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A SURVEY OF MEDICINAL PLANTS USED IN KIENI FOREST, KIAMBU COUNTY, KENYA.

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Abstract

Medicinal plants are affordable and accessible, but documentation and evidence of their effectiveness is limited. A study was conducted in Kieni forest, Kiambu County with the aim of documenting the therapeutic plants, exploring their phytochemical properties and potential antimicrobial properties. Semi-structured questionnaires administered via the snowball technique was used in the survey. Phytochemical investigations was carried out using standard methods while disc diffusion method was used to evaluate antimicrobial properties. The study identified 28 medicinal plants from 25 families. The most common conditions treated by the plants were noted to be skin infections (31 %). The primary sources of medicinal plant knowledge were from parents and grandparents (63%). Plant parts that were most utilized were the leaves (27%), while only 2% of the seeds were used. Qualitative analysis of methanol extracts from *Croton macrostachyus*, *Cordia africana*, *Elaeodendron buchananii*, *Senna didymobotrya*, and *Coleus barbatus* identified phytochemicals such as terpenoids, flavonoids and tannins. The microbial sensitivity assay showed that the plant extracts displayed efficacy against Gram-positive bacteria, such as *Bacillus subtilis, Streptococcus pyogenes* and *Staphylococcus aureus*. However, limited efficacy was observed against isolates of *Salmonella typhi* and *Escherichia coli* although the former exhibited susceptibility to *E. buchananii* leaves extracts. All the tested plant extracts were unable to inhibit the growth of *Candida albicans*. *E. buchananii* emerged as the most effective species against tested microorganisms with a mean zone of inhibition of 9.90 (SD 0.391), while *C. macrostachyus* and *C. africana* were relatively less effective. Minimum Inhibitory Concentration (MIC) revealed *S. pyogenes* as the most susceptible microorganism, with an average MIC of 65.63 mg/ml. *S. aureus* and *B. subtilis* had a MIC of 81.25 mg/ml and 125.00 mg/ml respectively. Conducting further research on isolated phytochemical compounds and properties could potentially lead to the development of cost-effective and less toxic drugs for managing microbial infections.

Key words: Ethnobotany, antimicrobial, phytochemical properties, documentation

Introduction

Throughout history, man has always relied on plants for food, clothes and shelter. Later on, man sought remedies for injuries and diseases from plants. Srivastava (2018) stated that historical records reveal that plants have been cultivated and used for therapeutic purposes for the past 60,000 years. The utilization of plants as medicine in India, China, and Egypt dates back to almost 5,000 years ago, while in Greece and Central Asia, it goes back to at least 2,500 years (Jamshidi-Kia *et al*., 2017).

Ethnobotanical studies provide a database of medicinal plants that are found in a given region, serving as a valuable database for research and exploring the commercial value of the identified medicinal plants. In Africa over 5,000 plant species have been documented as medicinal plants, however, only a few have been validated (Taylor *et al* 2001). Numerous reports document the uses of therapeutic plants by the diverse communities in Kenya. For example, a study done by Nankaya *et al*. (2020) focused on identifying therapeutic plants utilized by the Loita Maasai. Wanjohi *et al*. (2020) recorded the medicinal plants used by the Marakwet community in Marakwet County, while Mbuni *et al*. (2020) identified the medicinal plants utilized by the communities living around Cherangani hills.

Traditional medicine remains significant in health promotion and management of chronic illnesses (Peltzer and Pengpid, 2019). For example, medicinal plants such as the bulbs of *Eucomis autumnalis* have been scientifically proven to have anti-inflammatory activity (Obakiro, *et al*., 2021). Investigations into the inhibitory effect of the plant's crude extract on Cyclooxygenase revealed significant anti-inflammatory activity, with IC_{50} values calculated at 72 µg/ml (Alaribe *et al*., 2020). In vivo studies done on rats using methanolic extract of *Artemisia absinthium* L showed antioxidant activity at a dose of 100 or 200mg/kg. This antioxidant activity can be used in the treatment of cerebral ischemia (Michel *et al*., 2020). The drug vinblastine extracted from *Catharanthus roseus* has been effectively used to treat various cancers such as Hodgkin's disease, non-Hodgkin's lymphomas, neck cancer and leukemia in children (Khan and Ahmad 2019). The anticancer activities are due to the alkaloids that can stall DNA replication resulting in cell death (Dhyani *et al*., 2022). These therapeutic properties of medicinal plants can be credited in part, to the development of secondary metabolites as a defensive tactic against environmental pressures. Examples of such compounds include alkaloids, cardiac glycosides, tannins, flavonoids and terpenoids.

Alkaloids are naturally occurring specialized metabolites that contain a nitrogen atom (Bhambhani *et al*., 2021). They are colourless, soluble in water and non-polar (Bhat, 2021). They have been found to have antiviral, anticancer and antibacterial properties in addition to their anti-inflammatory activities (Roy 2017; Martino *et al*., 2018; Adamski *et al*., 2020). Plant families that have been identified to have alkaloids include Amaryllidaceae, Berberidaceae, Leguminoceae, Solanaceae, Liliaceae, Ranunculaceae and Papaveraceae (Bhambhani *et al*., 2020). Cardiac glycosides are naturally occurring steroid compounds found in plants and the toad venom (Škubník *et al*., 2021). They are made up of a lactone ring, steroid ring and sugar moiety (Botelho *et al*., 2019. Cardiac glycosides are utilized in treating several cardiac diseases and possess antitumor effects due to their senolytic activity (Schneider *et al*., 2017; Ayogu and Odoh, 2020). The most common cardiac glycosides identified are digitoxin, digoxin and ouabain (Botelho *et al*., 2019). Tannins are polyphenolic compounds with aromatic rings (De Melo *et al*., 2023). They exhibit antimicrobial activity against bacteria and some fungi such as yeast. Their antimicrobial activity is due to their ability to inhibit cell wall synthesis, inhibit fatty acid biosynthetic pathways and by iron chelation (Farha *et al*., 2020). Flavonoids are polyphenol compounds found in plants (Wang *et al*., 2017). They have been identified to possess numerous beneficial effects such as anti-neoplastic, antibacterial, antioxidant, antiviral and cell-killing properties (Jucá *et al*., 2020; Mutha *et al*., 2021; Vaou *et al.,* 2021). Phenolic antioxidant property is based on their ability to combine with metal ions, improving endogenous antioxidant system thus avoiding the formation of free ions (Michel *et al*., 2020). Terpenoids constitute the largest class of naturally occurring hydrocarbon compounds and are derivatives of mevaronic acid made up of five carbon isoprene unit (Zeng *et al*., 2020). Chen *et al*. (2021) noted that terpenoids are a major area of focus in cancer research due to their potential as a treatment for the disease. For instance, the essential oil of *Curcumae rhizoma* has been recognized to have terpenoids that have proved promising in the treatment of cancer.

Rapid population growth driven by job opportunities in urban areas has led to a housing shortage in Nairobi. As a result, Kiambu County has become an overflow area for housing, leading to developers clearing fertile land for construction, resulting in environmental degradation. Additionally, excessive tree cutting for charcoal production and unregulated logging have depleted natural forests, accelerating the destruction of ecosystems and diminishing knowledge on medicinal plants. Therefore, it is crucial to prioritize the documentation and conservation of traditional medicinal plant knowledge amid these environmental issues.

This study documents some medicinal plants found in Kieni forest based on information from respondents, it identifies the phytochemical compounds present in some of the plants and their antimicrobial activities. Conducting further research on these compounds and properties could potentially lead to the development of cost-effective and less toxic drugs for managing microbial infections.

Methodology

The ethnobotanical study was carried out in Kieni forest, Kiambu County, Kenya.

Study area

The forest lies on either side of the Kamae–Kieni-Thika road (Latitude 1°10' S and Longitude 36°49' E) (Figure 1). It is the largest part of the Kikuyu escarpment forest covering 13,724 hectares (Nyukuri, 2012). The area lies in Lari Sub County that has a population density of 195 people per square kilometer. The major tribe in the area is the Kikuyu community. The populace is primarily concentrated in regions within a 3-kilometer radius of the forest.

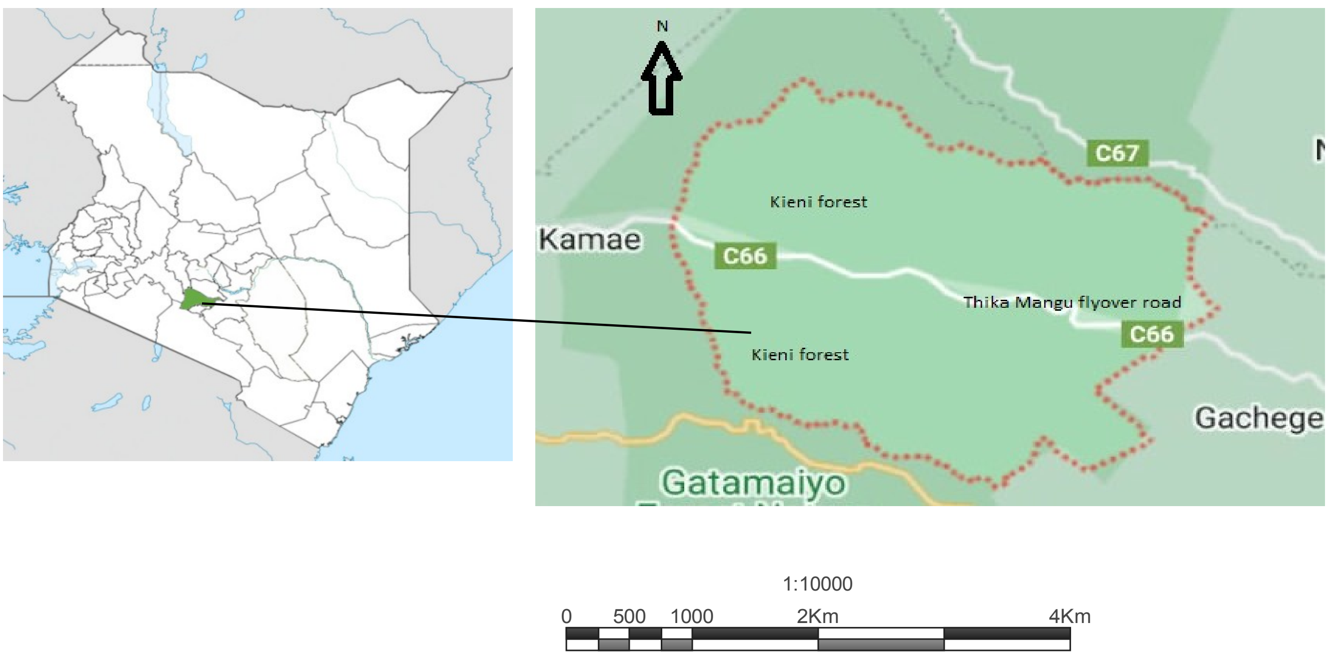

Figure 1: Map of Kenya showing the study area (google map)

Sampling

Ethical approval was obtained from Kenyatta University Ethical Review Committee (Reference number PKU/2757/11882) and the research licence was obtained from National Commission for Science Technology and Innovation (Licence No. NACOSTI/P/23/28501). Oral interviews and questionnaires were used to collected data on the commonly used medicinal plant species. Snowballing technique was employed to distribute the questionnaires. Informants were asked to specify their age, gender, academic level, names of therapeutic plants used, the ailments they cure, the plant components they use, and the plants sources. The sample size of 210 was determined using Bukhari sample size calculator (Bukhari, 2020) set at a 95% confidence level. Butt *et al* (2015) formula was used to determine the relative frequency of citation (RFC) RFC= FC/N. FC is the number of respondent and N is the total number of respondent in the study.

Plants mentioned by the respondents were identified by a taxonomist before collecting. Five medicinal plants species were randomly selected for phytochemical and antimicrobial screening. Voucher specimen of the plants selected were prepared and deposited in the Plant sciences department herbarium at Kenyatta University.

Extraction and concentration of active ingredient

Fresh samples of approximately 20-30 pieces of each identified medicinal plant part were collected, washed with distilled water, and left to dry naturally away from direct sunlight, for a duration of four weeks. The plants were extracted using the methodology outlined by Kumar *et al*., (2010). A laboratory grinding mill was used to pulverize the dried samples into fine granules. A conical flask was used to soak approximately 100 grams of the pulverized plant samples in 350 cm^3 of methanol for a duration of 8 hours. The mixture was then subjected to intermittent shaking, set at 25 °C at 150 revolutions, for 48 hours to guarantee the complete removal of the compounds. Whatman filter paper was utilized to filter the extracts and the filtrate was then subjected to a concentration process using a rotary evaporator maintained at a temperature of 50°C. Phytochemical and antimicrobial tests were then conducted on each methanolic plant extract. Qualitative analysis of the phytochemicals present was done on the methanolic extracts using standard procedures by Ezeonu and Ejikeme (2016), De Silva *et al*. (2017) and

Karthikeyan and Vidya, (2019). The methanolic extracts were analysed for alkaloids, cardiac glycosides, flavonoids, saponins, terpenoids, tannins and steroids.

Phytochemical tests

Alkaloids assay (Wagner's test). One milliliter of the filtrate obtained from the plant extract was mixed with 6 milliliters of the Wagner's reagent. A yellow or orange coloration indicated the presence of alkaloids.

Cardiac glycosides assay (Keller-Kiliani test). To two millilitres of the filtrate, four millilitres of glacial acetic acid, one milliliter of concentrated sulphuric acid and one mililitre of 2.0 % Iron (III) chloride was incorporated. The appearance of a green-blue colour indicated the presence of cardiac glycosides.

Flavonoids assay (Alkaline reagent test). To three millilitres of the methanol plant extract, an equal amount of ammonia solution was added. Then, 2ml of concentrated sulphuric (VI) acid was then added to the resulting mixture. A yellow hue that vanished after sometime indicated the existence of flavonoids.

Saponins assay (frothing test). To five mililitres of water, two millilitres of methanolic extract were added and agitated vigorously. The development of stable foam of at least one centimeter indicated the presence of saponins.

Terpenoids assay (Salkowski test)**.** Three drops of chloroform were added to five milliliters of the methanolic extract. To the resulting mixture, two drops of concentrated sulphuric VI acid was then added carefully to create a separate layer. A positive reaction was noted upon observing red-brown discoloration.

Tannins assay (iron (lll) chloride test). Three milliliters of iron (III) chloride solution was added to three milliliters of the methanol extract. Tannin presence was demonstrated by blue-black precipitate.

Steroids assay (Liebermann-Burchard's test). The methanol extract, weighing 2 grams, was mixed with 10 cubic centimeters of chloroform in a boiling tube and the resultant mixture was filtered. Afterwards, 2 milliliters of the filtrate were combined with an equal volume of acetic acid. One millilitre of concentrated sulphuric VI acid was thereafter added. Presence of a blue-green ring indicated the presence of steroids.

Antimicrobial assays

Test microorganisms

The ethnobotanical information collected from the study was used to identify the appropriate microorganisms to be tested. The microorganisms selected were clinical isolates of *Candida albicans, Escherichia coli, Staphylococcus aureus*, *Salmonella typhi*, S*treptococcus pyogenes*, *Bacillus subtilis* and *Enterococcus faecalis*. The microorganisms were obtained from the laboratory at Kenyatta University, department of Biochemistry, Microbiology, and Biotechnology.

Antimicrobial assay using disc diffusion method

The standard method by Elgayyar *et al*. (2001) was employed to screen for antibacterial activities in the methanolic extract. For bacterial culture, Muller Hinton agar was prepared following the instructions provided by the manufacturer. Fungi were cultured using Potato Dextrose Agar (PDA). Ten ml of the prepared mixtures was poured into petri dishes. Freshly prepared microbial culture that matched McFarland tube number 0.5 (1.5×10^{8} CFU/ ml) were inoculated onto the petri dish using cotton swabs (Hemeg *et al*., 2020). To 1ml of dimethyl sulfoxide, 0.5 grams of the plant extracts was dissolved. Sterile discs with a radius of 3 millimetres were immersed in the plant extract then placed on the upper layer of the petri dishes. The plates were then incubated at 37 °C for 24 hours. Negative control discs were immersed in dimethyl sulfoxide, while fluconazole (250mg) and amoxicillin (250 mg) were utilized as reference standards for fungi and bacteria, correspondingly. The experiment was repeated three times, and the average results were recorded. An inhibitory zone of 9 millimeters or greater around the discs signified antibacterial activity.

Minimum inhibitory concentration determination

Medicinal plants that showed high antimicrobial properties (zone of inhibition ≥ 9 mm) during preliminary screening were further bio-assayed using the two-fold series broth microdilution method to determine their minimum inhibitory concentration (Omwenga *et al*., 2011). The plants tested in this study were *Coleus barbatus, Senna didymobotrya,* and *Elaeodendron buchananii*.

Standard procedure according to Clinical Laboratory Standards Institute (CLSI) 2012 protocol were followed. Two milliliters of Muller Hinton agar broth was poured into the wells. 0.5 grams of the plant extracts were dissolved in 1 milliliter of dimethyl sulfoxide (DMSO). The resulting solution was then subjected to serial dilution in separate wells to achieve a final concentration range spanning from 500 to 3.59 milligrams of plant extract per milliliter of solution. Each well was inoculated with 50 µL of the microbial suspension. Amoxicillin 250 mg was used as positive control while dimethyl sulfoxide was used as the negative control. Afterward, the plates were put in an incubator at a temperature of 37°C for a duration of 24 hours. Minimum inhibitory concentration was considered as the lowest concentration that prevented growth.

A 20ml dilution of the plant extract that showed no bacterial growth after 24 hour incubation were obtained from the well and the content inoculate in a petri dish containing 30 ml of Muller Hilton agar for 24 hours. The first dilution that showed no growth after 24 hour incubation was considered as the MBC (Rajakumar *et al*., 2022).

Data analysis

The study utilized SAS JMP Pro 14 (USA) to determine how respondents' age, gender, and education level influenced the quantity of therapeutic plants identified. The statistical analysis involved conducting an ANOVA at a 95% confidence level. This was to investigate the significant differences in the number of therapeutic plants identified by informants based on these demographic factors. The Turkey test was then utilized to compare the means and identify any significant differences between the groups. The average zones of inhibitions were determined for each antimicrobial assay test, Analysis of Variance was carried out to establish the level of significance. P-value of less than 0.05 indicated statistical significance. Turkey's HSD test was employed to detect any significant disparity among the means. The results were illustrated using graphs and tables.

Results

The total number of respondents interviewed were 210. This comprised 111 (52.85 %) males and 99 (47.14 %) females. Out of the people interviewed, 3 (1.43 %) were practicing herbalists identified by the area residents.

The study shows that the knowledge of medicinal plants was mainly acquired from parents and grandparents 132/210 (62.85 %), while herbalist were the least likely source of knowledge of medicinal plants 6/210 (2.85%) (figure 1).

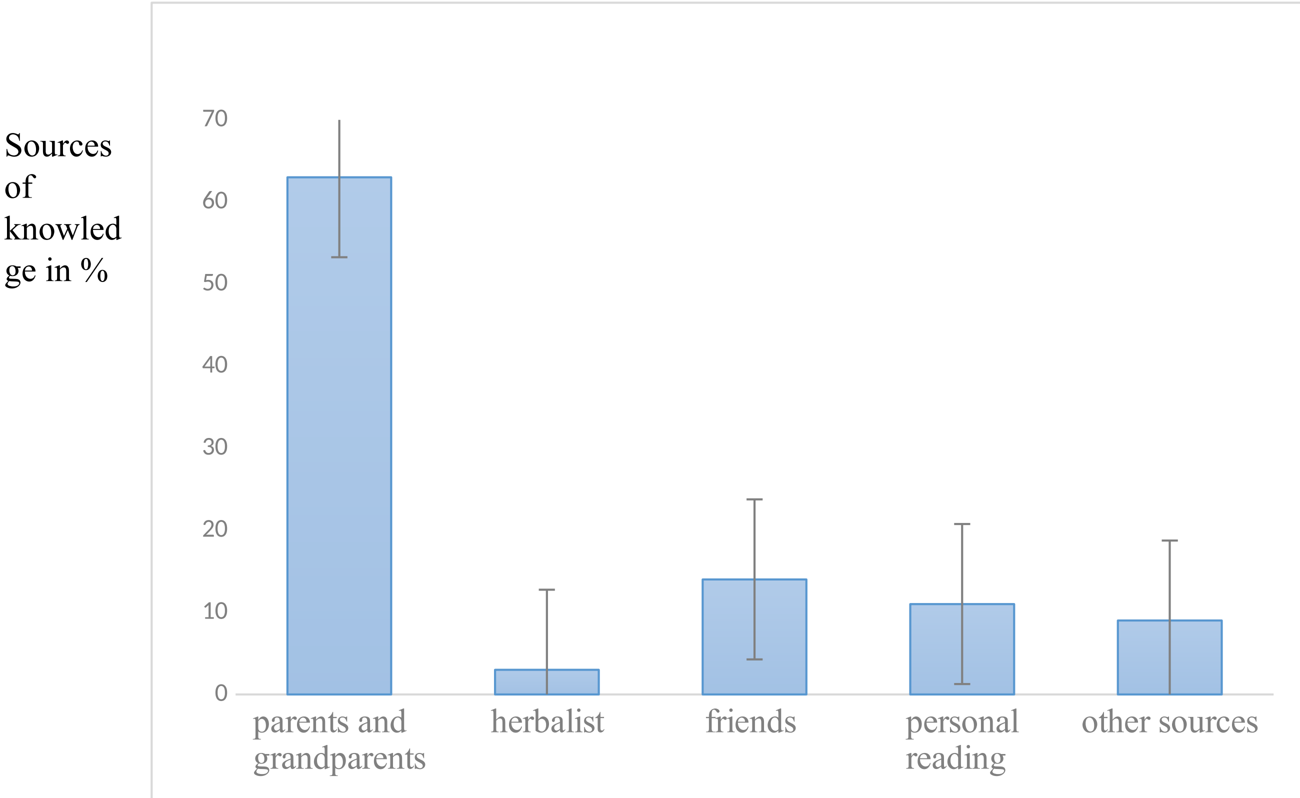

Figure 1: Sources of knowledge in medicinal plants in percentage

The level of knowledge on medicinal plants differed statistically ($p = 0.047$). The females seem to know more medicinal plants than the male with a mean of 5.18 and 4.70 respectively. Respondents with primary level of education were able to name more medicinal plants (mean 3.07 SD ± 0.12) as compared to those with secondary (mean 2.97 SD ± 0.10) or university level of education (mean 2.66 SD ± 2.66).

There was a significant difference between the age and the number of medicinal plants named ($P < 0.001$). Respondents of 71 years and above were able to name an average of 3.81 (35.71%) of the identified medicinal plants. 51-70 years named an average of 3.00 (28.57 %), 31-50 years named an average of 2.99 (21.42%) and less than 30 years named 2.53 (14%). Thus the level of knowledge of therapeutic plants increased with age.

The study indicates that more than one species was used in the treatment of the same disease. The common diseases treated were skin ailments 31.25%, malaria 18.75%, stomach disorders 16.67%, upper respiratory tract infections 14.58 % and dental problems 4.17%.

Decoction was the most preferred method of preparing medicinal plants. Other methods of preparation were infusion, poultice, maceration and use of plant sap**.** The study shows that 58% of the medicinal plants used were harvested from the forest, while 38% were grown in gardens. Additionally, 13% were obtained from traders, while 1% came from other sources. The availability of the plants seemed to vary according to the plant species. For instance, some plant species, like *Prunus africana*, were difficult to find, while others, such as *Croton* species, were common and easier to locate. This highlights the importance of understanding the availability and accessibility of different medicinal plants, as well as the potential impact of harvesting on their natural populations.

The most preferred plant part for medicinal use was identified to be the leaves. Other plant parts used include bark, roots, fruits and whole plant (Figure 2).

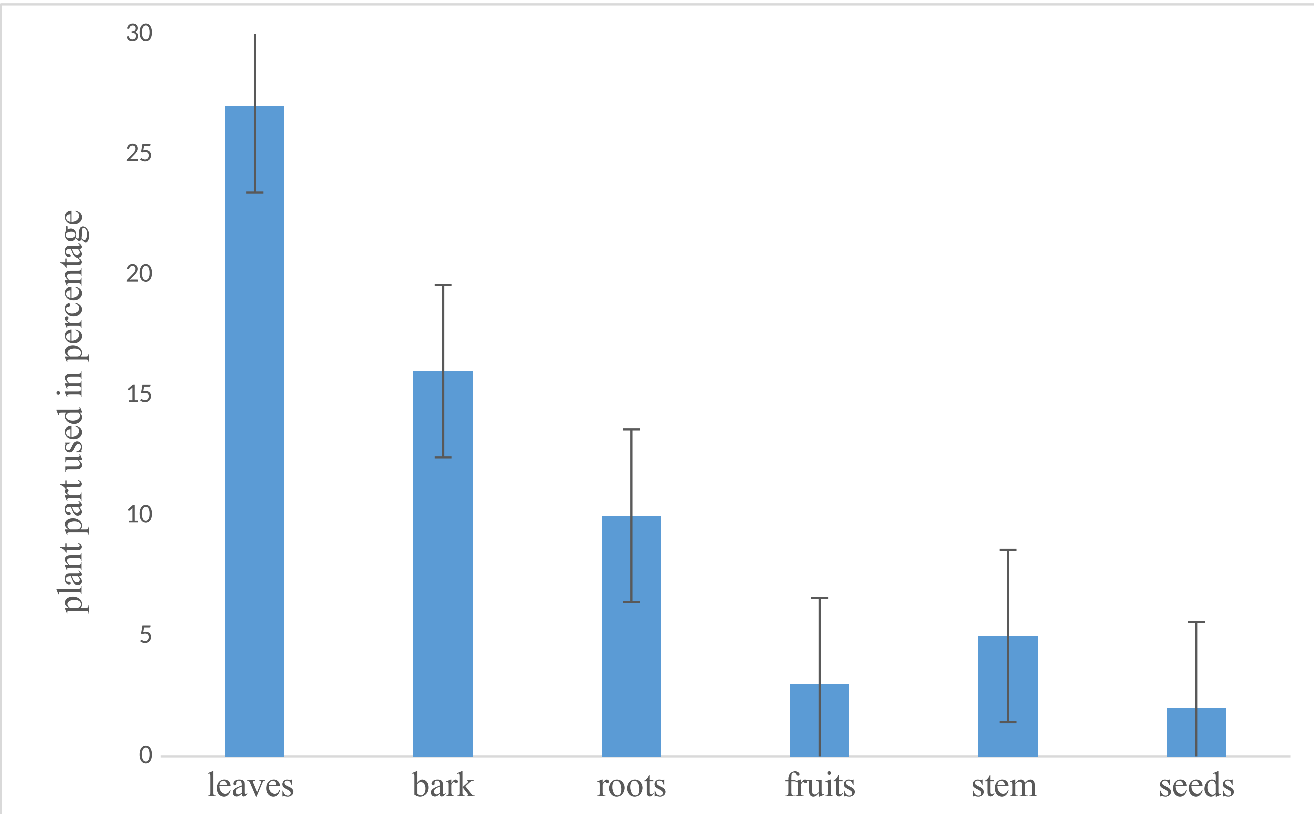

Figure 2: Parts of medicinal plant used in percentage

Table 1: Medicinal plant species used in Kieni forest, Kiambu County

Family and species name	Common name (Kikuyu)	Relative frequency of citation	Diseases managed	Plant component used and method of preparation	Documented ethnobotanical uses from literature
Aloaceae *Aloe lateritia* Engl.	Kiruma	0.20	Cough, Cold, Malaria Wounds, Chicken pox.	Leaves infusion. Leaves sap.	Malaria (Bjorå *et al*.,2015), asthma, boils, hernia, female infertility, cough (Anywar *et al*.,2022)
Aloaceae *Aloe kedongensis* Reynolds	Mugwa-nugu	0.18	Wound Worms, Fever and Malaria.	Leaves sap. Leaves decoction.	Typhoid, malaria, sexually transmitted diseases, abdominal pain, ringworms, ear infections, cold flu and pneumonia (Anywar *et al*., 2022.)
Asteraceae *Vernonia lasiopus* O.Hoffm	Mucatha	0.13	Malaria, fever	Leaves and seeds decoction.	Epilepsy, fever, abdominal pain, diarrhea, malaria. (Machocho *et al*., 2020; Gitahi *et al*., 2021)
Boraginaceae *Cordia africana* Lam	Muringa	0.25	Wounds, skin diseases and Stomach disorders	Powdered leaves and bark infusion.	Malaria, skin diseases (Wondafrash *et al*., 2019), gastric ulcers (Yismaw *et al*., 2020)
Bromeliaceae *Ananas comosus (L.)* Merr.	Inanathi	0.16	Cough, Constipation, Boils, Burns and Itches.	Sap from leaves and fruits.	Blood clotting, diarrhea, cardiovascular, tumor (Wali 2019) bacterial infections, burns (Paixão, *et*

					al.,2021)
Burseraceae *Commiphora eminii* Engl.	Mukungugu	0.005	Malaria, Stomach disorders and Typhoid fever Toothache Snake bite and Wounds	Bark decoction. Bark and Fruits are chewed. Sap from fruits.	Snakebites, constipation toothaches (Fern, 2024)
Caesalpiniaceae *Caesalpinia volkensii* Harms	Mubuthi	0.13	Stomach diseases, Malaria Reduce pain during pregnancy	Leaves decoction. Poultice from leaves.	Diarrhea, ophthalmic diseases, diabetes mellitus complications (Machocho *et al*., 2020; Githinji, 2018)
Canellaceae *Warburgia ugandensis* Sprague	Muthiga	0.18	Malaria, Epilepsy, Stomachache Diarrhoea, Malaria and Chest Pain Wounds Toothache	Bark and Leaves decoction. Leaves sap. Bark is chewed for toothache.	Malaria, bronchial infections, diarrhea, snake bites, measles, weakened joints. Stomachache (Okello, and Kang, 2021). HIV treatment (Anywar *et al* 2021)
Celastraceae *Mystroxylon aethiopicum* (Thunb.) Loes	Rurigi	0.07	Wounds and Burns Cough, Anaemia Stomach Pain	Leaves sap. Decoction of leaves and bark.	Urinary tract infections, wounds, diarrhea (Simugomwa *et al*., 2022) joint pain (Githinji, 2018,Muchonjo, 2021)
Celastraceae *Elaeodendron buchananii* (Loes.) Loes.	Mutanga	0.05	Stomach, infections, Wounds	Roots and bark infusion. Poultice made of leaves or roots sap is used.	Wound, syphilis and digestive disorders, urinary tract infections (Omwenga *et al*.,2015; Odak *et al*.,2018)
Euphorbiaceae	Mukinduri	0.23	Stomach worms	Leaves decoction.	Cough, wounds, typhoid,

Croton megalocarpus Hutch.			Wounds Severe cold and Pneumonia	Leaves sap applied to wound. Bark decoction	pneumonia, anthelmintic agent (Langat *et al*., 2020; Kathare *et al*., 2021)
Euphorbiaceae *Croton macrostachyus* Hochst. ex Delil	Mutundu	0.24	Wounds, warts Cough Rashes in newborn babies	Leaves sap. Leaves and bark decoction. Babies bathed in bark decoction.	Diabetes, wounds ringworms, malaria, hemorrhoids, stomachache (Gebrehiwot *et al*., 2018; Meresa, 2019) anti HIV (Terefe, 2022)
Lamiaceae *Senna didymobotrya* (Fresen.) H.S..irwin and Barneby	Mwinu	0.19	Abdominal pain, Abscesses and Malaria	Stem and leaves decoction.	Treatment of helminthes (Githinji, 2018) sexually transmitted infections (Maema *et al*., 2019)
Lamiaceae *Coleus barbatus* (Andrews) Benth	Maigoya	0.18	Worms, Boils, Sexually transmitted diseases, Stomach worms, Antimalarial, Eczema, Skin Diseases, Burns Wounds	Leaves and roots decoction	Leaves used as emmenogauge, diuretics, treatment of intestinal disorders. Honey is added to leaves decoction and drunk as remedy for leukorrhea (Mir *et al.,* 2021), skin ailments, heart disorders (Joshi, 2021).
Lauraceae *Ocotea usambarensis* Engl.	Muthaiti	0.09	Stomach worms Wounds	Leaves decoction. Bark powder applied to wounds.	Applied to swellings, treating whooping cough, measles,

			Malaria and Backache	Roots infusion.	wounds malaria, backache and as an anti-fungal malaria (Orwa *et al* 2009; Amri and Kisangau 2012)
Meliaceae *Azadirachta indica* A. Juss.	Muarubaini	0.17	Malaria and Stomach worms. Dental diseases. Spermicide and skin diseases	Decoction of leaves, stem and roots. The twigs are chewed. Oil extracted from the seeds.	Head-ache, backache, fever, insecticide, malaria, stomach-ache (Amri and Kisangau 2012) diabetes control, cancer (Islas *et al*., 2020)
Moraceae *Ficus sycomorus* L.	Mukuyu	0.18	Malaria. Ringworms Laxative Snake bite	Leaves decoction. Milky latex from the bark. Root decoction. Macerated leaves.	Menstrual cycle, women infertility (Amri and Kisangau 2012)

The respondents were able to identify 28 medicinal plants from 25 different families, which are commonly used to treat various ailments in humans (Table 1). Among the families, Euphorbiaceae, Oleaceae, and Solanaceae had two species each while the other families had only one species each (Table 1). Species with the highest frequencies of citation were, *Cordia africana* Lam, *Croton macrostachyus* Hochst ex Delil, *Croton megalocarpus* Hutch, *Senna didymobotrya*, *Azadirachta indica* A.Juss, and *Aloe lateritia* Engl

Plate 1: Some of the commonly used medicinal plants

a) Croton macrostachyus

b) *Cordia africana*

c). *Elaedendron buchananii*

e). *Coleus barbatus*

d). *Senna didymobotrya*

Phytochemical analysis

Five plant species obtained from the ethnobotanical study were randomly selected for phytochemical analysis and antimicrobial screening. These species were *Cordia africana, Croton macrostachyus, Coleus barbatus, Elaeodendron buchananii* and *Senna didymobotrya* (plate 1). The study indicated that the methanolic extracts of the selected plants contained various phytochemicals, including alkaloids, tannins, cardiac glycosides, flavonoids, saponins and steroids. Alkaloids were detected in most of the tested plant extracts except *Coleus barbatus* roots which tested negative for alkaloids. The least common phytochemicals were cardiac glycosides and steroids. Cardiac glycosides were found in *Elaeodendron buchananii* leaves and steroids were only found in *Coleus barbatus* roots.

Elaeodendron buchananii leaves had the highest concentration of phytochemicals followed by Senna *didymobotrya*, *Croton macrostachyus* leaves and bark and *Cordia africana* leaves and bark had the lowest concentration (Table 2).

Table 2: Phytochemical test results of methanol plant extracts

	Alkaloids	Terpenoids	Saponins	cardiac glycoside	flavonoid	Steroids	Tannins
Cordia africana (bark)	+	+	-	-	+	-	+
Cordia africana (leaves)	+	+	+	-	+	-	+
Croton macrostachyus (bark)	+	+	+	-	+	-	+
Croton macrostachyus (leaves)	+	+	+	-	+	-	+
Senna didymobotrya	+	+	+	-	+	-	+
Coleus barbatus barbatus leaves	+	+	-	-	+	_	+
Coleus barbatus (Andrews)Benth (roots)	_	+	+	-	+	+	+
Elaeodendron buchananii (leaves)	+	+	+	+	+	_	+
Elaeodendron buchananii (bark)	+	+	+	_	+	_	+

Key + Present
-Absent

Antimicrobial activity

The antimicrobial activity of the five selected plants was determined using the disc diffusion method. The average areas of inhibition of the plant extracts against specific bacterial and fungal isolate are summarized in Table 3.

Staphylococcus aureus growth was inhibited by all plant extracts except *Croton macrostachyus* leaves and *Cordia africana* leaves. The study found that *Senna didymobotrya* (plate 2) and *Elaeodendron buchananii* barks exhibited some activity of antimicrobial potency towards *Staphylococcus aureus*, with average zones of inhibition of 13.00 mm.

In addition, *Elaeodendron buchananii* leaves and bark exhibited the highest level of antimicrobial activity against *Streptococcus pyogenes*, with an average zone of inhibition of 16.33 and 14.67 mm respectively. However, *Senna didymobotrya*, *Croton macrostachyus* leaves and bark, as well as *Cordia africana* leaves and bark, were unable to inhibit the growth of *S. pyogenes.*

Only the leaves extracts of *Elaeodendron buchananii* inhibited the growth of *Salmonella typhi* with a mean inhibition zone of 12.00 millimeters.

Two plant species (*Croton macrostachyus* and *Cordia africana*) which had been identified to be used in herbal medicine were ineffective against all the tested organisms.

The plant extracts showed high microbial activity against Gram-positive bacteria, including *Staphylococcus aureus*, *Bacillus subtilis* and *Streptococcus pyogenes*. Nevertheless, the plant extracts were ineffective in inhibiting growth of *Salmonella typhi, Enterococcus faecalis* and *Escherichia coli*. Furthermore, none of the plant extracts exhibited any inhibitory activity against *Candida albicans*.

Plates 2: Zone of inhibition of the plant extracts against *Staphylococcus aureus*

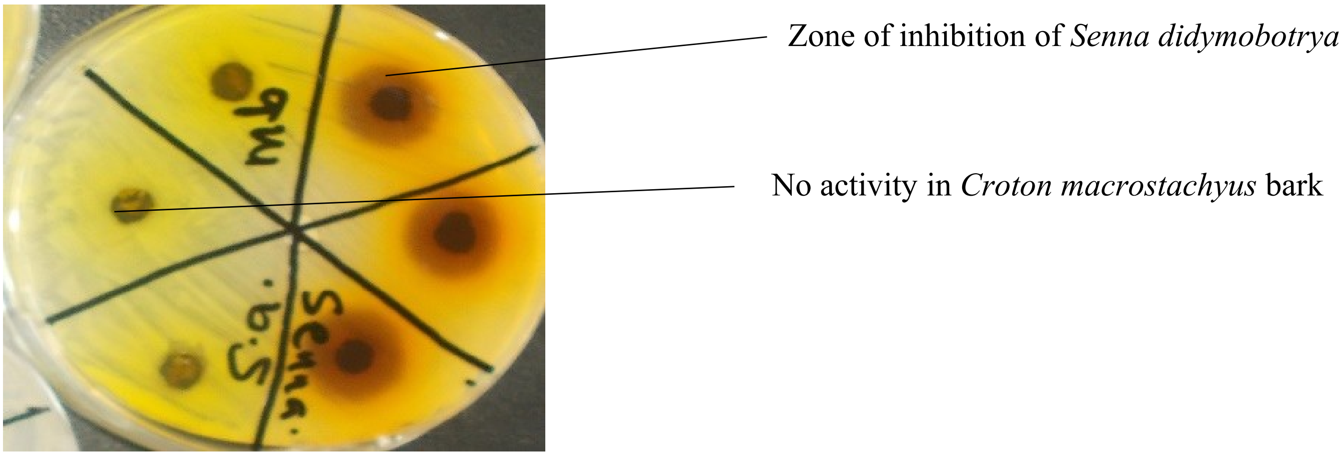

Table 3: The mean zones of inhibition (in millimeters) of the selected medicinal plants against the test cultures (SD error ± 0.1546**)**

Medicinal plants	*S. aureus*	*B. subtilis*	*E. faecalis*	*S. pyogenes*	*E. coli*	*S. typhi*	*C. albicans*	mean
Elaeodendron buchananii (leaves)	12.67	10.00	6.00	16.33	6.00	12.00	6.00	9.904 b
Elaeodendron buchananii (bark)	13.00	9.67	6.00	14.67	6.00	6.00	6.00	8.286 c
Coleus barbatus roots	10.33	10.33	6.00	11.00	6.00	6.00	6.00	7.951d
Croton macrostachyus (bark)	8.00	6.00	6.00	6.00	6.00	6.00	6.00	6.285 gh
Croton macrostachyus (leaves)	6.33	6.00	6.00	6.00	6.00	6.00	6.00	6.047hi
Senna didymobotya whole plant	12.00	8.00	6.00	7.00	6.00	6.00	6.00	7.285f
Cordia africana leaves	6.33	6.00	6.00	6.00	6.00	6.00	6.00	6.000 i
Cordia africana bark	9.66	6.00	6.00	6.00	6.00	6.00	6.00	6.499 gh
Positive control	23.00	18.00	21.00	20.00	17.00	10.00	18.00	17.857 a
Negative control	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.000 i

Key -mean inhibition values not connected by the same letter are significantly different

Minimum Inhibitory Concentration (MIC)

MIC was investigated for *Cordia africana* bark, *Senna didymobotrya* whole plant, *Coleus barbatus* leaves and roots and *E. buchananii* leaves and bark. These plants showed a region of inhibition of ≥ 9mm after 24 hour incubation.

The study identified that at a concentration of 31.25 mg/ml, *E. buchananii* leaves were able to deter the growth of *Staphylococcus aureus* while *Senna didymobotrya* and *Coleus barbatus* leaves inhibited growth at concentration of 62.50 mg/ml. *Coleus barbatus* root and *E. buchananii* bark had a similar MIC of 125.00 mg/ml. While *Cordia africana* extract deterred the proliferation of *S. aureus* at a concentration of 250.00 mg/ml.

Senna didymobotrya inhibited growth of *B. subtilis* at a very high concentration of 250.00 mg/ml. *Coleus barbatus* leaves and *Elaeodendron buchananii* bark inhibited growth at a concentration of 125.00 mg/ml while *Coleus barbatus* roots and *Elaeodendron buchananii* leaves had similar MIC of 62.50 mg/ml against *Bacillus subtilis.*

Elaeodendron buchananii leaves were able to inhibit the proliferation of *S. typhi* at a strength of 125.00 mg/ml. All other plant extracts did not show significant antimicrobial activity against S. *typhi*.

Elaeodendron buchananii leaves had the lowest inhibitory concentration against *S. pyogenes* (15.63mg/ml). While *Coleus barbatus* leaves and root and *E. buchananii* bark had similar mic values of 62.50 mg/ml. *S. didymobotrya* had the highest inhibitory concentration of 125.00 Milligrams per milliliter (Table 4). Minimum Inhibitory Concentration (MIC) revealed *S. pyogenes* as the most susceptible microorganism, with an average MIC of 65.63 mg/ml, followed by *S. aureus* at 81.25 mg/ml and *B. subtilis* at 125.00 mg/ml.

Table 4: MIC of the methanolic plant extract (mg/ml**) against selected microorganisms.**

Species name	*S. pyogenes*		*S. aureus*		*B. subtilis*		MIC *Salmonella typhi*	
	MIC mg/ml	MBC mg/ml	MIC mg/ ml	MBC mg/ ml	MIC mg/ml	MBC mg/ ml	MIC mg/ml	MBC mg/ml
E. buchananii leaves	15.62 3	15.62 5	31.25	31.25	62.5	62.5	125	125
E. buchanaii bark	62.5	62.5	62.5	62.5	125	250	ND	ND
C. barbatus leaves	62.5	62.5	62.5	125	125	250	ND	ND
Coleus barbatus roots	62.5	62.5	125	125	62.5	62.5	ND	ND
Senna didymobotrya	125	250	62.5	125	250	500	ND	ND
Cordia africana bark	ND		250	250	ND	ND	ND	ND
positive	15.625		15.625		31.25		31.25	

DISCUSSION

The study identified 28 medicinal plants in 25 families that are utilized in treating different illnesses in humans. This shows that plants remain significant in the treatment of various human diseases. This can be accounted for by their rich source of phytochemicals. The common diseases treated were upper respiratory tract infections, malaria and skin infections. These results agree with a prior investigation carried out among the Loita Maasai community in Kenya, which revealed that respiratory and gastrointestinal infections were the primary ailments treated with herbal medicine (Nankaya, 2020). A study done by Phumthum *et al*., (2018) in Thailand also produced comparable outcome.

Female respondents seem to know more medicinal plants than the males with a mean of 5.18 and 4.70 respectively. These results agrees with other studies that also demonstrated that women have greater ethnobotanical knowledge than their male counterparts (Da Costa *et al*., 2021). This could be because they are the primary family health caregivers (Alqethami *et al*., 2017). However, a study done around the Cheragani hills forest reserve (Mbuni *et al*., 2020) showed that there was no statistical difference between men's and women's knowledge of therapeutic plants. This implies that both men and women living in that community have a close relationship with the forest. Medicinal plants knowledge depended on culture and occupation of the community. Respondents of more than 71 years were able to name more medicinal plants than any of the other age groups. This is because knowledge accumulates with increase in age and with continued interaction with the natural environment. Young people below the age of 30 were less knowledgeable about plants of medicinal value. This could be attributed to urbanization where the young people do not spend time with the older generation so as to acquire knowledge and skills of medicinal plant. Studies done in different parts of the world have produced comparable results (Odongo *et al*., 2018). This puts the knowledge of herbal medicine at risk because as older people die so does the knowledge of herbal medicine unless documented.

Respondents with university and college level of education had less knowledge of medicinal plants as compared to those of primary school level of education. The educated preferred to use modern medicine as opposed to traditional medicinal plants. Similar findings were observed in a study done by Ahmad *et al*. (2017). Their study showed that people with lower levels of education have more experience using medicinal plants than high level intellectuals.

The study showed that 63% of the knowledge acquired on plants of medicinal value was mainly acquired from parents including grandparents. These results are consistent with the study done by Tefera and Yihune (2018) which showed that traditional knowledge was transferred from the parent to the children. However, knowledge of plants used for medicinal purposes was a secret affair only passed on to the male child according to seniority and level of trustworthiness. If the first son was not trustworthy it was given to the second son. A study done by Eskedar (2011) established similar findings.

According to the study, 58 % of the plants used were harvested from the wild. This finding is in line with the one by Nankaya *et al*. (2020) who reported that the Loita Maasai harvested medicinal plants from the wild due to their proximity to the forest. According to him the community did not show signs of cultivating the plants. Wild plants are normally preferred compared to the cultivated species because they require minimum investment, are free from pesticides, and are natural. In addition they have more efficacy than cultivated species. The study also identified that some of the species such as *Prunus africana* were difficult to find due to over-harvesting of its bark. The study showed that the most prevalent method of preparation of the herbal drugs was by boiling the part of the plant in hot water. These results agree with other studies such as those by Randrianarivony *et al.* (2017), Awang *et al*. (2018) and Mahmoud *et al*. (2019). This method is commonly used because hot water has the ability to extract most compounds present in the plant (Mahmoud *et al*., 2020)

The leaves were identified to be the most frequently used plant part. These findings are in agreement with those done by Mutwiwa *et al*. (2018) in Mwala Sub County, Machakos, Kenya and also among the Ijebus of southwestern Nigeria (Segun *et al*., 2018). Studies done among the Ayata community in Dinalupihan, Philippines (Tantengco *et al*., 2018) and Ngadisari village in Indonesia also showed similar findings. The assumption in the use of the leaves is that they rejuvenate quickly, they are easy to harvest and prepare into concoctions, decoctions or infusions compared to the stem barks and roots.

Phytochemical screening of methanol extracts of *Cordia africana* were found to have alkaloids, flavonoids, terpenoids and tannins in the leaves and bark of the plants. Saponins showed positive results only in the leaves. The results are in line with those of Alhadi *et al*. (2015) and Sabry *et al*. (2022) who found that the leaves and barks contain tannins, triterpenes and flavonoids.

Croton macrostachyus tested positive for alkaloids, flavonoids, terpenoids, tannins and saponins. The results agree with those of Kiristos *et al*. (2018) who isolated alkaloids, tannins, saponins and terpenoids from the barks of the species. Alemu *et al*. (2017) also reported that methanol leaf extracts contain tannins, alkaloids, terpenoids, saponins, and flavonoids.

Croton macrostachyus and *Cordia africana* methanolic extracts were found to be ineffective against the selected microorganisms. Studies done by Alhadi *et al*. (2015) using methanolic stem bark extract of *Croton macrostachyus* have produced similar results. However, studies done by Kiristos *et al*. (2018) using ethanol extracts of *Croton macrostachyus* stem bark 100 mg/ml exhibited antimicrobial activity against *S. aureus*, *S. typhi* and *E. coli.* This showed that the nature of the solvent could determine the microbial activity of the extract. Ethanol could have extracted more or different types of phytochemicals than methanol.

Senna didymobotrya methanol extracts were found to contain alkaloids, saponins, flavonoids, terpenoids and tannins. This agrees with a study done by Korir *et al*. (2012) where they identified terpenoids, saponins and flavonoids in the leaves. Muiru *et al*. (2019) also confirmed the presence of tannins, alkaloids, terpenoids and flavonoids in the methanol leaf extracts of *S. didymobotrya*. *S. didymobotya* methanol extract at a concentration of 62.50 mg/ml fairly inhibited the growth of *S. aureus*. A study done by Jeruto *et al*. (2017) demonstrated that methanol extracts of the stem bark inhibited growth at a concentration of 75.00 mg/ml. This disparity could result from the fact that when the entire plant is used, more phytochemicals are extracted compared to when only one part of the plant is utilized. Studies carried out by Muiru *et al*. (2019) showed that methanol extracts had moderate activity against *Candida albicans* which is in contrast with this study. This disparity could be caused by the age of the plant, time of collection of the plant, type of soil and method of extraction (Arama *et al*., 2017). Antimicrobial activities against *S. aureus* were associated with the presence of phytochemicals such as alkaloids and tannins (Ngure *et al*., 2013).

The roots and leaves of *Coleus barbatus* were identified to have alkaloids, flavonoids, terpenoids and tannins. These findings are in line with those of Ezeonwumelu *et al*. (2019) who isolated flavonoids and tannins as well as simple phenolics, phenolic acids and coumarins from the same plant. The major active principle isolated from *Coleus barbatus* is forskolin (Mitra *et al*., 2020). It is one of the more than 67 diterpenoids isolated from the plant that has pharmacological effects. Nevertheless, forskolin is insoluble in water and this limits its clinical usefulness. The phytochemicals present may justify most of the traditional uses of *Coleus barbatus*.

C. barbatus showed activity against *S. aureus*, *B. subtilis*, and *S. pyogenes*, but weak or no activity against *E. coli*, *S. typhi*, *Enterococcus faecalis* and *Candida albicans*. These results are consistent with those of Matu and Van Staden (2003) who investigated the action of methanolic, hexanoic and aqueous extract of *Coleus barbatus* against *Bacillus subtilis, Staphylococcus aureus* and *E. coli.* Their study showed that methanoic extract had activity against all the Gram positive isolates. Studies also carried out by Nguta and Kiraithe (2019) also showed that methanol extracts showed activity against *S. aureus* and *Candida albicans* at a concentration of 500 mg/ml but there was no activity against *E. coli*. However, a study done by Santos *et al*. (2014) on crude ethanoic leaf extracts showed activity against *E. coli* at a concentration of 6.25 g/ml. Therefore, the type of solvent used can determine the type and quantity of phytochemicals extracted.

The study identified the presence of tannins, alkaloids, cardiac glycoside and terpenoids in the bark and leaves of *E. buchananii* in various concentrations. Phytochemical analysis on other species of *Elaeodendron* revealed the presence of phenols, flavonoids, triterpenes and tannins (Maroyi and Semenya, 2019). *E. buchananii* leaves were the most effective against the selected bacteria with mean inhibition zone of 9.90 mm, SD ± 0.155. The bark and leaves extract of *E. buchananii* showed activity against *S. pyogenes*, *B. subtilis* and *S. aureus* but little or no activity against *C. albicans*. At a concentration of 31.25 mg/ml, *E buchananii* leaves showed inhibition against *S. aureus* while the *E. buchananii* bark had a MIC of 62.50 mg/ml. However, studies done by Jenipher *et al*. (2018) using methanolic extracts of the species showed activity against *S. aureus* at a concentration of 31.25 mg/ml. Studies done by Hamza *et al*. (2006) showed that methanolic extract of the bark of *Elaeodendron buchananii* showed strong activity against *C. albicans* at a concentration of 15.62 µg/ml. This is in contrast to this study where methanol extracts of the plant were not active against the fungus. The difference could be attributed to differences in climate, soil chemistry, topography and rainfall. These factors affect the concentration of phytochemicals and thus usage of the plant.

The Gram-positive bacteria *S. pyogenes*, *S. aureus* and *B. subtilis* were fairly sensitive to the plant extracts as compared to the Gram-negative *Escherichia coli,* and *Salmonella typhi*. This is because the Gram-positive bacteria have a cell wall with a considerably thick peptidoglycan layer which is made up of a polymer that permits numerous compounds to pass through, consequently making the bacteria more susceptible to the methanolic plant extracts (Kitonde, *et al*., 2013). *Candida albicans* was resistant to all the methanol plant extracts. This is because *C. albicans* has the ability to produce biofilms on synthetic materials. These biofilms are extremely hard to eradicate and the cause of drug resistance (Ponde *et al*., 2021).

Conclusions and recommendations

The study showed that people living around Kieni forest, Kiambu County were able to identify 28 medicinal plant species and that knowledge of medicinal plants was passed on from parent to offspring. This shows that plants continue to play a key role in the health care system and that the family units are important in passing on of medicinal plant knowledge from one generation to the next. It is thus important to understand how this knowledge is transmitted to the next generation so as to safe guard this knowledge against possible loss. In addition, research on children ethnobotanical knowledge needs to be carried out.

Euphorbiaceae, Oleaceae, and Solanaceae were identified to be the most utilized plant species with the leaves being the most utilized plant part. Future research should entail identifying other species within these families that have antimicrobial properties. In addition, the abundance of species in this families need to be determined to ensure that the harvesting strategies in place are sustainable.

The most common phytochemicals identified were flavonoids, tannins and terpenoids. The chemical composition of these plants can thus be determined so as to understand their mode of action.

Elaeodendron buchananii, *Senna didymobotrya*, and *Coleus barbatus* exhibited the highest level of effectiveness on bacteria types, *Staphylococcus aureus*, *Bacillus subtilis* and *Streptococcus pyogenes*. These results form a basis for further research into phytotherapeutic properties of these medicinal plants with the aim of uncovering new and effective drugs for the treatment of microbial diseases.

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