Antimicrobial Activity of Kenyan Laikipia County *Leonotis mollissima* Plant Extract

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Abstract

The use of herbal medicine is expanding rapidly across the world due to the high bills involved in the importation of modern medicinal drugs. About 80 percent of the African population use traditional medicine from plants to treat common infectious diseases caused by microorganisms. The main objective of this research was to determine the antimicrobial activity of crude extracts and isolated compounds of Laikipia University *Leonotis mollissima* (Lion's ear) from the *Lamiaceae* family. The plant was studied in this research due to its wide use by local communities of Kenya for medicinal remedies. Its decoction is used by the Marakwets of Kenya to treat microbial diseases. The plant was sampled from Laikipia University Kenya, identified, air dried and voucher specimen kept for reference in the Biological Department of Egerton University in Kenya. Crude extracts from dry powder of the leaves were successively extracted with hexane, dichloromethane, ethyl acetate and methanol for seventy-two hours. With repeated column chromatography, *Leonotis mollissima* dichloromethane leaves (79.69 g) crude extract yielded compounds **1** (Sederin), **2** (20-hydroxylucidenicacid D2) and **3** (labdane). Identification of pure compounds was achieved by ^{1}H and ^{13}C NMR (500 MHz) spectroscopy. Chemical shifts (δ) were expressed in ppm relative to tetramethylsilane (TMS) as internal standard and coupling (*J*) in Hz. On screening for antimicrobial activity, all crude extracts showed an **MIC** (Minimum Inhibition Concentration) of < 0.1 mg/mL to > 0.5 mg/mL on all test microorganism (*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* and *Candidas albican*. Compounds **1** and **3** had an **MIC** of > 0.16 mg/mL on all test microorganisms while **2** compound had an **MIC** of 0.10 mg/mL. The **IC_{50}** (Inhibition Concentration that reduces the effect of microorganisms by 50%) for all crude extracts and isolated compounds was less than that of Amoxil® and Doxycycline® antibiotics on all test micro-organisms on calculation with Graphpad Prism 8 free download computer program at different concentrations. This is a confirmation that *Leonotis mollissima* contains compounds that can be isolated and used as drugs to treat various diseases including microbial infectious diseases.

Keywords: Kenya, Laikipia County, *leonotis mollissima*, minimum inhibition concentration (MIC)

Introduction

Many people in the world depend on plants for food, clothing, shelter and health care. Historically, plant medicines were discovered by trial and error (Facchini et al., 2000). Many higher plants have been the source of medical agents since the earliest times and today, they continue to play a dominant role in the primary health care of about 80 percent of the world's population (Tilburt &

Kaptchuk, 2008). In Africa, up to 60 percent of the population consult one of an estimated 200,000 traditional healers especially in rural areas where these healers are more numerous and accessible than allopathic physicians (Van Wyk et al., 2000). The people in Asia, North and South America, as well as Australia and New Zealand have used concoctions prepared from a wide range of medicinal plants for treating the sick. The information on which plant and what part of the plant cures which disease was passed on from generation to generation. This rich heritage of traditional medicinal practices was looked down upon following the slicing of third World countries into fragmented pockets with European spheres of influence. It was branded as primitive although many pharmaceutical drugs and medicinal syrups administered to patients in modern hospitals are of plant origin (De Sa' Ferraira & Ferrao, 1999).

Medicinal agents derived from plants are also an essential feature in the health care system of the remaining 80 percent of the population residing mainly in developing countries. Of the world's twenty-five best-selling pharmaceutical agents, twelve are derived from natural products, which continue to play an important role in drug discovery programmes of the pharmaceutical industry and other research organizations (Chen et al., 2016). Without plants, most medicines taken would not exist. Over 40 percent of medicines now prescribed in USA contain chemicals derived from plants extracts (Facchini et al., 2000).

Throughout the world, botanists and biochemists search the plant kingdom for new medicines. For example, the native Pacific yew was burned as trash generated by logging operations in the Pacific Northwest. In 1975, a substance in its bark, taxol, was found to reduce the production of cancerous tumours (Facchini et al., 2000). A comprehensive search of known plants for medicinal chemicals is an enormous task. Of the estimated 250,000 plant species on earth, only 2 percent have been thoroughly screened for chemicals with potential medicinal use. Many native plant habitats are destroyed almost daily and therefore many medicinally valuable plants will be gone before scientists can investigate them (Facchini et al., 2000). Although plant extracts have been used in the treatment of diseases, research has shown some secondary metabolites present in these medicinal plants to be potentially toxic and carcinogenic, thus care should be taken before use (De Sa' Ferraira & Ferrao, 1999). Secondary metabolites are molecules that are not necessary for the growth and reproduction of a plant. They may serve some role in herbivore deterrence due to astringency or they may act as phytoalexins, killing bacteria that the plant recognizes as a threat. They are often involved in key interactions between plants and their abiotic and biotic environments that influence them (Facchini et al., 2000).

Lion's ear (*Leonotis mollissima)* belongs to the mint family: Lamiaceae, genus *Leonotis* that comprises about 10 species (Nurdan & Aysel, 2007). The plant is known to treat cold, cough, fever, headache and asthma (Fowler, 2006). Among the Marakwets, the root decoction is used to treat wounds, festering sore and intestinal worms. Young leaves and buds are used to treat conjunctivitis and indigestion and are also chewed for cramp in the stomach (Kokwaro, 1976). However, no antimicrobial activity has been reported on the Kenyan Laikipia *Leonotis mollissima* crude extract. This research, sought to extract and isolate chemical compounds from the *Leonotis mollissima* leaves, and test their antimicrobial activity on selected bacteria.

Materials and Methods

Leaf samples of *Leonotis mollissima* were obtained from Laikipia University Laikipia county in Kenya. These were put in sample bags in June 2014, and air dried in shade. The air dried and ground leaves were sequentially extracted using hexane, dichloromethane, ethyl acetate and methanol for 72 hours. Exactly 10 μL to 50 μL of 10 mg/mL crude extracts and 5 μL to 40 μL of

4mg/mL isolated compounds were tested for antimicrobial activity on *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 10876 and an isolate of *Candida albicans* in duplicates. Extraction solvent was used as negative control (Oshomoh, 2012). The crude dichloromethane extract showed more compounds and its fraction was purified by step gradient isolation (dichloromethane/methanol) followed by repeated column chromatography (ethyl acetate/hexane).

Determination of **MIC** (Minimum Inhibition Concentration) was carried out for all the crude extracts and isolated compounds in a serial dilution. Methanol was used as negative control (Oshomoh, 2012). The lowest concentration with the smallest inhibition zone was taken as the **MIC.** For the **IC_{50}**, different concentrations of Amoxil® and Doxycycline® antibiotics (10.000 mg/L, 4.000 mg/L, 1.000 mg/L, 0.400 mg/L, 0.100 mg/L, 0.040 mg/L, 0.010 mg/L and 0.004 mg/L in methanol) were prepared using serial dilutions method. The IC_{50} for Amoxil® and Doxycycline® antibiotic was determined using probit analysis software (GraphPad Prism was used to plot inhibition zone against log of concentration of Amoxil® and Doxycycline® antibiotics). The IC_{50} for the crude extracts and the pure compounds were determined in a similar way. Their IC_{50} were then compared with the IC_{50} for Amoxil® and Doxycycline® antibiotics (Oshomoh, 2012).

Results

From dichloromethane crude extract of Laikipia leaves sample (79.69 g), three compounds **1 (**Siderin, white pow 7.70 mg)**, 2** (20-hydroxylucidenicacid D2, 7.10 mg) and **3** (13R)-19*α*,13*α*-epoxylabda-6*β*(19).16(15)-dioldilactone, 21.20 mg) were isolated with repeated CC and Thin Layer Chromatography (TLC) (Kinuthia et al., 2018) (see figure 1).

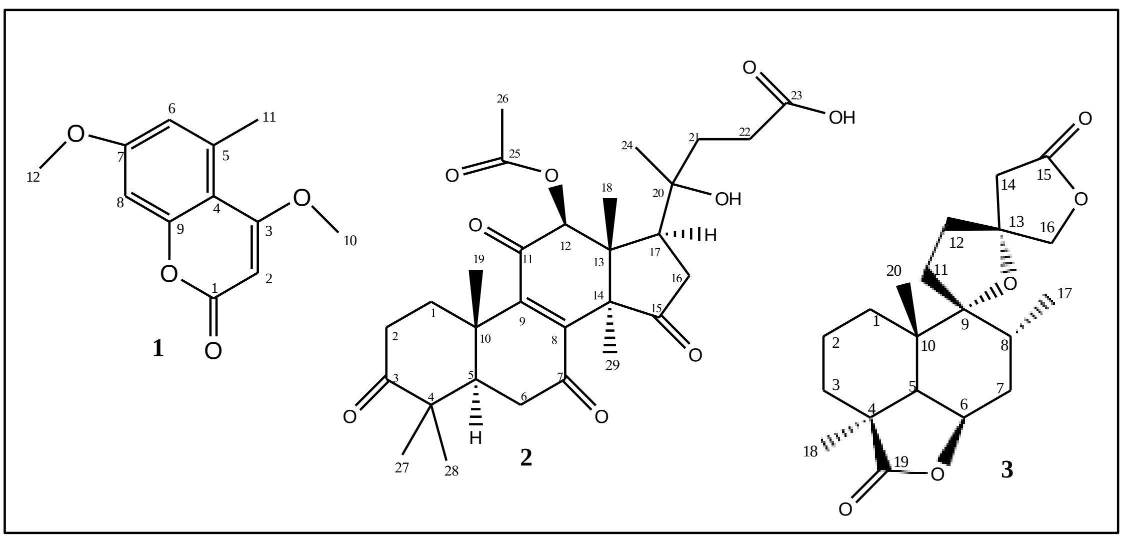

Fig. 1: Structures of Compounds Isolates from Laikipia County, *leonotis mollissima*

All the crude extracts had very significant antimicrobial activity on *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus cereus* and *Candida albicans* at a concentration of 1mg/mL as indicated in table 1. All the organic extraction solvents did not show any activity. Of all the compounds isolated, only compound **2** showed significant antimicrobial activity on *Escherichia coli* at a concentration of 0.4 mg/mL as indicated in table 2.

Table 1: Inhibition Zone (mm) of Crude Extracts at a Concentration of 1 mg/mL

Sample	Micro-organism					Control
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella typhimurium*	*Candida albicans*	
Hexane extract	16	16	9	17	11	-
Dichloro-methane extract	12	20	12	20	11	-
Ethyl acetate extract	9	14	12	12	10	-
Methanol extract	I0	11	11	15	11	-

Table 2: Inhibition Zone (mm) of Compound 2 (20 Hydroxylucidenicacid D2) at Different Concentrations

Micro-oganism	Concentrations (mg/mL)							
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Bacillus cereus	-	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-	-
Escherichia coli	-	-	-	-	6	6	7	9
Salmonella typhimurium	-	-	-	-	-	-	-	-
Candida albicans	-	-	-	-	-	-	-	-

The crude extracts had an MIC of < 0.1 mg/mL to > 0.5 mg/mL on all test microorganism as indicated in table 3. Compounds **1** (Siderin) and **3** (Labdane) had an MIC of > 0.16 mg/mL on all microorganisms while compound **2** (20-hydroxylucidenicacid D2) had an MIC of 0.08 mg/mL on *Escherichia coli* as indicated in table 3. Siderin is known to have a variety of bioactivities including anticoagulant, estrogenic, dermal photosensitizing, anti-microbial, vasodilator, molluscacidal, antihelmintic, sedative and hypnotic, analgesic and hypothermic activity (Divakar & Parminder, 2017). Lanostene-tetracyclic triterpenes possess anti-tumor, anti-inflammation, antioxidant, antimicrobial and blood fat reducing effects (Qing et al., 2014).

Additionally, a variety of biological activities have been encountered in labdane diterpenes such as antibacterial, antifungal, antiprotozoal, enzyme inducing, anti-inflammatory activities and modulation of immune cell functions. They also exhibit significant cytotoxic and cytostatic effects against leukemic cell lines of human origin (Costas & Konstantinos, 2001). This is an indication that *Leonotis mollissima* have compounds that can be developed to treat infectious microbial diseases.

Table 3: MIC of Crude Extracts and Pure Compounds on Test Micro-organism

Sample	MIC (Minimum Inhibition Zone) mg/mL				
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella typhimurium*	*Candida albicans*
Hexane extract	< 0.1	0.1	0.1	< 0.1	> 0.5
Dichloromethane extract	< 0.1	< 0.1	0.1	0.2	>0.5
Ethyl acetate extract	> 0.5	0.3	< 0.1	0.2	>0.5
Methanol extract	0.4	> 0.5	< 0.1	0.1	>0.5
Siderin **(1)**	> 0.16	> 0.16	> 0.16	> 0.16	> 0.16
20-hydroxylucidenicacid D2 **(2)**	> 0.16	> 0.16	0.08	> 0.16	> 0.16
Labdane **(3)**	> 0.16	> 0.16	> 0.16	> 0.16	> 0.16

The IC_{50} for dichloromethane crude leave extracts (see figure 1 and also table 4) was 4 and 12 times less that of Amoxil® antibiotic on *Bacillus cereus* (figure 2). The IC_{50} for Doxycycline® antibiotic on *Escherichia coli* was found to be 4.5 and 4.7 times more on comparison with both dichloromethane and ethyl acetate crude extracts (table 4). These concentrations were too low compared to those of the two antibiotics. Compound **2** (20-hydroxylucidenicacid D2) had an IC_{50} of 0.141 mg/mL (see figure 3 and also table 4) on *EC* which was 11 times less that of Amoxil® antibiotic and 1,600 times that of Doxycycline® antibiotic (table 4). This is an indication that compound *Leonotis mollissima* has compounds that can be used to treat diseases caused by *Escherichia coli*.

Table 4: IC50 of Crude Extracts and Pure Compounds on Test Micro-organism

Sample	IC_{50} (mg/mL)				
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella typhimurium*	*Candida albicans*
DCM extract	0.210	52.602	52.602		
EtOAc extract			49.889		
Siderin **(1)**					
(20-hydroxylucidenicacid D2 **(2)**			0.141		
Labdane **(3)**					
Amoxil® antibiotic	0.775	1.178	1.486	3.811	1.776
Doxycycline® antibiotic	0.044	1.200	233.884	1.276	0.632

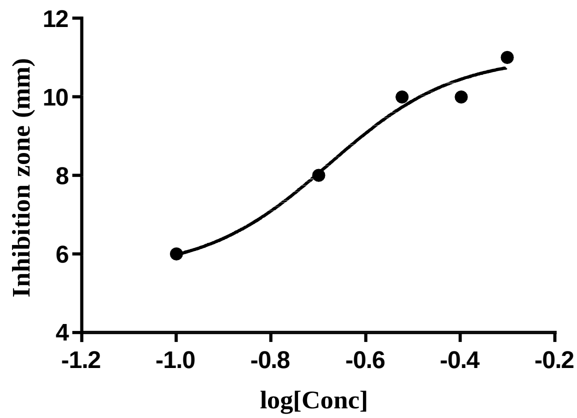

Fig. 2: Dichloromethane Crude Extract IC50 on Bacillus cereus = 0.210 mg/mL

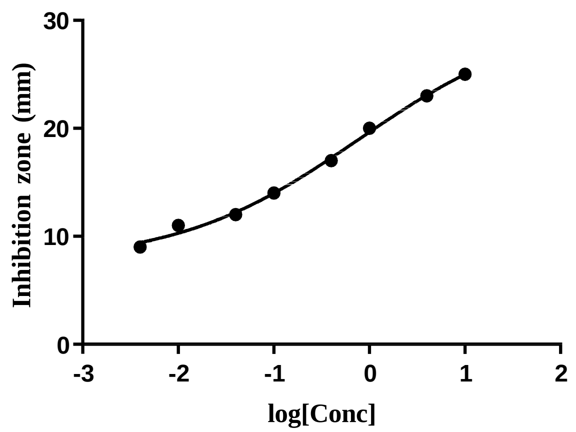

Fig. 3: IC50 of Amoxil® Antibiotic on Bacillus cereus = 0.775 mg/mL

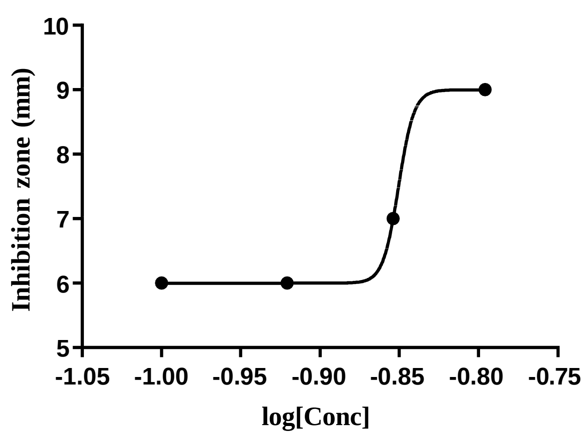

Fig. 4: IC_{50} of Compound 2 on *Escherichia coli* = 0.141 mg/mL

Conclusion

Leonotis mollissima crude extracts showed significant antimicrobial activity on all the test micro-organism (*Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella typhimurium* and *Candida albicans)* at a concentration of 1 mg/ml. They had lower MIC (Minimum Inhibition Concentration) and IC_{50} (Inhibition Concentration that reduces the effect of microorganisms by 50%) as compared to the Amoxil® and Doxycycline® antibiotics that were used as positive control for comparison.

From dichloromethane leaves crude extract, three compounds **1 (**Siderin)**, 2** (20-hydroxylucidenicacid D2) and **3** (13R)-19*α*,13*α*-epoxylabda-6*β*(19).16(15)-dioldilactone were isolated (Kinuthia et al., 2018) (Figure.1). All had an MIC of > 0.16 mg/ml. Compound **2** had significant $\mathbf{IC_{50}}$ of 0.141 mg/ml on *Escherichia coli*. Their IC_{50} was lower than for Amoxil® and Doxycycline® antibiotics. Methanol was used as negative control.

Antimicrobial activity of all the compounds isolated were lower as compared to the crude extracts. This is a confirmation that the plant contains compounds that can be isolated and used as drugs to treat various diseases including microbial infectious diseases.

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