 ± 4.03
TR	NA	NA	20.0 ± 0.00	23.00 ± 0.82
AN	NA	NA	14.0 ± 0.50	14.67 ± 1.64
CA	NA	NA	13.0 ± 3.50	24.33 ± 1.73

Values are mean inhibition zone (mm) ± S.D of two replicate cork borer 6mm

KEY**: A=** Ethanolic stem-bark extract, B= Ethanolic stem-bark extract, TM= *Trychophyton Mentagrophyte*, TR= *Trychophyton rubrum*, AN= *Aspergillus niger*, CA=*Candida albican*, FLUC=Fluconazole

Table 2 Susceptibility of the test fungi to *A. nilotica* of crude Leaf extract, Fluconazole and 50% Honey

Organisms	Diameter zone of inhibition (mm)			
	Leaves extract			
A (1000mg/ml)	B (125mg/ml)	50% Honey	FLUC (125µg/ml)	
TM	16.00 ± 0.00	15.00 ± 1.00	13.50 ± 0.50	26.67 ± 4.03
TR	16.00 ± 0.00	12.50 ± 0.50	20.00 ± 0.00	23.00 ± 0.82
AN	17.00 ± 1.00	15.00 ± 7.50	14.00 ± 0.50	14.67 ± 1.64
CA	18.50 ± 0.50	13.50 ± 0.50	14.50 ± 3.50	24.33 ± 1.73

Values are mean inhibition zone (mm) ± S.D of two replicate cork borer -6mm

KEY**: A=** Ethanolic leaf extract, B= Ethanolic leaf extract, TM= *Trychophyton Mentagrophyte*, TR= *Trychophyton rubrum*, AN= *Aspergillus niger*, CA=*Candida albican*, FLUC=Fluconazole

Table 3 Susceptibility of the test fungi to *Acacia nilotica* stem-bark fractions

Organism	Diameter zone of inhibition (mm)		
	Stem-bark fraction (100mg/ml)		
	A (1000mg/ml)	B (1000mg/ml)	C (1000mg/ml)
TM	NA	NA	NA
TR	NA	NA	NA
AN	NA	NA	NA
CA	NA	NA	NA

KEY: A= Ethanolic stem-bark extract, B= n-hexane stem-bark extract, C= Ethylacetate stem-bark extract, TM= *Trychophyton Mentagrophyte*, TR= *Trychophyton rubrum*, AN= *Aspergillus niger*, CA=*Candida*
NA = Not active

Table 4 Susceptibility of the test fungi to *Acacia nilotica* Leaf fractions

Organism	Diameter zone of inhibition (mm)		
	Leaves fractions (1000mg/ml)		
	A (1000mg/ml)	B (1000mg/ml)	C (1000mg/ml)
TM	12.50	13.50	19.50
TR	12.50	13.50	18.00
AN	13.50	NA	NA
CA	14.50	12.50	15.00

KEY: A= Ethanolic leaf extract, B=n-hexane leaf extract, C= Ethylacetate leaf extract, TM= *Trychophyton Mentagrophyte*, TR= *Trychophyton rubrum*, AN= *Aspergillus niger*, CA=*Candida albican*, NA = Not active

Table 5 Minimum inhibitory concentration (MIC) of Honey, Leaf and Stem-bark extracts of *Acacia nilotica* with Fluconazole

ORG	Extracts			
	A (mg/ml)	B (mg/ml)	HONEY	FLUC (µg/ml)
TM	250.00	NA	50.00	64.00
TR	31.25	NA	12.50	32.00
AN	15.63	NA	25.00	128.00
CA	62.50	NA	6.25	32.00

KEY: A=Ethanolic leaf extract, B=Ethanolic bark extract, FLUC=Fluconazole, TM=*Trychophyton Mentagrophyte*,TR=*Trychophyton rubrum*, AN= *Aspergillus niger*, CA=*Candida albican*, ORG=organisms

Table 6 Minimum Fungicidal Concentration (MFC) of Honey, the leaf and stem-bark extracts of *Acacia nilotica* with fluconazole

ORG	Extracts			
	A (mg/ml)	B (mg/ml)	Honey (% v/v)	FLUC (µg/ml)
TM	250.00	NA	50.00	128.00
TR	62.50	NA	25.00	32.00
AN	31.25	NA	50.00	128.00
CA	125.00	NA	12.50	64.00

KEY: A=Ethanolic leaf extract, B=Ethanolic bark extract, FLUC=Fluconazole, TM=*Trychophyton Mentagrophyte*, TR=*Trychophyton rubrum*, AN= *Aspergillus niger*, CA=*Candida albican*, ORG= organisms

DISCUSSION

Plant based drugs are gaining popularity because of several advantages such as fewer side effect, better patient tolerance, relatively less expensive and acceptance due to a long history of use, especially herbal medicines which provide rational means for the treatment of many diseases that are incurable in other system of medicine (Sinkar and Samarth, 2019). Antimicrobial drugs also provide the main basis for treatment of various microbial infections, however, the high genetic variability of some microorganisms enable them to rapidly develop antimicrobial resistance. Thus, there has been a continuing search for new potent antimicrobials.

The results of the present study provide a scientific validation for the popular use of the medicinal plants studied and serve as a guide which may help in selection of plants with antimicrobial activities for further phytochemical work on the isolation and the identification of the active compounds.

The present study showed that *A. nilotica* L. leaves extracts and honey were effective inhibitors of fungal growth. The leaves extracts of the plant and honey showed varying degrees of activity against the test fungi. The leaves extracts showed inhibitory activity ranging from 12.5 to 18.5 mm against the four clinical fungal pathogens while honey at 50%v/v concentration showed inhibitory activity ranging from 13.5-20mm. Whereas, at 1000 and 125mg/ml of ethanolic stem-bark extracts of A. *nilotica* L, all the fungal pathogens did not show any activity. Satish *et al*. (2008) found that ethanolic leaves extract of the *A. nioltica* L. exhibited significant study against *Candida albican* and *Trychophyton rubrum*. *A. nilotica* L. showed diameter zones of inhibition from 9 to 35 mm, whereas in this study, zones of inhibitions were from 12.5-18.5mm, and the extracts was of ethanol solvent. The results revealed that ethanol leaves extract was more effective against all test organisms than stem- bark extract. This may be due to the ability of the ethanol to extract a wide range of chemical compounds of the plant leaves which might be responsible for the antifungal activity.

The leaves ethanolic extract of A. *nilotica* showed MIC ranging from 15.63-250mg/ml against the test fungal while the MIC for the honey ranges from 6.25-50mg/ml, whereas there were no activity in all the fungal pathogens to the ethanolic stem-bark of the same plant. It is clear from Table 4 that fluconazole 125µg/ml showed marked activity (MDIZ: 14.67-26.67 mm) against four fungal pathogens. The antifungal efficiency of fluconazole on the test fungal pathogens was higher than the activity on the honey and ethanolic leaves extracts of *Acacia nilotica*. While the antifungal activity of honey and leaves extract nearly equal to activity of fluconazole 125µg/ml This result is relatively in agreement with the findings of Al-Fatimi *et al*. (2007) because they found that the methanolic extract of *A. nilotica* L. gave antifungal activity against *Candida spp* and *Trichophyton mentagrophytes*, *Candida krusei*, and *A. fumigatus* respectively.

CONCLUSION

From the studies, traditional plants may represent new sources of anti-microbial with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine (Gandhiraj and Soman, 2014). Fluconazole (conventional agent) was found to be more effective and more potent than the leaves extract and honey. Results indicate the

potential of this plant for further work on isolation and characterization of the active principle responsible for antifungal activity and its exploitation as therapeutic agent. There is need for phytochemical as well as biological activities of plants. There is need for developing drugs from plants as microorganisms are becoming resistant to antibiotics thereby creating health problems.

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