Ndegwa FK, et al., J Altern Complement Integr Med 2023, 9: 347
DOI: 10.24966/ACIM-7562/100347

HSOA Journal of
Alternative, Complementary & Integrative Medicine

Research Article

Kenyan Traditional Medicine: Exploring Solutions to the Modern Antibacterial Crises through Natural Products Chemistry

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Abstract

Background: Medicinal plants are used to treat various ailments in Kenya. This study describes the antibacterial activity of medicinal plant extracts used in traditional medicine in Kenya. Seven medicinal plants were studied to determine antimicrobial effect of plant extracts *in vitro* after phytochemical screening.

Materials and methods: Dry materials of the plants, *A. remota, B. micrantha, S. didymobotrya, C. africana, P. peruviana, P. africana, and A. annua* were sequentially extracted using three solvents - water, methanol, and hexane. Antimicrobial activity of plant extracts against *E. coli, P. aeruginosa, B. cereus* and *M. smegmatis* was determined by Kirby Bauer disc diffusion assay.

Results: The indiscriminate use of antibiotics has resulted in widespread development of antimicrobial resistance, compounded by the lack of discovery of new antibiotics pose serious threat to humankind. Phytochemicals hold great promise for the discovery and isolation of new chemotherapeutic agents. In this study, plant extracts extracted with different solvents from seven medicinal plants from Kenya, inhibited *E. coli, P. aeruginosa, B. cereus and M. smegmatis*. Most of the extracts were highly effective against the two Gram + bacteria, *B. cereus and M. smegmatis*.

Conclusion: Plant extracts of Kenyan medicinal plants inhibited *E. coli, P. aeruginosa, B. cereus and M. smegmatis*. Further investigation of plant extracts may lead to new therapeutics against infectious bacteria.

Keywords: Antimicrobial; Medicinal plants; Phytochemicals; Plant extracts; Traditional medicine

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Citation: Ndegwa FK, Kondam C, Ghose D, Esan TE, Waxali ZS, et al. (2023) Kenyan Traditional Medicine: Exploring Solutions to the Modern Antibacterial Crises through Natural Products Chemistry. J Altern Complement Integr Med 9: 347.

Received: April 17, 2023; **Accepted:** April 24, 2023; **Published:** May 01, 2023

List of Abbreviations

A. remota: Ajuga remota

B. micrantha: Bridelia micrantha

S. didymobotrya: Senna didybomotrya

C. africana: Cordia Africana

P. peruviana: Physalis peruviana

P. africana: Prunus Africana

E. coli: Escherichia coli

P. aeruginosa: Pseudomonas aeruginosa

B. cereus: Bacillus cereus

M. smegmatis: Mycobacterium smegmatis

Introduction

Universal right to health care is part of the constitution of the World Health Organization (WHO) and yet an estimated 5.7 million people die every year due to treatable infectious diseases [1]. Worldwide deaths due to antibiotic resistant organisms is reported to be about 750,000 and expected to reach around 10 million by the year 2050 [2,3]. A very small proportion of these deaths are in developed countries. According to the Centers for Disease Control and Prevention (CDC) there were more than 35,900 deaths in the USA [4] and the European One Health Action Plan against Antimicrobial Resistance reported that there were 25,000 deaths in the European Union are due to antibiotic resistance [5].

In many developing countries around 70-95% of the people rely on plants for the treatment of various diseases [3,6]. Traditional medicine based on plants has been practiced for thousands of years. The first written records are the Mesopotamian clay tablets in cuneiform from around 2600 BC. Plant derived materials such as oils of cedar and cypress, licorice, myrrh, and poppy juice are all in use even today [7]. The Ebers Papyrus from 1500 B.C., contains about 700 drugs administered as gargles, snuffs, poultices, infusions, pills, and ointments,

with beer, milk, honey, and wine. Traditional medicine was practiced by the Chinese for centuries with the first written record from 1500 B.C. and the Ayurvedic system of India is documented from about 1000 B.C. [7,8] in addition to prehistoric times, also deals with the medicines used during ancient times, middle Ages, dark ages, Greek, Roman, early modern, and 19th and 20th century. Folklore medicine which is passed on by word of mouth over the millennia is practiced in many developing countries as an alternative to conventional medicine since it is not available or affordable for the people [9-12].

There are approximately 374,000 species of plants in the world today [13] and according to the Medicinal Plant Names Services [14], 28,187 species are used in medicine, representing nearly 7.5% of all plant life. Thus, the vast majority of plant life remains unexplored for antimicrobials [15]. According to a recent review on plants with antibacterial activities, the top five countries by the number of species examined were South Africa, Cameroon, Brazil, India, and Iran [3]. These authors attributed this to a strong combination of plant diversity, ethnobotanical tradition, scientific training and equipment and other facilities available.

In Kenya, the use of medicinal plants for the treatment of various ailments is quite common. It is estimated that about 70% of the Kenyan population uses medicinal plants for primary health care. Divergent Kenyan communities like Ogiek, Taita, and Maasai use medicinal plants as therapeutics since they live far away from modern medical facilities. In the Ogiek community, 96 % of the population uses medicinal plants as their major therapeutic agents [16]. Various studies have reported the antimicrobial properties and efficacy of Kenyan medicinal plants [17]. Evaluation of medicinal properties of such plants can lead to the discovery and isolation of new therapeutic agents for the treatment of infectious diseases.

In this study, seven different Kenyan medicinal plants, *Artemisia annua, Ajuga remota, Bridelia micrantha, Cordia africana, Physalis peruviana, Prunus africanus,* and *Senna didymobotrya* were chosen because these plants are commonly used for the treatment of malaria, pneumonia, diarrhea, and other diseases. Extracts were made from various plant parts with water, methanol, acetone, and hexane as solvents and antibacterial activity was determined against *Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus* and *Mycobacterium smegmatis*.

Materials and Methods

Plant materials used in this study

Dry materials of the plants, *A. remota, B. micrantha*, *S. didymobotrya, C. africana*, *P. peruviana*, *P. africana,* and *A. annua* were obtained in the powdered form from the Department of Pharmacognosy and Pharmaceutical Chemistry, Kenyatta University, Nairobi, Kenya. The medicinal uses of these plants and the parts of each plant used in this study are listed in table 1.

Preparation of Plant Extracts

The dried powdered plant materials were sequentially extracted using three solvents - water, methanol, and hexane. Water was used for the first extraction by decoction and then sequentially extracted using methanol and hexane in a Soxhlet apparatus. The plant materials were also extracted non-sequentially in acetone using Soxhlet apparatus. Each sample was extracted in one of the four solvents – water, methanol, hexane, and acetone. Water was evaporated from the extracts by lyophilization, and the other three solvents were removed by drying under nitrogen gas.

Phytochemical analysis

Extractions were performed in a Soxhlet apparatus. Thin-layer chromatography employed silica gel plates F 1500/LS from Merck. Samples were spotted on the plates about 5µL using capillary tubes. Chromatographic spots were visualized using ultraviolet lamp emitting at 254 and 365 nm. All solvents and reagents were of analytical reagent grade.

Qualitative phytochemical characterization

Anisaldehyde/ Sulphuric acid reagent for Steroids; Dragendorff reagent for alkaloids; potassium hydroxide (Bornträger reaction) for coumarins (UV 365 nm) and anthraquinones (vis. and UV 365nm); 5% ethanolic solution of H_2SO_4 for cardiac glycosides (vis. and UV 365nm); Aluminum chloride solution (1% ethanolic $AlCl_3$) for flavonoids (UV 365 nm); Iron (III) chloride reagent (3% $FeCl_3$) for tannins and phenolic compounds; Ninhydrin for amino acids, amines, and amino sugars (0.2% ethanolic ninhydrin solution); Phenol / sulfuric acid solution for carbohydrates (Touchstone, 2019); Vanillin / H_2SO_4 solution for Terpenes/Terpenoids. The qualitative phytochemical characterization and analysis data is shown in table 2.

Thin-layer chromatography

The volume of the spots applied on the chromatographic plates was about 5µL. Then TLC plates were treated with the reagents for colorimetric detection of phytochemicals.

Family Name	Botanical Name	Common Name	Part Used	Preparation	Traditional use
Asteraceae	*Artemisia annua*	Sweet wormwood	Leaf, flower	Decoction	Malaria, Psoriasis, Infections
Lamiaceae	*Ajuga remota*	Bugleweed	Leaf	Decoction	Malaria, Chest pains
Solanaceae	*Physalis peruviana*	Cape gooseberry	Leaf	Decoction	Typhoid, Pneumonia
Rosaceae	*Prunus africana*	Red stinkwood	Stem bark	Decoction	Pneumonia / chest pain, loss of appetite
Boraginaceae	*Cordia africana*	Giku	Bark, leaf	Decoction	fatigue, anti-inflammatory
Fabaceae	*Senna didimobotrya*	Candelabra tree	Leaf	Decoction, steam	Pneumonia
Euphorbiacce	*Bridelia micrantha*	Mitzeeeri	Stem bark	Chew	Chest pains

Table 1: Medicinal plants tested for their antibacterial activity in the study.

Antimicrobial Activity

Bacterial Strains: The cultures of *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 23846), *M. smegmatis* (NRRL-B-24020) and *B. cereus* (Department of Biological Sciences stock), were kept stored in glycerated Luria-Bertani (LB) broth at -75°C until used.

Plant Extract	Steroids	Alkaloids	Tannins /Phenols	Carbohydrates	Proteins /Amino acids	Cardiac glycosides	Flavonoids	Anthraquinones	Terpenes/Terpenoid
Ajuga remota									
Water	+++	-	+	+++	+++	+++	+++	+++	+++
Methanol	++	-	+	++	+	++	++	++	++
Acetone	++	-	-	-	-	+	-	+	++
Hexane	+	-	-	-	-	-	-	-	++
Artemisia annua									
Methanol	++	-	++	+	+	+	++	+++	++
Acetone	+++	-	++	+	-	+	+	+++	++
Hexane	+	-	-	-	-	-	-	-	++
Prunus africana									
Methanol	+	-	+	+	-	+	+	+	+
Acetone	+++	-	++	+	-	+++	+	+++	+
Hexane	-	-	-	-	-	-	-	-	+
Physalis peruviana									
Acetone	+++	-	+	++	+	+	++	+	+++
Hexane	+	-	-	-	-	-	-	-	+++
Cordia africana									
Water	++	-	++	++	++	+	-	+	-
Methanol	+++	-	-	+	-	+	-	-	-
Hexane	+	-	-	-	-	+	-	-	+
Senna didymibotrya									
Water	+++	-	+	+++	+	+++	+	+	-
Methanol	++	+	++	++	-	++	++	+++	-
Acetone	+++	-	-	-	-	+	+	+	-
Hexane	-	-	-	-	-	-	-	-	+
Bridelia micrantha									
Water	++	+	+++	+	+	-	-	+	-
Methanol	+++	++	+++	++	+	++	-	+++	-
Acetone	+++	-	++	-	-	-	-	+	-
Hexane	-	-	-	-	-	-	-	-	+

Table 2: Phytochemical analysis of plant extracts.

+ = low presence ++ = moderately present, +++ = highly present, - = absent.

Antibacterial activity of extracts

For the assay, the frozen cultures were streaked on LB plates and incubated at 37°C or 28°C depending on the optimum growth temperature for each bacterium. A single colony from these plates was used to inoculate 5ml of LB broth and incubated with shaking at 200 RPM overnight. These fresh overnight cultures were used for the disc diffusion assay [18,19]. Each plant extract was tested at 0.2 mg/mL, and 2 mg/mL concentrations against the bacteria listed previously. The plates were then incubated at 37°C for 24-48 hours depending on the organism and the diameter (in mm.) of the zone of inhibition was measured. As controls, in addition to traditional antibiotics kanamycin and nalidixic acid, an established plant-based extract that is extensively used as a pest and disease control agent *Azadirachta indica* (Neem oil) was also used.

Results and Discussion

The family names of the plants, their botanical names, native language names, the parts of the plants used, the preparation and the ailments treated are presented in table 2. Each plant sample was extracted with at least one or more of the following solvents: water, methanol, acetone, and hexane. Depending on the availability, the samples were tested for antibacterial activity and the results are presented in table 3. The antibiotics kanamycin and nalidixic acid, and neem oil (*Azadirachta indica*) were used as positive controls.

Extracts of *A. annua, A. remota, B. micrantha, C. africana, P. peruviana, P. africanus,* and *S. didymobotrya* prepared with various solvents inhibited growth of *E. coli, P. aeruginosa, B. cereus* and *M. smegmatis* as determined by disc diffusion method table 3. The *A. annua* extracts prepared with water were effective against all four or

Extract	E. coli		P. aeruginosa		B. cereus		M. smegmatis	
Dose in mg/mL	**2.0**	**0.2**	**2.0**	**0.2**	**2.0**	**0.2**	**2.0**	**0.2**
A. annua (water)	11	0	11	0	10	0	11	0
A. annua (acetone)	10.5	0	19	0	18.5	7.5	0	0
A. annua (hexane)	8.0	0	0	0	15	0	12	0
A. remota (water)	0	0	8.0	0	7.0	0	0	0
B. micrantha (water)	10	0	10.5	0	16	0	16	0
B. micrantha (methanol)	11	0	14	0	17.5	7.5	11.5	0
B. micrantha (acetone)	0	0	12	0	16	0	15	0
B. micrantha (hexane)	8.5	0	12.5	0	15	0	19	0
C. africana (water)	0	0	9.5	0	7.5	0	9.0	0
C. africana (methanol)	0	0	13	0	10	0	12	0
C. africana (hexane)	10	0	0	0	17.5	7.0	9.0	0
P. peruviana (water)	0	0	12	0	7.0	0	11.5	0
P. africana (water)	0	0	0	0	17	0	12.5	0
P. africana (acetone)	10	0	10.5	0	20	0	0	0
P. africana (hexane)	0	0	12.5	0	0	0	12	0
S. didymobotrya (water)	8.5	0	11.5	0	19.5	9.0	11	0
S.didymobotrya (methanol)	0	0	0	0	19	8.5	12	0
S. didymobotrya (hexane)	0	0	0	0	11	0	11	0
Kanamycin (control)	23	13	0	0	27	20	27	18
Nalidixic acid (control)	10	0	10	0	33	25	9.0	0
Azadirachta indica (Neem oil) (control)	13	0	12	0	10	0	12	0

Table 3: Antibacterial Activity of plant extracts.

ganisms at 2.0 mg/ml. The acetone extract was twice as effective as the water extract against *P. aeruginosa* and *B. cereus*. *B. cereus* was very sensitive to acetone extract since it was inhibited even at 0.2 mg/ml. The hexane extract was ineffective against *P. aeruginosa*.

B. micrantha extracts prepared with water, methanol and hexane were highly effective against all the four organisms. *B. cereus* was highly sensitive to methanol extract since it was inhibited at the low concentration of 0.2 mg/ml. Surprisingly, *E. coli* was resistant to acetone extract in contrast to the other three organisms.

The *C. africana* extracts in water or methanol were active against *P. aeruginosa*, *B. cereus* and *M. smegmatis* while *E. coli* was resistant. In contrast, the hexane extract was active against all organisms except *P. aeruginosa. B. cereus* was very sensitive to the hexane extract since it was effective even at the low concentration of 0.2 mg/ml. The pattern of inhibition exhibited by the water extract of *P. peruviana* is like that of *C. africana* methanol extract in that they both inhibited *P. aeruginosa, B. cereus* and *M. smegmatis* but not *E. coli.*

Depending on the solvent used, the extracts of *P. africanus* showed wide variation in their effect on the bacteria tested. The water extract inhibited only the two Gram positive *B. cereus* and *M. smegmatis.* Acetone extract was inhibitory to *E. coli, P. aeruginosa,* and *B. cereus* while, *M. smegmatis* was resistant. The sensitivity of Gram-negative *P. aeruginosa* and Gram-positive *M. smegmatis* for the hexane extract was about the same as shown by the diameter of the zone of inhibition, while the other two organisms were resistant.

The water extract of *S. didymobotrya* inhibited all the four organisms. In contrast, the hexane and methanol extracts were effective against the two Gram-positive bacteria. The methanol extract was very effective against *B. cereus* since it was inhibitory even at the low concentration of 0.2 mg/ml.

Every one of the plants, extracted with one or more of the solvents was effective against all the four organisms at a concentration of 2 mg/ml. In total there were eighteen samples and only nine inhibited *E. coli* while thirteen inhibited the other Gram-negative bacterium *P. aeruginosa*. The two Gram-positive bacteria, *M. smegmatis* and *B. cereus* were inhibited by sixteen and seventeen preparations respectively. There were a few differences between the two organisms; M. *smegmatis* was resistant to three extracts to which *B. cereus* was susceptible, namely, the acetone extracts of *A. annua,* and *P. africanus* and the water extract of *A. remota*. *B. cereus* was resistant to a single extract, that of hexane from *P. africanus* to which *M. smegmatis* was susceptible. *B. cereus* was the only bacterium that was inhibited by the low concentration of 0.2 mg/mL of the methanol extracts of *B. micrantha,* and *S. didymobotrya* and acetone extract of *A. annua*.

The importance of the solvents used and the temperature optimum for the preparation of extracts was demonstrated by the discovery of the anti-malarial drug Artemisinin from *A. annua* by Nobel laureate Youyou Tu. After trying out extraction by several different solvents, it was discovered that ether extraction at lower temperature results in the isolation of the product [20].

Antibiotic resistant *E. coli* causes common infections such as urinary tract infections as well as life threatening bloodstream infections. Antibiotic resistance rates are rapidly rising, especially to third and fourth generation cephalosporins, and fluoroquinolones [21].

P. aeruginosa infections are common in patients with cystic fibrosis, burn patients, HIV infected as well as immunocompromised individuals. It can also infect many anatomic sites, including skin, subcutaneous tissue, bone, ears, eyes, urinary tract, lungs, and heart valves with serious consequences [22]. Infections of *P. aeruginosa* are becoming more difficult to treat because of increasing antibiotic resistance. Some multidrug-resistant strains are resistant to nearly all antibiotics, including carbapenems. In 2017, multidrug-resistant *P. aeruginosa* caused an estimated 32,600 infections among hospitalized patients and 2,700 estimated deaths in the United States [23].

Mycobacterium tuberculosis is responsible for one of the top 10 causes of death worldwide and it is also a leading cause of death from a single infectious agent (ranking above HIV/AIDS). It has been reported that in 2019, about 10 million people were infected resulting in the death of around 1.4 million people. Drug-resistant TB is a great public health threat and worldwide close to half a million people developed rifampicin-resistant TB and of those, 78% had multidrug-resistant TB [24].

The acid-fast bacterium, *M. smegmatis* is usually used as a surrogate for screening and primary evaluation of anti-mycobacterial activity because of its similarity in cell wall composition with *M. tuberculosis*. It also shares several important properties with *M. tuberculosis*, including similar resistance to certain macrolide antibiotics and several synthetic compounds [25,26]. As pointed out above, seventeen preparations in different solvents from the six plants (Table 2) showed anti *M. smegmatis* activity. These extracts should be tested against *M. tuberculosis*.

B. cereus is ubiquitous in its distribution and is present in soils, plants and the intestinal tracts of mammals and insects. The endospores can persist in the environment and can be transmitted via farm produce such as vegetables, fruits, nuts etc. The bacterium can cause two types of gastrointestinal diseases, either the emetic or the diarrheal, due to different types of toxins [27]. The organism can also cause several systemic and local infections especially in immunologically compromised individuals, newborns, intravenous drug users, patients with traumatic or surgical wounds, and those with catheters [28].

In 2004 it was discovered that *B. cereus* can cause anthrax, until then, *Bacillus anthracis* was considered the sole etiologic agent causing the fatal mammalian disease anthrax. The pathogenicity of *B. anthracis* is due to two plasmids, pXO1 and pXO2, encoding the toxin complex and the poly-γ-D-glutamic acid capsule, respectively [29]. Two types of anthrax causing *B. cereus* strains has been identified. One strain designated as Bcbva (*B. cereus* biovar anthracis) possess both the pBCXO1 and pBCXO2 plasmids and the other atypical strain that possess only the pBCXO1 plasmid. These plasmids are more than 99% similar to the pXO1 and pXO2 plasmids of *B. anthracis*. The Bcbva strain produces the toxin complex and the poly-γ-D-glutamic acid capsule like *B. anthracis* and the atypical strains may produce a unique exopolysaccharide dependent on the plasmids harbored [30].

Anthrax is considered a disease of great public health threat due to its ability to infect wide range of domestic and wild animals, as well as humans. The stability of spores made *B. anthracis* a great concern for its potential use in a bioterrorist attack, as happened in Japan in 1993 and the United States in 2001 [31]. After the attacks, Centers for Disease Control and Prevention (CDC) in the Department of Health and Human Services (HHS) created a list of select agents and toxins and added *B. anthracis* as Tier 1 select agent. In 2017 *B. cereus* Biovar *anthracis* was added to this list [32].

The results of this study demonstrated that the plant extracts examined were far more effective against the two Gram positive *B. subtilis* and *M. smegmatis* than the two Gram-negative bacteria. These plant extracts can be used for the development of new anti-bacterial agents that may be useful in the treatment of Methicillin-resistant *S. aureus* (MRSA) and multi drug resistant *M. tuberculosis*.

Some of the plants described above were the subject of previous studies. Rolta et al., 2021 [33] investigated the antimicrobial activity of Methanolic (ME) and Petroleum Ether (PE) extracts of *A. annua* against *E. coli, S. aureus* and the yeast, *Candida albicans*. The extracts were effective against all the three organisms. They further showed that these extracts had synergetic effect in combination with antibiotics and antifungal agents resulting in the reduction of Minimum Inhibitory Concentration (MIC). Qualitative phytochemical screening showed the presence of compounds such as phenolics, flavonoids, saponins, Phyto steroids, alkaloids, glycosides, carbohydrates, proteins, and free amino acids in both ME and PE extracts.

Preliminary phytochemical screening of aqueous and 70% ethanol extracts of *A. remota* leaves showed the presence of phenolic compounds, flavonoids, saponins, tannins, and steroids and they did not contain alkaloids and anthraquinones [34]. From a methanolic extract of aerial parts of *A. remota*, a triterpene, ergosterol-5,8-endoperoxide was isolated and shown to be an inhibitor of *Mycobacterium tuberculosis* [35].

B. micrantha, ethyl acetate extract of stem bark contained, alkaloids, tannins, anthraquinones, steroids and flavonoids and were reported to exhibit antibacterial activity against *S. aureus, Shigella sonnei, Salmonella typhimurium,* and *Helicobacter pylori* [36].

Chemical analysis of methanolic extracts of the seeds of *C. africana* showed the presence of flavonoids, tannins, steroids, phenols and saponins. The extracts exhibited, anti-inflammatory and antioxidant activity. Further, it was reported to exhibit antiulcer activity in pyloric ligated rats [37]. Ethyl acetate fractions of leaves and stems showed antibacterial and antifungal activity while chloroform water and methanol extracts were reported to be inactive [38].

P. peruviana methanolic extracts contained tannins, steroids, anthraquinones, flavonoids, terpenoids by phytochemical analysis. Extracts from fruit, root, stem and leaves of this plant exhibited anti-bacterial and anti-fungal activity [39]. Aqueous and dichloromethane extracts from various parts of the plant were reported to have anti-bacterial activity against Gram + and Gram bacteria as well as anti-fungal activity. Phytochemical analysis of fruit, root, stem, and leaves revealed the presence of tannins, saponins, steroids, flavonoids, and alkaloids. However, anthraquinone was absent in all the extracts [40].

P. africana bark extract is used traditionally for the treatment of various conditions such as fever, malaria, prostate cancer, and benign prostatic hyperplasia. The stem bark of the plant was evaluated for its phytochemical constituents and acute toxicity on fifteen female wistar rats. The result of the qualitative phytochemical screening of the methanolic bark extract showed the presence of carbohydrates, triterpenes, glycosides, tannins, flavonoids, alkaloids and saponins [41]. In connection with their studies on anti-inflammatory compounds in

the stem bark, dichloromethane extract of *P. africana* was analyzed by qualitative phytochemical methods and found to contain tannins, saponins, flavonoids, alkaloids, quinones, cardiac glycosides, terpenoids, phenolics and coumarins [42].

Methanolic crude extracts of *S. didymobotrya* leaves, flowers, stem bark, immature pods and root bark were assayed for antibacterial activity against *E. coli* and *S. aureus*. Most of the extracts were effective against both organisms, depending on the sample and the concentrations used. Phytochemical analysis of the methanolic extracts revealed the presence of terpenoids, alkaloids, phenols flavonoids and steroids [43].

In an extensive review of plants with antibacterial activities published between 1946 and 2019 with emphasis on the period 2012 and 2019 it was reported that the plant families, *Asteraceae, Fabaceae* and *Lamiaceae* were the most studied [3]. The genus *Artemisia* is the most studied plant genus since the discovery of the antimalarial drug artemisinin, and its derivatives in the species *A. annua* by Tu, 2016 [44]. Five different *Artemisia* species have been investigated as a source of antimicrobial compounds and reported to exhibit antibacterial activity. Crude extraction of leaves in methanol was the most common method used in most phytochemical studies [3]. In our studies the three most studied families of plants *Asteraceae, Fabaceae* and *Lamiaceae* are represented respectively by the three genera *Artemisia, Senna* and *Ajuga,* however, none of the species except for *A. annua* are represented.

Screening of botanical extracts leads to the identification of plants with anti-bacterial, anti-fungal or anti-protozoal activity. It has been pointed out that screening for single active compounds by fractionation based on antimicrobial assays is insufficient to capture the potential of phytochemical compounds. The extracts may contain hundreds or even thousands of compounds at varying quantities and identifying the compounds responsible for a given anti-microbial activity represents a great challenge. It is very often assumed that the activity of a mixture is attributed to the presence of just a few known constituents. However, several studies have shown that the observed activity of plant extracts can result from mixtures of compounds with synergistic, additive, or antagonistic activity [45,46]. In addition to investigating these interactions within herbal extracts, many recent studies involve finding phytochemicals that synergize with existing antibiotics, and act as resistance-modifying agents against drug-resistant bacteria. The great potential of synergy was demonstrated by studies involving methicillin-resistant *Staphylococcus aureus* (MRSA).

Extracts of leaves, flowers, and twigs of the plant *Cytisus striatus* were tested by the disk diffusion method for synergy with ciprofloxacin and erythromycin. It was found that the leaf extracts increased the effectiveness of both antibiotics against all the seven strains of MRSA examined. In addition, 22 isoflavonoids were also tested as antibiotic adjuvants and it was found that there was a clear synergy between isoflavonoids and the two tested antibiotics, showing their great potential for clinical therapy of infections of antibiotic-resistant microorganisms such as MRSA [47]. An extract of the plant *Callicarpa americana*, was found to synergize with oxacillin against methicillin-resistant *S. aureus* and it was shown that the active compound was the clerodane diterpene, 12(S),16ξ-dihydroxycleroda-3,13-dien-15,16-olide. [48].

Conflict of Interest

The authors declare no conflict of interest.

Author's Contribution

FKN, NKG, PKM, POO, Designed experiments, provided ethnobotanical information and plant material. CK, TEE, ZSW, MEM: Performed the extractions, phytochemical experiments, and analysis. DG, RM, Designed and performed the bacterial experiments, analyzed the data, and co-wrote the paper. TJH: Designed the experiments and co-wrote the paper.

References

1. Daulaire N, Bang A, Tomson G, Kalyango JN, Cars O (2015) Universal access to effective antibiotics is essential for tackling antibiotic resistance. J Law Med & Ethics 43: 17-21.
2. O'Neill J (2016) Tackling drug-resistant infections globally: final report and recommendations. The review on antimicrobial resistance, UK.
3. Chassagne F, Samarakoon T, Porras G, Lyles JT, Dettweiler M, et al. (2021) A Systematic Review of Plants with Antibacterial Activities: A Taxonomic and Phylogenetic Perspective. Front Pharmacol 11: 586548.
4. Center for Disease Control (2019) Antibiotic Resistance Threats in the United States. Department of Health and Human Services, CDC, USA.
5. European Union (2017) A European One Health Action Plan against Antimicrobial Resistance. European Union: European Commission, Maastricht, Netherlands.
6. Willis KJ (2017) State of the World's plants, 2017. Royal Botanic Gardens, UK.
7. Cragg GM, Newman DJ (2005) Biodiversity: a continuing source of novel drug leads. Pure Appl Chem 77: 7-24.
8. Petrovska B (2012) Historical review of medicinal plants' usage. Pharmacognosy Reviews 6: 1-5.
9. Dias DA, Urban S, Roessner U (2012) A historical overview of natural products in drug discovery. Metabolites 2: 303-336.
10. Coronado-Aceves EW, Sánchez-Escalante JJ, López-Cervantes J, Robles-Zepeda RE, Velázquez C, et al. (2016) Antimycobacterial activity of medicinal plants used by the Mayo people of Sonora, Mexico. J Ethnopharmacol 190: 106-115.
11. Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12: 564-582.
12. Gautam R, Saklani A, Jachak SM (2007) Indian medicinal plants as a source of antimycobacterial agents. J Ethnopharmacol 110: 200-234.
13. Christenhusz M, Byng J (2016) The Number of Known Plant Species in the World and Its Annual Increase. Phytotaxa 261: 201-217.
14. MPNS (2020) Medicinal Plant Names Services (MPNS). Royal Botanic Gardens, UK.
15. Porras G, Chassagne F, Lyles JT, Marquez L, Dettweiler M, et al. (2021) Ethnobotany and the Role of Plant Natural Products in Antibiotic Drug Discovery. Chem Rev 121: 3495-3560.
16. Ndegwa FK (2008) Use, Conservation and Harvesting of Medicinal Plants by the Ogiek of East Mau Forest, Nakuru District, Kenya. Kenya. Kenyatta University, Kenya.
17. Nankaya J, Gichuki N, Lukhoba C, Balslev H (2019) Medicinal plants of the Maasai of Kenya: A review. Plants (Basel) 9: 44.

18. Anas A, Jiya J, Rameez MJ, Anand PB, Anantharaman MR, et al. (2013) Sequential interactions of silver–silica nanocomposite (Ag–SiO_2NC) with cell wall, metabolism and genetic stability of *Pseudomonas aeruginosa*, a multiple antibiotic-resistant bacterium. Lett Appl Microbiol 56: 57-62.

19. Goshu GM, Ghose D, Bain JM, Pierce PG, Begley DW, et al. (2015). Synthesis and Biological Evaluation of Pyrazolopyrimidines as Potential Antibacterial Agents. Bioorg. Med Chem Lett 25: 5699-5704.

20. Miller LH, Su X (2011) Artemisinin: Discovery from the Chinese herbal garden. Cell 146: 855-858.

21. Collignon P (2009) Resistant *Escherichia coli*—we are what we eat. Clin Infect Dis 15: 202-204.

22. Bush LM, Vazquez-Pertejo MT (2020) Pseudomonas and Related Infections. Kenilworth, New Jersey: Merck Manual, Professional Version, USA.

23. Center for Disease Control (2019) Pseudomonas aeruginosa in Healthcare Settings. Centers for Disease Control and Prevention, USA.

24. World Health Organization (2020) Global Tuberculosis Report 2020. World Health Organization, Geneva, Switzerland.

25. Chaturvedi V, Dwivedi N, Tripathi RP, Sinha S (2007) Evaluation of *Mycobacterium smegmatis* as a possible surrogate screen for selecting molecules active against multi-drug resistant *Mycobacterium tuberculosis*. J Gen Appl Microbiol 53: 333-337.

26. McGaw LJ, Lall N, Hlokwe TM, Michel AL, Meyer JJ, et al. (2008) Purified compounds and extracts from *Euclea* species with anti-mycobacterial activity against *Mycobacterium bovis* and fast-growing Mycobacteria. Biol Pharm Bull 31: 1429-1433.

27. Arnesen LPS, Fagerlund A, Granum PE (2008) From soil to gut: *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol Rev 32: 579-606.

28. Bottone EJ (2010) *Bacillus cereus*, a Volatile Human Pathogen. Clinical Microbiol Rev 23: 382-398.

29. Hoffmaster AR, Ravel J, Rasko DA, Chapman GD, Chute MD, et al. (2004) Identification of anthrax toxin genes in a *Bacillus cereus* associated with an illness resembling inhalation anthrax. Proc Natl Acad Sci 101: 8449-8454.

30. Baldwin VM (2020) You can't *B. cereus* – a review of *Bacillus cereus* strains that cause anthrax-like disease. Front Microbiol 11: 1731.

31. Salter SJ (2011) You cannot *B. cereus*. Nat Rev Microbiol 9: 83.

32. Centers for Disease Control and Prevention (CDC), Department of Health and Human Services (HHS) (2016) Possession, Use, and Transfer of Select Agents and Toxins-- Addition of Bacillus Cereus Biovar Anthracis to the HHS List of Select Agents and Toxins. Interim final rule and request for comments. Fed Regist 81: 63138-63143.

33. Rolta R, Sharma A, Sourirajan A, Mallikarjunan PK, Dev K (2021) Combination between Antibacterial and Antifungal Antibiotics with Phytocompounds of Artemisia Annua L: A Strategy to Control Drug Resistance Pathogens. J Ethnopharmacol 266: 113420.

34. Tafesse TB, Hymete A, Mekonnen Y, Tadesse M (2017) Antidiabetic activity and phytochemical screening of extracts of the leaves of Ajuga remota Benth on alloxan-induced diabetic mice. BMC Complement Altern Med 17: 243.

35. Cantrell CL, Rajab MS, Franzblau SG, Fronczek FR, Fischer NH (1999) Antimycobacterial ergosterol-5,8-endoperoxide from *Ajuga remota*. Planta Med 65: 732-734.

36. Adefuye AO, Ndip RN (2013) Phytochemical analysis and antibacterial evaluation of the ethyl acetate extract of the stem bark of *Bridelia micrantha*. Pharmacogn Mag 9: 45-50.

37. Yismaw YE, Abdelwuhab M, Ambikar DB, Yismaw AE, Derebe D (2020) Phytochemical and Antiulcer Activity Screening of Seed Extract of *Cordia africana* Lam (Boraginaceae) in Pyloric Ligated Rats. Clin Pharmacol 12: 67-73.

38. Alhadi EA, Khalid HS, Alhassan MS, Gader HA, Kabbashi AS, et al. (2015) Comparison of antimicrobial activity and thin layer chromatography profile of ethyl acetate fraction of leaves and stem of *Cordia Africana* (Boraginaceae). Int J Multidiscip Res Dev 2: 665-669.

39. Kamau PK, Njeruh FM, Kirira PG, Kolawole OA, Kolawole OE, et al. (2020) An Assessment of Antibacterial and Antifungal Activity of Methanolic Extracts of Kenyan *Physalis peruviana*. Inst J Tradit Med 10: 1-12.

40. Kamau PK, Gakio P, Njeruh FM, Thuita J (2017) Antimicrobial evaluation and phytochemical screening of aqueous and dichloromethane crude extracts of Kenyan *Physalis peruviana*. IOSR J Dent and Med Sci 16: 101-109.

41. Mwangi KJ, Kariuki KJ, Reuben T, Kibe KG (2018) The phytochemical components and acute toxicity of methanolic stem bark extract of *Prunus africana*. IOSR J Pharm 8: 39-45.

42. Mutuma GG, Ngeranwa J, G'ori MAK, Kiruki S (2020) Phytochemical and Anti-Inflammatory Analysis of *Prunus africana* Bark Extract. Res J Pharmacog 7: 31-38.

43. Jeruto P, Arama PF, Anyango B, Maroa G (2017) Phytochemical screening and antibacterial investigations of crude methanol extracts of *Senna didymobotrya* (Fresen. HS Irwin and Barneby. J Appl Biosci 114: 11357-11367.

44. Tu Y (2016) Artemisinin-A gift from traditional Chinese medicine to the world (Nobel lecture). Angew Chem Int Ed 55: 10210-10226.

45. Abreu AC, Mcbain AJ, Simões M (2012) Plants as sources of new antimicrobials and resistance-modifying agents. Nat Prod Rep 29: 1007-1021.

46. Caesar LK, Cech NB (2019) Synergy and Antagonism in Natural Product Extracts: When 1+1 Does Not Equal 2. Nat Prod Rep 36: 869-888.

47. Abreu AC, Coqueiro A, Sultan AR, Lemmens N, Kim HK, et al. (2017) Looking to nature for a new concept in antimicrobial treatments: isoflavonoids from *Cytisus striatus* as antibiotic ajuvants against MRSA. Sci Rep 7: 3777.

48. Dettweiler M, Melander RJ, Porras G, Risener C, Marquez L, et al. (2020) A clerodane diterpene from *Callicarpa americana* resensitizes methicillin-resistant *Staphylococcus aureus* to β-lactam antibiotics. ACS Infect Dis 6: 1667-1673.

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