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Validation of the Ability of Selected Medicinal Plants to Control Bacterial Diseases as Applied by Local Herbalists in Kaya Tsolokero and Kaya Kauma of Kenya

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Abstract

An ethnobotanical study was carried out in the sacred Kaya forests of Kauma and Tsolokero of Kilifi County in Kenya. Ethnobotanical data on useful and medicinal plants was collected. Communities living around these two forests depend on the diversity of flora for their livelihood. The local herbalists use indigenous trees and shrubs to heal a variety of diseases. The communities have knowledge to prepare a variety of medicinal formulations to treat diseases which have been passed down through generations. In this study, twelve medicinal plants were selected and tested to validate their ability to control conditions caused by *Escherichia coli* and *Staphylococcus aureus*. Formulations from these twelve plants were prepared using a protocol that was adopted from the local medicinal practitioners. The formulations were used in the laboratory to inhibit the growth of *S. aureus* and *E. coli* using the dual plating method. Some of the perceived medicinal plants by the practitioners and the community exhibited medicinal properties with very clear growth inhibition zones. *Mildbraedia carpinifolia* (Mfundiran'gambi), *Vernonia homilantha* (Mlazakoma), *Solanun incannun* (Mtondo) and *Senna siamea* (Mchilifi) against *S. aureus* while, *Zanthoxylum chalybeum* (Mdungu) and *Croton pseudophulchellum* (Myama wa nyika) had activity against Escherichia coli. Results in this study validated the medicinal properties of the local formulations of some of the tested plants as applied by the local herbalists.

Keywords: Sacred forest; Indigenous medicinal species; Medicinal plants; Herbalists

Introduction

Plant resources provide vital materials for survival to humanity. They serve in fulfilling economic, medicinal, forage, constructional, apiary and more importantly medicinal applications to man. Plant products [1] are considered to be the most important components of diet for good health. They also preserve cultural heritage, biological information and indigenous knowledge on their utility [2]. The traditional African people have used indigenous medicinal plants for ages to survive and maintain good health. These traditional plant concoctions have been around for much longer than western medicine. Complex interactions and effects from using different plants in traditional medicinal formulations have been known for thousands of years [3]. Various means are used to traditionally administer plant medicines: typically drinking infusions or decoctions, steaming, enemas and smoking [4]. All these preparations have their position for specific conditions. While steaming medicines is good for nervous complaints, and relaxes a person quickly, being absorbed through the mucosal membranes of the nose makes them quite effective. Other treatments such as drinking ibises and cleansing mixtures are taken for longer periods to work internally to rebalance the digestive system [4].

In 1993, the inception of the pivotal Indigenous Plant Use Forum (IPUF) through Anthony Cunningham stimulated networking among researchers. This was followed by a great thrust in ethnos pharmacological studies driven by Professor J Van Staden [5]. The earliest recorded uses of medicinal plant formulations are found in Babylon circa 1770 BC in the Code of Hammurabi and in ancient Egypt circa 1550 BC. In fact, ancient Egyptians believed medicinal plants have utility even in the afterlife of their pharaohs. Plants have been recovered from the Giza pyramids and can be found on display at the Excellence Resource Centre in Cairo Museum.

Kaya is a sacred forest of the Mijikenda people in the Coastal region of Kenya. The Mijikenda is a closely related ethnic group with very similar intonations in their language and interrelated cultural practises. They include nine tribes believed to have emanated from Shingwaya in Tanzania. The nine tribe include, Giriama, Digo, Chyoni, Duruma, Jibana, Kambe, Kauma, Rabai and Ribe. The history of Mijikenda tribe connects to Southern region of Somalia where these tribes were attacked by Cushitic and Oromo tribe and took shelter in the forests of coastal region to form Kaya forest meaning a forest with home. The literal meaning of Kaya in their language was 'Home'. The Kaya forest is considered to be an intrinsic source of ritual power and the origin of cultural identity among the Mijikenda community besides being a place of prayer. The Kaya forests of coastal Kenya are thus one example of a phenomenon that has been described from many other African countries and from other continents; the sacred forest or sacred grove [6-8]. Sacred groves range from areas of several hundred hectares to clusters of trees or even individual tree, and there are many reasons for their sacred status. Some are recognized as the home of a deity, or of ancestors or other benevolent or malevolent spirits. They may have originated as the burial sites for some ancestral founding figures, or of revered community elders. Some sacred groves are former battle grounds or sites at which a community leader first established title to the location. The settlement, ritual centre, and fortified enclosure associated with the forest are also part of the Kaya. Eleven of the approximately 30 separate Kayas were grouped together and inscribed as the Sacred Mijikenda Kaya Forests, of UNESCO World Heritage

Sites in 2008 [9]. Today Kayas are sacred places used as burial places for elders as well as for veneration ceremonies or other traditional ceremonies by the community members. Kaya Kauma is situated in the Jaribuni location, of Ganze Division in the Kilifi County of Kenya. It is primarily a Kaya of the Kauma people and occupies an area of over 100 hectares with geographical co-ordinates at S 03°:37'14" and E 39°44'10". Kaya Kauma is a Primary Kaya forest sitting at 120 m above the sea level [8]. The forest type exhibits a deciduous forest which slopes down in the north to Nzovuni river. Kauma forest slopes down at the back of Jaribuni village to Nzovuni River on the west. The surrounding areas have scrubby vegetation and are inhabited by villages and farms and the soils exhibit a rich content of iron-ore which has made the area a prominent site for iron-ore mining. Limestone quarrying is also quite prevalent here. The herbalists are greatly dependent on the medicinal flora prevalent in and around the forest for treatment of various ailments.

Kaya Tsolokero belongs to the Jibana people, is a natural forest which is located in the Junju location in Kikambala division of Kilifi County, at an altitude of 135 m above sea level and geographical positioned at S03°50' and E 39°44'34". It covers an average area of 25 acres. Kaya Tsolokero is a Secondary Kaya Forest, an extension of Kaya Jibana. This forest is highly degraded and a threat to the indigenous plants species. The vegetation exhibits an evergreen thick forest with a variety of floral diversity and rich culture nursed by the communities. The culture and traditions of the Mijikenda have saved the forest in the last decades from the expanding tourism industry, and the increasing demand for land due to a growing population besides the increasing demand for natural resources such as firewood. The population around the sacred forests of Kaya Kauma and Kaya Tsolokero exhibit a great dependency on the medicinal plants to treat a variety of diseases.

Plants have been used in traditional medicine for several years. Medicinal plants have been used to treat the spiritual origins of disease as well as the physical symptoms [9]. The vast knowledge of such plants is now getting acknowledged by the modern world and the role played by indigenous people as custodians of the world's genetic heritage is now recognized [10]. Local medicinal knowledge has led towards development of therapeutic concepts which has accelerated some drug discoveries. This phenomenon is now referred to as reverse pharmacology [11].

Preliminary study has shown that fifty five plant species from Kaya Kauma and forty seven from Kaya Tsolokero were claimed to be of great medicinal value [12]. The crude methods used for extraction of medicinal concoctions were reported as boil, grind, smoke, infusions from sun-dried or raw plant tissues and direct applications on the body. The common diseases treated by the communities using the localized flora included muscle pains, malaria, skin diseases, asthma, measles, eye treatment, stomach ache, diarrhea, immune booster, intestinal worms, blood pressure, gonorrhea, cough, yellow fever, jaundice, treatment of boils, wounds and croissants, diarrhea in livestock, ease of delivery, treatment of bones, epilepsy, diseases in poultry, treatment of snakebites, blood enhancer, blood clotting, chest pains, diphtheria, anemia and scabies.

In this study, medicinal plants that were reported to treat bacteria related cases were identified. Their ability to treat bacterial related diseases was rationalized in the laboratory where infusions prepared using methodologies adopted from those used by the local herbalists were tested.

Materials and Methods

Selection of plant material

Semi-structured questionnaires were circulated among the villages surrounding the two Kaya forests to interrogate the population and the local herbalists on plant species perceived by the community to have medicinal properties. Plants species cited in this exercise were recorded. From the list, a total of 11 plant species that were perceived to treat bacteria related ailments were shortlisted for laboratory evaluation (Table 1). The traditional way of preparing medicinal concoctions was recorded as explained by the local herbalists.

Site	Local Name of Plant	Scientific Name	Plant Part claimed as	Disease treated
Kaya Kauma	Mudungu	*Zanthoxylum chalybeum*	Roots	Chlorea
	Mvumo	*Premna chrvsoclada*	Roots	Diptheria
	Mkulu	*Diospyros cornii*	Bark	Skin disease/Scabies
	Myama wa nyilca	*Croton pseudopulchellus*	Stem	Malaria
	Mdungu	*Zanthoxylum chalybeum*	Stem	Typhoid Fever
	Mtsalafu	*Senna occidentalis*	leaves	Stomach ache
Kaya Tsolokero	Mdunguhitswa	*Zanthoxylum holtziannum*	Roots	Brucellosis
	Mfundiragambi	*Milbraedia carpinifolia*	Bark	Cough
	Mfundiragambi	*Milbraedia carpinifolia*	Roots	Cough
	Clubiriti	*Pslilotrichum sclerenthum*	leaves	Listeriosis
	Mclungulutswa	*Zanthoxylum holtziannum*	Roots	Brucellosis
	Mfundiragambi	*Milbraedia carpinifolia*	Bark	Cough
	Mfundiragambi	*Milbraedia carpinifolia*	Roots	Cough

	Clubiriti	*Pshlotrichum sclerenthum*	leaves	Listeriosis
	Mlazakoma	*Vernonia hornilantha*	leaves	Stomach ache
	Mkwamba	*Fluggea virosa*	Roots	Leprosy

Table 1: List of medicinal plants selected for laboratory analysis.

Preparation of medicinal concoctions: Laboratory analysis of the eleven selected plants was done. Concoctions were prepared in the laboratory where the plant part was soaked in water in the ratio of 1:1 (wt/vol). The methods of preparing concoctions for each plant was adopted from the practice of the local herbalist with a few adjustment as described in Table 2.

Site	Local Name	Scientific Name	Ways of treatment of the part of the Plant	Age of harvest of Plant
Kaya Kauma	Mdungu	*Zanthoxylum chalvbeum*	Boil Roots and Stem, Filter the water and drink	Mature
	Mvumo	*Premna chrysoclada*	Scratch the top layer of the roots :mix with oil and apply directly on the place infected with Candidiasis	Mature
	Mkulu	*Diospyros cornii*	Scratch the bark and apply the powder on the infection	Mature
	Myama wa nyika	*Croton pseudo pulchellus*	Boil the roots /stem and drink the water	Anytime
	Mdungu	*Zanthorylum chalvbeum*	Boil Roots and Stem, Filter the water and drink	Mature
	Mtsalafu	*Senna occidentalis*	Raw leaves juice to drink	Anytime
Kaya Tsolokero	Mdungulutswa	*Zanthacylum holtziannum*	Boil roots and drink water	Anytime
	Mfundiragambi	*Milbraedia carpinifolia*	Scratch the bark of the stem and top layer of the root to chew	Anytime
	Chibiriti	*Pslilotrichum sclerenthum thwaites*	Ash of the leaves are used	Anytime
	Mlazakoma	*Vernonia homilantha*	Boil the leaves and drink water	Sapling to mature
	Mkwamba	*Fluggea virosa*	Boil the leaves and drink water	Anytime
	Mchewa/Mpewa	*Flagelleria guineensis*	Roast and apply on the surface of the body above the kidneys	Mature

Table 2: Crude Way of Treatment of Aforementioned Plant Parts by Local herbalists in Kaya Kauma and Kaya Tsolokero.

Concoctions prepared from roasted plant samples 100 g of the plant samples were roasted in Maffol Furnace at 450°C for 1 hour. The resultant ash was dissolved in 100 ml SDW to maintain the ratio 1:1. Similarly for boiled samples 100 g was boiled in 200 ml of SDW until the volume reduced to 100 ml. Samples that did not require boiling were directly introduced into SDW in the ratio of 1:1. All the prepared concoctions were allowed to cool at 4°C overnight. The prepared concoctions were filtered using blotting paper and thereafter sterilised through 0.2 µm Millipore filter using a syringe in a biological safety chamber. The samples were stored at 4°C until further use.

Test Pathogen: The bacteria *Staphylococcus aureus* that causes minor skin infection, abscesses, pneumonia, meningitis, bacteria derma [13] and many more diseases similar to those described by the local herbalist was chosen to validate the efficacy of the selected plant species. Similarly, *Escherichia coli* that causes bloody diarrhea, stomach upset, anaemia, [14] urinary tract infections and subsequently kidney failure was also chosen as a test pathogen. *E. coli* accession number AP001918 and *S. aureus* accession number AP003367 were selected for these tests.

Bioassay

Nutrient Agar (NA) and MacConkey Agar media were sterilized at 121°C for 15 minutes and introduced into Petri plates under sterile environment. Penicillin (Ampiclox) was dissolved in DSW and a concentration of 25 mg/ml was used in the trials as a positive control. *E. coli* and *S. aureus* were cultured on MacConkey agar at 25°C overnight. Using wire loop a scoop of the developed colony was introduce into 10 ml of SDW and vortexed to mix. The suspension was then serially diluted in SDW to $\times 10^{-7}$ Form the $\times 10^{-7}$ suspension, 100 µl was drawn and plated on nutrient agar. The bacterial suspension was then spread to dry using a sterile glass rod and allowed to dry under sterile environment.

The prepared plant concoctions were serially diluted in SDW to $\times 10^{-3}$. For each petri plate, the following treatments were made; 1 was Penicillin (Ampiclox) at 25 mg/ml, 2 was SDW, 3,4,5 and 6 were plant concoctions at straight concentration, diluted at $\times 10^{-1}$; $\times 10^{-2}$; $\times 10^{-3}$, respectively. Using a micropipette 30 µl of the test samples were loaded onto sterile discs, allowed to dry then mounted on the Petri dish inoculated with *S. aureus* and *E. coli* as shown in Figure 1. The petri

plates were then sealed with Para film and incubated at 28°C for 24 hours in the incubator. Three replicates for each plant species and bacterial isolates were prepared.

It was then allowed to dry and the discs were placed equidistant on the inoculated Petri plates as described above.

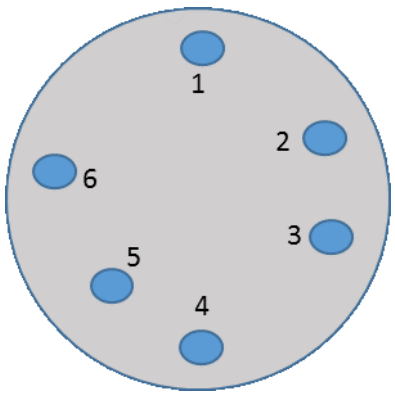

Figure 1: Zone of inhibition. [1]Penicillin (Ampiclox), [2]SDW, [3]Straight concentration, [4]$\times 10^{-1}$ diluted concoction, [5]$\times 10^{-2}$ diluted concoction, [6]$\times 101540^{-3}$ diluted concoction.

Results and Discussion

Kara	Medicinal	Food	Constructional	Fuel firewood and Bee Keeping	Recreational	Total
auma	73	43	15	25 10	10	176
Tsolokero	120	74	64	15 2	29	304
Total	193	117	79	40 12	39	480

Table 3: Number of useful plant species identified by residents of Kaya Kauma and Tsolokero.

Residents from Kaya Kauma and Kaya Tsolokero identified about 480 plant species as useful. Out of the total identified useful plants 40% were medicinal species. From the medicinal plants identified, the herbalists reported to utilize 66 medicinal species in Kaya Kauma and 60 in Kaya Tsolokero (Data not shown).

Kaya	Bark	Stem	Leaves	Root	Fruit
Kauma	26%	10%	68%	30%	6%
Tsolokero	11%	12%	43%	30%	16%

Table 4: Percentage of different Plant parts utilized by the Herbalist in the study area.

Out of all the plant parts reported, leaves were the most utilized part by the herbalist at 68% and 43% for Kaya Kauma and Kaya Tsolokero, respectively (Table4). The Bark was used more to prepare medicinal concoctions in Kaya Kauma compared to Kaya Tsolokero. Despite the high biodiversity exhibited in Kaya Tsolokero, plants were used more as medicine by the herbalist of Kaya Kauma (41%) compared to Kaya Tsolokero at 38%. The raw fruits were used more for medicinal purposes by the herbalist in Kaya Tsolokero compared to Kaya Kauma as in Figure 2.

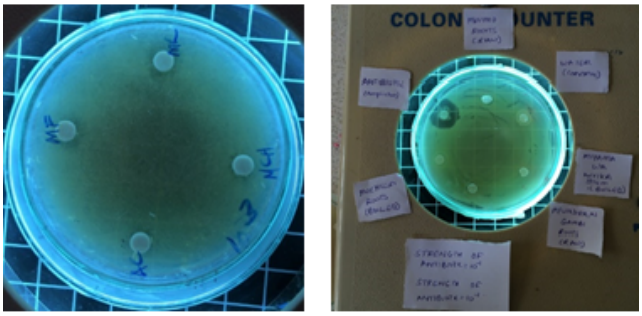

Figure 2: Inhibition zone shown by different plant extracts against the growth of certain bacteria.

No.	Local name of the plant	Genus	Species	Name of Microbe	Activity	Name of the Microbe	Activity Part	Part Harvested	Mode of Preparation
1	Mfundiranigambi	*Mildbraedia*	*carpinifolia.*	*E. coli*	-	*Staphylococus aureus*	-	Bark	Raw
2	Mfundiranigambi	*Mildbraedia*	*carpinifolia.*	*E. coli*	-	*Staphylococus aureus*	++	Root	Raw
3	Mlazakoma	*Vernonia*	*homilantha*	*E. coli*	-	*Staphylococus aureus*	++	Leaves	Boil
4	Mchewa'Mpewa	*Flagelleria*	*guinebsis*	*E. coli*	-	*Staphylococus aureus*	-	Leaves	Roast
5	Mtondo	*Solanum*	*incannum*	*E. coli*	-	*Staphylococus aureus*	++	Roots/Fruits	Raw
6	Mvumo	*Premna*	*chrysoclada*	*E. coli*	-	*Staphylococus aureus*	-	Root	Raw

7	MIculu	*Diospyros*	*cornii*	*E. coli*	-	*Staphylococus aureus*	-	Bark	Ash
8	Mushomoro	*Lantana*	*camara*	*E. coli*	-	*Staphylococus aureus*	-	Leaves	Raw
9	Mdungu	*Zanthacylum*	*chalybeum*	*E. coli*	++	*Staphylococus aureus*	-	Root	Boil
10	Mdungu	*Zanthacylum*	*chalybeum*	*E. coli*	-	*Staphylococus aureus*	-	Stem	Boil.
11	Mdungulutswa	*Zanthacylum*	*sps*	*E. coli*	-	*Staphylococus aureus*	-	Root	Boil
12	Muchilffi	*Senna*	*siamea*	*E. coli*	-	*Staphylococus aureus*	++	Root	Boil.
13	Myama wa nyika	*Croton*	*psetuiopchyllum*	*E. coli*	-	*Staphylococus aureus*	-	Stem	Boil.
14	Mchewa/Mpewa	*Flagelleria*	*guinensis*	*E. coli*	-	*Staphylococus aureus*	-	Leaves	Ash
15	Mdungu	*Zantharvlum*	*chalvbeum*	*E. coli*	++	*Staphylococus aureus*	-	Stem	Boil.

Table 5: Bioactivity of selected medicinal plant species against *E. coli* and *S. aureus*.

Boiled roots of *Zanthoxylem chalybeum* (Mdungu) inhibited the growth of *E. coli* but didn't inhibit *Staphylococcus aureus* at the applied concentration.*S. aureus* was inhibited by concoctions prepared from raw roots of *Mildbraedia carpinifolia* (Mfundiragambi); boiled leaves of *Vernonia homilantha* (Mlazakoma), Raw roots and fruits of *Solanum incannum* and boiled roots of *Senna siamea* (Mchilifi). Applied volumes in our tests was 30 µl but the actual recommended doze by the herbalist may range from one to three times a day which is approximately 200 ml for one glass. In essence the actual doze is 2000 times more than what was used in our tests. Probably the other plants that tested negative for respective bacteria would have been effective if higher concentration was applied. Our results however proved the efficacy of some species to control bacterial populations *in vitro* which is corroborates the medicinal properties of the plants.

The efficacy of *Zanthoxylem chalybeum* to control cholera (Vibrio cholera) was demonstrated by Olilia. In this study *Z. chalebyum* effectively suppressed *E. coli*. It was also evident of the medicinal traits by the suppression of *E. coli* by this plant extract. A significant reduction in blood glucose was observed within treatment in a group of rats after oral administration of glucose from time zero [15]. he cytotoxicity of two diterpenes from *Premna schimperi* and *Premna oligotricha* (Verbenaceae) was studied using the MTT assay. heir cytotoxic activity against three human (HeLa, SK.N.SH, and ECV 304) and two murine (L929 and RAW 264.7) carcinoma cell lines varied between 1.5 to 35 micrograms/ml and was comparable with azauridine and chlorambucil [16]. *Premna corymbosa* can be recommended for the liver disorders [17]. Nine known compounds have been isolated from the stem bark of *Premna integrifolia* [18]. Species under Croton genus are found in the different parts of the world and are widely used for the treatment of bacterial infections [19]. From different Croton species different methods were used to extract the active contents from roots, leaves, stem-bark and seeds). his inhibition was also evident in the laboratory analysis. Bactericidal and antifungal assays were done using extracts derived from *Z. chalybeum* and *W. ugandensis* (agar well diffusion, disc diffusion and colony count assays). Most reports suggested that extracts of the seed and stem were most often used both the crude and the purified fractions of *Z. chalybeum* had no antibacterial activity against *E. coli* and *S. aureus* (Table 1). his was true in all assays i.e., paper disc, agar well diffusion as well as the colony count assay. [19] The results obtained showed that *Senna occidentalis* (L.) leaf extracts have interesting pharmacological active compounds with great radical scavenging and antimicrobial effects and as such could be used in ethno medicine for treatment of some infections and ailments. Antioxidant Antimicrobial *Senna occidentalis* (L.) Phytochemicals Organisms Ethno medicine [20]. Staphylococcus aureus and *Escherichia coli*, equally determined to evaluate the antioxidant activities of the extracts. Pharmacological properties and pharmacodynamic Properties of *Zanthoxylem* was recognized through in vitro Experiments on Antimicrobial activity he antibacterial and antifungal activities of fruit essential oil; leave and roots bark were demonstrated [20,21] Anti-parasitic activity Roots extracts were found to be significantly active against the intracellular form of *Leish maniamajo* parasite [22]; while leaves extract has presented lowest anthelminthic activities on *Ascaris lumbricoides* [23]. he non-polar fractions from crude alkaloid was displayed a good anti-plasmodial (W2) with a IC50 ranging 1.91 to 4.32 µg/ml [24]. he ethanolic root bark extract (150 mg/kg to 500 mg/kg, p.o.) has gastroprotective effect in Sprague-Dawley rats working possibly via antimuscarinic or antihistaminic mechanism [25]. he leaves powder (3.2 g/kg to 4.8 g/kg p.o.) demonstated a moderate antihelmintic effect [26,27] *Flueggea virosa* . heir structures were assigned via spectroscopic methods with the absolute configurations of 1 and 2 being established by X-ray diffraction analysis and calculated electronic circular dichroism data, respectively. Both alkaloids showed mild in vitro anti-HIV activity [28]. Four new securinega-type alkaloids, fluevirines A-D (1-4), along with ten known ones, were isolated from the twigs and leaves of *Flueggea virosa* [29,30]. Among them, fluevirine at (1) was a novel C, C-linked dimeric indolizidine alkaloid and showed weak antimicrobial activity against *Staphylococcus aureus* [31]. he inhibitory activity on pancreatic lipase enzyme of Diospyros kaki fruit

and Citrus unshiu peel mixture extract (PCM) was evaluated *in vitro* and its anti-obesity effects were studied based on the serum lipid parameters analysis from high-fat diet- (HFD) fed mice *in vivo* [32]. No there is report on *D. cornii* recorded till date. No extensive work done on carpinifolia species however the species is identified in Anthelmintic activity of medicinal plants for treating parasitic diseases in Cote d'Ivore. Further studies are necessary to isolate the active components from these extracts as stated in Springer-Verlag [33]. No work has been done on the species *Pslilotrichum sclerenthum* hwaites (Chibiriti) regarding the phytochemicals presence. However the species has been recorded in the records of Synonymic Notes on African Dynasties [34]. No report on this species of *Vernonia homilantha* (Mlazakoma). However the genera "Vernonia" is known for its medicinal importance and ethno pharmacological relevance. Vernonia species were identified to be used in ethno veterinary medicine while two species are used in self-medication practices by chimpanzees and gorillas [34]. *Flagellaria guineensis* (Mchewa) hasn't been worked much. However this plant has been recognised by the PROTA (Plant Resources of Tropical Africa) as its occurrence in the subtropical indigenous forests in the coastal districts [35]. In general, the potential exists in this district to promote the basket industry by intensifying the harvesting of *F. guineensis* [36].

The medicinal effects of these raw plants could be more effective with the enzymes in the human body. But their claim on the medicinal species to heal certain diseases couldn't be contradicted. It also proves that the residents and the local practitioners do possess a very rich indigenous knowledge towards their flourishing flora. The efficacy of many chosen species has been known for its medicinal traits and in treatment of variety of diseases as cited above. However the chosen medicinal species whose scientific analysis hasn't been done also indicates its medicinal importance from the genera it belongs to which has been identified by other researchers.

Conclusion

The results of this study have revealed that different medicinal species as claimed by the herbalists of Kaya Kauma and Kaya Tsolokero has been validated truly for their medicinal properties. It can treat a variety of bacterial and other disease. The knowledge of the local practitioners is true and ethical. The dwelling population around these Kaya forests are actually benefited from these Herbalists.

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