Science World Journal Vol. 21(No 1) 2026 https://dx.doi.org/10.4314/swj.v21i1.34
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Full Length Research Article

PHYTOCHEMICAL SCREENING AND ANTIFUNGAL ACTIVITY OF *ACACIA NILOTICA* (*vachellia nilotica)* METHANOLIC LEAF EXTRACT

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ABSTRACT
This work investigates the phytochemical analysis and antifungal activities of the methanolic leaf extract of *Acacia nilotica*. The phytochemical screening was carried out using a standard procedure to detect the presence of secondary metabolite which include: Alkaloids, Tannins, Flavonoids, Steroid and Anthraquinones. The extract undergoes thin-layer (TLC) and column chromatography to determine the active components. The fraction that appears more undergoes FT-IR analysis, which showed the most active compounds present in the *Acacia nilotica*. The antifungal assay of the active compound was conducted which inhibited the growth of *Candida albicans* by measuring the zone of inhibition at different concentration ranging from 0.25 mg/mL, 0.125 mg/ml, 0.0625 mg/mL and 0.03125 mg/mL respectively, fluconazole was used as positive control in *Candida albicans*, antifungal activities in *Candida albicans* showed zone of inhibition of 19 mm, 16 mm, 14 mm, 9 mm respectively and 34 mm at control. Thus, the analyses conducted confirmed the presence of various phytochemicals and antifungal properties of the plant.

Keywords: *Acacia nilotica*; antifungal; *Candida albicans*; phytochemical screening; secondary metabolites.

INTRODUCTION

People have used various plants, microalgae and their derivatives for medical purposes, including the treatment of infectious diseases. The use of plants and microalgae as sources for novel antimicrobial as well as antioxidant agents offers advantages in terms of nutraceutical, medicinal, and energy purposes (Cheesman *et al.*, 2017; Sobri *et al.*, 2024; Musa *et al.*, 2025). Plants are readily accessible and inexpensive; extracts or compounds from plant sources often demonstrate high-level activity against microbes (Zango *et al.*, 2023; El-Saadony *et al.*, 2025). They are particularly active against bacteria, fungi, and viruses with rarely severe side effects (Cheesman *et al.*, 2017; Gorlenko *et al.*, 2020; Castronovo *et al.*, 2021). Most pharmacological activities of medicinal plants are traced to their secondary metabolites. Secondary Metabolites are smaller molecules when compared to the constituents of primary metabolites like proteins, carbohydrates, and lipids (Gorlenko *et al.*, 2020). Furthermore, herbal products from medicinal plants are produced through a number of processes such as extraction, fractionation, purification, and concentration of plant materials (Abubakar and Haque, 2020; Kapadia *et al.*, 2022; Labaran *et al.*, 2024). According to recent studies conducted by the World Health Organisation (WHO), about 80% of the world's population relies on traditional medicine (Organization, 2019; Kithinji *et al.*, 2025).

Acacia species are multipurpose trees distributed worldwide, which comprise nearly 1200 species of the Fabaceae family belonging to the tribe Acaciacea (Jeevitha *et al.*, 2021; Botella *et al.*, 2023). Approximately 152 chemical components have been discovered from the Acacia species during the last seven decades (Muhaisen, 2021; Batiha *et al.*, 2022). The main factors for their possible biomedical values are the isolated key compounds (flavonoids, terpenoids, phytosterols, phenolic acids, hydrocarbons, fatty acids, and other substances) accumulated primarily on leaves, stem barks, and pods. Protein and mineral content are abundant in numerous species, especially in the leaves, twigs, and pods (Amoussa, Sanni, and Lagnika, 2020; Barthelemy, Jean, and Bolou, 2023). Acacia species have been extensively investigated because of their widespread ecological amplitude, dietary, and beneficial properties (Tiwari *et al.*, 2023; Saha and Dey, 2024).

Acacia nilotica, especially other Acacia species, are used in local traditional medicine by people as a remedy for various health problems like cancers of (ear, eye, or testicles), and indurations of liver and spleen, condylomas, and excess flesh (Saeedi, Sultana and Rahman, 2020; Kumari and Swer, 2025). It is also used for colds, congestion, coughs, diarrhea, dysentery, fever, gallbladder, hemorrhage, hemorrhoids, leucorrhea, ophthalmia, sclerosis, smallpox, and tuberculosis (Kumari and Swer, 2025). The increasing resistance by disease agencies to antifungal drugs led to new strategies and incentives toward research on treatment, prevention, and development of new drugs against this microbial organism. Therefore, the present record became necessary for the reason that environmental condition genetic variation may affect the production of phytochemicals by plants.

The present study evaluates the phytochemical and antifungal activation of *Acacia nilotica* leaves using *Candida albicans* as the organism of interest. It employs the use of methanolic extracts of the plants for the phytochemical screening and antifungal analysis. FTIR spectrum of the extract has been conducted, and deductions have been made based on the various analyses conducted.

MATERIALS AND METHODS

The *Acacia nilotica* leaves was obtained from Dabaibayawa ward, located within latitude of 12° 51' 27" N (12.8575°) and longitude of 7° 35' 2" E (7.5841°) in Batagarawa Local government Area of

Katsina state. The leaves were washed under running tap water to eliminate dust and other foreign particles and dried under shade for two weeks and later ground to powder form. The reagents and Thin Layer Chromatography (TLC) precoated plates, Silica gel were purchased from Merck Pharmaceutical. The reagents were of analytical grade. Potato dextrose agar (PDA) was obtained from Bristol Scientific Company Limited. The incubator was Eurotherm: Model No. 2216E incubator. Fluconazole was used as positive control and obtained from Merck Pharmaceutical

Test Organisms
The inoculated *Candida albicans* fungal culture was collected from the Department of Medical Microbiology Federal Teaching Hospital Katsina, Nigeria.

Preparation of Methanolic Leaves Extract
For the methanolic extract preparation, about 10 g of the powdered sample was dissolved in 100 mL of methanol in conical flacks that contain magnetic bar and it was covered by foil and set on a magnetic stirrer for regular stirring, for 24 hours. After that, the mixture was filtered using 11 µm Whatman filter paper. The filtrate was stored into a refrigerator. The residue undergoes second and third round with the same volume of solvent. The filtrate was combined in a beaker and dried in an oven at 70 °C for few hours to evaporate the solvent, and then the extract was scrapped and weighed.

Culture media and preparation of inocula
For the culture media preparation, 5.8 g of potato dextrose agar (PDA) was dissolved in 150 mL of distilled water. The media was autoclaved for 15 min at 15 psi pressure at 120 °C. The PDA plates were prepared by pouring 15 mL of media in sterile Petri plates and the plates were allowed to solidify. 0.1% of the inoculum suspension was swabbed uniformly in the Petri plates and the inoculum was allowed to dry.

Test for Alkaloids
For the determination of alkaloid content in the extract, two milliliters (2 mL) of the extract was measured and stirred with 2 mL of 10% aqueous hydrochloric acid (HCl). A few drops of Wagner's reagent were added to the extract. A reddish-brown precipitate ascertains the alkaloids presence (Harborne, 1998).

Test for Tannins
For the analysis of tannins, about 2-3 mL of extract was measured. Then, 5% of $FeCl_3$ was added dropwise to the extract and stirred. A dark green colored complex was formed, indicating the presence of condensed tannins in the extract (Momoh *et al*., 2021).

Test for Saponin
For the analysis of saponins, two milliliters (2 mL) of the extract and 2 mL of distilled water were mixed and shaken thoroughly. The whole tube was filled with froth that lasted for several minutes, indicating the presence of saponin in the extract (Harborne, 1998).

Test for Flavonoid
For the test of flavonoids, 3 mL of the aliquot of the filtrate and 1mL of 10% of NaOH were mixed. A yellow color that was developed indicates the presence of flavonoids in the extract (Harborne, 1998).

Test for Steroids
For the determination of the steroids, 2 mL of the extract was measured in a beaker and mixed with 2 mL of chloroform. To the mixture, 2 mL of H_2SO_4 was carefully added to form a lower layer. A reddish-brown color formed at the interface indicates the presence of a steroidal ring (Harborne, 1998).

Test for Anthraquinone
For the detection of anthraquinone, 5 mL of the extract was placed in a test-tube and 5 mL of benzene was added and shaken vigorously. The mixture was then treated with an additional 5 mL of 10% ammonia. A persistent color in the benzene (organic) layer after phase separation, such as a deep red, purple, or orange hue, will be observed, depending on the specific anthraquinone derivative present (Harborne, 1998).

Thin-layer chromatography (TLC)
The TLC was conducted as a free test to observe the fractions that are present in the leaves of *Acacia nilotica*. The TLC of the crude extracts was carried out using a precoated plate in methanol. The precoated TLC plates were placed with a tapering mark towards one end of the plate. Horizontally, a line is traced with a pencil that is 2 to 3 cm from the mark's angle. Using a capillary tube, 1 drop of the extract of the pigment is applied in the midsection of the line and was allowed to dry. The process was repeated by adding a drop and allowing it to dry for 4 - 5 times. In the chromatographic chamber, the methanol solvent was poured. The precoated plate was suspended in the chamber. The loading spot remains about 1 cm above the level of the solvent. The chamber remains uninterrupted for a while. The solvent passes along the plate, scattering various pigments of the blend to different distances. After the solvent reaches $3/4^{th}$ of the precoated plate, the plate was carefully taken off out of the chamber and it was allowed to dry.

Column Chromatography
The column was prepared by wet loading. 20 g of the silica gel was weighed into a 250 mL beaker and mixed with methanol. The solvent used in TLC is stirred to form a slurry. The slurry was allowed to stand for 2 minutes to eliminate air bubbles. The bottom of the column was packed with pieces of sand, followed by a bed of cotton wool. The column was loaded with the slurry by pouring it directly after vigorous stirring. Sides of the column were washed with the solvent mixture to push down the stock silica gel from the slurry. The tap of the column was open to drain the solvent and ensure good packing of the silica gel. The tap was closed as the solvent front just level with the top of the silica gel. The size of the column was 10 cm in length and 2 cm in diameter. 0.5 g of the methanol extract was dissolved in methanol. A small amount of the silica gel was added and stirred. The column was loaded with the sample by placing it directly. Sides of the column tube were washed to push down the sample gently without disturbing the column. Another piece of cotton wool was placed on the loaded sample. The mobile phase methanol was poured gently into the column. The tap was then opened, and the mixture was allowed to elute. 4 fractions, in various volumes each, were collected in test tubes, in which the most active fraction was utilized for characterization via FTIR and further antibacterial activities.

Fourier transform infrared (FTIR) spectroscopy
The FTIR spectroscopy of the methanol extracts was carried out in order to determine the types of functional groups present. The FTIR

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spectra of *Acacia nilotica* leaves extract were recorded using a Perkin Elmer System 2000 (Waltham, MA, USA), with a wavelength range of 4000 to 400 cm^{-1} at 4 cm^{-1} resolution and 16 scans. The potassium bromide technique (KBr) was applied to the samples with the proportion of 1:20 (w/w).

Antifungal activity

For the antifungal analysis, 4 pieces of Whatman filter papers were immersed in the four different concentrations of the extract, ranging from 0.25 mg/mL, 0.125 mg/mL, 0.0625 mg/mL, and 0.03125 mg/mL were allowed to soak for 20 minutes using sterilized forceps and a wire loop. Each piece of Whatman filter paper from the four different concentrations was arranged in each of the prepared PDA plates containing *Candida albicans*. Fluconazole was used as a fungal positive control. The plates were incubated for up to 24 to 48 hours, which is suitable for fungal growth, and it was observed after 48 hours.

Minimum Inhibitory Concentration (MIC)

The MIC was determined by identifying the lowest concentration of the extract that inhibits the growth of the fungal culture. The minimum inhibitory concentration of each plate was measured in milliliters using a meter rule, and the result was compared with the CLAIS breakpoint of fungal sensitivity testing, and it was recorded.

RESULTS AND DISCUSSION

Percentage Yield of *Acacia nilotica*

The **percentage yield** of the extract is an important parameter in phytochemical and pharmacological research because it reflects the efficiency of the extraction process and the number of bioactive compounds that can be obtained from a given quantity of plant material. A high yield indicates that the solvent system used was effective in extracting soluble constituents, whereas a low yield may suggest that the plant contains fewer soluble compounds in that solvent or that the extraction method was less efficient. The result of the % yield of *Acacia nilotica* extract was presented in the table 1 below.

Table 1: The % yield of *Acacia nilotica* extract

Plant name	Solvent	% Yield
***Acacia nilotica* leaves**	Methanol	36.7

Phytochemical Analysis

Phytochemical analysis of the methanolic extract of *Acacia nilotica* revealed the presence of tannins, flavonoids, alkaloids, anthraquinones, and steroids. Saponins were not present in the extracts (Table 2). The chemical constituents present in the extract have been reported to possess many therapeutic values. This result is contrary to the findings of Mohammed *et al*. (2019), where they found that tannins, saponins, flavonoids, carbohydrate where present, and Anthraquinones, alkaloids, terpenes, and steroids were not (Auwal *et al*., 2014). The tannins can evoke an antidiarrheal effect, and these substances may precipitate proteins on the enterocytes, reducing peristaltic movement and intestinal secretion (Narayana *et al*., 2001).

The flavonoids have attracted a great deal of attention due to their potential health benefits. Over the past few years, several experimental studies have demonstrated biological and pharmacological properties of many flavonoids, especially their antimicrobial activity, anti-inflammatory, antioxidant, and anti-tumor effects, which are associated with free radical-scavenging action (Middleton Jr, Kandaswami and Theoharides, 2000). The Flavonoids have also been reported to possess hypoglycemic and antidiabetic effects (Sok Yen *et al*., 2021). Flavonoids have antioxidant activity, protect cells against oxidative damage, and reduce the risk of developing certain types of cancers (Hernández-Rodríguez, Baquero, and Larrota, 2019). The result was presented in Table 2.

Table 2: Phytochemical analysis of *Acacia nilotica* leaves
Key: + Present, - Absent

S/N	Phytochemical	Result
1	Alkaloids	+
2	Tannins	+
3	Saponins	-
4	Flavonoids	+
5	Steroids	+
6	Anthraquinones	+

Tin Layer Chromatography

The TLC result indicated the separation of three distinct fractions: a yellow color, a green color, and a reddish-brown color. Among these, the yellow color was predominant. While the green and reddish-brown fractions appeared in smaller proportions. This suggests that the crude sample contained a major component, yellow, and at least two minor components, green and reddish-brown, which were successfully resolved on the TLC plate. The difference in color intensities might correlate with the relative abundance of each compound in the mixture. These findings are in agreement with the general principle of TLC separation, where compounds migrate at different rates depending on their polarity, affinity to the stationary phase silica/alumina, and solubility in the mobile phase solvent system. The TLC can effectively distinguish compounds by producing bands of different colors and Rf values, reflecting differences in polarity and molecular structure (Kowalska and Sajewicz, 2022; Akash and Rehman, 2025).

The predominance of the yellow fraction is comparable with observations by Harborne (2018), who reported that plant extracts often show a dominant band corresponding to flavonoid or carotenoid derivatives, which are usually yellow under visible or UV light (Harborne, 1998). The phytochemical extracts typically display a major yellow or orange band accompanied by minor green or brown bands, corresponding to secondary metabolites such as alkaloids, phenolics, or chlorophyll derivatives (Altemimi *et al*., 2017; Alamgir, 2018). Furthermore, relative intensity and frequency of occurrence of colored bands on TLC reflect the chemical profile of the extract (de Souza *et al*., 2018).

In line with this, the abundant yellow fraction in the present result might represent the major class of compounds in the mixture, while the green and reddish-brown fractions indicate less abundant but chemically distinct constituents. Overall, the TLC result demonstrates successful separation and preliminary identification of at least three chemical groups. The presence of multiple fractions agrees with other scholars' findings that natural extracts or complex chemical mixtures rarely consist of a single compound

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www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
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but rather contain a spectrum of metabolites, each migrating differently on TLC depending on their polarity and interactions with the stationary phase.

Table 3: Thin Layer Chromatography

Sample	Result
***Acacia nilotica* extract**	Yellow, Green, and Reddish brown

Column Chromatography

The column chromatography of the extract of *Acacia nilotica* leaves produced three distinct fractions, characterized by yellow, green, and reddish-brown bands. Among these, the yellow fraction appeared most prominently, while the green and reddish-brown fractions were present in smaller proportions. The dominance of the yellow fraction suggests that it represents the major active compound(s) within the leaves, while the other fractions likely correspond to minor phytochemical constituents. This result is consistent with the principle of column chromatography, where compounds are separated based on their polarity, adsorption to the stationary phase, and differential solubility in the mobile phase. The strong elution of the yellow fraction indicates a compound of intermediate polarity, which is typical of many biologically active flavonoids and phenolic compounds that have been reported in *Acacia nilotica*.

Findings from previous studies support this observation. Harborne reported that the yellow coloration in plant chromatographic fractions (Harborne, 1998), which is often associated with flavonoids and tannins, is abundant in *Acacia* species. In agreement, Edeoga et al. identified yellow fractions in chromatographic separations of medicinal plants as rich in phenolic compounds with known antimicrobial and antioxidant activities (Edeoga *et al*, 2005). Similarly, Orwa et al. noted that *Acacia nilotica* contains a high concentration of flavonoids and condensed tannins, which often appear as yellowish fractions during chromatographic separation (Orwa, 2009).

The presence of the green fraction could be attributed to chlorophyll derivatives or other pigments, while the reddish-brown fraction likely represents alkaloids or tannin-related compounds, which have also been documented in *Acacia nilotica* leaves (Sharma *et al*., 2014). The predominance of the yellow fraction therefore not only confirms the phytochemical richness of the plant, but also highlights it as the most probable source of the active bio-compounds responsible for the medicinal properties of *Acacia nilotica*. Overall, the chromatographic results are in agreement with the findings of earlier scholars who have emphasized that *Acacia nilotica* leaves contain a dominant class of flavonoids and phenolic compounds, accompanied by smaller fractions of other phytochemical groups. The separation of three visible fractions in this study underscores the complex phytochemical profile of the plant and supports its traditional use in herbal medicine. Table 4 below shows the result of column chromatography of *Acacia nilotica*

Table 4: Column Chromatography

Sample	Result
***Acacia nilotica* extract**	Yellow, Green and Reddish brown

Fourier Transform Infrared Spectroscopy

The FTIR analysis of the extract revealed the presence of several functional groups that are indicative of biologically active phytochemicals. The characteristic absorption at 1602 cm^{-1}, corresponding to C=C stretching vibrations, together with C–H bending absorptions at 760, 805, and 864 cm^{-1}, confirms the presence of aromatic compounds. Aromatic compounds are widely reported to exhibit diverse biological activities, including antimicrobial, antioxidant, and anti-inflammatory properties. Many naturally occurring aromatics are known to contribute to the medicinal value of plants, particularly due to their stability and ability to interact with biological targets. In addition, a strong O–H stretching band at 3280 cm^{-1}, along with C–N stretching absorptions at 1192 and 1312 cm^{-1} indicates the presence of alcohols and possibly phenolic groups. Alcohols and phenolics are recognized for their antioxidant activity, and several studies have demonstrated their role in the prevention of oxidative stress-related diseases. These findings are consistent with previous reports that alcohol and phenol-containing compounds in medicinal plants contribute to antibacterial and antifungal properties (Edeoga, Okwu, and Mbaebie, 2005).

Furthermore, the detection of C=O functional groups is significant, as these groups are commonly found in alkaloids and flavonoids. For instance, alkaloids such as caffeine and theobromine contain carbonyl functionalities, which are essential for their pharmacological actions. Similarly, flavonoids such as quercetin and kaempferol possess C=O groups that are integral to their structure and biological activity. Flavonoids are well documented for their antioxidant, anti-inflammatory, and antimicrobial effects (Al-Khayri *et al*., 2022; Chagas *et al*., 2022), while alkaloids are known for a wide range of therapeutic applications, including analgesic and stimulant properties (Rajput *et al*, 2022).

The FTIR result in **Figure 1** confirms that the extract of *Acacia nilotica* leaves contains key functional groups associated with aromatic compounds, alcohols, phenolics, alkaloids, and flavonoids. This aligns with the findings of other scholars who have reported that *Acacia nilotica* is rich in phenolic compounds, flavonoids, and alkaloids, which account for its broad-spectrum medicinal properties (Thangum *et al*., 2018; Al-Nour *et al*, 2019). Thus, the spectroscopic evidence strongly supports the traditional use of *Acacia nilotica* in herbal medicine and highlights its potential as a source of bioactive compounds.

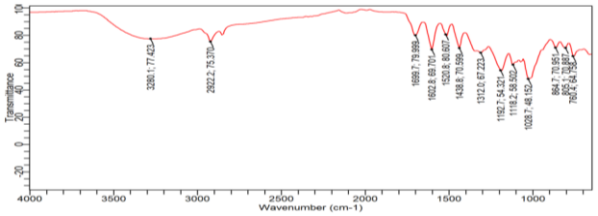

Figure 1. FTIR spectrum of *Acacia nilotica* leaf extracts

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www.scienceworldjournal.org
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Table 5: FT-IR analysis of *Acacia nilotica* leaf extracts

Peak Number	Wavenumber (cm^{-1})	Functional Group	Vibration mode	Possible Compound
1	760	C-H	Bending (out of plane)	Aromatic compound
2	805	C-H	Bending (out of plane)	Aromatic compound or Alkenes
3	864	C-H	Bending (out of plane)	Aromatic compound or Alkenes
4	1028	C-O	Stretching	Alcohols or ethers
5	1118	C-O	Stretching	Alcohols or ethers
6	1192	C-O or C-N	Stretching	Alcohols or ethers
7	1312	C-N or CH_2/CH_3	Stretching or bending	Amines or Alkanes
8	1438	CH_2/CH_3	Bending	Alkanes or Alkenes
9	152	N-O or C=C	Stretching	Nitro compounds or alkenes
10	1602	C=C	Stretching	Aromatic compound or alkenes
11	1699	C=O	Stretching	Aldehyde, Ketones, acids
12	2922	C-H	Stretching	Alkanes or Alkenes
13	3280	N-H or O-H	Stretching	Amines, Amides, or alcohols

Antifungal activities of *Acacia nilotica* leaves against Candida albicans

The result for antifungal sensitivity test was found to be effective. A zone of inhibition of 19 mm indicates that the fungal isolate is Susceptible to the antifungal agent. This result suggests that: The antifungal agent is active: The fungal isolate is likely to respond to treatment with the antifungal agent. Infection treatment is likely successful: The antifungal agent is likely to inhibit the growth of the fungal isolate, making treatment successful. In general, a zone of inhibition of 19 mm indicates that the fungal isolate is susceptible to the antifungal agent and treatment with this agent may be effective (Sadiq *et al.*, 2015). A zone of inhibition of 15 – 18 mm indicates that the fungal isolate is Susceptible Dose-Dependent (SDD) to the antifungal agent. This result suggests that dose adjustment may be necessary. The fungal isolate may respond to the antifungal agent, but higher doses or more frequent administration may be required to achieve optimal effect. Treatment outcome may depend on dosing: The effectiveness of treatment may depend on achieving adequate drug concentrations at the site of infection. SDD results indicate that the antifungal agent may still be effective, but careful consideration of dosing and monitoring of treatment response is necessary.

A zone of inhibition of 14 mm indicates that the fungal isolate is Resistant (R) to the antifungal agent, thus suggests that antifungal agent is unlikely to be effective: However, when fungal isolate is likely to continue growing despite treatment with the antifungal agent, Alternative treatment options are needed: A different antifungal agent or treatment approach be necessary to effectively manage the infection. Resistance results indicate that the antifungal agent is unlikely to be an effective treatment options should be considered (Pfaller, Diekema, and Sheehan, 2006). The antifungal activities of *Acacia nilotica* leaves are shown in Table 6.

Table 6: Antifungal Sensitivity Test of *Acacia nilotica* leaves against *Candida albicans*

Name of organism	**Concentration**				
Candida albicans **Zone of Inhibition**	A (0.25 mg/mL)	B (0.125 mg/mL)	C (0.0625 mg/mL)	D (0.03125 mg/mL)	Control 34 mm
	19 mm	16 mm	14 mm	9 mm	

Key: (+ve) = positive, positive control = (Fluconazole), (mm) = millimeters

Conclusion

The leaves of *Acacia nilotica* appear rich in secondary metabolites. These leaves are used extensively in traditional medicine to heal different health problems. It is suggested that the methanol extract of *Acacia nilotica* leaves revealed a significant scope to develop a novel broad spectrum of antifungal drug formulation and can be used to carry out further pharmacological evaluation to be used as antifungal agents/drugs. More research is needed to investigate toxicological studies of the extracts, and quantitative phytochemical analysis of *Acacia nilotica* needs to be examined.

Acknowledgement

The authors appreciate the Departments of Chemistry, Microbiology, Basic and Applied Sciences of the Hassan Usman Katsina Polytechnic, for the provision of Laboratory equipment and other facilities used throughout the research.

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