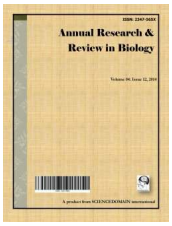

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Antimicrobial Activity of *Acacia nilotica* ssp. *nilotica* against Some Causative Agents of Urogenital Infections

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Authors' contributions

This work was carried out in collaboration between both authors. Author KSAU designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author HHEK managed the literature searches and revised the first version of this study. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: To assess and compare the antimicrobial efficacy of methanol and aqueous extracts derived from *Acacia nilotica* ssp. *nilotica* against standard strains of microorganisms and clinical isolates.
Study Design: An experimental study which was carried out at the microbiology laboratory, Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan, during the period from September 2012 to March 2013.
Methodology: Leaves and barks methanol extracts of *A. n.* ssp. *nilotica* at concentration of 100 mg/ml were subjected to antimicrobial screening against six standard strains and 133 clinical isolates including bacterial and fungal species. Screening for antimicrobial activity of extracts, standard antibiotics and antifungal drug were detected by the agar well diffusion method. The Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum

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Fungicidal Concentration (MFC) of tested extracts were determined using the broth dilution method. The MDIZs of clinical isolates were analyzed statistically using One- Way analysis of variance.

Results: The highest antibacterial activity of leaves and barks methanol extracts of *A. n.* ssp. *nilotica* on standard microorganisms was noted against *Pseudomonas aeruginosa* with zone of inhibition of 40 mm and the minimum activity was showed against *Escherichia coli* with zone of inhibition of 27 mm. The antifungal activity of leaves and barks methanol extracts was ranged from 23 mm to 24 mm. The MIC showed by the methanol leaves and barks extracts on standard bacterial strains was ranged from 3.125 mg/ml to 12.50 mg/ml and the MBC was ranged from 6.25 mg/ml to 50 mg/ml. On the other hand, the MIC of methanol leaves and barks extracts toward standard fungi was 6.25 mg/ml, while MFC was 12.50 mg/ml. Statistical analysis showed that, there are significant differences between effects of methanol and aqueous extracts of *A. n.* ssp. *nilotica* on the standard microorganisms (*P*=.000). The highest MDIZ produced by leaves methanol extract was against clinical isolates of *Escherichia coli* which was 25.03 ±3.59 mm, while the lowest MDIZ was obtained by *Klebsiella pnuemoniae* which was 22.67±7.83 mm. Clinical isolates of *Staphylococcus saprophyticus* showed higher MDIZ toward barks methanol extract which was 25.71±1.98 mm, while the lowest MDIZ for the same extract was revealed by *Enterococcus* spp. which was 22.82±7.85 mm. There is highly statistical significant differences between the effect of leaves and barks methanol extracts and Gentamicin 20 µg/ml on the clinical isolates (*P* = .000). The MIC exhibited by methanol leaves and barks extracts on the bacterial isolates was ranged from 3.125 mg/ml to 12.50 mg/ml, the MBC was ranged from 6.25 mg/ml to 25 mg/ml. The MIC of methanol leaves and barks extracts toward *Candida albicans* was 6.25 mg/ml, whereas the MFC was 12.50 mg/ml.

Conclusion: Methanol extracts of leaves and barks of *A. n.* ssp. *nilotica* were found to be effective against both standard microorganisms and clinical isolates.

Keywords: Antimicrobial activity; Acacia nilotica ssp. Nilotica; barks extracts; clinical isolates; leaves extract; antifungal activity.

1. INTRODUCTION

The irrational use of broad-spectrum antimicrobial therapies used in the treatment of urogenital infections represents one of those mechanisms that increase the appearance of multi-drugs resistant microbial strains [1,2,3]. The use of medicinal plants is not just a custom of the distant past. However, approximately 80- 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicines [4]. In developing countries, low income people such as farmers, workers, people of isolated villages and native communities use the herbal medicine as the primary source of medicine [5]. As well as, in such communities; synthetic drugs are expensive and sometimes inadequate for the treatment of the infectious diseases. Moreover, synthetic chemotherapeutic agents may cause undesired side effects. Therefore, there is an urgent need to search new safer and effective natural medicinal products to fight and control the microbial infections [6,7,8]. It was known that the plant which naturally synthesis and accumulate some secondary metabolites like alkaloids, glycosides, tannins, saponins and volatile oils, possess medicinal properties. A vast knowledge of how to use the plant against different illnesses may be expected to have accumulated in areas where the use the plants still of great importance [9,10]. *Acacia nilotica* (L.) Willd. Ex. Del. is a genus of shrubs and trees [11]. It is moderate to large size tree that reaches a height of 10 to 20 meters. The barks are blackish grey or dark brown in mature trees and deeply grooved, with longitudinal fissures [12]. The leaves are twice compound, they consist of 5-11 feather-like pairs of pinnae [13]. The flower stalks are hairy. The pods are very characteristic, resembling a beaded necklace. *A. n.* ssp. *nilotica* spread around the tropical to warm-temperate regions of both hemispheres, including Europe, Africa, southern Asia, and the Americas [14]. In Sudan, this subspecies occurs in drained situations along water courses such as on the river banks of the White and Blue Niles [15]. Regarding traditional medicine uses, A. n. ssp. *nilotica* differs from ethnic group to another Zulu use barks for cough; Chipi has use roots for tuberculosis and root decoction, said to impart courage, even aphrodisia, and the root is used to cure impotence [16]. The barks used for diarrhea, dysentery, and leprosy. Decoction of pods used as gargle in urogenital diseases [17]. The barks, gum, leaves, and pods used medicinally in West Africa. Barks, leaves, and young pods are strongly astringent due to tannin, and had chewed in Senegal as anti-scorbutic, in

Ethiopia as lactogogue. Bark decoction drunk for intestinal pains and diarrhea. Other preparations used for coughs, gargle, toothache, ophthalmia, syphilitic ulcers and gonorrhoea [18]. Some of rural communities in Sudan use different parts of *A. n.* ssp. *nilotica* to cure dysentery, leprosy, cancer, impotence, colds, congestion, coughs, diarrhea, dysentery, fever, gallbladder disease, hemorrhage, hemorrhoids, leucorrhea, ophthalmic, sclerosis, smallpox, diabetes and tuberculosis [10,16]. In this work the antimicrobial properties of methanol and aqueous extracts of *A. n.* ssp. *nilotica* leaves and stem barks have been investigated against microorganisms cause urogenital infections.

2. MATERIALS AND METHODS

2.1 Sample Collection

Leaves and stem barks of *A. n.* ssp. *nilotica* were collected in September 2012 from SHAMBAT Area. It is situated in Khartoum North, Khartoum State, Sudan, and its geographical coordinates are latitude 15° 40' 5" North and longitude 32° 32' 1" East. The plant parts were spread, air-dried in the shade for twenty days and their botanical identification was kindly made by Professor Hatil Hashim El-Kamali, Department of Botany – Faculty of Science and Technology, Omdurman Islamic University (OIU), Omdurman, Sudan. Voucher specimens of the plant (HHK 376) have been deposited at Faculty of Science and Technology. Botany Department Herbarium, OIU. The microbiological works has been carried out in the microbiology laboratory, Medicinal and aromatic plants research Institute (MAPRI), Khartoum, Sudan.

2.2 Preparation of Plant Extract and Standard Antimicrobial Agents

2.2.1 Preparation of aqueous extract

Aqueous extracts were prepared according to the method described by EL-Kamali and Awad EL-Karim, [19]. Exactly 100 g of the plant powder poured in 1 liter (1000 Milliliters) of hot sterile distilled water and left for 24 hours at room temperature. The mother liquor was filtered through What-man No.2 filter paper (Sigma-Aldrish, Inc. USA). Extracts were kept in deep freezer for 48 hours, then introduced in freeze dryer till completely dried. The dried plant extracts were crushed from the flask using a spatula. Each residue was weighed and the yield percentage was determined. The crude extracts were stored in dark dry sterile containers in the microbiology laboratory, MAPRI, Khartoum, Sudan, until use for antimicrobial screening. At the time of testing, the aqueous residue (2 g) was dissolved in sterile distilled water 20 ml (con. 100 milligram/milliliter), and kept in refrigerator until used.

2.2.2 Preparation of methanol extract

The plant parts washed with distilled water, then dried at 60℃ over night, cut into small pieces and crushed in a mechanical mortar to a coarse powder. Powder sample (100 g) were soaked in 200 ml of 98 % methanol (Chem. Lab. Ltd. Belgium) for 3 days at room temperature and then filtered through What-Man No.2 filter paper. The methanol extract was filtered and evaporated under reduced pressure again using Rotary Evaporator to complete dryness. Each residue was weighed and the yield percentage was determined. Then, the residue was stored dry in sterile containers in the microbiology laboratory, (MAPRI), until use for antimicrobial testing [19].

2.2.3 Preparation of standard antibiotics and antifungal drugs

Gentamicin (Lunik Pharma Pvt. Ltd., India), ampicillin (Therapeutic Pharmaceutical, India) and candizole (The United Pharmaceutical. Ltd. Jordan) were prepared immediately before used by diluting them in sterile de-ionized water. A series of concentrations were done by double fold dilution method from the original concentration to get 40 µg /ml, 20 µg /ml, 10 µg /ml and 5 µg /ml for each reference antibiotic, whereas the antifungal drug (Candizole) was prepared at concentration of 5 mg/ml, 10 mg/ml, 20 mg/ml and 40 mg/ml.

2.3 Preparation of Culture Media and Inoculum

Bacteria were grown in enriched and selective culture media. Mueller-Hinton agar (Oxoid, Ltd, England) was used as base medium for screening of antibacterial activity. Sabouraud's dextrose agar (Oxoid, Ltd, England) was used as a medium for identification and it is used for screening of antifungal activity [20].

2.3.1 Preparation of Mcfarland standard

0.5 McFarland standard was prepared by mixing 9.95 ml of 1% Sulphuric acid (Shree Pushkar Chemicals & Fertilizers Ltd. India) with 0.05 ml of

1% anhydrous barium chloride in distilled water in order to estimate the approximate bacterial density [21]. The tube was use for comparison of bacterial suspension with the standard inoculum whenever required.

2.3.2 Preparation of bacterial inoculum

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto Mueller-Hinton agar slopes and incubated at 37ºC for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing approximately 10^7 CFU/ ml. The suspension was stored in the refrigerator at 4℃ until used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique [22]. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micropipette onto the surface of dried Mueller-Hinton agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37℃ for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared; all the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained [23].

2.3.3 Preparation of fungal inoculum

The inoculum of *C. albicans* was prepared as previously described by Cormican and Pfaller, [24]. Briefly, *C. albicans* was grown on Sabouraud's Dextrose Agar (SDA) plates for 24 hr. For each isolate, five colonies were grown until their diameters were at least 1 mm. Then, the colonies were picked off and suspended in 0.85% saline solution. The suspension was adjusted to the turbidity of a 0.5 McFarland standard at a wavelength of 530 nm. Quantitative colony plate counts were determined on SDA to verify the inoculum size. The final inoculum concentration was standardized to approximately 1×10^6 CFU/ml. The suspension was stored in the refrigerator at 4℃ until used [25].

2.4 Purification of Cultures by Streaking Plate Method

The bacteriological and fungal techniques followed in the present work were described by Mackie and McCartney, [20], Koneman et al. [21], Kavanagh, [23]; Cruickshank et al. [26]; Arvidson et al. [27]; Cheesbrough, [28].

Once the primary inoculum from the clinical specimens was made, a wire loop was use to spread the material into the four quadrants of the plate, as described by Cheesbrough, [28] and Koneman et al. [21]. The wire loop was sterilized between each successive quadrant streak. The inoculated plates were incubated at 37℃ for 24 hours. The purpose of this technique is to dilute the inoculum on the surface of the Agar medium, so that single isolated colonies of bacteria and fungi, known as colony forming units, can be isolated. We used this method for sub-culturing the standard strains and obtained clinical isolates on Nutrient agar, MacConkey's Agar, Mannitol salt agar, Blood Agar, Chocolate Agar, Nutrient Agar and Sabouraud's dextrose agar (Oxoid limited, England).

2.5 Sources and Maintenance of Tested Microorganisms

2.5.1 Sources of standard microorganisms

Five standard strains of bacteria and one species of fungi were obtained from American Type Culture Collection (ATCC) Rockville, Maryland, USA.Those reference strains include *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 53657), *Proteus vulgaris* (ATCC 6380), *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 7596).The strains were activated and sub-cultured three successive times and stored at 4℃.

2.5.2 Sources of the clinical isolates

The clinical isolates were obtained from urogenital infections specimens of Sudanese patients (urines, urethral discharges and high-vaginal swabs). These isolates were collected from different General and Private Hospitals of Khartoum State, Sudan. After explaining the purpose of the study, verbal approval from the directors of these hospitals has been taken. Clinical isolates were maintained frozen in Trypton Soya Agar TSA, (Oxoid Ltd. England).

2.5.3 Identification of clinical isolates

Each clinical isolate was inoculated on a suitable culture media and incubated aerobically and the other an aerobically. The obtained isolates were then purified by streaking on plates containing the appropriate selective and differential culture media that mentioned above. The purified isolates were then identified by microscopic examination, cultural characters and biochemical tests and stored in a refrigerator until they were used.

2.6 *In vitro* Screening for Antimicrobial Activity

2.6.1 Testing of antimicrobial activity of standard antibiotics and antifungal against standard microorganisms

The agar well diffusion method was followed to determine the antimicrobial activity of standard antibiotics and antifungal drug against standard microorganisms. 20 ml of two culture media; Mueller-Hinton agar and Sabouraud's dextrose agar were poured on a glass Petri-dish of same size and allowed to solidify. Agar surface of each plate was streaked by a sterile cotton swab with the standard strain. Agar plate was punched with a sterile cork borer (No.4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each antibiotic using automatic micro-pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 18 hours. After incubation the diameters of the resultant growth inhibition zones were measured [20,26].

2.6.2 Determination of the antibacterial activity of the extracts

The agar well diffusion method [20] was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension 10^7 CFU/ ml were thoroughly mixed with 100ml of cooled molten sterile Mueller-Hinton agar which was maintained at 45ºC. 20 ml aliquots of the inoculated Mueller-Hinton agar were distributed into sterile Petri-dishes. The agar was left to set and in each of these plates 4 cups (10 mm in diameter) was cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each extracts using automatic micropipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 18 hours. Two replicates were carried out for each extract against each of the test organisms. After incubation, the diameters of the resultant growth inhibition zones were measured averaged and the mean values were tabulated.

2.6.3 Determination of antifungal activity of the extracts

Testing of antifungal activity of extracts was performed by the agar well diffusion method with minor modifications. Two ml of the standardized *Candida albicans* stock suspension 10^6 CFU/ ml were thoroughly mixed with 100 ml of cooled molten sterile Sabouraud's Dextrose agar which was maintained at 45ºC. 20 ml aliquots of the inoculated Sabouraud's Dextrose agar were distributed into sterile Petri-dishes. The agar was left to solidify and in each of these plates 4 cups was cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of each extracts using automatic micropipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 24 - 48 hours. Two replicates were carried out for each extract against each of the test fungus. After incubation, the diameters of the resultant growth inhibition zones were measured averaged and the mean values were tabulated [29].

2.6.4 Determination of minimum inhibitory concentration (MIC) for standard microorganisms and clinical isolates

The plant extract that was found active, as antimicrobial agent, was later tested to determine the MIC values for each strain. Mueller-Hinton broth medium was used to test the inhibitory effect of tested extracts on standard bacteria and clinical isolates, while Sabouraud's dextrose broth was used to determine the MFC of extracts on the standard strains and clinical isolates of *Candida albicans*. MIC was determined using broth dilution method as described by Mackie and McCartney [20]. Tubes were prepared in the series of increasing concentrations of the plant extracts. The extracts were double-fold diluted to give the final concentrations of 3.125, 6.25, 12.50, 25, and 50 mg/ml. The organisms tested were growing in broth over night to contain 10^7 CFU/ml. A loop-full of diluted culture was spots with a standard wire loop that delivers 0.001 ml of inoculum and inoculated in tubes with equal volume of Mueller Hinton broth, Sabouraud's dextrose broth and the plant extracts. The tubes

were incubated aerobically at 35℃ for 24-48 hours. Three control tubes were maintained for each strain (media control, organism control and extract control). The lowest concentration (highest dilution) of the extract that produced no visible growth (no turbidity) in the first 24 hours when compared with the control tubes was considered as initial MIC.

2.6.5 Determination of minimum bactericidal and minimum fungicidal concentrations (MIC&MFC) for standard micro-organisms and clinical isolates

The Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) determine the lowest concentrations that completely kill bacteria and fungi after 48 hours of incubation at 35℃. MBC and MFC were determined for each standard organism and clinical isolate after determination of MICs of microorganisms has completed. The streaks were taken from the two lowest concentrations of the plant extract plates that exhibiting invisible growth (from inhibition zone of MIC plates) and subcultures onto the Mueller-Hinton agar plates (for bacteria) and Sabouraud's dextrose agar plates (for fungi). The plates were incubated at 35℃ for 24 hours then examined for bacterial and fungal growth in corresponding to plant extract concentration. MBC and MFC were taken as the concentration of the plant extract that did not exhibiting any bacterial or fungal growth on the freshly inoculated agar plates [21].

2.7 Statistical Analysis

Statistical analysis was carried out using the Statistical Package for Social Sciences, SPSS Version 20 (SPSS, IBM, Corporation, New York, USA). The diameters of inhibition zones of tested organisms were expressed as mean±SD. The data were subjected to one-way analysis of variance (ANOVA). The significance of differences between means was assessed, where appropriate. A two-tailed P-value of less than 0.05 was considered an evidence of statistical significance.

2.8 Ethical Consideration

Approval was obtained from Medicinal and Aromatic Plants Research Institute (MAPRI), National Centre for Research (NCR), Khartoum, Sudan to conduct the laboratory work.

3. RESULTS AND DISCUSSION

The antimicrobial activity of methanol and aqueous extracts of the leaves and stem barkss of *A. n.* ssp. *nilotica* at concentration of 100 mg/ml were assessed against five standard strains of bacteria and one species of fungi. As well as, the methanol extracts were tested against 133 clinical isolates obtained from urogenital infections. On the basis of the results of identification tests, it was found that the 133 clinical isolates were distributed as follows 29 *Escherichia coli*, 18 *Pseudomonas aeruginosa,* 16 *Proteus mirabilis*, 14 *Staphylococcus saprophyticus*, 13 *Neisseria gonorrhoeae*, 12 *Klebsiella pneumoniae*, 11 *Enterococcus* spp. and 20 *Candida albicans* (Table 1).

3.1 The Antimicrobial Activity of *A. n.* ssp. *nilotica* Extracts on the Standard Microorganisms

The yield percentages of leaves and methanol barks extracts of *A. n.* ssp. *nilotica* was 5.6% and 3.8% for each which was higher compared to the yield percentages of aqueous extracts of the same parts which produced 2.5% and 2.2%. The results of susceptibility were interpreted as active (> 18mm), moderate active (14-18 mm), and inactive (< 14 mm) [21,30,31]. The leaves and methanol barks extracts showed high activity against standard strains where the diameters of inhibition zones ranged from 24 mm to 40 mm, while the leaves and barks aqueous extract showed lower varied activity which was ranged from 16 mm to 19 mm (Table 2). These results are similar to those findings of El-Kamali and Awad El-Karim, [19], Shanab [32] Mashram et al. [33] and not parallel to the finding of Khan et al. [34].

It was noted from Table 2 that the leaves methanol extract *A. n.* ssp. *nilotica* followed by methanol barks extract exhibited higher levels of inhibitory effects than aqueous extracts against all standard microorganisms (Fig. 1). These results coincide with Dabur et al. [35]. Methanol extracts of leaves and barks of *A. n.* ssp. *nilotica* revealed high antifungal activity on standard strain of *Candida albicans*, while the aqueous extracts of the same parts showed lowest antifungal activity (Fig. 2). There are approximately equal effects for extracts of *A. n.* ssp. *nilotica* in both Gram negative and Gram positive tested bacteria. The results were supported that, the patterns of inhibition not

varied with plant part only, but also the solvent system and method of extraction may affect both the yielded extract and antimicrobial activity on the tested organism. These findings are in agreement with Saba et al. [36] and Mahesh et al. [17].

3.2 Comparison between Effects of Standard Antibiotics and Tested Extracts on the Standard Bacteria and Fungi

The results of statistical analysis showed that there are no statistical significant differences between effects of Gentamicin 40 µg/ml and 100 mg/ml of methanol and aqueous extracts of *A. n.* ssp. *nilotica* on the standard bacterial strains (*P*= 1.000).There was also no statistical significant differences between effects of Gentamicin 40 µg/ml and Gentamicin 20 µg/ml (*P* = 1.000). The effect of Gentamicin 20 µg/ml on the standard bacterial strains compared to the effects of 100 mg/ml of methanol and aqueous extracts of *A. n.* ssp. *nilotica* revealed no statistical significant differences (*P* = 0.124), whereas there is statistical significant differences between effects of Gentamicin 20 µg/ml and Ampicillin20 µg/ml on standard bacteria (*P*=0.019). The effect of Ampicillin 40 µg/ml compared to leaves and methanol barks extracts of *A. n.* ssp. *nilotica* revealed no statistical significant differences (*P* = 1.000). Among the tested extracts the leaves and barks methanol extracts of *A. n.* ssp. *nilotica* showed significant statistical differences compared to the leaves and barks aqueous extracts of the same plant (*P* = .001) (Table 3). Regarding antifungal activity of leaves and barks methanol extracts of *A. n.* ssp. *nilotica* showed high activity towards *Candida albicans* which were higher than activity of 10 mg /ml of Candizole, while the aqueous extract of the same parts showed moderate antifungal activity against *Candida albicans*, which were lower than activity of 5 mg/ml of Candizole (Table 4).

Table 1. Biochemical properties of examined clinical isolates

Character	Tested clinical isolates							
	E.c.	**P.a.**	**P.m.**	**S.sa.**	**N.g.**	**K.p.**	**E.sp.**	**C.a.**
Gram stain	G-ve	G-ve	G-ve	G+ve	G-ve	G-ve	G+ve	ND
Aerobic growth	+	+	+	+	+	+	+	+
Motility Test	+	+	+	-	-	-	-	ND
Catalase Test	+	+	+	+	+	+	-	ND
Coagulase Test	ND	ND	ND	-	ND	ND	ND	ND
Indole Test	+	-	-	-	-	-	-	ND
Methyl red Test	+	-	-	+	-	-	-	ND
Voges Proskauer	-	-	+/-	-	ND	+	+	ND
Oxidase Test	-	+	-	-	+	-	-	-
Urease Test	-	-	+	+	-	+	-	-
Citrate Test	-	+	+/-	-	ND	+	-	-
DNase Test	-	-	-	-	-	-	-	-
Nitrate Test	+	+	+	-	-	+	+	-
Germ tube Test	ND	ND	ND	ND	ND	ND	ND	+
Novobiocin(5µg)	S	ND	ND	R	ND	S	S	ND
Bacitracin	ND	ND	ND	ND	ND	R	ND	ND
Mannitol	+	+	-	+	-	+	-	-
Sucrose	+/-	-	-	+	-	+	-	+
Lactose	+	-	-	+	-	+	+	-
Acid from glucose	-	+	+	+	+	+	+	+
Gas from glucose	+	-	+	-	+	+	-	-

Key: E.c.: Escherichia coli; P.a.:Pseudomonas aeruginosa; P.m.: Proteus mirabilis; S.sa.: Staphylococcus saprophyticus; N.g.: Neisseria gonorrhoeae K.p.: Klebsiella pneumoniae; E.sp.: Enterococcus spp.; C.a.: Candida albicans; +: more than 80% of isolates had positive reaction. ; - : more than 80% of isolates had negative reaction. +/-: variable character. ; ND: not determined; R: Resistant S: Sensitive; G-ve: Gram negative reaction. ; G+ve: Gram positive reaction.

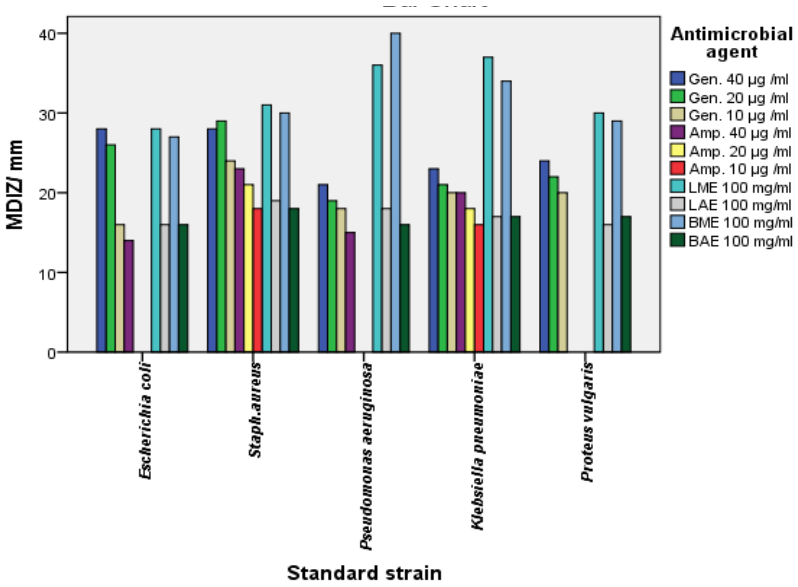

Fig. 1. Activity of standard antibiotics and tested extracts versus standard bacterial strains

Table 2. Antimicrobial activity of *Acacia nilotica* ssp. *nilotica* against standard strains

Part used	Solvent system	Conc./ mg/ml	Yield %	Tested standard strains /DIZ mm					
				E.c.	S.au.	Ps.a	K.p.	P.v.	C.a.
Leaves	Methanol	100	5.6	28	31	36	37	30	24
	Aqueous	100	2.5	16	19	18	17	16	17
Stem	Methanol	100	3.8	27	30	40	34	29	23
Barkss	Aqueous	100	2.2	16	18	16	17	17	16

*Key: E. c. = Escherichia coli (ATCC 25922), S. a. =Staphylococcus aureus (ATCC 25923), Ps. a. = Pseudomonas aeruginosa (ATCC 27853), K. p. = Klebsiella pneumoniae (ATCC 53657), P. v. = Proteus vulgaris (ATCC 6380) and C.a. =Candida albicans (ATCC 7596). Conc.= Concentration.**DIZ/ mm: Diameter of Inhibition Zone in Millimeters. mg.= milligram. µg /ml= microgram/milliliter*

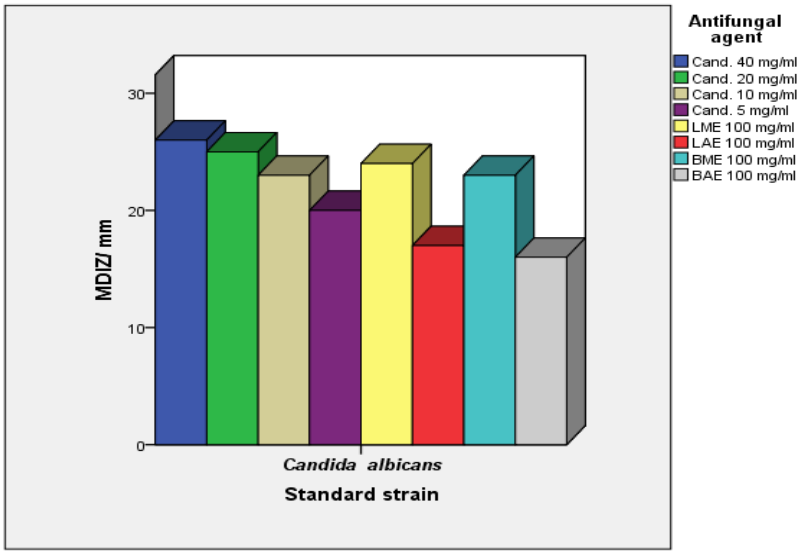

Fig. 2. Activity of standard antifungal drug and tested extracts versus standard fungs

Table 3. Antibacterial activity of standard antibiotics against the standard bacterial strains

Antibiotics	Concentration used µg /ml	*Tested standard strains /**DIZ mm				
		E.c.	S.au.	Ps.a.	K.p.	P.v.
Gentamicin	40	28	28	21	23	24
	20	26	29	19	21	22
	10	16	24	18	20	20
	5	_	20	17	18	18
Ampicillin	40	14	23	15	20	-
	20	-	21	-	18	-
	10	-	18	-	16	-
	5	-	14	-	14	-

*Key: E. c. = Escherichia coli (ATCC 25922), S. a. =Staphylococcus aureus (ATCC 25923), Ps. a. = Pseudomonas aeruginosa (ATCC 27853), K. p. = Klebsiella pneumoniae (ATCC 53657), P. v. = Proteus vulgaris (ATCC 6380) and C.a. =Candida albicans(ATCC 7596). **DIZ/ mm: Diameter of Inhibition Zone in Millimeters. µg /ml= microgram/milliliter*

Table 4. Antifungal activity against standard *Candida albicans*

Antifungal drug	Concentration mg/ml	*Candida albicans* DIZ (mm)
Candizole	40	26
	20	25
	10	23
	5	20

*Key: Candida albicans.**DIZ/ mm: Diameter of Inhibition Zone in Millimeters. mg.= milligram.*

3.3 The Antimicrobial Activity of *A. n.* ssp. *nilotica* Extracts on the Clinical Isolates

The methanol extracts of leaves and barks of *A. n.* ssp. *nilotica* were only assessed against clinical isolates because the results of antimicrobial activity of the aqueous extracts on the standard microorganisms were lower and not encouraging to continue in measuring further antibacterial activity screening for the aqueous extracts. The antimicrobial activity of extracts on the clinical isolates was expressed as MDIZ/ mm ± SD. The MDIZs obtained by leaves methanol extract on the clinical isolates was 25.03±3.59 mm for *Escherichia coli*, 23.55±8.26 mm for *Enterococcus* sp., while *Klebsiella pneumoniae* produced the least MDIZ which was 22.67±7.83 mm. The rest of clinical isolates obtained slightly varied inhibition zones which were 24.11±3.01 mm, 24.13±3.12 mm, 24.21±3.26 mm and 24.62±2.99 mm for *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus* saprophyticus and *Neisseria gonorrhoeae*, respectively. On the other hand, the barks methanol extract exhibited prominent activity against four clinical isolates compared to the rest strains which was 25.0±2.66 mm for *Pseudomonas aeruginosa*, 25.69±2.75 mm for *Proteus mirabilis*, 25.71±1.98 mm for *Staphylococcus saprophyticus* and 25.0±2.92 mm for *Neisseria gonorrhoeae*. Clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* showed MDIZs which was 24.86±2.60 mm and 23.67±7.62 mm for each, while the clinical isolates of *Enterococcus spp.* revealed the lowest MDIZ which was 22.82±7.85 mm (Table 5). The antifungal activity of *A. n*. ssp. *nilotica* methanol extracts on the clinical isolates of *Candida albicans* was 24.10± 2.86 mm for the leaves and 24.55±1.73 mm for the barks (Table 6).

3.4 Susceptibility of Clinical Isolates towards Methanol Extracts of *A. n.* ssp. *Nilotica*

Table 7 showed that all isolates of *Escherichia coli* (29) and *Candida albicans* (20) were inhibited by the two extracts and they have been inhibited by 100%. 12 isolates of *Neisseria gonorrhoeae* (92.3%) were sensitive to methanol leaves extract, whereas only 7.7% of them (one isolate) were found to be resistant. 84.6% (11 isolates) of them were sensitive to methanol barks extract, while 15.4% of them (two isolates) were found to be resistant to this extract. 13 isolates of *Staphylococcus saprophyticus* and 11 isolates of *Klebsiella penumoniae* (92.9%) for each were susceptible to methanol extracts of leaves and barks, while 7.7% (one isolate) from each bacteria was found to be resistant to the two extracts. All strains of *Enterococcus* spp. (11 isolates) were affected by the methanol barks extract been inhibited by 100%. 9 isolate of them (85%7) were affected by methanol leaves extracts, while two isolates (14.3%) were found

intermediately sensitive. All isolates of *Pseudomonas aeruginosa* (18 isolates) were affected by the methanol leaves extract and they have been inhibited by 100%.15 isolates out of them (78.6%) were sensitive to barks methanol extract, while two isolates (14.3%) were intermediately sensitive and one isolate (7.1%) was resistant to this extract. 15 isolates of *Proteus mirabilis* (93.3%) were susceptible to leaves and barks methanol extracts, while 6.7% (one isolate) was found resistant to these extracts (Table 7).

3.5 Effects of Standard Antibiotics Compared to the Tested Extracts on the Clinical Bacterial and Fungal Isolates

The outcomes of statistical analysis demonstrated that the effect of (100 mg/ml)

Table 5. Antibacterial activity of *A. n. ssp. nilotica* extracts and standard antibiotics towards clinical isolates

No. of clinical isolates	MDIZ/ mm ± SD of tested extracts and standard antimicrobial agents				
	LME 100 mg/ml	BME 100 mg/ml	Gent. 40 µg/ml	Gent. 20 µg/ml	Amp. 40 µg/ml
E. c. (29)	25.03 ±3.59	24.86±2.60	28.0	25.0	14.0
Ps. a. (18)	24.11±3.01	25.0±2.66	21.0	19.0	15.0
P. m. (16)	24.13±3.12	25.69±2.75	24.0	22.0	16.0
S. s. (14)	24.21±3.26	25.71±1.98	28.0	24.0	18.0
N. g. 13	24.62±2.99	25.0±2.92	21.85	18.5	14.0
K. p. 12	22.67±7.83	23.67±7.62	23.0	21.0	15.0
E. spp. 11	23.55±8.26	22.82±7.85	21.36	19.5	17.0
Total: 133					

Key: E.c. = Escherichia coli, Ps.a. = Pseudomonas aeruginosa. P.m = Proteus mirabilis; S.s. = Staphylococcus saprophyticus; N.g.= Neisseria gonorrhoeae.; K.p.= Klebsiella pneumoniae, E.spp.= Enterococcus sp.; C. a. = Candida albicans MDIZ/ mm: Mean Diameter of Inhibition Zone SD: Standard Deviation; LME: Leaves Methanol extract; BME: Barks Methanol extract; Gent.: Gentamicin; Amp.: Ampicillin.

Table 6. Antifungal activity of *A.n.ssp. nilotica* extracts and standard antifungal drug towards clinical isolates

No. of clinical isolates	MDIZ/ mm ± SD of tested extracts and standard antimicrobial agents				
	LME 100 mg/ml	BME 100 mg/ml	Cand. 40 mg/ml	Cand. 20 mg/ml	Cand. 10 mg/ml
Candida albicans (20)	24.10± 2.86	24.55±1.73	26	25	23

Key: LME: Leaves Methanol extract; BME: Barks Methanol extract; Cand. : Candizole.

Table 7. Susceptibility of clinical isolates to *Acacia nilotica* ssp. *nilotica* methanol extracts

Type and No. of clinical isolates	Methanol leaves extract 100 mg/ml			Methanol barkss extract 100 mg/ml		
	AC	MA	IA	AC	MA	IA
Escherichia coli (29)	100%	-	-	100	-	-
Neisseria gonorrhoeae (13)	92.3%	-	7.7%	84.6%	-	15.4%
Staphylococcus saprophyticus (14)	92.9%	3.6%	7.1%	92.9%	-	7.1%
Klebsiella penumoniae (12)	92.9%	-	7.7%	92.9%	-	7.1%
Enterococcus spp. (11)	85.7%	14.3%	-	100 %	-	-
Pseudomonas aeruginosa(18)	100%	-	-	78.6%	14.3%	7.1%
Proteus mirabilis (16)	93.3%	-	6.7%	93.3%	-	6.7%
Candida albicans (20)	100%	-	-	100%	-	-

MDIZ: > 18mm =active; MDIZ: 14-18mm= moderately active; MDIZ: < 14 mm=inactive; (-) = not determined; AC: Active; MA: moderate active; IA: Inactive.

Table 8. MIC, MBC and MFC reading of methanol extracts of *A. n.* ssp. *nilotica* on the standard microorganisms

Standard organisms	MICs, MBCs and MFC values per mg /ml											
	E.c.		Ps.a.		P.v.		S.au.		K.p.		C.a.	
Antimicrobial assay	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
Leaves extract	6.25	12.50	6.25	12.50	12.50	50	12.50	50	3.125	6.25	6.25	12.50
Barks extract	6.25	12.50	3.125	6.25	6.25	12.50	6.25	12.50	6.25	12.50	6.25	12.50

Key: MIC: Minimum inhibitory concentration; MBC: Minimum Bactericidal concentration; MFC: Minimum Fungicidal concentrations. E.c. =Escherichia coli (ATCC 25922), S. a. =Staphylococcus aureus (ATCC 25923), Ps. a. = Pseudomonas aeruginosa (ATCC 27853), K. p. = Klebsiella pneumoniae (ATCC 53657), P. v. = Proteus vulgaris (ATCC 6380) and C.a. =Candida albicans (ATCC 7596).

Table 9. MIC and MBC readings of methanol extracts of *A. n.* ssp. *nilotica* against clinical bacterial isolates

Plant part	MICs and MBCs values of bacterial clinical isolates per mg /ml													
	E.c.		Ps.a.		P.m.		S.sa.		N.g.		K.p.		Ent. spp.	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Leaves extract	3.125	6.25	6.25	12.50	12.50	25	12.50	25	3.125	6.25	12.50	25	3.125	6.25
Barks extract	6.25	12.50	12.50	12.50	6.25	12.50	6.25	12.50	6.25	12.50	12.50	25	6.25	12.50

Key: E.c. = Escherichia coli, Ps.a. = Pseudomonas aeruginosa. P.m. = Proteus mirabilis; S.sap. = Staphylococcus saprophyticus; N.g.= Neisseria gonorrhoeae.; K.p.= Klebsiella pneumoniae; E.spp.= Enterococcus spp.

leaves and barks methanol extracts on the clinical isolates was stronger compared to effect of Ampicillin 40 µg/ml and there is highly statistical significant differences between their effects (P = 0.000). There was also statistical significant difference between the effect of barks methanol extract and Gentamicin 20 µg/ml on the clinical isolates (P = 0.023) (Table 5). These findings coincide with Saba et al. [18]. The high antimicrobial activity of both leaves and barks methanol extracts of A. n. ssp. *nilotica* could be due to its abundant constituents of tannins, alkaloids cyanogenic glycosides, saponins and phenolic compounds [36].

3.6 Results of MIC, MBC and MFC of Extracts of *A. n.* ssp. *nilotica* on Standard Microorganisms

The MIC showed by the methanol leaves extract toward standard bacterial strains was ranged from 3.125 mg/ml to 12.50 mg/ml, while the MIC of methanol barks extract on standard bacterial strains was ranged from 3.125 mg/ml to 6.25 mg/ml. On the other hand, the MIC of methanol leaves and barks extracts toward standard fungi was 6.25 mg/ml. The MBC of methanol leaves extract on standard bacterial strains was ranged from 6.25 mg/ml to 50 mg/ml, whereas MBC of methanol barks extract on standard bacterial strains was ranged from 6.25 mg/ml to 12.50 mg/ml. The MFC of methanol leaves and barks extracts toward standard fungi was 12.50 mg/ml (Table 8).These results in agreement with the findings of Mashram et al. [34].

3.7 Results of MIC, MBC and MFC of Extracts of *A. n.* ssp. *nilotica* on Clinical Isolates

The MIC exhibited by methanol leaves extract on the bacterial isolates was ranged from 3.125 mg/ml to 12.50 mg/ml, while the MIC of methanol barks extract on bacterial isolates was ranged from 6.25 mg/ml to 12.50 mg/ml. The MBC of methanol leaves extract on bacterial isolates was ranged from 6.25 mg/ml to 25 mg/ml, whereas MBC of methanol barks extract on standard bacterial strains was ranged from 12.50 mg/ml to 25 mg/ml (Table 9). The MIC of methanol leaves and barks extracts toward *Candida albicans* was 6.25 mg/ml. The MFC of methanol leaves and barks extracts toward standard fungi was 12.50 mg/ml (Table 10).

Table 10. MIC and MFC of methanol extracts of *A. n.* ssp. *nilotica* on the clinical isolates of *Candida albicans*

Plant part	MIC and MFC values of clinical isolates of *Candida albicans* per mg /ml	
	MIC	**MFC**
Leaves extract	6.25	12.50
Barks extract	6.25	12.50

MFC: Minimum Fungicidal Concentration

4. CONCLUSION

This study elucidated the efficacy of *A. n.* ssp. *nilotica* as a source of potent antimicrobial substances. The medicinal value of this plant may relies in the presence of certain chemical substances such as alkaloids, tannin, flavonoids and phenolic compounds that are responsible for production of definite physiological changes on the human body. Most of standard strains and clinical isolates been inhibited by the two methanol extracts of *A. n.* ssp. *nilotica*. Depending on the results of this study, there is an urgent need to perform phytochemical studies and clinical trials to explain how the *A. n.* ssp. *nilotica* extracts kill the microorganisms.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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