

Antimicrobial Potential of *Acacia nilotica*, Subspecies, *tomentosa* against some Microorganisms that cause Urogenital Infections.

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ABSTRACT

Background: For centuries, aromatic plants with therapeutic properties were used to treat human illnesses

Objective: The aim of this study is to investigate and compare the antimicrobial susceptibility of microorganisms that cause urogenital infections to leaves and stem bark extracts of *Acacia nilotica* spp. *Tomentosa*, using standard strains and clinical isolates.

Result: The minimum inhibitory concentration (MIC) showed by leaves and bark methanol extracts on the standard bacteria ranged from 3.125 mg/ml to 12.50 mg/ml and the minimum bactericidal concentration (MBC) ranged from 6.25 mg/ml to 50 mg/ml. where the Mean Diameter of Inhibition Zone (MDIZ) ranged from 22.367.40 mm to 25.542.47 mm. On the other hand, isolates of *Candida albicans* showed MDIZ which was 23.203.44 mm for leaves methanol extract and 25.251.92 mm for bark methanol extract. The MIC exhibited by leaves and bark methanol extracts on bacterial isolates ranged from 3.125 mg/ml to 12.50 mg/ml and the MBC was ranged from 6.25 mg/ml to 25 mg/ml.

Conclusion: The study revealed that the extracts of *A. n.* ssp. *tomentosa* possess significant antimicrobial compounds, which showed formidable effectiveness against both standard strains and clinical isolates.

Keywords: Antimicrobial potential; *Acacia nilotica* subsp. *tomentosa*; leaves extracts; Bark extract; Antifungal activity.

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INTRODUCTION:

In developing and under-developed countries low-income people, such as farmers, workers, people of isolated villages and native communities use folkloric medicine for treatment of their common infections ^[1]. As well as, in the poor communities, synthetic drugs are expensive and sometimes inadequate for the treatment of some infectious diseases. On the other hand, indiscriminate use of broad-spectrum antimicrobial agents used in the treatment of urogenital infections represents one of those mechanisms that increase the development of multi-drug resistant microbial strains ^[2,3]. 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 ^[4,5,6]. Plants that used for traditional medicine contains a wide range of substances that can be used to treat chronic infections as well as acute infectious diseases. A perfect knowledge of how to use a plant against different illnesses may be expected to have been accumulated in areas where the use of the plant remains to be of great importance. [7] A wide range of phytochemical compounds contained in plants are known to inhibit microbial pathogens [8,9]. Successful determination of such biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Organic solvents such as ethanol, acetone, and methanol are often used to extract bioactive compounds [10]. *Acacia nilotica*

(L.) Willd. Ex. Del. is a genus of shrubs and trees [11]. It is a moderate to large size tree that reaches a height of 10 to 20 meters. The barks are blackish grey [12]. The leaves are twice compound, [13]. This genus is spread around the tropical to warm-temperate regions including Europe, Africa, Southern Asia, and the Americas [14]. In Sudan, it grows in drained locations along water courses such as on the river banks of the White and Blue Nile [15]. Regarding traditional medicine uses, *Acacia nilotica* differs from ethnic group to another; the Zulu use barks for cough; the Chipi use roots for tuberculosis and root decoction, and is believed to impart courage [16]. The barks are used for diarrhea, dysentery, and leprosy. Decoction of pods is used as gargle in urogenital diseases [17].

The barks, gum, leaves and pods are strongly astringent due to tannin and are used medicinally in the East and West Africa. Some of rural communities in Sudan use different parts of *Acacia nilotica* spp. *tomentosa* to cure dysentery, leprosy, impotence, colds, congestion, coughs, diarrhea, dysentery, fever, gallbladder disease, hemorrhage, hemorrhoids, ophthalmic, diabetes and tuberculosis [16,18]. This study was conducted to investigate and compare the antimicrobial activity of methanolic and aqueous extracts of *A. n.* subsp. *tomentosa* on some pathogens recovered from urogenital infections.

MATERIALS AND METHODS

Sample Collection:

Leaves and fruits of *A. n.* spp. *tomentosa* were collected in

September 2012 from SHAMBAT Area. It is located 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 383) 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.

Preparation of Plant Extract and Standard Antimicrobial Agents;

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 Whatman No.2 filter paper (Sigma-Aldrich, 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 the 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.

Preparation of Methanol

Extract: The plant parts were washed with distilled water, then dried at 60°C 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].

Preparation of Standard Antibiotics and Antifungal

Drugs: entamicin (Lunik Pharma Pvt. Ltd., India), ampicillin (Therapeutic Pharmaceutical, India) and candizole (The United Pharmaceutical. Ltd. Jordan) were prepared immediately before use by diluting them in sterile de-ionized water. A series of concentrations were prepared 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.

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 is used for screening of antifungal activity [20].

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 used for comparison of bacterial suspension with the

standard inoculum whenever required.

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 37°C 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 Colony forming Units, (CFU), per ml. The suspension was stored in the refrigerator at 4°C 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°C 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].

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°C until used [25].

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 used 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°C 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).

Sources and Maintenance of tested Microorganisms Sources of Standard Microorganisms:

Five standard strains of bacteria and one fungus 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°C.

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, an ethical verbal approval from the directors of these Hospitals has been taken. Clinical isolates were maintained frozen in Trypton Soya Agar TSA, (Oxoid Ltd. England).

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.

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 37°C for 18 hours. After incubation the diameters of the resultant growth inhibition zones were measured [20,26].

Antibacterial Activity of

Extracts' Determination:

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 45°C. 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 37°C 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.

Antifungal Activity of Extracts' Determination:

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 45°C. 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 37°C 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].

Determination (MIC) for standard strains and clinical isolates:

The plant extract that was found active, as antimicrobial agent, and 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 Minimum Fungicidal Concentrations, (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 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°C 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 that were considered as the initial MIC.

Determination (MIC&MFC) for standard strains 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°C. 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°C 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].

Statistical analysis: The data analysis was carried out using SPSS version 20 (IBM, Corporation, New York, USA). The mean diameter of inhibition zone between different categories was compared using one-way analysis of variance and Tukey's *post hoc* test. The mean value between extract and antibiotics was compared using independent sample t-test. The statistical significance was fixed at 0.05.

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.

Results and Discussion:

In this study the antimicrobial potential of methanol and aqueous extracts of the leaves and stem barks of *A. n. spp. tomentosa* at concentration of 100 mg/ml were assessed against standard strains and 133 clinical isolates obtained from urogenital infections. The results of identification tests, showed 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).

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.

Activity of *A. n. spp. tomentosa* extracts on the standard strains

The yield percentages of leaves and barks methanol extracts of *A. n. spp. tomentosa* was 9.8% and 8.4% for each which was higher compared to the yield percentages of aqueous extracts of the same parts which produced 4% and 3.6%. The results of susceptibility were interpreted as active (> 18mm), moderate active (14-18 mm), and inactive (< 14 mm) [21, 30]. The leaves and barks methanol extracts showed high activity against standard strains where the diameters of inhibition zones ranged from 20 mm to 39 mm, while the leaves and barks aqueous extract

showed lower varied activity which was ranged from 20 mm to 23 mm (Table 2). The antimicrobial activity of *A. n. spp. tomentosa* extracts could be due to presence of bioactive components such as alkaloids, flavonoids, terpenoids, glycosides tannis, and phenolic compounds [31]. It is obvious that leaves and barks extracts of *A. n. spp. tomentosa* inhibited all the standard strains examined in this study. These results are pararell to those findings of El-Kamali and Awad El-Karim, [19], Saba *et al.* [18].

Table (2): Antimicrobial activity of *A. n. spp. tomentosa* against standard strains

Part Used	Solvent system	Conc./ mg/ml	Yield %	*Test standard strains MDIZ/mm** of 100 mg /ml extract.					
				E.c	S.a	P.a	K.P	P.v	C.a
Leaves	Methanol	100	9.8	27	33	39	36	30	21

	Aqueous	100	4	20	23	22	23	20	22
Stem Barks	Methanol	100	8.4	28	30	38	30	28	20
	Aqueous	100	3.6	22	21	23	22	21	21

Key: MDIZ: Mean Diameter of Inhibition Zone. *E.c. = *Escherichia coli* (ATCC 25922), Ps.a. = *Pseudomonas aeruginosa* (ATCC 27853), K.p.=*Klebsiella pneumoniae* (ATTCC 53657), P.v. = *Proteus vulgaris* (ATCC 6380), S.a. = *Staphylococcus aureus* (ATCC 25923) and C. a. = *Candida albicans* (ATCC 7596).

Standard antibiotics vs tested extracts on the standard strains:

Statistical analysis revealed that there were highly statistical significant differences between activity of 100 mg/ml from *A. n. spp. tomentosa* extracts compared to the effect of gentamicin 40 µg/ml on the standard bacteria ($P = 0.000$). There was also statistical significant difference between effects of gentamicin 20 µg/ml and effect of *A. n. spp. tomentosa* extracts on the standard bacteria ($P = 0.000$). Extracts *A. n. spp. tomentosa* exhibited antibacterial activity which was higher than activity of

ampicillin 40 µg/ml on the standard bacteria, this difference in the activity was highly statistically significant ($P = 0.000$). The activity of leaves and barks methanol extracts were found to be higher compared to the activity of leaves and barks aqueous extracts, where these differences were statistically significant ($P = 0.000$). There was no statistical significant difference between effects of leaves aqueous extract and effect of barks aqueous extract on the standard bacteria ($P = 1.000$) (Table 3, fig 1). Regarding antifungal activity, *A. n. spp. tomentosa* leaves and barks

extracts showed activity towards mg/ml and 10 mg /ml of standard strain of *Candida albicans* which falls between 5 Candizole (Table 4, fig 2).

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	<i>Candida albicans</i> DIZ (mm)
Candizole	40	26
	20	25
	10	23
	5	20

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

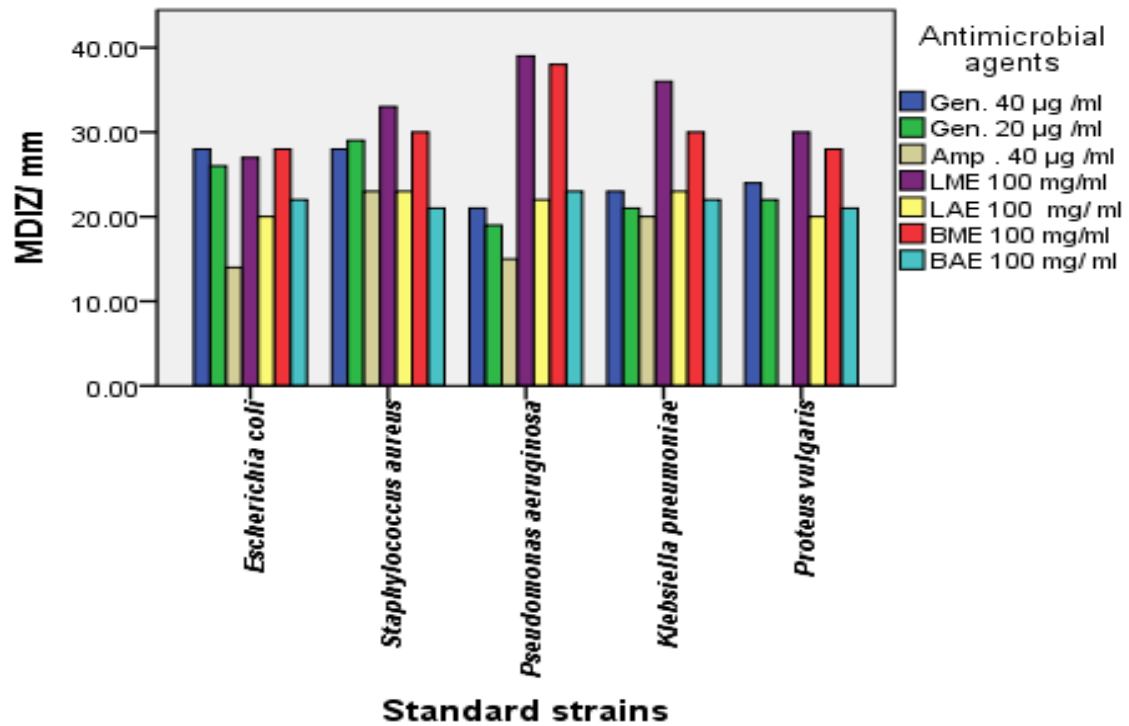


Fig. 1: Activity of standard antibiotics and tested extracts versus standard strains

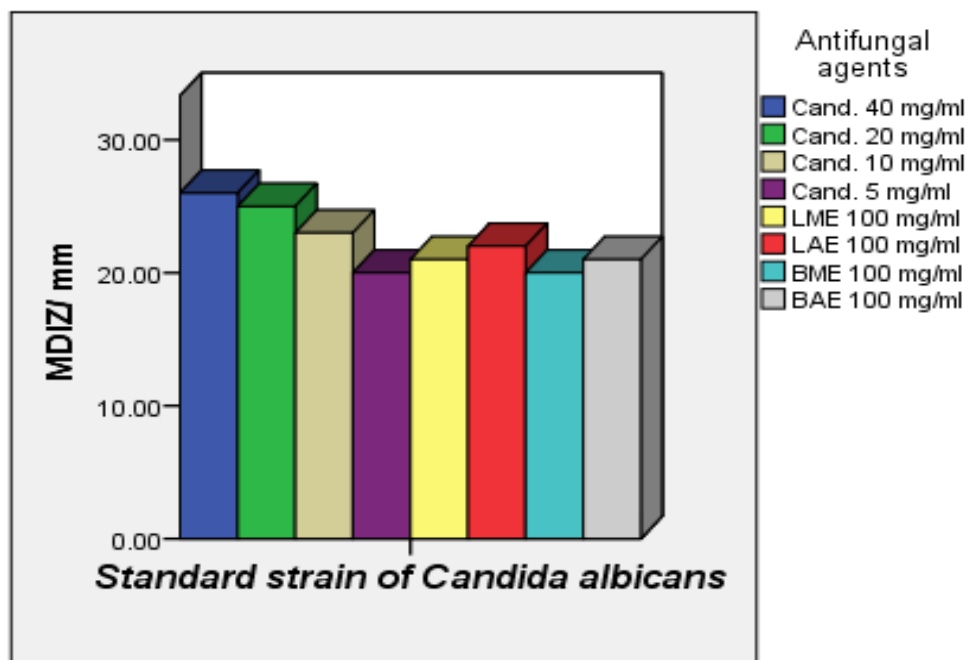


Fig. 2: Activity of standard antifungal agents and tested extracts versus standard fungus

Activity of *A. n. spp. tomentosa* extracts on the clinical isolates

The methanol extracts of leaves and barks of *A. n. spp. tomentosa* were only assessed against clinical isolates. The antimicrobial activity of extracts on the clinical isolates was expressed as MDIZ/ mm \pm SD. The highest mean diameters of inhibition zones obtained by leaves methanol extract on the clinical isolates was 24.10 \pm 2.94 mm for *Escherichia coli*, 23.92 \pm 3.95 mm for *Neisseria gonorrhoeae*, 23.45 \pm 8.31 mm for *Enterococcus* spp. and 23.00 \pm 8.15 mm for *Klebsiella pneumoniae*, while the rest isolates showed semi-equal activity which was 22.94 \pm 6.84 mm, 22.72 \pm 4.97 mm and 22.36 \pm 7.40 mm for *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus saprophyticus*, respectively. Clinical isolates of *Neisseria*

gonorrhoeae revealed higher susceptibility toward barks methanol extract which was 25.54 \pm 2.47 mm, whereas the lower susceptibility was shown by *Enterococcus* spp. which was 23.36 \pm 8.18 mm. Barks methanol extract showed equivalent MDIZs on the rest isolates which was 24.24 \pm 3.27 mm, 24.17 \pm 4.13 mm, 24.06 \pm 6.73 mm and 24.00 \pm 7.83 mm for *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus saprophyticus* and *Klebsiella pneumoniae* (Table 5). Antifungal activity of *A. n. spp. tomentosa* methanol extracts on the clinical isolates of *Candida albicans* was 23.20 \pm 3.44 mm for the leaves and 25.25 \pm 1.92 mm for the barks, where this activity was higher than 10 mg/ml of candizole (Table 6).

Table (5): Antibacterial activity of *A. n. spp. tomentosa* extracts and standard antibiotics on clinical isolates

No. of Clinical isolates	MDIZ/ mm \pm SD of tested extracts and standard antimicrobial agents				
	LME 100 mg/ml	BME 100 mg/ml	Gent. 40 μ g/ml	Gent. 20 μ g/ml	Amp. 40 μ g/ml
<i>E. c.</i> (29)	24.10 \pm 2.94	24.24 \pm 3.27	28.0	25.0	14.0
<i>Ps. a.</i> (18)	22.72 \pm 4.97	24.17 \pm 4.13	21.0	19.0	15.0
<i>P. m.</i> (16)	22.94 \pm 6.84	24.06 \pm 6.73	24.0	22.0	16.0
<i>S. s.</i> (14)	22.36 \pm 7.40	24.00 \pm 7.31	28.0	24.0	18.0
<i>N. g.</i> 13	23.92 \pm 3.95	25.54 \pm 2.47	21.85	18.5	14.0
<i>K. p.</i> 12	23.00 \pm 8.15	24.00 \pm 7.83	23.0	21.0	15.0
<i>E. spp.</i> 11	23.45 \pm 8.31	23.36 \pm 8.18	21.36	19.5	17.0
Total: 113					

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. spp. tomentosa* extracts and standard antifungal drug on clinical isolates

No. of Clinical isolates	MDIZ/ mm \pm 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
<i>Candida albicans</i> (20)	23.20 \pm 3.44	25.25 \pm 1.92	26	25	23

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

Effects of standard antibiotics compared to the tested extracts on the clinical isolates

The outcomes of statistical analysis illustrated that there are highly significant differences between the effects of (100 mg/ml) methanol extracts of *A. n. spp. tomentosa* and the effect of standard antibiotics on the clinical isolates ($P= 0.000$). The effect of gentamicin 40 $\mu\text{g/ml}$ was stronger compared to the effect of leaves and barks methanol extracts on the clinical isolates where the differences were statistically significant ($P = 0.964$ for leaves and $P = 0.998$ for barks). The effect of leaves and barks methanol extracts on the clinical isolates was stronger compared to effect of ampicillin 40 $\mu\text{g/ml}$ and the difference was statistically

significant ($P = 0.000$). The effect of barks methanol extract on the clinical isolates was also stronger compared to the effect of leaves methanol extract where the difference was statistically significant ($P = 0.871$) (Fig. 3).

Results of MIC, MBC and MFC of *A. n. spp. tomentosa* extracts on standard strains

The MIC showed by the methanol leaves extract toward standard bacteria was ranged from 6.25 mg/ml to 12.50 mg/ml, while the MIC of barks methanol was ranged from 3.125 mg/ml to 6.25 mg/ml. On the other hand, the MBC of leaves methanol extract on the standard bacteria was ranged from 12.50 mg/ml to 25 mg/ml, whereas MBC of methanol barks extract was 6.25 mg/ml to 12.50 mg/ml. The MIC

showed by leaves and barks extracts on standard fungi was 6.25 mg/ml for while the MFC of the same extracts 12.50 mg/ml for leaves and barks (Table 7).

Results of MIC, MBC and MFC of *A. n. spp. tomentosa* extracts on the clinical isolates

The MIC exhibited by leaves methanol extract on the bacterial isolates was ranged from 3.125 mg/ml to 12.50 mg/ml, while the MIC of the barks extract was

ranged from 3.125 mg/ml to 6.25 mg/ml. On the other hand, the MBC of the leaves was ranged from 6.25 mg/ml to 25 mg/ml, whereas the MBC of the bark was ranged from 6.25 mg/ml to 12.50 mg/ml (Table 8). The MIC of methanol extracts toward *Candida albicans* was 6.25 mg/ml for leaves and bark, while the MFC of methanol extracts on the standard fungi was 12.25 mg/ml (Table 9).

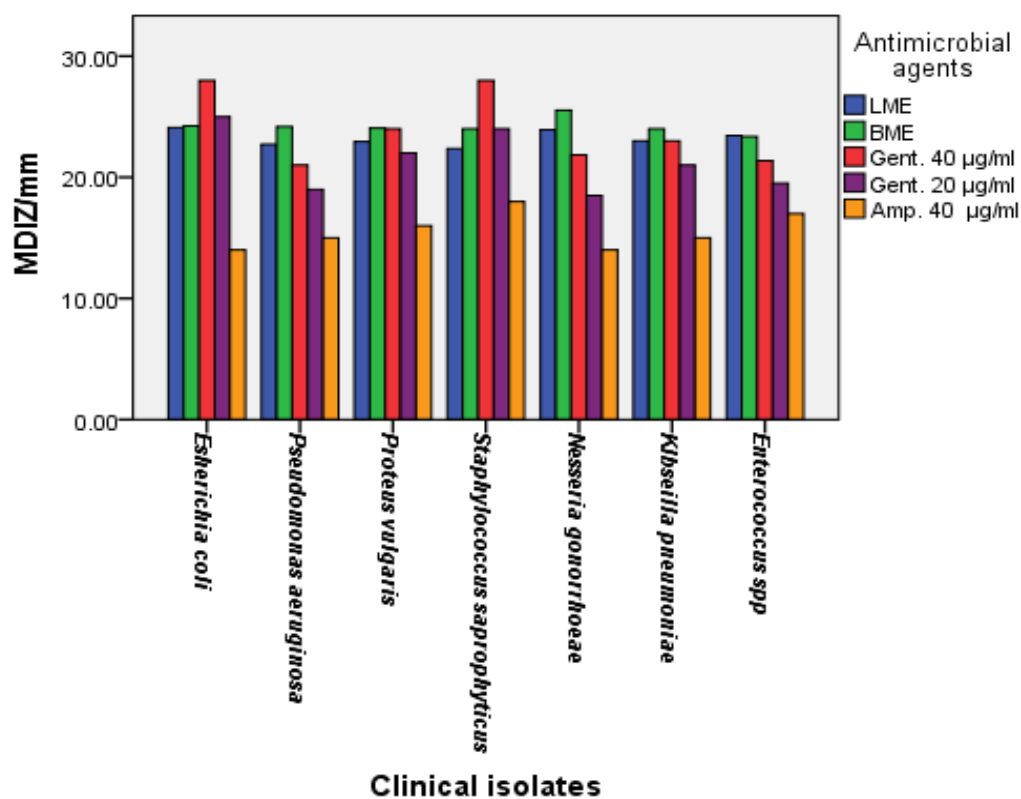


Fig. 3: Activity of tested extracts and standard antibiotics on the clinical isolates

Table (7): MIC, MBC and MFC readings of methanol extracts of *A. n. spp. tomentosa* on the standard strains.

Standard organisms	MICs, MBCs and MFC values per mg /ml											
	E.c.		Ps.a.		P.v.		S.au.		K.p.		C.a.	
Antimicrobial assay	MI	M	MI	M	MI	M	MI	M	MI	M	MI	M
Leaves extract	6.2	12.	12.	25	12.	25	12.	25	12.	25	6.2	12.
	5	50	50		50		50		50		5	50

Barks	6.2	12.	3.1	6.2	6.2	12.	6.2	12.	6.2	12.	6.2	12.
extract	5	50	25	5	5	50	5	50	5	50	5	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 (8): MIC and MBC readings of methanol extracts of *A. n. spp. tomentosa* 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	MB	MIC	MB	MIC	MB	MIC	MB	MIC	MB	MIC	MBC	MIC	MB
Leaves extract	3.1	6.25	3.12	6.25	12.5	25	3.12	6.25	3.125	6.25	12.5	25	3.12	6.25
Barks extract	6.25	12.5	3.12	6.25	3.12	6.25	3.12	6.25	3.125	6.25	3.12	6.25	6.25	12.5
		0	5		5		5				5			0

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.*

Table (9): MIC and MFC of methanol extracts of *A. n. spp. tomentosa* on the clinical isolates of *Candida albicans*.

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

MFC: Minimum Fungicidal Concentration

Conclusion

Leaves and barks methanol extracts of *A. n. spp. tomentosa*

exhibited remarkable activity on the standard strains and clinical isolates, however, bark methanol extracts of was found to be more effective than leaves extract. This may elucidate the effectiveness of the plant part as a source of natural remedy. The antimicrobial efficacy of this plant may relies on 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 microorganisms. Further phytochemical studies and clinical trials are needed to explain how the plant extracts kill the microorganisms.

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