



(RESEARCH ARTICLE)



Antibacterial activities and antioxidant potential of *Adansonia digitata* leaves

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Abstract

Resistance to synthetic antibiotics remains a significant global health challenge, prompting interest in natural alternatives such as phytochemicals. This study evaluated the antimicrobial potential of methanol and aqueous extracts from *Adansonia digitata* leaves against various pathogenic bacteria. Quantitative analysis revealed higher concentrations of alkaloids (5.60% in methanol extract), flavonoids, tannins, terpenoids (3.2% in methanol extract), and phenols in the methanol extract compared to the aqueous extract. The methanol extract demonstrated notable antibacterial activity, particularly against *Serratia marcescens* and *Staphylococcus aureus*, with a maximum zone of inhibition of 24.00 mm. In contrast, the aqueous extract showed no significant antibacterial effect. However, several bacteria, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Citrobacter freundii*, exhibited resistance to both extracts, despite exposure to concentrations below the Minimum Inhibitory Concentration (MIC). MIC values ranged from 62.50 mg/mL to 500 mg/mL for different bacterial strains, indicating varying susceptibility levels. This resistance highlights the complex way bacteria respond to phytochemicals and indicates that more research is necessary to identify the precise bioactive substances causing antimicrobial action as well as potential resistance pathways.

Keywords: *Adansonia digitata*; Antimicrobial; Phytochemicals; Pathogenic bacteria; Antibiotics.

1. Introduction

As a leading cause of death worldwide and a major source of morbidity and mortality, infectious illnesses persist (WHO, 2000; De Rycker *et al.*, 2019). Of the 57 million deaths globally each year, infectious diseases account for about 15 million (more than 25%) of the deaths (WHO, 2004). The poor are disproportionately affected by the morbidity and mortality linked to infectious diseases. The most vulnerable are infants and children. According to Morens *et al.* (2004), infectious illnesses also disproportionately affect indigenous and underprivileged populations in developed nations.

One of the greatest medical advances in human history has been the introduction of antibiotics. However, in the last several years, the ongoing, fast-paced emergence of antibiotic resistance has become one of the most significant health care concerns, both in community and hospital settings (Rice, 2009). The treatment of infectious diseases has become a significant issue in the modern period due to the emergence of antibiotic resistance and the significant side effects associated with conventional treatments (Yadav *et al.*, 2016). Because they contain various phytoconstituents that can treat a wide range of ailments, including infectious diseases, more than 35,000 plants from all around the world have been used for medical purposes (Abidullah *et al.*, 2021). The existence of secondary metabolites in plants gives them a great deal of promise as antibacterial agents. There are countless opportunities for the creation of novel drugs to treat resurgent infectious diseases due to the diversity of these natural compounds (Demain & Fang, 2000; Wink, 2015). Antioxidant and antibacterial qualities can be found in *Adansonia digitata* (Baobab) leaf extracts (Lanzotti, 2006).

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Temperature programmed desorption (TPD) studies can be applied to *Adansonia digitata* leaves to identify and analyze the desorption of bioactive compounds at different temperatures, which can reveal specific temperatures at which antibacterial and antioxidant compounds are released. TPD helps identify the compounds responsible for antibacterial activity by analyzing desorbed gases, understanding optimal release temperatures for effective extraction, and studying the mechanisms of action against pathogens. Similarly, TPD can identify antioxidant compounds, provide insights into their thermal stability and release, and optimize extraction processes to preserve their properties. In practice, TPD involves heating samples in a controlled environment and analyzing the desorbed gases with a mass spectrometer, allowing researchers to correlate the data with antibacterial and antioxidant assays to determine the efficacy of the identified compounds (Onivefu, 2023; Oluwayomi, 2024).

Aim

The aim of this investigation was to determine if bacteria exposed to *Adansonia digitata* leaves at concentrations lower than the minimum inhibitory concentration (MIC) may become resistant to the phytochemicals.

Specific Objectives

The study's specific objectives are to:

- Identify and measure the phytochemicals contained in each extract from the dried, powdered *Adansonia digitata* leaves;
- Assess the extracts' bacteriostatic and bactericidal effects on chosen pathogenic indicator organisms;
- Find the extracts' minimum inhibitory concentration; and
- Evaluate how well the previously exposed bacteria adjust over time to the antibacterial activity of the *Adansonia digitata* leaf extracts.

2. Literature Review

2.1. Antimicrobial resistance

Antimicrobial resistance (AMR) is one of the top ten global public health issues that has to be addressed right away. According to estimates from the CDC (2019), illnesses linked to antibiotic resistance would kill 1.27 million people worldwide in 2019. Recent decades have seen observations of decreased susceptibility of isolates to commonly used biocides among significant pathogenic bacteria species, especially in healthcare settings (Griffiths *et al.*, 1997). This was often seen in tandem with an increase in the incidence of antibiotic resistance. Consequently, worries have been raised that bacteria may develop resistance to specific biocide regimens in specific situations or that a decreased susceptibility to biocides can encourage a higher level or frequency of antimicrobial resistance (Fraise, 2002). Although the initial focus of these concerns was on healthcare settings, where biocides and antibiotics were frequently used along with frequent monitoring, the investigations' scope has expanded to include other domains where the use of biocides has increased, such as household cleaning and hygiene products (Gilbert and McBain, 2003). Microorganisms (including bacteria, fungi, viruses, and parasites) react to antimicrobial drugs (such as antibiotics, antifungals, and antivirals) by developing antimicrobial resistance (AMR). Antimicrobial drugs gradually become less effective as a result. Transmission risk is increased by infections that linger in the human body (Zhu *et al.*, 2022).

2.2. Antibiotics and Antibiotics Resistance

One of the greatest medical achievements of humanity has been the creation of antibiotics. However, in the last few years, the rapid rise of antibiotic resistance has become a major concern for healthcare, both in the community and in hospital settings (Aarestrup, 2005; Rice, 2009). The ubiquity of the issue and the urgent need for more international surveillance are demonstrated by the resistance elements that are currently emerging from the US, China, India, and most developing nations (Marston *et al.*, 2016). Antimicrobial resistance is a global concern that is becoming a greater hazard to health care because to the lack of availability of effective antimicrobials. The widespread use of antibiotics is causing a multitude of resistance mechanisms to emerge, which is seriously endangering our capacity to treat bacterial infections (Sebe *et al.*, 2023c). At therapeutically high antibiotic concentrations, resistant bacteria can be selected; however, the role that considerably lower antibiotic concentrations, which are ubiquitous in many ecosystems, play in selection remains unclear (Gullberg *et al.*, 2011). The recent global enrichment and spread of highly resistant pathogenic bacteria in the microbiosphere has been largely attributed to human activities, most notably the widespread and excessive use of antibiotics in agriculture, veterinary medicine, and human health (Aarestrup, 2005; Babatunde *et al.*, 2022; Cabello, 2006; Martinez, 2008; Sebe *et al.*, 2023b).

Furthermore, conventional medications frequently cause immunosuppression and severe allergic reactions, among other possible adverse effects. According to O'Neill's (2015) description, the number of deaths caused by resistant illnesses was 700,000 annually, and by 2050, there will be 50 million infectious individuals worldwide. On the one hand, the rate of development of novel antibiotics is not keeping up with the rise in AMR infection prevalence. Conversely, global antibiotic usage selectively amplifies antimicrobial resistance (AMR) infections, increasing the risk to public health. Moreover, AMR would compromise the efficacy of numerous illness treatments, including chemotherapy for cancer, HIV medication, and malaria treatment (Zhu *et al.*, 2022).

2.3. Phytochemicals

Plants were a natural source of maintaining human health before the use of antibiotics or pharmaceutically produced medications (Bolaji *et al.*, 2024). The issue of drug (antibiotic) resistance in microbes, especially bacteria, has inspired research into alternative treatments, with a focus on plants that contain antimicrobial chemicals (a vital source of biologically active metabolites), from which new medications may be developed or manufactured (Copp, 2003; Babatunde *et al.*, 2023). Both the natural bioactive compounds in medicinal plants and the plants themselves are extremely valuable sources of antibacterial medication because of their wide biological diversity, exposing new chemical structures that may function on a range of biochemical pathways, leading to the development of new antimicrobial medications.

"Plant-chemicals" is the literal translation of "phytochemicals." These are nonnutritive chemical components derived from plants that have a host of health advantages, including the ability to prevent sickness (Durodola *et al.*, 2024; Coulibaly *et al.*, 2014). Numerous diseases have been discovered to benefit from the use of phytochemicals, a broad class of compounds produced by plants that have strong antioxidant properties (Nwozo *et al.*, 2023). Their nutrients are non-essential, meaning the body does not need them to survive (Rahim *et al.*, 2022). Plants create these substances in order to maintain life, and when humans consume them, they have positive health effects (Atia and Abdullah, 2014; Ahaotu *et al.*, 2020). Plants that contain bioactive substances, such as flavonoids, may naturally include phenolic compounds that have beneficial impacts on human health and function as antioxidants to neutralize free radicals. (Datsugwai *et al.*, 2017). Primary and secondary constituents can be distinguished among the more than a thousand identified phytochemicals based on their roles in plant metabolism. Because of the adverse effects of these antibiotics and the recurring antibiotic resistance of harmful microorganisms, research into alternate sources of antimicrobials, such as medicinal plants, for their antimicrobial characteristics, is gathering momentum. Plant-produced phytochemicals, or secondary metabolites, have demonstrated potential as antibacterials both by themselves and in conjunction with currently available antibacterial drugs.

Furthermore, these secondary metabolites protect the plants against infections by bacteria, fungi, and viruses (El-Mahmood and Ameh, 2007). Treatment of resistant bacteria may benefit from the use of phytochemicals since they often function through distinct mechanisms than traditional antibiotics. In order for a plant to survive in its surroundings, secondary metabolism is essential.

2.4. Baobab (*Adansonia digitata*) as a source of phytochemical

In rural areas, especially in developing and underdeveloped nations, traditional and age-old remedies are used to treat infectious diseases because they are known to be less expensive, more effective, and to have few or no side effects in comparison to synthetic treatments. Numerous secondary metabolite classes were extracted and separated from Bombacoideae plant species through phytochemical studies; volatiles and fatty acids were also reported (Refaat *et al.*, 2012). Some compounds were identified from Bombacoideae species. Several members of Bombacoideae are used as fiber and other utilities and some are also used as ornamental plants. *Adansonia digitata* is a multipurpose plant with various economic and social values (Chadare *et al.*, 2008). *Adansonia digitata* is a popular plant with over three hundred traditional uses in African nations (Buchmann *et al.*, 2010).

2.5. Phytochemical components of Baobab and functions

Many different types of phytochemicals have been discovered, indicating that this family is a significant source of phytochemicals. Alkaloids, anthocyanins, coumarins, flavonoids, lignans and neolignans, sesquiterpenes and sesquiterpene lactones, sterols, tannins, and triterpenes have all been isolated using the Bombacoideae subfamily (Osman, 2004). *Adansonia digitata* is one of the most studied species due to its therapeutic effects against antipyretics, diarrhea, dysentery, and as a substitute for cinchona in traditional pharmaceutical compositions (Paula *et al.*, 1997). In various countries, different parts of the tree have long been used to cure a variety of clinical ailments, such as dysentery and diarrhea. Phytochemical screening of *Adansonia digitata* leaf extract revealed the presence of flavonoids, saponins, mucilage, steroids, and alkaloids (Lanzotti, 2006).

The chemical makeup of *A. digitata* leaf methanol extracts, as described by Suliman and Nour (2017), is displayed in Table I. *Adansonia digitata* has been found to have significant therapeutic potency based on surveys; however, this claim has not been thoroughly investigated ((Paula *et al.*, 1997). Squalene (27%) and phytol (13%), palmitic acid (37%) and oleic acid (21%) were the main components in the leaves, whereas palmitic acid (30%) and oleic acid (24%) were discovered to be the main ingredients in the bark (Suliman & Nour, 2017).

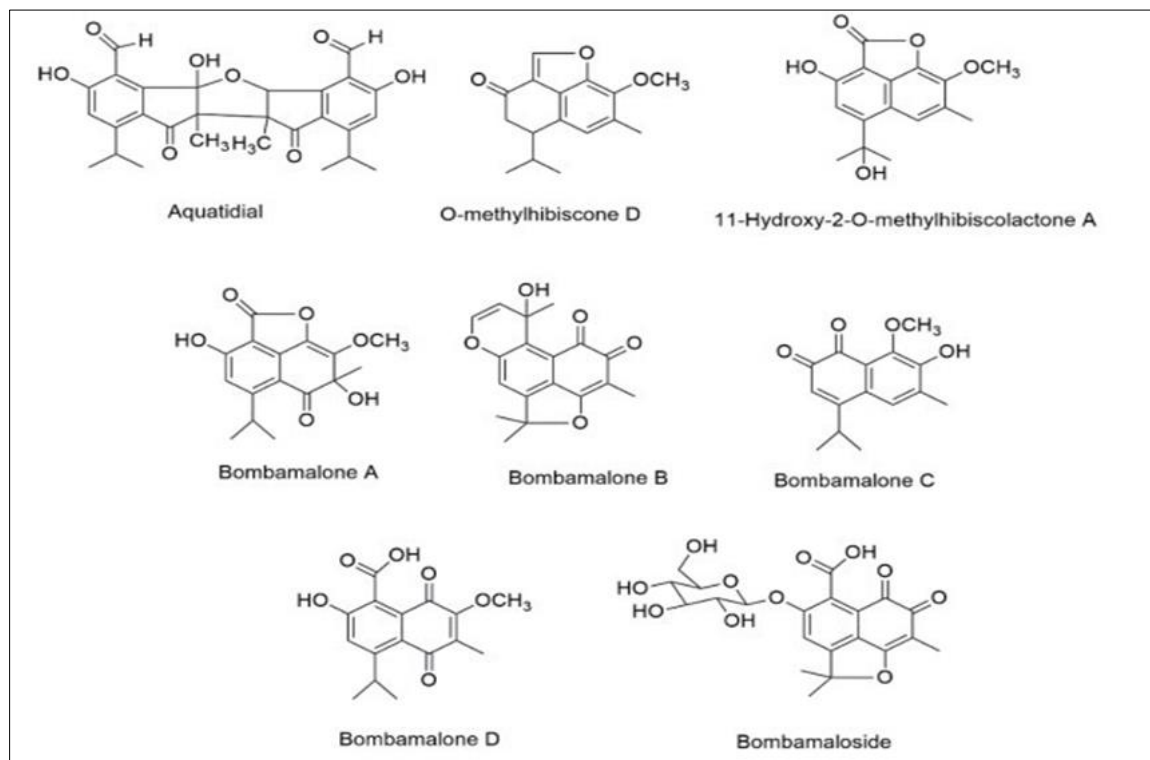


Figure 1 The chemical structures of new isolated compounds from Bombacoideae species (Das *et al.*, 2021)

Many tropical countries use plants in the Bombacoideae family for traditional medicine because to its pharmacological properties, which include anti-inflammatory, astringent, antibacterial, stimulant, antipyretic, analgesic, and diuretic effects (Refaat *et al.*, 2012). According to Sidibe and Williams (2002), the leaves are used to treat otitis, ophthalmia, urinary tract infections, internal pain, and bug bites. It is also recommended to use a mixture made of the leaves, roots, flowers, and bark to cure asthma, colic, and other gastrointestinal ailments. The leaf infusions are used to treat fever, diarrhea, kidney problems, inflammation, asthma, and blood purification (Zhang *et al.*, 2019). According to De Caluwe *et al.* (2010), the baobab's constituent parts are used to treat a variety of illnesses, including diarrhea, malaria, and bacterial infections.

The tree has certain portions that are used medicinally. *A. digitata*'s antibacterial activity against pathogenic bacteria, such as *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Enterobacter aerogenus*, has been investigated using methanolic extracts of its leaves, flowers, and fruit walls. The results showed that all of the extracts of *A. digitata* displayed antibacterial activity against all of the pathogenic bacteria under study, with the exception of the fruit wall extracts, which show no inhibition against *K. pneumoniae* (Samatha *et al.*, 2017). Additionally, plant extracts have been shown to exhibit anti-viral, anti-bacterial, and anti-trypanosomal properties (Anani *et al.*, 2000; Atawodi *et al.*, 2003). The *in vitro* trypanocidal activity of baobab was investigated microscopically against *T. brucei brucei* and *T. congolense*, the trypanosomes that cause haggard in animals. The methanol root extract significantly affected the motility of *T. brucei brucei* and *T. congolense* after 50 and 55 minutes, respectively (Atawodi *et al.*, 2003).

Table 1 Chemical composition of methanol extracts of *A. digitata* leaves (Suliman and Nour, 2017)

No.	Compound	Chemical Formula	RT	Molecular weight	Percentage (%)
Leave crude methanol extract					
1	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀	25.133	280	1.06
2	Palmitic acid	C ₁₆ H ₃₂ O ₂	27.427	256	8.95
3	Linolenic acid	C ₁₈ H ₃₀ O ₂	32.182	278	1.70
4	Phytol	C ₂₀ H ₄₀ O	32.348	296	13.28
5	Stearic acid	C ₁₈ H ₃₆ O ₂	32.491	284	2.10
6	Pentacosane	C ₂₅ H ₅₂	36.771	352	0.95
7	Octacosane	C ₂₈ H ₅₈	39.827	394	5.26
8	Squalene	C ₃₀ H ₅₀	42.762	410	27.06
9	Nonacosane	C ₂₉ H ₆₀	44.324	408	5.05
10	Tetratriacontane	C ₃₄ H ₇₀	51.311	478	4.38
11	Vitamin E	C ₂₉ H ₅₀ O ₂	53.29	430	1.10
12	γ-Sitosterol	C ₂₉ H ₅₀ O	63.453	414	4.98
13	Friedelin	C ₃₀ H ₅₀ O	64.288	426	3.60
14	β-Amyrin	C ₃₀ H ₅₀ O	65.467	426	4.24
15	Germanicol	C ₃₀ H ₅₀ O	65.987	426	4.08
16	α-Amyrin	C ₃₀ H ₅₀ O	68.631	426	7.02
17	Germanicol	C ₃₀ H ₅₀ O	71.709	426	1.54

There have been reports of some antibiotic activity against *Mycobacterium phlei*, *Bacillus subtilis*, *Escherichia coli*, *Streptococcus faecalis*, and *Staphylococcus aureus* (Atawodi *et al.*, 2003). In addition to having viricidal activity (direct inactivation of virus particles) and intracellular antiviral activity (which may be the result of several antiviral compounds acting in concert or one compound having multiple actions), methanol leaf extracts from *A. digitata* have shown to be highly effective at fighting the viruses Sindbis, Herpes simplex, and polio (Anani *et al.*, 2000).

Many human diseases, such as gastroenteritis, typhoid fever, upset stomach, diarrhea, and dysentery, are caused by bacteria such as *E. Coli*, *S. aureus*, *S. Typhi*, and others. Among other reasons, low environmental hygiene standards and restricted access to clean water contribute to the high prevalence of these illnesses in Africa. Thus, the study determines the phytochemical characteristics of the baobab tree and the antimicrobial efficacy of extracts made from its stem bark and leaves (Bashir *et al.*, 2021). The global economy, food security, modern health care systems' viability, and society are all seriously threatened by antibiotic resistance (O'Neill, 2014; O'Neill, 2015; Sebe, 2023a). The presence of such active components in the *A. digitata* extracts is expected to have a substantial role in the antibacterial activity. The phytol compound (monounsaturated diterpene alcohol) was discovered to have antimicrobial effects against *S. aureus* (Inoue *et al.*, 2005).

2.6. Purpose for more research on phytochemicals

For thousands of years, humans have used natural products derived from plants, animals, and microorganisms, either as pure substances or as unrefined extracts (Parekh and Chanda, 2007). In wealthy countries, about 80% of individuals practice traditional plant-based medicine. The antibacterial qualities of many plants, which are primarily produced during secondary metabolism, have led to their application. Thus, more research on these plants is necessary to fully comprehend their characteristics, safety, and effectiveness (Bakht, 2011; Sebe *et al.*, 2023a). Furthermore, synthetic medications have terrible negative effects when used to cure illnesses. For this reason, the practice of traditional

medicine in conjunction with contemporary medicine is becoming more widely accepted worldwide (Ra *et al.*, 2020). Furthermore, because bacteria are becoming more resistant to synthetic drugs every day, herbal remedy may be a useful tool for treating illnesses with fewer adverse effects (Sharma *et al.*, 2011).

3. Material and methods

3.1. Collection of samples

Fresh leaves of *A. digitata* were collected from Awe in Oyo state identified at the Department of Botany, University of Ibadan, Ibadan, Nigeria and air dried under shade at ambient room temperature of 25 °C ± 2 °C and smoothed using blender for the experiment as described by Yakubu *et al.* (2014).

3.2. Sterilization of Materials

The glassware and media utilized in this work were autoclaved at 15 pounds per square inch steam pressure for 15 minutes at 121 °C and sterilized in a hot air oven for two hours at 180 °C. Sterilizing the inoculating loops involved dipping them in ethanol and heating them to a red-hot temperature using a Bunsen burner. Flask, tube, bottle, and beaker openings were flamed both before and after use. Every day, the entire workstation was cleaned with 70% ethanol both before and after the inoculation.

3.3. Media Preparation

The preparation of Mueller Hinton Agar, Nutrient Broth, and Nutrient Agar followed the manufacturer's instructions. Prior to sterilization, the media were homogenized at 121 °C for 15 minutes, and then cooled to 45 °C.

3.4. Plant Extraction

Using methanol and distilled water in a maceration process, baobab sample leaves were extracted. 50 g of the ground sample was extracted in 500 mL of solvent, and each sample was combined with its solvent (distilled water, 95% methanol [5 mL water and 95 mL methanol]) at a ratio of 1:10 (w/v). After being homogenized, the plant part combinations were placed on a rotary shaker set at 120 rpm for two days. The homogenized mixtures underwent a 30-minute centrifugation at 4000 rpm (SE-CFTDZ-WS, Labkits, U-Therm International (Hong Kong) Limited) at room temperature (22±2 °C). The collected supernatant was sieved using a double layer of muslin cloth, and then filtered through Whatman No. 4 filter paper. Using a rotary evaporator (IKA® RV10, Artisan Technology Group, Champaign, US) set to 40 °C for methanol and 90 °C for water, the solvents were extracted (concentrated) under vacuum. The concentrated extracts were dried down even more to form a paste, which was then placed in a sterile, closed container and frozen at 4 °C for later usage (Turker *et al.*, 2009; Ghassan *et al.*, 2012).

3.5. Qualitative phytochemical Screening of *Adansonia digitata* leaf

Plant extracts were tested for the presence of different phytochemicals utilizing a variety of techniques and precise measurements, as Sofowora (1993) describes. A brownish-green precipitate was obtained by boiling 5 milliliters of extracts with 10 milliliters of water, filtering the mixture, and adding a few drops of 0.1% ferric chloride. By combining 5 mL of extracts with 2 mL of chloroform and 3 mL of strong sulfuric acid, which resulted in a reddish-brown tint at the interface, terpenoids were found. By mixing 1 mL of strong sulfuric acid and 5 mL of ammonia with 5 mL of extracts, flavonoids were temporarily colored yellow. When five milliliters of extract were shaken with five milliliters of distilled water, a steady froth formed, which was indicative of saponins. Finally, 5 mL of extracts were diluted with 10 mL of alcohol, boiled, filtered, and then 2 mL of ammonia, 5 mL of chloroform, 10 mL of acetic acid, and Wagner's reagent were added in turn, resulting in a reddish-brown precipitate. This process proved the presence of alkaloids.

3.6. Quantitative phytochemical analysis of *Adansonia digitata* leaf

Precise measurements and processes were used to determine the different phytochemicals present in plant samples. To extract the alkaloids, 5 g of the plant sample was combined with 200 mL of 10% ethanoic acid in ethanol. The mixture was then allowed to stand for 4 hours, filtered, concentrated to 1/4 of its original volume, and concentrated ammonium hydroxide was added to precipitate the alkaloids. The plant sample was then collected, dried, cleaned, and weighed. According to Williamson and Manach (2005), 10 g of plant material was extracted for flavonoids using 100 mL of 80% aqueous methanol at room temperature, filtered, and the filtrate evaporated to dryness and weighed. 20 g of plant samples were heated for 4 hours at 55°C with 100 mL of 20% ethanol to extract saponins. The samples were then filtered, re-extracted with 200 mL of 20% ethanol, concentrated, and purified using diethyl ether and n-butanol, and dried to a constant weight (Cheesbrough, 2000). By combining 0.1 mL of sample extract with reagents, detecting

absorbance at 700 nm, and expressing the tannin content in terms of mg of tannic acid equivalents per g of dried sample, the tannin content was calculated using the Folin-Ciocalteu method (Tyler, 1994).

3.7. *In-vitro* screening of antimicrobial activity of *Adansonia digitata* leaf

3.7.1. Source of microorganisms

Pure cultures of *Citrobacter freundii*, *Escherichia coli*, *Staphylococcus aureus*, *Serratia marcescens*, and *Klebsiella pneumoniae* were obtained from the University of Ibadan's Department of Microbiology in Nigeria. The isolates were then cultivated on nutrient agar slants and kept in a refrigerator at 4 °C for the duration of the study (Cheesbrough, 2000). The organisms were sub-cultured on nutrient agar in plates within 24 hours at 37 °C.

3.7.2. Preparation of Graded Concentration of the *Adansonia digitata* leaf

For correct dissolving to yield 1000 mg/mL, one gram of each of the extracts from baobab leaves was weighed and dissolved in one milliliter of 20% dimethyl sulphoxide (20 milliliters of DMSO in 80 milliliters of distilled water) as the extraction solvent. Additional concentrations, which included 750 mg/mL, 500 mg/mL, 250 mg/mL, 200 mg/mL, 125 mg/mL, 100 mg/mL, 50 mg/mL, 40 mg/mL, 25 mg/mL, 20 mg/mL, and 12.5 mg/mL, were produced by dilution with 20% DMSO.

3.7.3. Preparation of 0.5 McFarland turbidity standards

One milliliter of concentrated H₃SO₄ was combined with ninety-nine milliliters of water to create a one percent sulfuric acid solution. Separately, 0.5 g of dehydrated barium chloride (BaCl₂) was dissolved in 50 mL of distilled water to provide a 1% solution of the substance. Using a magnetic stirrer to guarantee homogeneity and avoid clumping, 0.5 mL of the barium chloride solution and 9.5 mL of the sulfuric acid solution were combined to make a 1.0% barium sulfate suspension. For standard comparison, the resultant turbid mixture was thereafter placed in a transparent bottle with a cap and stored at room temperature. This turbid solution, which weighed about 5 milliliters, was put into a stopped test tube—the kind used to prepare the test and control inoculums—and kept at 25 °C in a dark environment. According to Baron and Yolken (1999) and Vallekobia *et al.* (2001), McFarland provides an approximate density of bacteria of 1x10⁻⁸ Colony Forming Units (CFU).

3.7.4. Preparation and standardization of inoculum suspension

One gram-positive bacterium, *Staphylococcus aureus* (ATCC 25923), and five gram-negative bacteria, *Escherichia coli* (ATCC 25922), *Citrobacter freundii*, *Serratia marcescens*, *Staphylococcus aureus* (ATCC 20613), and *Klebsiella pneumoniae* (ATCC 27853), were among the microorganisms obtained from the microbiology department. The test strains' viability and purity were biochemically confirmed before to use (Elgayyar *et al.*, 2000). A test tube containing general colonies of the test organisms was filled with a small volume of sterile water. The suspension was then adjusted by adding distilled water to match the 0.5 McFarland standard (10⁻⁸ CFU/mL), simulating the look of an overnight broth culture (Azu *et al.*, 2007). Suspensions of bacterial inoculum were made using Coyle's (2005) methodology. Enough material from an overnight broth culture of the test organisms was transferred into a tube holding 2.0 mL of normal saline using a sterile cotton swab. After that, the turbidity was changed to make each isolate equal the 0.5 McFarland standard, or roughly 1.5 x 10⁻⁸ CFU/mL.

3.7.5. Determination of antagonistic activity of *A. digitata* using agar well diffusion assay

The antibacterial activity of the baobab tree leaf extracts was evaluated using the agar well diffusion technique, in accordance with Biradar *et al.* (2007). Mueller-Hinton Aseptic conditions were followed in the preparation, sterilization, cooling, and pouring of agar into sterile Petri plates to a depth of approximately 4 mm. The agar was then left to firm for half an hour. Using a sterile cotton swab, standardized bacterial inoculum suspensions were applied to the sterile agar plates. A sterile cork borer was used to aseptically bore three 6.0 mm diameter wells into each plate after 15 minutes. First, florfenicol and cefpodoxime were pipetted into the second well as positive controls, followed by 0.3 mL of leaf extract at 1000 mg/mL and 20% DMSO as a negative control in the third well. After giving the extract two hours to permeate the agar, the plates were incubated upright for twenty-four hours at 37°C. For concentrations of 750 mg/mL, 500 mg/mL, 250 mg/mL, 200 mg/mL, 100 mg/mL, and 50 mg/mL, this process was repeated. Using a slide caliper, the diameters of the inhibition zones were measured after 24 hours to evaluate the effects. The results were reported to the closest millimeter in accordance with the guidelines set forth by the National Committee for Clinical Laboratory Standards (2003).

3.7.6. Determination of minimum inhibitory concentration (MIC)

To verify microbial resistance and track novel antimicrobial drugs, minimum inhibitory concentrations, or MICs, are crucial in diagnostic laboratories (Arti-Singh and Arun, 2011). The minimum inhibitory concentration, or MIC, is the antimicrobial concentration at which, during an overnight incubation period, no discernible growth is inhibited. As per the National Committee for Clinical Laboratory Standards methodology, the MICs of Baobab extracts were ascertained by means of the broth dilution method. 500 mg/mL, 400 mg/mL, 250 mg/mL, 125 mg/mL, and 62.5 mg/mL of extract were present in five tubes containing sterile Muller-Hinton broth. A broth culture ($\sim 1.5 \times 10^8$ cfu/mL) of each test organism was added to test tubes 1-4, with tube 5 serving as the negative control. Bacterial growth was assessed following a 24-hour incubation period at 37 °C. The minimum inhibitory concentration (MIC) was found to be the lowest concentration that impeded growth (Magashi and Abdulmalik, 2018).

3.7.7. Determination of minimum bactericidal concentration (MBC)

The number of sterile test tubes that showed no discernible growth based on the minimum inhibitory concentration (MIC) was determined, and five milliliters of sterilised Mueller-Hinton Broth were poured into each tube. Each new tube containing 5 mL of Mueller-Hinton Broth received 0.1 mL of culture from these tubes, which were then labeled and stored on a test-tube rack. Using a sterile bent glass rod, 0.1 mL from each culture tube was placed over the surface of the sterilized Mueller-Hinton agar on Petri dishes after it had set. For twenty-four hours, tubes and plates were incubated at 37 °C. The minimum bactericidal concentration (MBC) was ascertained by looking for turbidity in the broth and bacterial colonies on agar plates (Vollekobia *et al.*, 2001).

3.7.8. Screening for adaptation

Five bacteria were treated to sub-MIC concentrations of plant extracts in nutritional broth to see if concentrations below the MIC may cause resistance. Following a 24-hour observation period, a loopful of cells from each condition were placed in 4 mL of new media with the same sub-MIC concentration, where they were left for another 24 hours of exposure, for a total of 48 hours of exposure. Every day, the procedure was repeated with a 72-hour exposure period and new media transfers. Overnight cultures of separate colonies of bacteria were used. By plating the bacteria onto Mueller-Hinton agar with wells containing the active extract concentration and monitoring the clear zones, the development of resistance was tracked (Gullberg *et al.*, 2011).

4. Results

The physical characteristics of *Adansonia digitata*, such as its texture, color, and percentage yield, were noted. The methanol extract of *A. digitata* leaf had a percentage yield of 20.57%; the aqueous leaf extract had a high percentage yield of 20.57% and the methanol leaf extract of 11.69%, respectively); the color of these extracts were brown.

The results are displayed in Table II.

The phytochemical qualitative result in Table III indicates the presence of terpenoids, steroids, anthraquinones, saponins, tannins, cardiac glycosides, flavonoids, and alkaloids in the methanol extract of *Adansonia digitata* leaf. The aqueous extract of *A. digitata* leaf did not contain any of these components.

Figure 2 shows the number of phytochemical contents present in both aqueous and methanol leaf extracts of *Adansonia digitata*

Table 2 Physical properties of crude extracts of *Adansonia digitata* leaf

Physical parameters	<i>Adansonia digitata</i>	
	MEAD	AEAD
Weight extracted (g)	140	150
Weight of extract (g)	13.40	11.05
Percentage yield (%)	20.57	11.69
Colour	Brown	Brown
Texture	Sticky	Sticky

LEGEND- MEAD: Methanol extract of *Adansonia digitata* Leaf; AEAD: Aqueous extract of *Adansonia digitata* Leaf

Table 3 Qualitative phytochemical constituents of the leaf of *Adansonia digitata*

Phytochemical Compounds	<i>Adansonia digitata</i>	
	MEAD	AEAD
Terpenoids	++	+
Steroids	++	-
Anthraquinones	+	-
Saponins	+	+
Tannins	++	+
Cardiac Glycosides	+	-
Flavonoids	++	++
Alkaloids	++	++
Phenol	++	+

Legend-MEAD: Methanol extract of *Adansonia digitata* Leaf; AEAD: Aqueous extract of *Adansonia digitata* Leaf; ++ : abundantly present; Present: +; Negative/ Not detected: -

The following compounds are present in the *A. digitata* leaf methanol extract, as shown in Figure 2: 0.85% saponins, 2.23% flavonoids, 0.76% phenol, 1.18% tannins, 1.65% terpenoids, and 5.60% alkaloids. *A. digitata* leaf aqueous extract also included 7.80% alkaloids, 0.70% saponins, 0.86% flavonoids, 0.74% phenol, 1.06% tannins, and 0.40% terpenoids, as Figure 2 illustrates. Consequently, the phytochemical content of the *Adansonia digitata* leaf extract in methanol was higher than that of the leaf extract in water. The terpenoid concentrations of *Adansonia digitata* leaf methanol and aqueous extracts are 1.65% and 0.90%, respectively. The aqueous extract of *A. digitata* leaves had a low saponin content of 0.70. In the methanol extract of *A. digitata* leaves, 0.85% of the leaves contained saponin. Figure 2 displays the graphical representation of this outcome.

The tannin contents of each sample at various intensities are displayed in Table IV. Each sample's tannin content rises with concentration, although the rates at which it does so vary. The lowest tannin level is found in the aqueous extract of *Adansonia digitata* leaf, which has 2.12 μg in 200 $\mu\text{g}/\text{mL}$ and 2.23 μg in 1000 $\mu\text{g}/\text{mL}$ at gallic acid equivalent.

The Concentration of flavonoid content (in μg) in the different extracts at quercetin (standard) equivalence was presented in Table V. The result of the flavonoid is similar to the trend of that of tannin in Table IV.

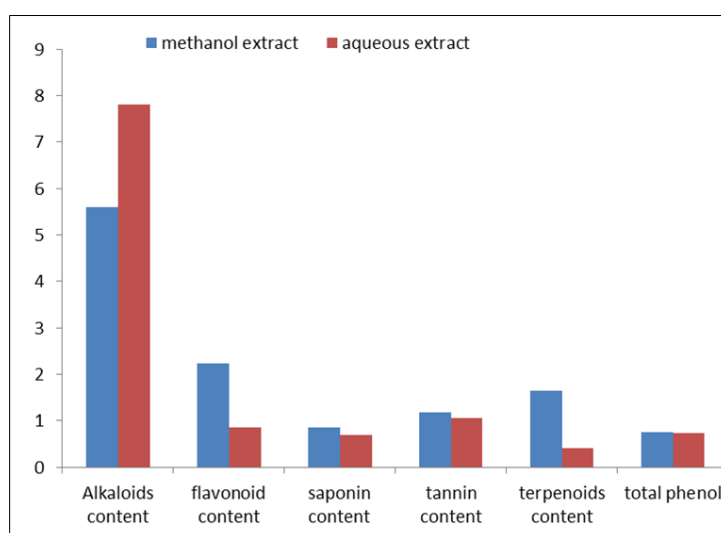
**Figure 2** Methanol extract of *Adansonia digitata* leaf: Aqueous extract of *Adansonia digitata* leaf

Table 4 Concentration of tannin (in μg) in the different plant extracts at gallic acid equivalence

Concentration ($\mu\text{g}/\text{mL}$)	MEAD (μg)	AEAD (μg)
200	2.35	2.12
400	2.63	2.21
600	2.64	2.28
800	2.74	2.28
1000	2.98	2.33

Legend- MEAD: Methanol extract of *Adansonia digitata* Leaf; AEAD: Aqueous extract of *Adansonia digitata* Leaf

Table 5 Concentration of flavonoid content (in μg) in the different plant extracts at quercetin (standard) equivalence

Concentration ($\mu\text{g}/\text{mL}$)	MEAD (μg)	AEAD (μg)
200 $\mu\text{g}/\text{mL}$	4.45	1.72
400 $\mu\text{g}/\text{mL}$	5.89	2.46
600 $\mu\text{g}/\text{mL}$	6.02	3.00
800 $\mu\text{g}/\text{mL}$	6.93	3.41
1000 $\mu\text{g}/\text{mL}$	7.96	3.50

Legend- MEAD: Methanol extract of *Adansonia digitata* Leaf; AEAD: Aqueous extract of *Adansonia digitata* Leaf

The leaf extract from *Adansonia digitata* has the lowest flavonoid content, with 1.72 μg at 200 $\mu\text{g}/\text{mL}$ and 3.50 μg at 1000 $\mu\text{g}/\text{mL}$ at quercetin equivalent, according to Table V's results.

The Total Phenol Content (in μg) of the various sample extracts at gallic acid equivalency is displayed in Table VI. The outcome also coincided with previous phytochemical component data, showing that the methanol extract of the identical sample had a larger content than the aqueous extract.

The antibacterial properties of *Adansonia digitata* extracts at various concentrations were evaluated against the indicator species. With the exception of *S. marcescens* and *S. aureus*, for which Cefpodoxime had no effect and *S. marcescens*, low effect, respectively, the results of the zone of inhibition comparison between the extracts and the synthetic antibiotics (Cefpodoxime and Florfenicol) showed that the synthetic antibiotics had significantly higher zones of inhibition than the extracts.

When tested against *S. marcescens* and *S. aureus*, the zone of inhibition for all extracts at 200 mg/ml and above was greater than that of Cefpodoxime, with the exception of the aqueous extract of *Adansonia digitata* leaf, which showed no inhibition zone at any concentration on any of the indicator organisms (Table VIII). With the control sample (dilution solvent: 20% DMSO), no zone of inhibition was seen for any of the indicator species.

Table 6 Total phenolic content of the different plant extracts (in μg) at gallic acid equivalence

Concentration ($\mu\text{g}/\text{mL}$)	MEAD (μg)	AEAD (μg)
200	1.51	1.47
400	1.78	1.48
600	1.80	1.52
800	1.87	1.53
1000	2.73	1.55

Legend- MEAD: Methanol extract of *Adansonia digitata* Leaf; AEAD: Aqueous extract of *Adansonia digitata* Leaf

Table 7 Antagonistic activity of methanol extract of *Adansonia digitata* leaf at different concentration against indicator organisms using agar well diffusion assay

MEAD(mg/mL)/ antibiotics	Test organisms				
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. marcescens</i>	<i>K. pneumoniae</i>	<i>C. freundii</i>
	Zone of inhibition in millimeters (mm)				
1000	24.00	NZ	24.00	NZ	NZ
750	21.00	NZ	22.00	NZ	NZ
500	23.00	NZ	22.00	NZ	NZ
250	22.00	NZ	22.00	NZ	NZ
200	21.00	NZ	21.00	NZ	NZ
100	19.00	NZ	20.00	NZ	NZ
50	18.00	NZ	18.00	NZ	NZ
FFC	26.00	24.00	29.00	12.00	14.00
CPD	6.00	22.00	NZ	NZ	20.00
D	NZ	NZ	NZ	NZ	NZ

Legend - MEAD: Methanol extract of *Adansonia digitata* leaf; FFC: Florfenicol; CPD: Cefpodoxime; D: Diluent (20% DMSO); NZ: No zone of Inhibition

Table 8 Antagonistic activity of aqueous extracts of *Adansonia digitata* leaf at different concentration against indicator organisms using agar well diffusion assay

AEAD(mg/mL)/	Test organisms				
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. marcescens</i>	<i>K. pneumoniae</i>	<i>C. freundii</i>
	Zone of inhibition in millimeters (mm)				
1000	NZ	NZ	NZ	NZ	NZ
750	NZ	NZ	NZ	NZ	NZ
500	NZ	NZ	NZ	NZ	NZ
250	NZ	NZ	NZ	NZ	NZ
200	NZ	NZ	NZ	NZ	NZ
100	NZ	NZ	NZ	NZ	NZ
50	NZ	NZ	NZ	NZ	NZ
FFC	26.00	24.00	29.00	12.00	14.00
CPD	6.00	22.00	NZ	NZ	20.00
D	NZ	NZ	NZ	NZ	NZ

Legend - AEAD: Aqueous extract of *Adansonia digitata* leaf ; FFC: Florfenicol; CPD: Cefpodoxime; D: Diluent (20% DMSO); NZ: No zone of Inhibition observed.

Two test organisms used in this research work were able to be inhibited from growing by the methanol extract of *Adansonia digitata* leaf (MEAD), resulting in a clear zone (Table IX). However, *Escherichia coli*, *Klebsiella pneumoniae*, and *Citrobacter freundii* were able to withstand the antagonistic effect of MEAD used in this research. The maximum zone of inhibition against *S. marcescens* was seen in the methanol extract of *Adansonia digitata* leaf, measuring 24.00 mm.

Table 9 Antagonistic activity of methanol extracts of *Adansonia digitata* leaf at 500 mg/mL concentration against indicator organisms using agar well diffusion assay before and after prior exposure to a concentration below MIC Zone of inhibition (mm)

Bacteria/ MEAD exposure concentration (mg/mL)	Hours	MEAD (500mg/ml)		CPD		FFC		Diluent	
		B	A	B	A	B	A	B	A
S. marcescens/ (25)	24	22	18	NZ	8	29	32	NZ	NZ
	48	22	16	NZ	7	29	30	NZ	NZ
	72	22	10	NZ	5	29	26	NZ	NZ
S. marcescens/ (12.5)	24	22	17	NZ	7	29	32	NZ	NZ
	48	22	14	NZ	6	29	29	NZ	NZ
	72	22	14	NZ	6	29	28	NZ	NZ
S. aureus/ (25)	24	23	25	6	NZ	26	32	NZ	NZ
	48	23	21	6	NZ	26	25	NZ	NZ
	72	23	19	6	NZ	26	25	NZ	NZ
S. aureus (12.5)	24	23	23	6	NZ	26	31	NZ	NZ
	48	23	20	6	NZ	26	27	NZ	NZ
	72	23	16	6	NZ	26	24	NZ	NZ

Legend: MEAD: Methanol extract of *Adansonia digitata* leaf; FFC: Florfenicol; CPD: Cefpodoxime; Diluent: (20% DMSO); NZ: No zone of Inhibition observed, B: Before exposure; A: After exposure

5. Discussion

This study looks into the phytochemical components and antibacterial properties of *Adansonia digitata* leaf extracts in relation to pathogenic bacteria such as *Escherichia coli*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Serratia marcescens*, and *Staphylococcus aureus*. According to earlier research, the phytochemical profiles of both extracts were comparable (Masola *et al.*, 2009). Though not as effective as Florfenicol, the methanol extract showed considerable antibacterial activity against a number of microorganisms (Samatha *et al.*, 2017). variances in geographic location, season, and environmental factors influencing phytochemical availability could account for variances in antibacterial efficacy (Biondi *et al.*, 2021).

Zones of inhibition were over 10 mm, indicating activity (Adeleke *et al.*, 2006); the aqueous extract indicated no antibacterial effect, varying from previous findings of effectiveness at higher concentrations (Danlami, 2017); the methanol extract showed broad-spectrum antibacterial activity, probably due to its higher polarity, enabling better extraction of bioactive compounds like polyphenols and flavonoids (Medini *et al.*, 2014). Regardless of its higher antibacterial activity, the methanol extract did not show bactericidal effects at the highest concentration, contrary to previous findings (Dharmananda, 2003; Datsugwai & Yusuf, 2017). Phytochemical analysis confirmed flavonoids in all extracts.

Bioactive phytochemicals including flavonoids, tannins, and alkaloids are responsible for the antibacterial properties of therapeutic plants. The effectiveness of these substances, however, depends on their precise kinds, concentrations, and interactions with other components; their identification alone does not ensure biological activity (Sharma & Singh, 2011). Tannins are used to cure diarrhea, burns, piles, gonorrhoea, leucorrhoea, and inflammation. They are also used as an antidote for alkaloidal poisoning, which suggests that plants high in tannins may suppress the bacteria that cause these illnesses (Buzzini *et al.*, 2008). All extracts contain terpenoids, which are active against viruses, bacteria, fungi, and protozoa. This activity is probably caused by lipophilic substances disrupting their membranes (Cowan, 1999).

Adansonia digitata extracts include alkaloids that have antimicrobial qualities. These include the ability to kill microbes like *Giardia* and *Entamoeba*, as well as potential antidiarrheal benefits via influencing the transit time of small intestines (Cowan, 1999). *Adansonia digitata*'s antibacterial effectiveness against certain pathogens demonstrates the diverse biological actions of saponins, which include antibacterial, antifungal, antiparasitic, antitumor/cytotoxic, antiviral, and

antioxidant properties (Sparg *et al.*, 2004). Perhaps as a result of their capacity to form complexes with soluble and extracellular proteins as well as with bacterial cell walls, flavonoid derivatives are strong antibacterial agents against a wide range of micro-organisms. They may be antibacterial because of their lipophilicity, which can break down microbial membranes (Cowan, 1999). According to Rasooli *et al.* (2008), phytochemicals can deteriorate microbial membranes, change the hydrophobicity of the surface of bacteria, hinder the synthesis of peptidoglycans, and modify quorum sensing.

Contrary to earlier findings, the investigation discovered that none of the extracts had bactericidal effects—only bacteriostatic ones. Clinical infections were successfully combated by *Adansonia digitata* leaf methanol extract at low concentrations (Cowan, 1999; Anani *et al.*, 2000). With the exception of *Serratia marcescens*, which only showed resistance to the extract after 72 hours, bacteria did, however, become resistant over time. Extended exposure decreased the antibacterial activity; initially, there was a synergistic effect with antibiotics, but this disappeared after 48 and 72 hours. This refers to the flexibility of bacteria and the possibility of an antibiotic-extract chemical synergy, especially against *Staphylococcus aureus*.

Variations in antibacterial effects are caused by the characteristics of the bacteria and their resistance mechanisms. Medicinal plants are becoming more widely acknowledged as supplemental therapies that improve antibiotic sensitivity (Sibanda & Okoh, 2007). They are useful in the fight against drug-resistant infections and are primary medicines for a large number of people worldwide (Aathira *et al.*, 2021). As pathogen resistance to synthetic drugs rises, herbal medicines provide efficient substitutes with fewer side effects (Sharma *et al.*, 2011). The use of natural products in traditional medicine dates back thousands of years and is still important today (Parekh & Chanda, 2007; Sofowora, 1993).

6. Conclusion

Adansonia digitata leaf is a good source of phytochemicals. The types of phytochemicals present determine their activities, and hence, the differences in the results obtained could be due to the type/class of flavonoid/tannin present in the sample and the quantity of that particular type present. The study investigated the phytochemical constituents and antibacterial activity of aqueous and methanol extracts of *Adansonia digitata* leaf. The findings revealed that the methanol extract exhibited significant antibacterial activity against selected pathogenic bacteria, while the aqueous extract showed no antibacterial effect. The methanol extract had higher yields of bioactive compounds, such as alkaloids, flavonoids, tannins, terpenoids, and phenols, which likely contributed to its antibacterial efficacy. The study demonstrated that:

- The methanol extract of *Adansonia digitata* leaf had a higher concentration of phytochemicals and showed substantial antibacterial activity against *Serratia marcescens* and *Staphylococcus aureus*.
- The aqueous extract had lower phytochemical content and did not exhibit any antibacterial activity.
- Methanol extracts exhibited broad-spectrum antibacterial activity, potentially due to the higher polarity of methanol, which allows for better extraction of bioactive compounds.
- The variations in antibacterial efficacy could be attributed to differences in geographical location, season, and environmental factors affecting the availability of phytochemicals.

These results suggest that the methanol extract of *Adansonia digitata* leaf holds promise as a source of natural antibacterial agents. Further research should focus on isolating and characterizing the specific bioactive compounds responsible for the observed antibacterial activity and assessing their potential for developing new antimicrobial therapies.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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