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This study focused on *Acacia nilotica* because of the plant's long history of ecological and folk medical applications. The Phytochemical compounds of *Acacia nilotica* were examined with the purpose of finding the antibacterial actions of this plant. Methanol was used to extract dried *Acacia nilotica* fruit. The phytochemical screening conducted on the *Acacia nilotica* fruit extract indicated high quantity of phenolic, Flavonoids and Tannin in methanol extract. Pods was analyzed by HPLC, there were 16 phenolic compounds rich in gallic acid as a major phenolic compound (24048.50 µg/g) and catechin as a major flavonoid (66162.88 µg/g). Seven commonly studied bacteria strains (*Escherichia coli, Bacillus subtilis, Bacillus cereus, Salmonella typhimurium, Staphylococcus aureus, Enterobacter cloacae, and Klebsiella pneumoniae*) were used to test the extract's antimicrobial properties. Antimicrobial testing revealed that the methanolic extract inhibited the development of every type of bacterium tested. The results may encourage baseline information for prospective use of these crude extracts in medication development programs in the pharmaceutical sectors as an example.

Keywords: *Acacia nilotica*; phytochemical composition; antimicrobial activity; flavonoids; tannin

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INTRODUCTION

Worldwide, there is still a trend towards the use of plants as phytoremediators —both traditional and modern—for both removal of bacterial contamination and illness prevention and treatment (Alam & Albalwai, 2020; Benzineb et al., 2019), these practices are popular and widespread (Alduhisa & Demayo, 2019; Morilla & Demayo, 2019). In the branch of medical treatment, antibiotic-resistant bacteria efficiency for novel diseases gives no effective treatment in contemporary medicine. So, the use of plants in the treatment of microbial diseases is growing (Aliero et al., 2023).

Natural medicinal plants can promote lifespan, perfect health, and self-healing. *Acacia nilotica*, is a multipurpose nitrogen-fixing tree legume that belongs to the Mimosaceae family. From Saudi Arabia, India, Sudan, Egypt and Sri Lanka to Mauritania and South Africa, it is widely distributed throughout subtropical and tropical Africa. In Asia, it is found eastward to Pakistan and India (Jame, 2018; Massey et al., 2017). Plant aerial parts were subjected to phytochemical examination, which revealed the presence of flavonoids and polyphenolic chemicals in the flowers. The fruits are said to include tannins, volatile oils, glycosides, coumarins, carbohydrates, and organic acids (Maldini et al., 2011). According to (El Toumy et al., 2011; Kamil, 2018; Omara et al., 2012), Acacia is rich in antioxidant phenolics, primarily condensed tannin and gallic acid, phlobatannins, protocatechuic acid, pyrocatechol (+) catechin (-) epigallocatechin-7-gallate, and (-) epigallocatechin-5, 7-digallate. Additionally, *Acacia nilotica* contains the following: l arabinose, catechol, galactan, galactoaraban, galactose, sulphoxides, pentosan, saponin and tannin (Bhushette & Annapure, 2017; Brahma N Singh et al., 2009). As a photocomposition panel of *Acacia nilotica*, it was found that presence of different types of secondary plant metabolites including polyphenols, mainly composed of condensed tannin and phlobatannin in addition to gallic acid, ellagic acid, catechin, epigallocatechin-7- gallate, flavonoids and gum. Aklso, different solvent extracts of the *Acacia nilotica* had been shown to have antimicrobial activities including anti-bacterial, anti-fungal, anti-viral and anti-amebic. By preventing the aggravation of oxidative stress states, feeding this antioxidant-rich medicinal herb helps prevent the development of numerous ailments (Rather & Mohammad, 2015).

The antimicrobial activity of *A. nilotica* has been assessed using extracts from its leaves and bark; however, little is known about how its individual microbiologically bioactive compounds affect drug-resistant bacteria, particularly bacterial pathogens that cause diarrhea. The global spread of resistant strains of *Shigella* and *Salmonella* as well as numerous

other ß-lactamase producers has emerged as a significant treatment issue (Teshome et al., 2019). There are more and more reports of multidrug resistant strains of bacteria, particularly in poor nations, in a comparative antibacterial investigation among acacia species, *Acacia nilotica* demonstrated strong antibiotic potential against *Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus and Escherichia coli* (Abdalla et al., 2020; Jabaka et al., 2019; Teshome et al., 2019). *Acacia nilotica* extracts in ethanol and petroleum ether demonstrated antibacterial activity against *Salmonella paratyphi*, *E. coli, Staphylococcus aureus, Proteus vulgaris, Proteus mirabilis*, and *Klebsiella pneumoniae* (Deshpande, 2013). Recently, several investigations on the discovery of new drugs from medicinal plants uses a multifaceted strategy combining molecular, botanical, and phytochemical methods. Thus, the current investigation focuses on the antibacterial ability of *Acacia nilotica* against seven bacterial strains as well as the phytochemical properties of *Acacia nilotica* utilizing various solvents.

MATERIALS & METHODS

Sample collection and preparation

The matured dried pods of *Acacia nilotica* fruits were purchased from the local markets for Medical Plants, Mansoura, Dakahlia Governorate. Specimen was identified by the Institute of Environmental Studies and Research, University of Sadat City, Egypt. Prior to examination, the dried pods were ground in an attrition mill, sieved through 200 µm wire mesh, placed in a plastic container, and stored at room temperature in a hermetically sealed with aluminum foil bag.

Chemical reagents for analysis

Folin-Ciocalteu reagent (Fluka, Biochemical Inc., Bucharest, Romania), Gallic acid (Biomedical Inc., Orange City, FL, USA), 1,1-Diphenyl-2-picrylhydrazyl (DPPH•), aluminum chloride, sodium hydroxide, sodium nitrite, catechin, vanillin, hydrochloric acid, ascorbic acid, and DMSO were purchased from Sigma Aldrich (St. Louis, USA). Sodium Carbonate, and tannic acid (El-Nasr Pharmaceutical Chemicals, Cairo, Egypt).

Preparation of plant Extract

Dried pods sample was extracted with methanol, depending on the method described by (Ogbadoyi et al., 2007), with some modifications. A blended 50 g sample of crushed pods was transferred into a beaker and 100 ml of 98% methanol was added at ambient temperature (28 ± 2°C). Then, agitation was done by rotary shaker. Extraction was allowed to proceed for 48h. The mixture was let to semi dry yelling thick crude. And plenty of concentration were prepared from this crude.

Determination of antioxidant activity using DPPH assay

Acacia samples were examined for their antioxidant capacities using the DPPH• assay with ascorbic acid as the reference, as described by (Kitts et al., 2000). One milliliter (mL) of each sample was combined with 1 mL of methanol to create a serial dilution. Each tube in the series was given a 1 mL dose of a 0.135 mM DPPH• solution. For 30 minutes, the tubes were stored at room temperature in the dark. The absorbance was measured at 517 nm and the % remaining DPPH• was calculated by the succeeding equation (Eq. (1):

$$\% \text{ DPPH}^{\bullet} \text{ remaining} = [\text{DPPH}^{\bullet}]_T / [\text{DPPH}^{\bullet}]_{T=0} \times 100 \quad \text{Eq. (1)}$$

To determine the effective concentration "IC50," the data of % DPPH remaining were plotted against mg extract/mL using an exponential curve. The number of antioxidants required to reduce the initial concentration of DPPH• solution by 50% was indicated by the IC_{50}. The IC_{50} results demonstrate a negative correlation with the sample's antioxidant apability (Parejo et al., 2000).

Phytochemical analysis
Total Tannin Contents

To analyses the tannin contents of the samples, the vanillin-hydrochloride assay (Broadhurst & Jones, 1978), which measures the samples' absorbance after they are treated with newly made vanillin-hydrochloride. The tested sample's achieved tannin content values were expressed as g of tannic acid/g dry plant. The tannic acid standard curve (y = 0.0009x; r2 = 0.955) was used to determine the tannin capacity of the samples under investigation.

Total phenolic contents

To determine the amount of phenolic content in the tested sample, the Folin-Ciocalteu (F-C) assay was performed in accordance with the methods described by (Issa et al., 2016; Wolfe et al., 2003). The characteristic values were determined as mg Gallic acid /g of the dried plant. A Gallic acid standard curve (y = 0.0062x, r2 = 0.987) was used in the procedure.

Total flavonoid contents

The number of flavonoids is expressed as mg catechin /g of the dried plant. The test was conducted on the

tested sample using the aluminum chloride colorimetric assay, in accordance with the protocol described by (Zhishen et al., 1999). Based on the following catechin "secondary metabolite" standard curve (y = 0.0028 x, r2 = 0.988), the total flavonoids were estimated.

HPLC analysis for Phenolic Compounds

An Agilent 1260 series was used for the HPLC analysis. Zorbax Eclipse Plus C8 column (4.6 mm x 250 mm i.d., 5 µm) was used for the separation. Water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) were combined to form the mobile phase, which was flowing at a rate of 0.9 ml/min. The following was the sequential linear gradient programming for the mobile phase: 18–22 min (82% A); 22–24 min (82% A); 0–1 min (82% A); 1–11 min (75% A); 11–18 min (60% A); and 0 min (82% A). At 280 nm, the multi-wavelength detector was observed. For every sample solution, there was one injection volume of five microliters. At 40 °C, the column temperature was kept constant.

Antibacterial activity of *Acacia nilotica* extracts (Agar well diffusion method)

The Agar well diffusion method is commonly used to determine the efficacy, during the experiment of Acacia fruit extract's antibacterial activity in inhibiting the growth of microorganisms. A volume of the microbial inoculum is dispersed throughout the entire agar surface, much like how the disk-diffusion method is utilized. After that, a 9 mm diameter hole is aseptically punched with a sterile cork borer or tip, and 50 µL of the antimicrobial agent or extract solution is injected into the well to achieve the necessary concentration. Then, agar plates are incubated in an environment that is optimal for the microorganism being tested (*Escherichia* coli, *Bacillus subtilis, Bacillus cereus, Salmonella typhimurium, Staphylococcus aureus, Enterobacter cloacae, and Klebsiella pneumoniae*). The evaluated microbial strain's development is stunted by the antimicrobial agent's diffusion in the agar media according to (Magaldi et al., 2004)**.** The widths of the acquired inhibitory zone surrounding the wells (in mm) were measured.

RESULTS

Phytochemical compounds of *A. nilotica*

The results regarding the total concentration of important phytochemical compounds (phenolics, flavonoids and tannins) of *A. nilotica* sample, are shown in Table 1 and Figure 1. *A. nilotica* fruits contain high amounts of phytochemical compounds as phenolics (568.50 mg GAE g^{-1}), flavonoids (210.769 mg quercetin g^{-1}) and tannins (152.005g of tannic g^{-1}).

Antioxidant activity measured by (DPPH assay)

The DPPH radical was used to assess the antioxidant activity of the *Acacia nilotica* fruit in this investigation. Damage to proteins, cell membranes, and DNA can all be caused by free radicals, which are thought to play a role in a wide range of pathologies. Maximum absorption of the DPPH radical occurs at a wavelength of 517 nm, making that value the standard by which it is quantified. When antioxidants are present, the hue shifts to a light yellow. Studies were conducted on the effects of inhibiting the DPPH radical at doses ranging from 0.003 mg/mL to 0.022 mg/mL. The reduction of the DPPH radical that occurred by the *Acacia nilotica* extract at different concentrations is recorded in Table 2 and Figure2. The results showed the DPPH scavenging activity of the *Acacia nilotica* extract in addition to the reference of ascorbic acid. The scavenging activity of the extract and the reference increased by increasing the concentration. The extract concentrates of 0.011 mg/ml caused DPPH inhibition percentage with values of 88.394 % for *Acacia nilotica* and concentration of 0.031 recorded DPPH inhibition percentage with values of 62.07%. The higher concentration of 0.022 and 0.062 mg/ml for *Acacia nilotica* and ascorbic acid, respectively, raised the inhibition percentage but the increase was not too much. So, it is preferable to use the lowest concentration to save the row material. Regarding the IC_{50}, the results showed significant results where their concentration were 0.0058 and 0.0222 mg/ml. These results indicated that both the peel and seed extracts are good antioxidants.

HPLC analysis of *Acacia nilotica* pods

Many naturally occurring components that are beneficial to health have been found in a variety of plant species, including phenolic compounds. Because of their potent antioxidant properties, phenolic component concentrations in plants have been positively linked to a host of health advantages. It has been observed that specific phenolic chemicals, such as flavonoids and phenolic acids, inhibit a variety of harmful bacteria and parasites. Fruits of *Acacia nilotica* contain phenolic and antioxidant components that have strong biological effects and are now considered a significant source of medicinal and therapeutic benefits. Phenolic compounds can be classified into compounds are known for their ability to scavenge excess free radicals and maintain the

Table 1. Phytochemical compounds of *Acacia nilotica* fruits

Compounds Sample	Phenolics (mg GAE g^{-1})	Flavonoids (mg quercetin g^{-1})	Tannins (g of tannic g^{-1})
A. nilotica fruits	568.50	210.77	152.01

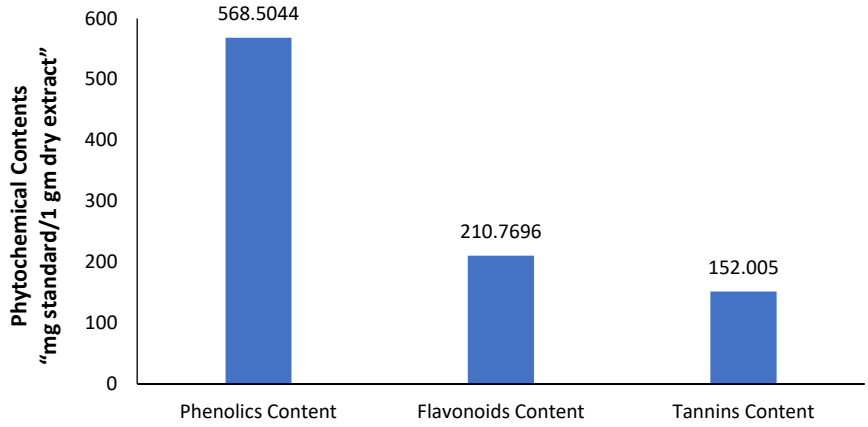

Fig 1. Phytochemical compounds of *Acacia nilotica* fruits

Table 2. The antioxidant results (% remaining DPPH, % scavenging activity and IC_{50} mg/mL) of the *Acacia nilotica* extract.

Sample	Concentrations (mg/mL)	% Remaining DPPH	% Scavenging activity	IC_{50} (mg/mL)
Sample	0.022	11.606	88.394	0.0058
	0.011	29.571	70.429	
	0.006	50.079	49.921	
	0.003	68.203	31.797	
Ascorbic acid	0.062	15.267	85.19	0.0222
	0.031	39.084	62.07	
	0.016	61.069	40.74	
	0.008	74.809	27.41	

balance of reactive oxygen species (ROS) in the human body. Table 3 and Figure 3 demonstrate the ret time and area of the multitude of guidelines. The bound phenolic compounds from *Acacia nilotica* pods, the predominant phenolic compounds are 16 different bound, were identified (Table 3 and Figure 4). Gallic acid represented about 24048.50 µg/g of total extract as a major phenolic compound but catechin 66162.88 µg/g is the major flavonoid as compared to standard compound.

Antibacterial activity of *Acacia nilotica* extract

The antimicrobial activity of different extracts of *Acacia nilotica* were tested against seven bacterial strains (*Escherichia* coli, *Bacillus subtilis, Bacillus cereus, Salmonella typhimurium, Staphylococcus aureus, Enterobacter cloacae, and Klebsiella pneumoniae*), the inhibition zone was measured by antibiotic zone reader (Table 4 and Fig 5). The methanolic crude extracts of fruits of *Acacia nilotica* showed the zone activities against tested bacterial pathogens ranged from 10.6 to 19.3 mm for *Enterobacter cloacae* and *Bacillus cereus,* respectively. The negative control (DMSO) had no activity against all tested bacteria as indicated in Table 3. As for the other bacterial strains were recorded a moderate zone between them (14.0, 15.0 and 17.0 mm) for *Salmonella typhimurium, Escherichia* coli and *Bacillus subtilis,* respectively. The zone was increased with *Staphylococcus aureus* and *Klebsiella pneumoniae* which recorded 19 mm for each one.

DISCUSSION

The greater antibacterial activity demonstrated in the *Acacia nilotica* extract has been attributed to the presence of significant phytochemical components. This result was similarly observed at the 10.6 mm lowest zone of inhibition. This is consistent with the findings of (Bauer et al., 1996), who reported that if the zone of inhibition in millimeters is less than 7, 7-9

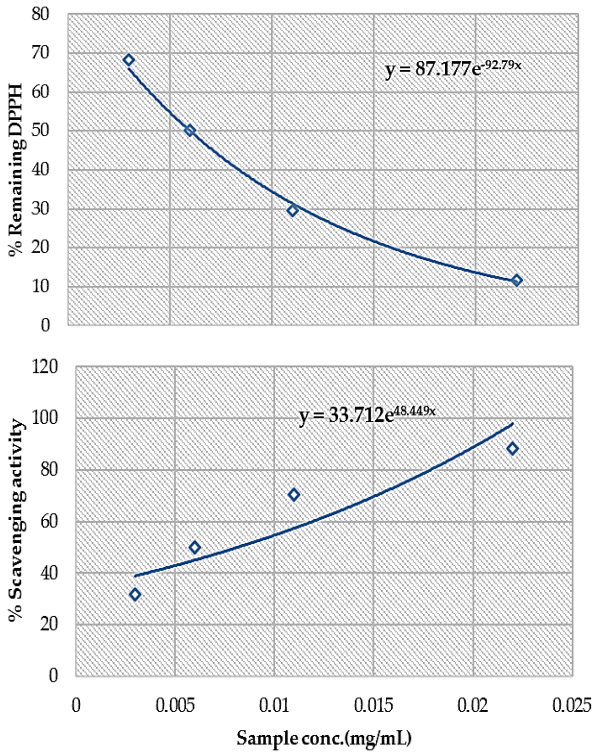

Figure 2. Exponential curves plotting the tested sample concentrations versus (a) % Remaining DPPH, and (b) % Scavenging activity.

mm intermediate, or greater than 10, mm, the microbicidal activity is classed as resistant or inactive, and if it is 10 mm and above, it is considered active or sensitive.

Saponins, glycosides, hydrolyzable tannins, triterpenoid, flavonoids, phenol, alkaloids, and volatile oils are among the significant phytochemicals found in extracts of *Acacia nilotica* that are widely used to treat a variety of medical conditions (Solomon-Wisdom & Shittu, 2010). Because of its capacity to scavenge free radicals, the high phenolic content found in the ethanolic extract of acacia leaves exhibits anti-oxidative and anti-cancerous characteristics (Kalaivani et al., 2011).

Given that phenol compounds have been shown to have antimicrobial action against pathogens, it is possible that the phenol compounds found in acacia extract are what give it its antibacterial properties (Hussein, 2012; Nyanyo & Akada, 2011). According to reports, tannins have also historically been used to treat dysentery and diarrhea (Adaramola et al., 2012). The outcome is consistent with the research of Deshpande (Deshpande, 2013), who studied the antibacterial activity of an ethanol and petroleum ether extract of *Acacia nilotica* stem bark *against Salmonella paratyphi, Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris*, and *Proteus mirabilis*. The outcomes demonstrated that both extracts had an inhibitory effect on the pathogens listed above. However, ethanol extract demonstrated increased activity as in contrast to the equivalent petroleum ether extract. Tannins, alkaloids, flavonoids, terpenoids, glycosides, steroids, and phenols were found to be positive in stem-bark crude extracts of *Acacia nilotica*, whereas saponins and anthraquinones were found to be negative. The plant's observed effects on *S. aureus* and *E. col*i may be due to the presence of these phytochemical substances in the ethanol and N Hexane extracts of *Acacia nilotica* (Jabaka et al., 2019). Consequently, the phytochemicals that make up this plant may have something to do with its therapeutic benefits. The medical benefit of medicinal plants is attributed to their chemical ingredients and secondary metabolites, often known as phytochemicals.

Naturally existing species with unpaired electrons, free radicals are created during regular metabolic activities. When their synthesis is excessive, it can lead to the oxidation of vital cell structure molecules, a condition known as oxidative stress that can cause several health problems. Employing the DPPH radical scavenging activity assay, our results corroborate the findings of (Singh & Arora, 2007; Sultana et al., 2007), who indicated that *Acacia nilotica* bark extracts showed a substantial antioxidant activity. It follows that the significant antioxidant activity of the studied extract, which is rich in polyphenolic chemicals, is not surprising. In addition, the antioxidant activity values of the DPPH assay for the samples of *Acacia nilotica* from Shahrak, acacia from Turbat, and acacia from Pedrak were 18.51851%, 4.47761%, and 8.25958%. According to the findings, Shahrak's acacia nilotica has the highest capacity to scavenge free radicals (Haroon et al., 2022).

When compared to standard, it indicates that certain plant species exhibit higher levels of antioxidant activity through the process of free radical scavenging. Thus, it may be concluded that *A. nilotica* possesses potential antioxidant action and may find application in medicine. The aforementioned results shown are in agreement with (Salem et al., 2011; Singh et al., 2009) that found phenolic acids (105 mg gallic acid/g), (1.3 mg caffeic acid/g), (18.2 mg ellagic acid /g), and (9.3 mg ferulic acid/g) as well as flavonoids (27.7 mg rutin /g).

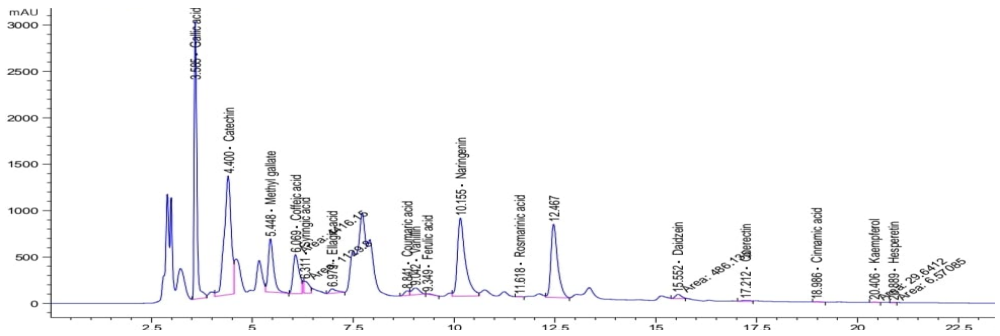

Figure 3. High-performance liquid chromatography chromatograms (bound phenolic compounds) of standards

Table 3. HPLC analysis of *Acacia nilotica* pods

Phenolic compounds	Standards		Phenolic compounds	*A. nilotica* pods (µg/g)
	Ret Time (min)	Area		
Gallic acid	3.60	226.11	Gallic acid	24048.50
Chlorogenic acid	4.25	385.33	Chlorogenic acid	ND
Catechin	4.49	347.64	Catechin	66162.88
Methyl gallate	5.50	297.70	Methyl gallate	5299.06
Coffeic acid	5.93	232.60	Coffeic acid	6834.83
Syringic acid	6.43	235.18	Syringic acid	1652.55
Pyro catechol	6.64	277.46	Pyro catechol	ND
Rutin	6.90	338.96	Rutin	ND
Ellagic acid	7.24	600.66	Ellagic acid	1103.56
Coumaric acid	8.68	561.98	Coumaric acid	298.67
Vanillin	9.10	347.12	Vanillin	736.82
Ferulic acid	9.74	344.29	Ferulic acid	76.72
Naringenin	10.40	328.22	Naringenin	21115.17
Rosmarinic acid	11.84	466.34	Rosmarinic acid	39.77
Daidzein	16.00	356.62	Daidzein	545.27
Querectin	17.32	296.35	Querectin	403.04
Cinnamic acid	19.26	558.41	Cinnamic acid	12.08
Kaempferol	20.62	317.06	Kaempferol	37.39
Hesperetin	21.21	406.79	Hesperetin	6.46
ND not detected				

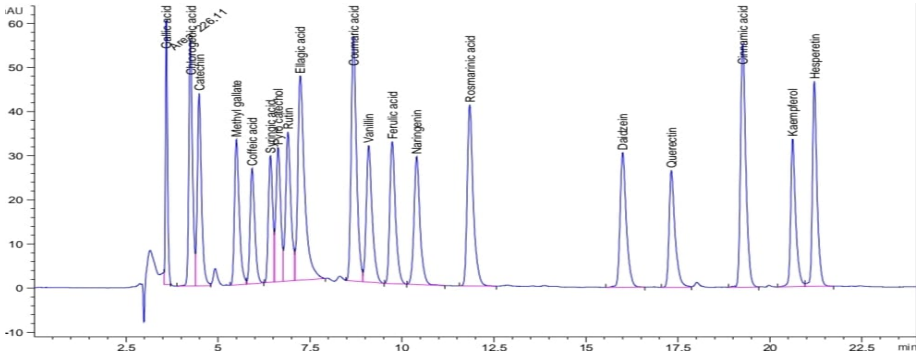

Figure 4. High-performance liquid chromatography chromatograms (bound phenolic compounds) of *Acacia nilotica* pods

Table 4. Antibacterial activity of *Acacia nilotica* methanol fruit extracts against the test bacteria

Test bacteria / Samples	Tested organisms						
	E. coli	*B. subtilis*	*B. cereus*	*Sal. typhi*	*S. aureus*	*Entero.*	*Klebsiella*
	Zone of inhibition in mm						
Acacia nilotica methanol	15 mm	17 mm	19.3 mm	14 mm	19 mm	10.6 mm	19 mm
Control (DMSO)	-ve	-ve	-ve	-ve	-ve	-ve	-ve

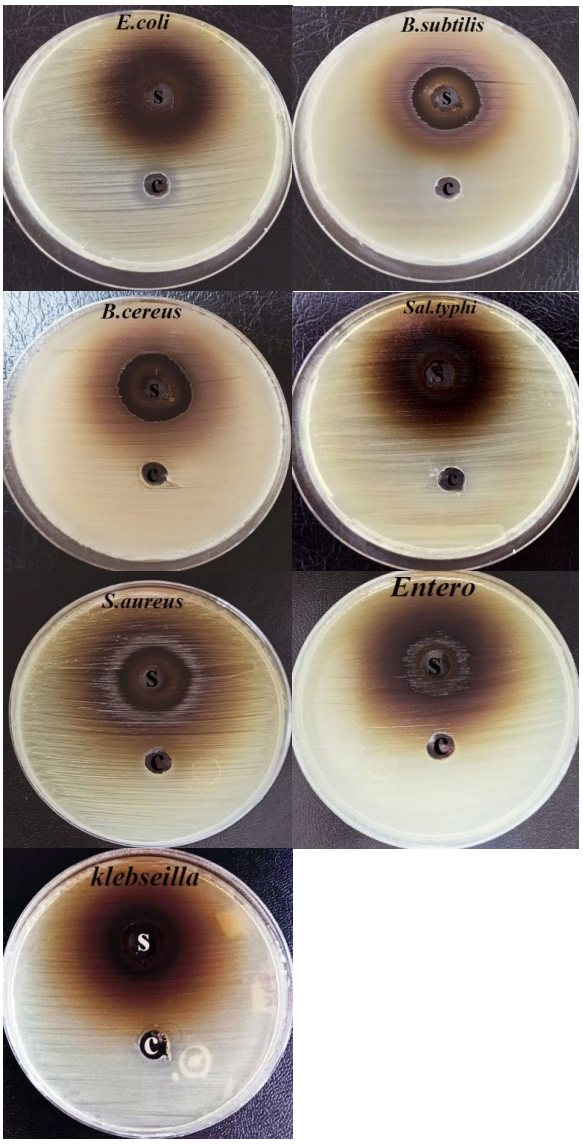

Figure 5. Antibacterial activity of *Acacia nilotica* methanol fruit extracts against the test bacteria

Also, (Salman et al., 2020) represented that about 227 mg Ethyl vanillin/g of total extract as a major phenolic compound and 48.70 mg catechin/g is the major flavonoid in Egyptian acacia brown pods. As bacterial pathogens become more resistant to modern medication, plants have been identified as one of the sources of natural compounds used to treat various diseases caused by these pathogens. The purpose of this study was to evaluate the antibacterial properties of *Acacia nilotica*'s active components against microorganisms that were resistant to multiple drugs. Various investigations have revealed the existence of various phytochemical elements from various extracts of *Acacia nilotica*, including Alkaloids, Saponins, Tannins, Phenols, Flavonoids, Terpenoids and Steroids (Abubakar et al., 2018; Jabaka et al., 2019; Manga et al., 2018). Data on the synergistic effects of various *Acacia nilotica* components against infections that cause multi-drug resistance are scarce.

The study's results were consistent with those of (Manga et al., 2018) found that the mean zone of inhibition of aqueous crude extracts of *Acacia nilotica* leaves against S. aureus and P. aeruginosa ranged from 6.7 ±1.15 to 13.7 ±1.15 mm. The mean antibacterial activity of *Acacia nilotica* crude extracts in methanol leaves was found to be higher than that of Gramme negative bacteria, measuring 25.67±2.08 and 33.00±0.45 mm, according to (Abubakar et al., 2018). This was less than that of (Sadiq et al., 2017), who found that the mean zone of inhibition of crude extracts of ethanolic leaves against a variety of dietary and clinical Salmonella species from Pakistan ranged from 11.3 ± 1.53 to 17.7 ± 0.58. The change in phytochemical contents may be caused by a variety of factors, including the type of bacteria present, their origins, the solvents utilized during the extraction process, and the geographic location of the plant sources. In a similar vein, (Jabaka et al., 2019) also observed a mean zone of inhibition of 3.67 ± 0.58 to 13.0 ± 0.00 mm for the ethanolic crude extract of *Acacia nilotica* stem back against E. coli. According to (Aliero et al., 2023), the mean zone activity of *Acacia nilotica* leaves in ethanolic crude extracts ranged from 6.33±0.67 to 23.2±0.30 mm to the studied bacterial pathogens. At 50 mg/ml, the extract had no effect against *Shigella flexneri*. It was found that when the concentration of the crude extract increases, so does the mean zone of inhibition for ethanolic crude extracts of *A. nilotica* leaves. The ethanolic crude extracts of *Acacia nilotica* leaves demonstrated a lower mean zone of activity against tested bacteria than the positive control, meropenem 30µg/ml. For every examined bacterium, the 10% DMSO negative control showed no action.

CONCLUSION

Acacia nilotica is known to be an antibacterial agent in removal of potent bacterial contamination, and it is used in numerous traditional medicines, it was selected for this study due to the increasing gene mutation of disease pathogens is the cause of the rise in antibiotic resistance. Following phytochemical screening, several secondary metabolites were determined as phenolics, flavonoids, and tannin in the plant. Using the agar well diffusion method, the crude extract was put through antimicrobial testing,

and the inhibition zone was quantified in (ml). When applied against bacterial species, the methanol extract showed positive effects.

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