

Available online on 15.10.2021 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Copyright © 2021 The Author(s): This is an open-access article distributed under the terms of the CC BY-NC 4.0 which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited



Open Access Full Text Article



Research Article

Antifungal Potential of silver nanoparticles from *Acacia nilotica* Pod against Dermatophytes

Yunusa Saheed

Microbiology Department, Yobe State University, Damaturu, Nigeria

Article Info:



Article History:

Received 14 August 2021
Reviewed 27 September 2021
Accepted 03 October 2021
Published 15 October 2021

Cite this article as:

Saheed Y, Antifungal Potential of silver nanoparticles from *Acacia nilotica* Pod against Dermatophytes, Journal of Drug Delivery and Therapeutics. 2021; 11(5-S):85-95

DOI: <http://dx.doi.org/10.22270/jddt.v11i5-S.5024>

*Address for Correspondence:

Yunusa Saheed, Microbiology Department, Yobe State University, Damaturu, Nigeria. E-mail Address: soy4kd@gmail.com

ORCID ID: <https://orcid.org/0000-0003-4570-4936>

Abstract

Background: Superficial fungal infections can lead to systemic infection in immune-compromised individuals. *Acacia nilotica* pod have been used ethnomedical to treat dermatophytes infection for ages. In this study, the anti-dermatophytes potential of silver nanoparticles biogenically synthesized using extracts from the pod of *A. nilotica* against dermatophytes isolated from secondary care hospital in Damaturu, North-East Nigeria. **Experimental:** Phytochemical screening and GC-MS analysis were conducted to screen the phytoconstituents of the plant material. The synthesized AgNPs were characterized by Uv-vis, FT-IR, and SEM. 133 samples (skin scraping) were screened for dermatophytes and antifungal susceptibility testing were conducted against the isolates using the aqueous, methanolic extracts, and AgNPs. **Results:** Phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, oxalate, quinones, phenols, saponins, terpenoids, GS-MS revealed the presence Polyphenolics including Hexadecenoic, Octadecanoic and Undecanoic acids, Catechol, pyrogallol, 3-methylpyridine, and methyl mannose. Uv-vis of synthesized AgNPs exhibited double sharp absorbance at 308nm and 311nm, FT-IR showed functional groups, thus, OH, C – H, C≡N, and C=O stretches of phenolics, alkenes, nitrile, and ketones respectively, and SEM showed various characteristic shapes and sizes. out of 133 samples collected, 54% were male and the age group with the highest clinical presentation were 51 – 60, followed by 1 – 10 years. according to clinical presentation, *Tinea pedis* (36%) and *T. capitis* (29%) were the commonest which may be due to constant contact with water and limited hair care. Aetiologic agents isolated include *Trichophyton mentagrophytes* (51%), *T. rubrum* (34%), and *T. tonsurans* (14%), although, there is no significant relationship between the clinical presentation and disease agent (p -value < 21.3 at 95% confidence level). AgNPs showed wider zones of inhibitions against all the isolates. **Discussion:** *A. nilotica* is indeed very rich in polyphenolics. Foot and hair infections commonest in the study could be due to current weather conditions but sharing of footwear, caps, and brushes should be discouraged. **Conclusion:** This study opens up possibilities for exploration of this eco and economic approach of improving the medicinal value of plants, an opportunity the pharmaceutical industries can utilize. This study is the first to report prevalence of dermatophytes in the area and explored the AgNPs against it.

Keywords: Damaturu, susceptibility, catechol, ethnomedicinal, *Acacia nilotica*, AgNPs

1. INTRODUCTION

Dermatophytes are fungal infections on the surface and deep-rooted to the skin, hair, and nails¹. Dermatophytes and *Candida* spp constitute a serious problem especially in tropical and subtropical developing countries where hot and humid weather predisposes to fungal infections by increased sweating and maceration of the skin. Other predisposing factors include poor socio-economic conditions, overcrowding, poor hygiene, and lack of adequate water supply. Dermatophyte infections can also be acquired directly through fomites such as upholstery, hairbrushes, and combs. Dermatophytes in pupils cause morbidity and lower their quality of life leading to poor school attendance and irritation in adults². They are believed to affect 20% to 25% of the world's population³, and the incidence continues to increase⁴ due to immunosuppression procedures and diseases. They consist of about 40 fungal species from three genera of *Trichophyton*, *Microsporum*, and *Epidermophyton*

leading to infections called dermatophytosis, ringworm, or *Tinea* (named according to the area of infection).

Due to the reduced efficacy of pharmaceutical products, possible side effects, resistance associated with some of the existing drugs, and increase pace of immunosuppressed individuals, management of dermatophytoses, though some considered it recurrent and seasonal, could be a public health concern shortly. Therefore, much of the attention has been paid to plant extracts to treat fungal infections, and treatment of these infections using nanoparticles is an alternative approach that can reduce treatment period and resistance⁵.

Natural medicinal plants promote self-healing, good health, and durability in ayurvedic medicine practices and have acknowledged that *A. nilotica* can provide the nutrients and therapeutic ingredients to prevent, mitigate or treat any diseases or conditions. *Acacia nilotica* pods are strongly constricted, white-grey, hairy, and thick⁶.

A. nilotica is a pantropical and subtropical genus with species abundant throughout Asia, Australia, Africa, and America⁷. It contains Polyphenol which can be of prime use to humans, the pods have potential antioxidants and are found effective in protecting plasmid DNA and human serum albumin protein oxidation induced by hydroxyl radicals though, the uses of Polyphenols in the plant is still vague⁸, other parts of the plant have been used for various purposes, the pod has been used ethnomedical for ages in the treatment of skin discomfort.

Silver Nanoparticle is environmentally friendly, non-toxic, chemically stable, low cost-effective when prepared green compared to other methods and, thus making it a cheaper alternative when available with the high potentiality to serve as coating agents to the phytochemicals in the plant extract for effective drug delivery and treatments.

The green synthesis does not utilize any potentially harmful chemicals that can add to global warming. If the enormous challenge is to develop an antifungal agent which has fungicidal properties and has the highest rate of mycological cure with the shortest duration of usage⁹ then, any drug developed using AgNPs synthesized from the pod of *Acacia nilotica* may be effective for the treatment of fungal diseases in plant and animal with the novelty of showing the possibilities of treatment success in the era of treatment failure.

This work is expected to scientifically back the use of the pod as anti-dermatophytoses, add to the existing literature about the advantages of Green AgNPs.

2. METHODOLOGY

2.1. Collection of plant material and preparation of *A. nilotica* pod extracts

Fresh, ripe green pods of *A. nilotica* were collected from Nguru, Identified and authenticated in Yobe State University Biological Garden. The air-dried pods were crushed with the aid of pestle and mortar, sieved, and extracted at 25g/250ml distilled water for 72hrs at 200rpm in a shaking incubator. The extracts were filtered using Whatman no. 1 paper, concentrated, and evaporated using a rotary evaporator, and lyophilized for 30hours in a freeze dryer. The methanolic extract was extracted exhaustively using a soxhlet extractor.

2.2 Phytochemical screening

Both aqueous and Methanolic extracts were tested for their chemical compositions. The tests carried out include:

2.2.1 Test for the presence of Alkaloids

About 0.5g of each extract was taken into a test tube and stirred with 3ml 1% aqueous hydrochloric acid on a steam bath. 1ml of the filtrate was treated with few drops of Mayer's reagent and picric acid solution. Precipitation with either reagent was taken as preliminary evidence for the presence of alkaloids¹⁰.

2.2.2 Test for the presence of Anthraquinones

Bontrager's test was used for the detection of anthraquinones. 0.5g of each extract was taken into a dry test tube and 5ml of chloroform was added and shaken for 5minutes. The extract was filtered and the filtrate was shaken with an equal volume of 100% ammonia solution. Pink, violet, or red colour in the lower layer indicate the presence of three anthraquinones¹¹.

2.2.3 Test for the presence of steroid

About 100mg of each extract was taken into a dry test tube and dissolve in 2ml of chloroform. Concentrated sulphuric acid was carefully added down the side of the test tube to form a lower layer. The formation of reddish-brown colour at the interphase is indicative of the presence of steroidal ring¹¹.

2.2.4 Test for the presence of flavonoids

Plant extract weighing 2.0g was taken into the test tube and detained with acetone on the water bath. The mixture was filtered while hot. The filtrate was cooled and used for sodium hydroxide test:

Sodium hydroxide test for flavonoids. 5ml of 20% sodium hydroxide was added to an equal volume of the detained plant extract. A yellow solution indicates the presence of flavonoids.

2.2.5 Test for the presence of glycosides

100mg (0.1g) of each plant extracts were dissolved in 1ml of glacial acetic acid containing one drop of Ferric chloride solution in a test tube. Then 1ml of concentrated sulphuric acid was added. A brown ring obtained indicate the presence of deoxy-sugar characteristics of cardenolides¹².

2.2.6 Test for the presence of saponins

0.5g of each extract was taken into a dry test tube and dissolved in distilled water in a test tube and was shaken vigorously for two minutes and observed for frothing which tube and was shaken vigorously for two minutes and observed for frothing which persisted on warming. This was taken as evidence for the presence of saponins.

2.2.7 Test for the presence of Tannins

About 0.5g of each extract was taken into a dry test tube and stirred with 1ml of distill water, it was filtered and Ferric chloride solution was added to the filtrate. A blue, a black, or green, or blue-green precipitate formed indicate the presence of Tannins¹².

2.2.8 Test for the presence of terpenoids

0.1g of each extract was taken into a test tube and dissolved in 1ml of chloroform and 1ml of acetic anhydride was added. Two drops of concentrated sulphuric acid were then added. A pink colour that changes to bluish green indicates the presence of terpenes.

2.3 GC-MS Analysis of *A. nilotica* Pod

GC-MS analysis of *A. nilotica* pod will be performed on Agilent GC-MSD (7890B-5977A) at the Research Laboratory in the Chemistry Department, Yobe State University.

2.4 Biosynthesis and Characterization studies on *A. nilotica* pod AgNPs.

2.4.1 Preparation: 0.0169g of AgNO₃ was dissolved in 100ml distilled water in a dark place.

Different ratios of 2:8 (2ml P.E +8ml AgNO₃ solution), 1:9, 7:3, 3:7, and 5:5. 10ml aqueous extract (10g in 100ml D. H₂O) of *A. nilotica* pod were added slowly to 90ml 1mM silver nitrate in 250ml conical flask⁵. This solution was incubated in the dark at 37°C until use. A control solution (without extract) was also incubated under the same condition¹³.

2.4.2 UV-Vis: Optical Density (OD) using UV-2401, India, performed between 200-800nm with a resolution of 1nm and Scanning speed of 300nm/min. The reduction of Ag⁺ ion was monitored by measuring the UV-vis spectrum of 1ml aliquots sample and 2ml deionized water in a quartz cell.

Silver Nitrate (1mM) was used to adjust the baseline as a blank¹⁴.

2.4.3 FTIR: Surface topology on the powder sample of AgNPs and the crude extract was carried out using IRAffinity-1S Spectrometer (Buck Scientific – 530). The AgNP solution was centrifuged at 10,000 rpm for 20 min. The solid residue obtained was then dried at room temperature, and the powder obtained was used for FTIR measurement using KBr.

2.4.4 SEM: Scanning Electron Microscopy (SEM) was taken using. The morphological properties of the as-synthesized Ag nanoparticles were studied using Phenom Pro-X 800-07334 operated at 25 kV. After the preparation of the nanoparticles, the suspension of nanoparticles in distilled water was used for SEM analysis by fabricating a drop of suspension onto a clean carbon-coated corer grid and allowing water to completely evaporate. The dried sample was then mounted with the aid of a sample holder^{15,16}. An Enlarged SEM image was observed⁵.

2.5 Isolation and identification of dermatophytes fungi

2.5.1 Sample collection: Skin Scraping will be collected, using a sterile surgical blade, placed in a clean labeled envelope, as described by¹⁷, and was transported to Yobe State University, Microbiology Laboratory.

2.5.2 Media preparation: The media (Dextrose Agar (PDA)) will be prepared according to the manufacturer's specifications. The media will be poured into sterile Petri dishes and allowed to solidify.

2.5.3 Inoculations: Specimens (scales) collected from each patient enrolled in the study will be inoculated onto the culture plates with PDA and incubated for 96 to 168 hours at room temperature (25°C to 35°C). The culture will be examined for the growth of dermatophytes. The colonies will be Sub-cultured to obtain typical growth of dermatophytes¹⁸.

2.5.4 Macroscopy: Positive cultures were examined microscopically for colour, the texture of colonies, their topography, and the pigmentation produced as described¹⁸.

2.5.5 Microscopy: Suspected cultures were also viewed microscopically the following staining with lactophenol cotton blue (LPCB) at 10x and 40x magnification for conidia as described by¹⁸.

2.5.6 Tease Mount Preparation: A clean glass slide will be placed on the workbench and a small drop of LPCB solution

in the middle of the slide, a fragment of a fungal colony was removed (approximately 1-2 mm from the periphery) with a wooden stick and place on the LPCB solution. The fragment will be teased gently with two wooden sticks until it has been separated. It was covered with a coverslip. The slide was carefully examined under low (x10) and high power (x40) objectives of the microscope for the characteristic shape and arrangement of the spores, hyphae, etc. as described by¹⁸.

2.6 In- vitro Antifungal Assay

The anti-dermatophytic activity of methanolic, aqueous extracts, and AgNPs of *A. nilotica* pod were determined using the Agar well diffusion method as described by⁵ and ¹⁹. Cork borer was used to bore holes on solidified agar, with the extracts dissolved in 5%DMSO with sterile saline differently at different concentrations of 400, 200, 100, 50, and 25 g/ml. Using Micro pipete, extracts were poured into the agar well of about 25ml of molten Potato Dextrose agar (HKM – HCM 050, Guanadong Huankai) plate containing suspension of 0.5 McFarland turbidity in 0.85% saline with a few colonies from a 24 -48hours old culture of isolates and the plates incubated at 35°C. Zones of inhibition (ZOI) that appear as clear areas surrounding the well from which the substances with antimicrobial activity diffused and the diameter of the ZOI measured in millimeters with a ruler.

3. RESULTS

3.1 Collection of Plant Materials, phytochemical screening, and GC-MS analysis of *Acacia nilotica* pod.

The *Acacia nilotica* od greenish and brownish when dried, it is brown glittering crystals were observed after lyophilization. The methanolic extract is a little darker when compared to the aqueous extract. The yield is presented in table 1.

Table 1: The yield

The plant parts	Solvent	Yield in percentage (%)
Fruit pod of <i>A. nilotica</i>	Aqueous	6.60
	Methanol	48

The percentage of yield is relatively close to the one earlier reported²⁰.

The aqueous extracts of *Acacia nilotica* Pod yielded



Figure 1: (a.) *A nilotica* pod (b.) *A. nilotica* pod extract

3.2 Phytochemical screening

Table 2: Phytochemical Screening of *Acacia nilotica* Pod

Phytochemicals	<i>Acacia nilotica</i> pod extract and Test Inference	
	Aqueous	Methanol
Alkaloid	+	-
Flavonoids	+	+
Glycosides	+	+
Oxalate	-	-
Phenols	+	+
Quinones	+	-
Saponins	+	+
Steroids	+	-
Tannins	+	+
Terpenoids	+	+

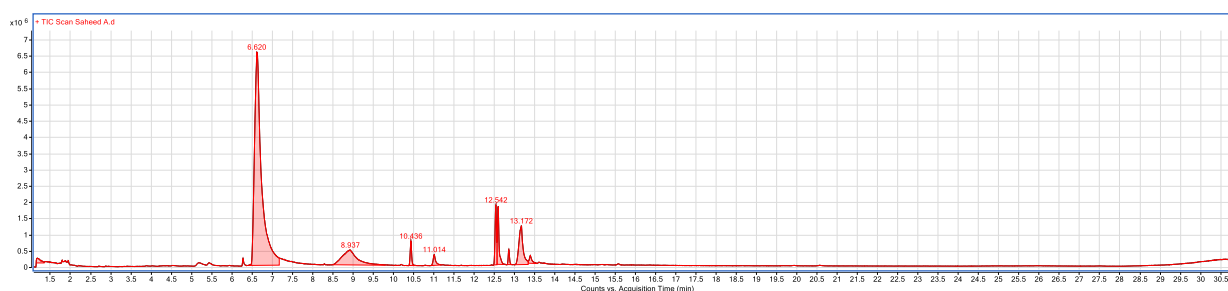
Table 2: shows the presence of Alkaloids, Flavonoids, Cardiac glycoside, phenols, Quinones, Saponings, steroids,

Tannins, and Terpenoids in Aqueous extract while all except alkaloids quinones and steroids were detected in methanolic extract of *A. nilotica* pod.

The results in this regard agree with the work of ²¹ for aqueous extracts. ²⁰ also identified Glycosides, Flavonoid, Terenoid, Saponins, and Tannins in both H₂O and Methanolic extracts.

Flavonoids are found in food, responsible for taste, colour, and phenolic compounds therapeutic potential has led to an upsurge in plants research²². They have also been found to contain antifungal activity, a wide range of antibiotics, they are a good coating agent, chelate free radicals and reactive oxygen species, increases the formation of capillaries and fibroblast, form complexes with proteins of cell wells²⁰. Phenols stimulate PGE formation based on their activities as a co-substrate for peroxidase reaction²¹. Polyphenols have excellent anti-fungal capabilities as well as a very good candidate for AgNPs. Ethanol extracts also showed all including sterol which was not observed in the methanolic extract in this study, this may be due to variation in the polarity of the extracting medium or reaction conditions^{23,24}.

3.3 GC – MS Analysis of *A. nilotica* pod

Figure 2: GC-MS spectrogram of *A. nilotica* podTable 3: Active fractions of *Acacia nilotica* pod from GC – MS.

Derivatives	RT	MW(g/mol)	Peak Area	MF
2,2'-Azoxybis[3-methylpyridine]	1.184	228	1015254.62	C ₁₂ H ₁₂ N ₄ O
1,2,3-Benzenetriol (pyrogallol)	6.62	126	79112701.84	C ₆ H ₆ O ₃
3-Methylmannoside	8.973	194	11856552.66	C ₇ H ₁₄ O ₆
Hexadecanoic acid, methyl ester	10.436	270	1904458.56	C ₁₇ H ₃₄ O ₂
Undecanoic acid	11.014	186	1434748.76	C ₁₁ H ₂₂ O ₂
9,12-Octadecadienoic acid, methyl ester	12.542	294	4622270.58	C ₁₉ H ₃₄ O ₂
6-Octadecenoic acid, methyl ester, (Z)-	12.599	296	4827313.37	C ₁₉ H ₃₆ O ₂
Methyl stearate	12.863	298	1023216.63	C ₁₉ H ₃₈ O ₂
9,12-Octadecadienoic acid (Z,Z)-	13.172	280	8493950.49	C ₁₈ H ₃₂ O ₂
Catechol	13.395	110	1079240.11	C ₆ H ₆ O ₂

The GC-MS spectrogram (Fig.2) led to the identification of some compounds (Table 3). A total of 10 peaks of different but closely related phytochemicals with their retention time and area presented. They are mostly polyunsaturated fatty acids. Including Azoxysis, 1,2,3 benzenetriol (pyrogallol), methylmannoside, hexadecanoic, undecanoic, octadecanoic, methyl stearate, pyrocatechol, and Linoleic acid.

The result in this study has been reported by previous scholars,^{25,26, 27} also, obtain similar phytochemicals from Methanol extract claiming their antibiotic potentials. Methyl stearate is an antifungal²⁸. Undecanoic acid - inhibit biofilm formation for some of the capric acids and also antibiotics. Capric and capric acids are chain fatty acids rarely found in food, it is also called pyrocatechol or 1, 2, -dihydroxy

benzene. Benzenediol isomers occurring along with polyphenols oxidase or catecholase.

According to²⁹, polyphenol oxidase was identified from propolis when purified from black poplar leaves. Catechol (O-diphenol) occurs naturally in fruits, vegetables, and plants PPO causes enzymes localized on the thylakoids of chloroplast with two distant possible catalyzes (1.) Hydroxylation of monophenols to O-diphenol and (2.) Oxidation of p and O quinones.

The same claim was supported by³⁰, after isolating catechol from poplar bud and argued that the origin of polyphenols oxidase is from poplar bud as they were not detected in other parts of bees. The vast majority of phenolic compounds

in higher lant vacuole could be involved in the production of O-quinones or catechol is oxidized into a quinone.

²⁴detected galloylated catechin and gallocatechin derivatives in Acacia extract using MS² fragmentation pattern. Benzene, hexadecenoic, and heptacosane extracts of *Acacia nilotica* have demonstrated Anti-microbial and mosquitocidal activities^{31,25}. Similar compounds and derivatives were also classified as asteroids – Octadecanoic acid, Tannins- ethyl gallate, pyrocatechol, and Terpenoids – Tetradecanoic acid, Hexadecanoic

³²demonstrated the catalytic role of *Acacia nilotica* pod using the phytoconstituent – gallic acid, ellagic acid, epicatechin, and rutin- suggesting them as well reducing agents for the synthesis of AgNPs and capping candidates. polyphenol and tannins from *A. nilotica* pod have potent antibacterial and

significant anti-biofilm activities against G+ve and G-ve bacteria as well as promoting wound healing efficiently within 15 days³³.

Flavonoids having catechol groups are fairly stable and various pyrcathechins have been suggested for therapeutic applications Octadecanoic serves as an anti-inflammatory³⁴.

Acacia extracts Secondary metabolites – are a complex mixture of active reducing biomolecules like antioxidant polyphenols, flavonoids, and phenolic acids, responsible for the reduction of metal ions and stabilization of Nano articles^{35,36}.

The polyphenols and phenolic acids which are the major constituents of *Acacia nilotica* pod have no doubt demonstrated nucleation with AgNO₃ solution indicating the reduction of Ag⁺ to Ag⁰.

3.4 Synthesis and Characterization of AgNPs

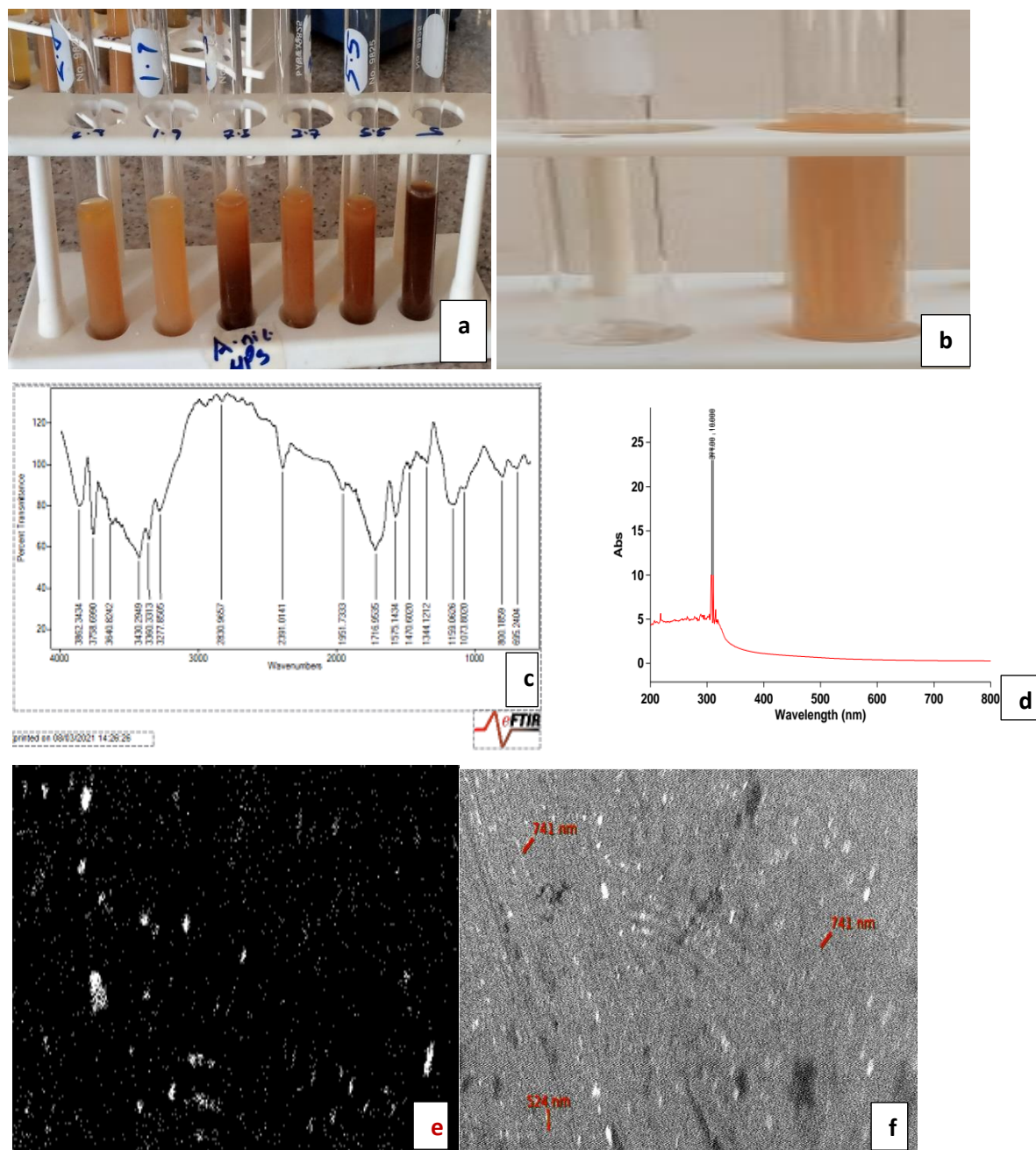


Figure 3: (a) AgNPs at different ratio, (b)1:9 AgNPs,(c) FTIR Spectrogram ,(d) Uv-vis spectrogram, (e)SEM image and (f) SEM image with ruler showing some enlarged particles.

3.4.1 Synthesized particles

The clear silver nitrate solutions showed colours change immediately after addition of the extracts as presented in Fig. 3. (a.) showed the colour of the particles at different ratios. 2:8, 1:9, 3:7, 7:3, 5:5, and the extract alone. 1:9 was considered for the rest of the study, fig.3(b). The colour change to light brown has been attributed to plasmons resonance formation on the surface of the molecules and oscillation of metal nanoparticles conduction electrons¹⁶.

3.4.2 Uv-Vis

The *A. nilotica* pod green nanoparticle analyzed using UV-2401, India. Uv-vis spectrophotometer confirmed the surface plasmon resonance bands with the sharp double peaks at 308 and 311nm (Fig. 3d). This indicates that the nanoparticle started to form immediately after the mixture and the double bands indicate that the Ag⁰ has been reduced to form a nanoparticle.

Lower double bands have been reported at 217 and 283nm³⁷, which is in contrast with the more common 430 – 450 ranges reported in other studies^{38,39&16}.

3.4.3 FTIR

The *A. nilotica* Pod extract was used as a reducing agent in synthesizing the nanoparticle. The functional groups indicating on the surfaces of AgNPs were confirmed using FTIR spectrogram (Fig. 3(c). The FTIR showed absorption

peak thus, 3428.3313 cm⁻¹, 2830.9657, 2391.0141, 1951.7333, 1716.9535, 1575.1434. This absorption indicates the possibility of bio-reduction and stability of the nanoparticles leading to the formation of surface plasmon resonance.

3428.3313 cm⁻¹ – OH bond stretching of phenolics⁴⁰. The double stretch between 2980 and 2830cm⁻¹ is due to the C-H stretching of nitrile or alkanes or C-H bending, at 2371, cm⁻¹ C≡N stretching of nitrile, C=O stretch of ketones or carbonyl group, and N-O symmetric stretch of nitrile.

It can be deduced from the FTIR that some of the phytochemicals present in the aqueous extract such as polyphenols have been involved in the redox reaction of silver salt to Ag⁰. The compounds would have capped and stabilized the solution by preventing the accumulation of the silver nanoparticles. As a good capping agent.

Other scholars have also reported similar peaks for *A. Senegal* and *A. tottilis*⁴¹ and *A. nilotica*³⁷.

3.4.4 SEM

Figure 3 (e and f) Shows the morphological properties of the nanoparticle as can be observed on the micrograph which confirmed that the nanoparticle development appeared to have close shapes and large surface diameters revealing the aggregation although only zoomed 500X. Other scholars have also reported diverse shapes and sizes in *A. nilotica* nanoparticles⁵.

3.5 Sample collection, isolation, and identification of Fungal Isolates

Table 4: Distribution of Dermatophytes according to Dermatographic Characteristics

Demographic Characteristics	Number of Sample	Percentage Distribution
1 – 10	21	15.8
11 – 20	18	13.5
21 – 30	14	10.5
31 – 40	10	07.5
41 – 50	21	15.8
51 – 60	33	24.8
61 – 70	09	06.8
71 – 80	04	03.0
81 – 90	03	02.3
Total	133	100
Sex		
Male	73	54.9
Female	60	45.1
Total	133	100

3.5.1 Distribution of Dermatophytes according to demographic Characteristics.

Out of 133 individuals enrolled in the study, 73(54%) were male while female counterparts took 60(45%) and the age group with the highest clinical presentation were 51 – 60 (24%) years followed by 1 -10 and 41 – 50 (15.8%) the lowest represented age groups were > 70 years with 7(5.3%) as shown in Table 4.

This agrees with the findings of ², establishing that males were more affected by dermatophytes than the female counterpart in their work sampling children from Bauchi state and disagree with the age group with the highest prevalence is 9 – 12years only because, they have enrolled children alone in their study. In Maiduguri,⁴¹ also reported that children 7 – 11 years (8.1%) are more infected followed by 4 – 6years (6.9%) and 12 – 16years (3.6%). The isolates recovered according to children also followed the same order at 64%, 25.4%, and 10.5%.

Table 5: Number of Samples Collected According to Clinical Presentation

Clinical Diseases	Number of Cases	Percentage Distribution (%)
<i>Tinea barbae</i>	01	0.8
<i>Tinea capitis</i>	39	29.3
<i>Tinea corporis</i>	13	9.8
<i>Tinea cruris</i>	04	3.0
<i>Tinea mannum</i>	11	8.3
<i>Tinea pedis</i>	49	36.8
<i>Tinea unguium</i>	16	12.0
Total	133	100

3.5.2 Number of Samples according to Clinical presentation

The data as presented in table 5, shows that the commonest clinical lesions were *Tinea. Pedis*, (36.8%), *T. capitis* (29.3%), *T. unguium* (12%), and the lowest representative were *T. barbae* (0.8%) and *T. cruris* (3%). Table 5. This result agrees with⁴².

3.5.3 Distribution and frequency of dermatophytic fungi Isolated

49 isolates of *Trichophyton mentagrophytes* 25(51%), *T. rubrum* 17(34.7%), and *Trichophyton tonsurans* 7(14.3%) were recovered according to infected sites as presented in table 6.

This corresponds to previous reports from the neighboring states of Bauchi, Borno, and Kano.⁴², 2020 described *Trichophyton* species (16%) as the leading cause of *tinea* in Kano and the only contrary result is that they reported *Tinea capitis* as the leading clinical disease while this report presented *Tinea corpori* and *T. unguium* ahead of *T. capitis*. *T. mentagrophytes* (16.7%) and *T. tonsurans* (10.5%) were among the isolates identified in Maiduguri ⁴¹.

This study disagrees with the reports that non-dermatophyte molds (51.7%) were mostly isolated from dermatophytosis cases in Bauchi².

Table 6. Distribution and Frequency dermatophytic fungi Isolated According to the Infected Sites

Diseases/ Isolates	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	<i>T. tonsurans</i>	Total
<i>Tinea barbae</i>	-	-	-	-
<i>Tinea capitis</i>	02	05	01	08
<i>Tinea corporis</i>	10	04	03	17
<i>Tinea cruris</i>	-	-	-	-
<i>Tinea mannum</i>	03	01	01	05
<i>Tinea pedis</i>	02	05	-	07
<i>Tinea unguium</i>	08	02	02	12
Frequency	25	17	07	49
Percentage	51	34.7	14.3	100

Table 7: Antifungal activities

ZOI(MM)	Number of positive and sensitive Isolates										
	Acacia Pod Aqueous Extract(mg/ml)					Acacia Pod Methanolic Extract(mg/ml)					Acacia pod AgNPs
	400	200	100	50	25	400	200	100	50	25	1:9
<i>Trichophyton Mentagrophytes</i> n=25											
<15	00	00	00	02	10	00	00	00	00	09	00
15 – 20	00	02	02	02	08	02	00	00	00	02	00
21 – 25	17	16	18	18	07	14	15	13	17	14	00
26 – 30	08	07	05	03	00	03	06	08	06	00	21
>30	00	00	00	00	00	06	04	01	00	00	04
<i>Trichophyton rubrum</i> n=17											
<15	00	00	00	00	00	00	00	00	00	00	00
15 – 20	00	00	00	00	03	00	00	00	00	1	00
21 – 25	06	07	05	03	07	06	08	04	03	07	03
26 – 30	06	08	12	14	07	05	05	13	14	06	13
>30	05	02	00	00	00	07	04	00	00	00	01
<i>Trichophyton tonsurans</i> n=7											
<15	00	00	00	00	01	00	00	00	00	00	00
15 – 20	00	00	00	02	02	00	00	00	00	00	00
21 – 25	02	03	04	04	04	03	03	04	04	04	03
26 – 30	02	03	03	00	00	01	04	03	03	03	04
>30	03	01	00	00	00	03	00	00	00	00	00

3.6 Antifungal Activities of *A. nilotica* pod Aqueous, Methanolic and AgNPs

Antifungal activities were expressed as an average diameter of the zones of inhibition calculated as the difference in diameter of the observed zones. The diameter of the wells is 6mm, only zones of inhibition greater than 6mm are regarded as a measure of antifungal activity⁴³, zones of inhibition less than 6mm are neglected⁴⁴.

All extracts (Aqueous, Methanolic and AgNps) were tested against 49 isolates of (*Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Trichophyton tonsurans*) at different dilutions (400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml, and 25mg/ml) as presented in table 7.

For Aqueous extracts – Antifungal activities against aqueous extract shows remarkable zones of inhibition – the highest zones observed (8) fall between 26-30mm against *T. Mentagrophytes* and maintenance activities between 21 - 25mm for all extract dilutions. *T. rubrum* shows zones of inhibition observed above 30mm at both 400mg/ml and 200mg/ml aqueous extracts. All extracts dilution showed activity with the lowest falling between 15 and 20mm at 25mg/ml. In *T. tonsurans*, the lowest dilution showed only one (1) zones of inhibition below 15mm. all dilution showed activity against Tt with 3 zones of inhibition observed at 400mg/ml and one at 200mg/ml. *T. tonsurans* is more susceptible to Aqueous extract compared to other extracts.

For Methanolic extract – methanolic extract showed zones of inhibition against *T. mentagrophytes* at all concentrations. Above 30mm zones of inhibition were observed for 40mg/ml, 200mg/ml, and 100 mg/ml, and the zones of inhibition between 21 -25 were observed at all tested

dilutions. *T. rubrum*, compared to *T. mentagrophytes* exhibited more susceptibility to the methanolic extract. *T. tonsurans*, only 3 zones of inhibition were above 30mm and most significant zones of inhibition were observed at 21-25 and 26-30 range for all extract dilutions.

For AgNPs – AgNPs didn't show antifungal activities below 26mm for both *T. mentagrophytes* and *T. rubrum*, except in the case of *T. tonsurans* were zones of inhibition between 21 - 25mm (03) and 26 30 (04) were exhibited and observed and no zones of inhibition above 30mm. AgNPs have shown broader and more consistent activities against all extracts.

Similar results have been reported^{36,37,45, 24, 46, 23,7, 5} and⁴⁷.

³⁶ using *A. nilotica* stem bark against *Shigella sonnei* and *Bacillus subtilis* observed antimicrobial activities with zones of inhibition ranging between 8.5mm and 18mm at 30mg/ml and 5mm and 16.5 at 25mg/ml.

³⁷ using FeNps synthesized from *A. nilotica* pod observed zones of inhibition between 11mm and 25mm at 3 concentrations (10, 40, and 60) against *E. coli*, *Salmonella*, *Staphylococcus aureus*, and *Candida albicans* as found by⁷. In other work,⁵ revealed that there is a difference of 3mm between AgNps synthesized from *A. nilotica* leaves against *Aspergillus niger*.

⁴⁵ used a methanolic extract from *A. nilotica* pod to obtain zones of inhibitions between 16mm and 19.5mm at 400mg/ml, 15mm and 19.5 mm at 200mg/ml against *S. aureus*, *Bacillus cereus*, *Escherichia coli*, and *Acinetobacter baumannii*. At 100mg/ml, 50mg/ml, and 25mg/ml,²⁴, reported Zones of inhibition of 22mm against clinical *E. coli* isolate, 21.7mm against *E. coli* isolated from fish, 21mm

against clinical isolate of *Salmonella* spp and 18mm against *salmonella* isolate from poultry meat. At 50mg/ml 18mm (clinical *E. coli*), 14.7mm (fish *E. coli*), 17.33mm (clinical *Salmonella*) and 18mm (*Meat salmonella*). At 25mg/ml 17.7mm (clinical *E. coli*), 11.33mm (Fish *E. coli*), 13.7mm (clinical *Salmonella*) and 12.7mm (meat *Salmonella*).

⁴⁷ revealed the antifungal efficacy of ethanolic *A. nilotica* pod extract as it causes weak effective growth inhibition by between 4.05 and 37.48% as the concentrations increased from 100 to 1000 ppm while [46], showed how hexane stem bark extract of the same plant inhibits mycelial growth by 46% (*Fusarium oxysporum*), 58% (*Rhizoctonia solani*) and 52% (*Alternaria brassicae*).



Figure 4: Sample Lates

4. CONCLUSION

Acacia nilotica pod extracted containing various secondary metabolites like polyphenols were used in the synthesis of AgNPs tested against dermatophytic fungi collected and isolated from clinical samples.

This investigation established the prevalence of dermatophyte infections in Damaturu with males more affected than females and the highest percentage found among 61-70 years, age group. Fungal infection of the foot is commonest, followed by a fungal infection of the hair commonly caused by fungal of *Trichophyton* species. The phytochemical screening suggests the presence of the phytochemicals supported by the GC-MS analysis of the same plant material. silver nanoparticles synthesized showed double absorption 311 and 308nm with FT-IR spectrogram showing stretches of alkane, alkenes, nitriles, ketones. The SEM shows the morphology of the AgNPs. Fungal species were susceptible to all the extracts. The green synthesized AgNPs showed improved and consistent antifungal efficacy against *T. mentagrophytes* and *T. tonsurans* than *T. rubrum*. This study has supported ethnomedicinal use of *Acacia nilotica* pod for treatment of dermatophytes, opened up the possibility of exploring more about the use of the eco and economic approach of improving medicinal values of the plants and the possibility of adding GC-MS analysis to the methods used the pre-synthesis analysis of AgNPs. Further exploration of these plants using the green synthesis approach, could open up more understanding and add value to medicinal plant research.

²³explained how *A. nilotica* pod induces cytopathogenicity, antiplatelet aggregation activity, antioxidant, decreases the arterial blood pressure, antituberculosis, antibacterial and antifungal activities against *C. albicans*, and *A. niger*.

It is said that silver interacts with thiol groups of protein on the cell membrane, which results in blocking respiration and producing ultimate death ⁴⁸. It has also been suggested that the interaction of silver nanoparticles with the cell wall increases the membrane permeability by forming pores or pits and thereby causing the death of microorganisms ⁴⁹ of the organisms ^{50,51}.

REFERENCES

- Ameen M, Epidemiology of superficial fungal infections. Journal of Clinics in dermatology, 2010; 28(2):197-201. <https://doi.org/10.1016/j.clindermatol.2009.12.005>
- Odeigah L, Rotifa S, Shittu R, Mutalub Y, Prevalence and risk factors of superficial fungal infections among primary school pupils in Ilorin, North Central, Nigeria. Annals of African Medical Research Journal, 2020; 3(100):23-27. <https://doi.org/10.4081/aamr.2020.100>
- Venkatesan B, Mahimainathan L, Das F, Gosh-Choudhury N, Choudhury GG, Journal of Cell Physiology. 2007; 211:457-467. <https://doi.org/10.1002/jcp.20953>
- Griffiths G, Simons K, The trans-Golgi Network: Sorting at the Exit Site of the Golgi Complex. Science Journal. 1986; 123:438-443. <https://doi.org/10.1126/science.2945253>
- Magesh R, Sivakumar K, Karthikeyan, et al., Invitro antifungal study of green synthesized silver nanoparticle from *A. nilotica* leaves extract against a plant and human pathogen. International Journal of Research in Pharmaceutical Sciences, 2018;10(1): 721-729.
- Baravkar AA, Kale RN, Patil RN, Sawant SD, Pharmaceutical and biological evaluation of formulated cream of methanolic extract of *Acacia nilotica* leaves. Res. J. Pharm. Technol., 2008;1(4):481-483
- Ali A, Akhtar N, Khan BA, Khan MS, et al., *Acacia nilotica*: A plant of multipurpose medicinal uses. Journal of Medicinal Plants Research. 2012; 6(9):1492-1496. <https://doi.org/10.5897/JMPR11.1275>
- Singh S, Padovani D, Leslie, R.A., Chiku, T and Banerjee R, Relative Contributions of Cystathionine Synthase and Cystathionase to H₂S Biogenesis via Alternative Trans-sulfuration Reactions. The Journal of Biological Chemistry,

- 2009; 284(33): 22457-22466.
<https://doi.org/10.1074/jbc.M109.010868>
9. Khanna S. and Tosh A, A clinician primer on the role of the microbiome in human health and diseases. Mayo Clinic Proceedings, 2014; 89(1):107-114.
<https://doi.org/10.1016/j.mayocp.2013.10.011>
 10. Evans WC, Trease and Evans Pharmacognosy, 12th Edition. WB Saunders Company. Td, London, England, 1996; p224-228.
 11. Sofowora A, Medicinal Plants and Traditional Medicine in Africa, 3rd Edition, Spectrum book Ltd., Ibadan, Nigeria, 2008;pp. 23-25.
 12. Trease GE and Evans WC, Pharmacognosy 15th Ed. Saunders Publishers, London, 2002;42 - 393.
 13. Parashar U, Kumar V, Bera T, Saxena P, Nath G, Srivastava S, et al., Study of mechanism of enhanced antibacterial activity by green synthesis of silver nanoparticles. Nanotechnology. 2011; 22, 102-114. <https://doi.org/10.1088/0957-4484/22/41/415104>
 14. Salem WM, Haridy M, Sayed WF, and Hassan NH, Antibacterial Activity of Silver Nanoparticles Synthesized from Latex and Leaf of *Ficus sycomorus*. Industrial Crops and Products. Elsevier. 2014;62:228-234.
<https://doi.org/10.1016/j.indcrop.2014.08.030>
 15. Bhagat M, Anand R, Datt R, Gupta V and Arya S, Green Synthesis of Silver Nanoparticles Using Aqueous Extract of *Rosa brunonii* Lindl and Their Morphological, Biological and Photocatalytic Characterizations. Journal of Inorganic and Organometallic Polymers and Materials. 2018;1:1-9.
 16. Saheed Y, Umar AF and Ilyyasu MY. Potential of Silver Nano Particles Synthesized from *Ficus sycomorus* Linn Against Multidrug-Resistant *Shigella* species Isolated from Clinical Specimens. American Journal of Life Sciences, 2020; 8(4):82-90. DOI: 10.11648/j.ajls.20200804.16.
<https://doi.org/10.11648/j.ajls.20200804.16>
 17. Cheesbrough M, District laboratory practice in tropical countries, Microbiological tests. Chapter 7. In: Cheesbrough M, Ed. pt 2. 2nd Ed. Cambridge: Cambridge University Press. 2012;pp: 9-267.
 18. Collins CH, Lyne PM, Grange GM, Collins and Lyne's Microbiological methods, 6th Edition. Butterworth, London. 1989. pp:214-401.
 19. Holder AI and Boyce ST, Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. Bums Journal. 1994; 20(5):426-429. [https://doi.org/10.1016/0305-4179\(94\)90035-3](https://doi.org/10.1016/0305-4179(94)90035-3)
 20. Abdallah A, Mustafa MI and Makhawi AM, Phytochemical screening and antimicrobial activities studies of *Acacia nilotica* fruit cover, 2020; CC-BY-NC-ND 4.0 International license. <https://doi.org/10.1101/2020.02.11.943456>: 1-16.
<https://doi.org/10.1101/2020.02.11.943456>
 21. Bansal VK and Goel RK, Gastroprotective effect of *Acacia nilotica* young seedless pod extract: Role of polyphenolic constituents. Asian Pacific Journal of Tropical Medicine (Elsevier). 2012:523-528. [https://doi.org/10.1016/S1995-7645\(12\)60092-3](https://doi.org/10.1016/S1995-7645(12)60092-3)
 22. Kumar S, and Pandey AK, Chemistry and Biological Activities of Flavonoids: An Overview. The Scientific World Journal. 2013;1:1-6. <https://doi.org/10.1155/2013/162750>
 23. Jame R. Phytochemical and Pharmacological Uses of *Acacia nilotica* - A Review. International Journal of Bioorganic Chemistry. 2018; 3(2): 6-10. DOI: 10.11648/j.ijbc.20180302.11.
 24. Sadiq BM, Hanpithakpong W, Tarning J and Anal AK, Phytochemicals and in-vitro evaluation antibacterial and antioxidant activities of leaves, pods, and stem bark extract of *Acacia nilotica* (L.) Del. Industrial crops and products. 2015;77:873-882.
<https://doi.org/10.1016/j.indcrop.2015.09.067>
 25. Pinto MEA, Araujo SG and Morais MI, Antifungal and antioxidant activity of fatty acid methyl esters from vegetable oils. Annals of the Brazilian Academy of Sciences Journal. 2017; 89(3):1671-1681. <https://doi.org/10.1590/0001-3765201720160908>
 26. Vivekanandhan P, Venkatesan R, Ramkumar G et al., Article Comparative Analysis of Major Mosquito Vectors Response to Seed-Derived Essential Oil and Seed Pod-Derived Extract from *Acacia nilotica*. International Journal of Environmental Research and Public Health. 2018; 15:388.
<https://doi.org/10.3390/ijerph15020388>
 27. Abdel-Hady H, El-Wakil EA, and Abdel-Gawad M, GC-MS Analysis, Antioxidant and Cytotoxic Activities of *Mentha spicata*. European Journal of Medicinal Plants. 2018; 26(1):1-12.
<https://doi.org/10.9734/EJMP/2018/45751>
 28. Shafaghat A, Antioxidant, Antimicrobial Activities and Fatty Acid Components of Flower, Leaf, Stem, and Seed of *Hypericum scabrum*. Journal of Natural Product Communication. 2011; 6(11):1739 -1742.
<https://doi.org/10.1177/1934578X1100601142>
 29. Kumari R, Mishra RC, Yadav A and Yadav JP, Screening of traditionally used medicinal plants for their antimicrobial efficacy against oral pathogens and GC-MS analysis of *Acacia nilotica* extract. Indian Journal of Traditional Knowledge. 2019; 18(1):162-168.
 30. Huang S, Zhang C-P, Li G Q. et al., Identification of Catechol as a New Marker for Detecting Propolis Adulteration. Molecules. 2014; 19:10208-10217.
<https://doi.org/10.3390/molecules190710208>
 31. Köse YB, Iscan G and Demirci B, Baser KHC and Celik C, Antimicrobial Activity of the Essential Oils Obtained from Flowering Aerial Parts of *Centaurea lycopifolia* Boiss. et Kotschy and *Centaurea cheirolopha* (Fenzl) Wagenitz from Turkey. Journal of Essential Oil Bearing Plants. 2007; 78:253-254.
 32. Edison TJI and Sethuraman MG, Electrocatalytic reduction of benzyl chloride by green synthesized silver nanoparticles using pod extract of *Acacia nilotica*. ACS Sustainable Chem. Eng Journal, 2013; 1:1 - 29. <https://doi.org/10.1021/sc4001725>
 33. Arya G, Kumari RM, Pundir R, Chatterjee S, Guta N, Kumar A, Chandra R, Kumar S, Versatile biomedical potential of biosynthesized silver nanoparticles from *Acacia nilotica* bark. Journal of Applied Biomedicine, 2019; 7(2):115-124.
<https://doi.org/10.32725/jab.2019.010>
 34. Kumar KM, Mandal BK, Tammina SK, Green synthesis of nano platinum using naturally occurring polyphenols, RSC. Advanced Journal, 2018; 3:4033-4039.
<https://doi.org/10.1039/c3ra22959a>
 35. Shah, Z., Hassan, S., Shaheen, K. et al., Synthesis of AgNPs coated with secondary metabolites of *Acacia nilotica*: An efficient antimicrobial and detoxification agent for environmental toxic organic pollutants. Materials Science & Engineering. 2020; 19:33034-6. <https://doi.org/10.1016/j.msec.2020.110829>
 36. Banso A, Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*. Journal of Medicinal Plants Research. 2008;3(2):082-085.
 37. Da'na E, Taha A and Afkar E, Green Synthesis of Iron Nanoparticles by *Acacia nilotica* Pods Extract and Its Catalytic, Adsorption, and Antibacterial Activities. Applied Science Journal. 2018; 8:1922. <https://doi.org/10.3390/app8101922>
 38. Niluxshun MCD, Masilamani K and Mathiventhan U, Green Synthesis of Silver Nanoparticles from the Extracts of Fruit Peel of Citrus tangerine, Citrus sinensis, and Citrus limon for Antibacterial Activities Journal, 2021: 1-8.
<https://doi.org/10.1155/2021/6695734>
 39. Jalilian F, Chahardoli A, Sadrjavadi K, Fattahi A, Shokoohinia Y, Green synthesized silver nanoparticle from *Allium ampeloprasum* aqueous extract: Characterization, antioxidant activities, antibacterial and cytotoxicity effects. Advanced Powder Technology Journal. 2020:1-10.
<https://doi.org/10.1016/j.apt.2020.01.011>

40. Emam R and Eassa N, Synthesis, Characterization, and Size Evaluation of Biosynthesized Silver Nanoparticles by UV- Vis Spectroscopy. African Journal of Engineering & Technology (AJET). 2021; 4(1):1-15. <https://doi.org/10.47959/AJET.2021.1.1.5>
41. Fatimah A, Antifungal Activity and Fourier Transform Infrared Spectrometric Characterization of Aqueous Extracts of Acacia senegal and Acacia tortilis on Phytopathogenic Fungi. JPRI, 2019; 31(2):1-11. <https://doi.org/10.9734/jpri/2019/v31i230295>
42. Nweze E. I. Etiology of dermatophytoses amongst children in northeastern Nigeria. Medical Mycology. 2001; 39:181-184. <https://doi.org/10.1080/mmy.39.2.181.184>
43. Diso SU, Adam JS, Mu'azu L, Abdallah MS. et al., Isolation and Characterization of Some Fungi Associated with Superficial Fungal Infections. ARC Journal of Dermatology, 2020; 5(1):12-16. <https://doi.org/10.20431/2456-0022.0501003>
44. Saheed Y, Nasir MU, Abbas B and Bello RY, GC-MS Analysis and Antimicrobial Spectrum of Stem Bark Extracts of Ficus sycomorus. Microbiology Research Journal International. 2020; 30(8):118-128. <https://doi.org/10.9734/mrji/2020/v30i830257>
45. Ramesh S, Vinitha UG, Anthony SP, Muthuraman MS, Pods of Acacia nilotica mediated synthesis of copper oxide nanoparticles and its in vitro biological applications. Materials Today: Proceedings. 2020:1-6. <https://doi.org/10.1016/j.matpr.2020.07.052>
46. Abdallah EM, Antibacterial Efficacy of Acacia nilotica (L.) Pods Growing in Sudan against Some Bacterial Pathogens. Int. J. Current Research Biosciences and Plant Biology, 2016; 3(3):6-11. <https://doi.org/10.20546/ijcrbp.2016.303.002>
47. Rai SP, Prasad MS, and Singh K, Evaluation of the Antifungal Activity of the Potent Fraction of Hexane Extract Obtained from the bark of Acacia nilotica. International Journal of Science and Research (IJSR), 2012; 10(3):730-738.
48. Abbassy MMS, Ibrahim HZ and Gab Alla MAA, Evaluating the Insecticidal and Fungicidal Efficiency of Acacia nilotica Pods Extract. J. Plant Prot. and Path., Mansoura Univ., 2018; 9(5):283 - 289. <https://doi.org/10.21608/jppp.2018.41410>
49. Sharman VK, Yngard RA and Lin Y, Adv. Colloid Surface Interface. 2009;145: 83. Shigella sonnei outbreak among men who have sex with men San Francisco, California, 2000-2001. MMWR Morb Mortal Wkly Rep 2001; 50:922-6.
50. Sondi I and Salopek-Sondi B, Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of Colloid Interface Science. 2004; 275:177-182. <https://doi.org/10.1016/j.jcis.2004.02.012>
51. Ontong JC, Paosena S, Shankar S, and Voravuthikunchai SP, Eco-friendly synthesis of silver nanoparticles using Senna alata bark extract and its antimicrobial mechanism through enhancement of bacterial membrane degradation. Journal of Microbiological Methods. 2019; 165:10569. <https://doi.org/10.1016/j.mimet.2019.105692>