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ANTIBACTERIAL ACTIVITY OF FIVE MEDICINAL PLANT EXTRACTS USED BY THE MAASAI PEOPLE OF KENYA

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ABSTRACT

Five locally known plants, *Solanum aculeastrum*, *Erythrina abyssinica*, *Carissa edulis*, *Croton megalocarpus* and *Myrica salicifolia* used by Maasai traditional healers for treatment of bacterial infections were extracted using chloroform, ethanol and water. The extracts obtained were then tested for *in-vitro* antibacterial activity on clinical isolates of gram-positive bacteria; *Staphylococcus aureus* and gram-negative bacteria; *Escherichia coli*, *Pseudomonas aerugenosa*, *Salmonella typhi* and *Krebsiella pneumonaie.* The activity of the water extracts of all the plants was the highest, followed by ethanol and chloroform extracts respectively. The water extract of *Croton megalocarpus* was observed to be the most active against all strains of bacteria.

KEYWORDS: Antibacterial Activity, *Erythrina abyssinica*, *Croton megalocarpus*, *Solanum aculeastrum*, *Myrica salicifolia*, *Carissa edulis*

INTRODUCTION

Plants are known as a major source of modern medicines. From ancient times, human have utilized plants for treatment and prevention of diseases, leading to the dawn of traditional medicine. This study presents the ethnobotany, ethnopharmacology of *E. abyssinica*, *C. megalocarpus*, *S. aculeastrum*, *M. salicifolia* and *C. edulis* as well as their potential therapeutic benefits as antimicrobials especially of their water extracts [Githiori *et al*, 2004].

Erythrina abyssinica commonly known as "the red hot poker tree" belongs to the family Leguminosae consisting of 730 genera and over 19,400 species distributed all over the world. The genus E*rythrina* comprises of approximately 120 species of which about 30 are found in continental Africa and 6 in Madagascar. Various species and sub-species from the genus *Erythrina* are found throughout east and southern Africa.

Traditionally, different parts of this plant are used by many communities to treat a number of ailments including infectious diseases such as gonorrhoea, syphilis, trachoma, conjunctivitis, dysentery. It is also used as an anthelmintic and to treat schistosomiasis. The ethyl acetate extract of the stem bark of *Erythrina abyssinica* has shown anti-plasmodial activity against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum* [Yenesew *et al*., 2005].

Myrica salicifolia is a shrub of 1 m in height but can grow into a tree of up to 20 m, usually aromatic and resinous. It belongs to the family *Myricaceae* found mostly in temperate to subtropical and tropical-montane regions of the world. Traditionally, the root extract of *M. salicifolia* was consumed by Maasai warriors to prime themselves for battle. They believed that uptake of this extract would produce feelings of detachment from the environment, invincibility, irritability, aggressiveness, overreaction to extraneous sounds and a tendency to keep a posture for a long time

[Njung'e, *et al*., 2002]. Studies have shown that the root extract of *M. salicifolia* is a non-hypnotic central nervous system (CNS) depressant with muscle reluctant, analgesic, hypothermic and antipyretic properties [Miaron, 2003].

Carrisa edulis belonging to *Apocynaceae* family comprises of about 155-200 genera and 2000 species, distributed primarily in the tropics and subtropics. Plants of *Apocynaceae* are often poisonous and rich in alkaloids or glycosides especially in the seeds and latex Some species are valuable sources of medicines, insecticides, fibers and rubber [Nick *et al*., 1995].

Different communities in Africa use parts of *C. edulis* to; alleviate pain, treat microbial infections in venereal diseases, for glandular inflammation, cause abortion, restore virility [Githiori *et a,l* 2004] and as an expectorant. In Kenya, a piece of the root is fixed into a hut roof as a snake repellent [Watt & Breyer-Brandwijk, 1962].

Studies have shown that its roots contain an active ingredient, carissin (a cardenolide), used in the treatment of sarcoma [Nastro *et al*, 2005]. The leaves significantly reduce blood glucose levels in STZ (streptozotocin) diabetic rates during the first three hours of treatment [El-Fiky *et al*, 1996]. *Carissa edulis* root bark extract possesses biologically active constituent(s) that have anticonvulsant activity which supports the ethnomedicinal claims of the use of the plant in the management of epilepsy [Miaron, 2003].

Solanum aculeastrum belongs to the family *Solanaceae* consisting of up to 80 genera and 3,000 species. *Solanum* is one of the largest genera with approximately 1,250 to 1,700 species that includes economically important species such as tomatoes, potatoes and egg plant [Liu Chang-Xiao, 1987]. The genus is widespread over the world although it is concentrated mainly in the tropics and subtropics. This plant is used as a hedge and the root decoction is used against gonorrhoea.

Croton megalocarpus belongs to the family *Euphorbiaceae* consisting of about 7,500 species in 300 genus. The genus Croton is pantropical with some species extending into temperate areas. The leaves, roots and bark are used to treat stomach problems and pneumonia. The seeds have high oil (32%) and protein contents. The oil extract is reported to be a forceful purgative [Thornsberry and Mcdougal 1983]. The bark decoction is used as a remedy for worms and whooping cough. Grounded roots are used for syphilis, anthrax, and snakebites [Kabir *et al*, 2005, Kavee, 2013] treatment.

In general, the five plants species are widely used traditionally by many communities to treat a number of ailments including infectious diseases in Kenya. A quick review shows that different extracts of these plants have been found to have activity on different bacterial assays, supporting their use by traditional medicine men.

Table 1: Ethno-Medicinal Uses of Four Plant Species by the Maasai Community

Species (Origin)	Plant Part	Uses	Reference
Myrica salicifolia			
Narok	Stem bark	Aphrodiasic, wounds boils, cough	[Miaron 2003, Kavee, 2013]
Erythrina abyssinica			
Kajiado	Whole plant	Colds, fever, influenza, eye infections, diarrhoea, wounds, boils, anthrax, typhoid	[Miaron 2003, Kavee, 2013]
Solanum aculeastrum			
Kajiado	Root bark	Cough, bronchitis, gonorrhoea, tonsolitis, boils, typhoid warts	[Miaron 2003, Kavee, 2013]
Croton megalocarpus			
Narok	Root bark	Cough, typhoid, antihelmintic	[Miaron 2003, Kavee, 2012]
Carissa edulis			
Narok	Stem bark	Gonorrhea, abdominal pains, back ache	[Miaron 2003, Kavee, 2012]

Source: *Maasai Traditional medicinemen*

The Maasai community's claim to the efficacy of these plants needs to be investigated and verified in order to elucidate the actual efficacy and their usefulness in treatment.

This study investigates the antimicrobial activities of the plant extracts on clinical isolates of *Escherichia coli, Salmonella typhi, Krebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus Aureus*. Solvents with varying polarities (chloroform, ethanol and water) were used for extraction to compare their potency on the microbes.

EXPERIMENTAL PROCEDURES

Plant Material

The four plant species recommended by the traditional medicine men in this study, were collected from Kajiado and Narok districts in the Rift Valley Province of Kenya on 20th February, 2009. The plants were identified by Mr. S. Mathenge of the University Herbarium, School of Biological Sciences, University of Nairobi, where a voucher specimen (Solanum- Mathenge-20/February, 2009) is deposited.

Extraction

The dried and ground plant parts (100g) of each of the following plants; *Solanum aculeastrum, Erythrina abyssinica, Carissa edulis, Croton megalocarpus* and *Myrica salicifolia* were each soaked in 150 ml of chloroform, ethanol and then water for only 12 hrs at room temperature (25°C). Removal of the solvent *in vacuo* resulted in twelve extracts which were subjected to different bacterial assays.

Test Bacteria Strains /Antibacterial Activity Assay

Five bacteria pathogenic clinical isolates, *Escherichia coli, Salmonella typhi, Krebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus Aureus*, were obtained from the Medical Microbiology Department of the Medical School, University of Nairobi. The strains were re-isolated three times on nutrient agar plates, identified and confirmed using the standard bacteriological methods [Obua *et al* 2002] at the Biotechnology Laboratory, Department of Veterinary Anatomy and Physiology, University of Nairobi. The organisms were maintained on nutrient agar slopes at 4^{o}C. The cultures were spread on nutrient agar plate and incubated aerobically at 37^{o}C overnight (18-24 hrs) before use. Using plate dilution method the cultures were adjusted to yield 1x10^{5} colony forming units per ml (cfu/ml).

Disc Impregnation

The plant extracts were transferred into a pre-weighed sample bottles by dissolving in acetone. A steady air current was used to dry the plant extracts for 24 hrs in, the sample bottle were weighed again to approximate the weight of the resulting crude extract (Table 1). A volume of 0.5 ml of 20% DMSO (BDH) was used to reconstitute the extracts. Sterile discs made from Whatman filter paper No. 1 were soaked in 100 µl of each extract to an approximate concentration of 20 mg/ml [Ibrahim *et al*, 2004] per disc. Discs were also soaked in 20% DMSO and sterile saline to act as control the impregnated disks were sterilized under ultra violet rays (UV) for 1 hr.

Seeding of Nutrient Agar Plates

0.5 ml of inoculums from each bacteria species was introduced onto the dry, sterile surface of the nutrient agar (Oxoid). The seeding rate was 1.0 x 10^{5} cfu/ml and a sterile glass spreader was used to make an evenly distributed culture. The plates were left to dry for 2 hrs.

Bacterial Susceptibility Testing

Sterile impregnated discs of known concentrations were carefully placed on the labeled seeded plates in duplicates. The plates were allowed to stand for 1 hr to allow diffusion to take place, then were incubated aerobically at 37^{o}C and examined for zones of inhibition after 24 hrs. The inhibition zones were measured with a ruler to the nearest millimeter. An average diameter of the readings was calculated. Discs impregnated with 20% DMSO and normal saline were also plated and examined as negative control. Extracts exhibiting apparent zones of inhibition were chosen for further evaluation. These experiments were repeated five times.

RESULTS AND DISCUSSIONS

Antibacterial Activities of the Plant Extracts

The antibacterial activities of the plant extracts were determined by measuring the inhibition diameters of the wells after 24 hrs of introducing the extracts to the colonies in the petri dishes. Different inhibition diameters were recorded for each of the five experiments per extract and an average reading was calculated. Table 2 below shows the average inhibition diameter readings of the extracts on the bacteria under test.

Table 2: Anti-Bacterial Activities of Various Plant Extracts

		Disc				
Sample	**Concentration Mg/Ml**	**1**	**2**	**3**	**4**	**5**
Chloroform Extracts						
Myrica salicifolia	20	6.86	-	5.50	-	8.25
Erythrina abyssinica		7.45	-	8.85	-	8.85
Solanum aculeastrum		7.50	7.13	8.25	-	9.45
Croton megalocarpu		6.30	7.38	7.38	-	8.25
Carissa edulis		-	8.25	-	-	-
Ethanol Extract						
Myrica salicifolia	20	8.57	-	9.14	-	6.57
Erythrina abyssinica		10.00	5.00	7.14	9.714	7.66
Solanum aculeastrum		10.00	7.72	9.00	2.57	7.00
Croton megalocarpu		6.29	7.09	6.57	8.29	7.71
Carissa edulis		6.29	-	2.57	3.00	6.57
Water Extract						
Myrica salicifolia	20	8.29	-	8.51	5.60	8.57
Erythrina abyssinica		8.61	-	8.57	6.57	8.57
Solanum aculeastrum		7.67	6.57	8.12	3.15	6.55
Croton megalocarpu		10.59	6.29	10.13	9.15	7.18
Carissa edulis		5.72	5.72	10.38	3.15	7.73
Amp	1.0 x10^{-1}	10.00	9.00	N/T	14.00	10.00
Ery	1.0 x10^{-1}	8.00	9.00	N/T	7.00	9.00
Amp	1.0 x10^{-2}	10.00	8.00	N/T	10.00	9.00
Erythromycin	1.0 x10^{-2}	6.00	9.00	N/T	7.00	8.60
Amp	1.0 x10^{-3}	10.00	9.00	N/T	9.00	9.00
Erythromycin	1.0 x10^{-3}	6.00	7.00	N/T	6.00	8.00

Microorganisms: 1= *Escherichia coli* (ATCC 25922), 2= *Staphylococcus aureus* (ATCC 29737). 3= *Krebsiella pneumoniae* (local strain) 4= *Pseudomonas aeruginosa* 5= *Salmonella typhi*.

"-" Not active.

[a] Inhibition zone in mm.

N/T= Not tested

The chloroform extracts of four plant species *Myrica salicifolia* (MSE), *Erythrina abyssinica* (S7c), *Solanum aculeastrum* (S13c) and *Croton megalocarpus* (S15c) showed activity on *E. coli*, *K. pneumonaie* and *S. typhi.* The highest activity was exhibited by S13c, S7c and S13c against *E. coli*, *K. pneumonaie* and *S. typhi* respectively. All the extracts were inactive against *P. aerugenosa. S. aureus* was sensitive to only three plant extracts; S13c, S15c and S17c two of which exhibited similar activity. *C. edulis* was active only on *S. aureus* recording an inhibition diameter of 8.25mm.

Ethanol extracts, S7e, S13e and S15e were sensitive to all bacteria strains with varying activities. The highest activity against *E. coli* was shown by S7e and S13e extracts with inhibition diameter of *ca* 10 mm. However, three bacteria strains*; Escherichia coli Krebsiella pneumonaie* and *Salmonella typhi* were sensitive to all ethanol extracts. The highest activity was exhibited by S7e and S13e against *E.coli.* Most bacteria strains were sensitive to ethanol extracts. However, *S. aureus* was not sensitive to both *Myrica salicifolia* (MSEe) and *C. edulis* ethanol extracts while *P. aerugenosa* was not sensitive to *Myrica salicifolia* (MSEe). The highest activity was exhibited by S7e and S13e against *E. coli.*

The bacteria were very responsive to the water extracts relative to the other two extracting solvents used. The water extracts of all the plant species except *Myrica salicifolia* (MSEw) and *Erythrina abyssinica* (S7w) were sensitive to all strains of bacteria. The extract of S15w showed the highest activity against *E. coli* with an inhibition diameter of 10.59 mm followed by S7w, MSEw, S13w and S17w respectively. The most sensitive extract against *K. pneumonaie* was S17w with an inhibition diameter of 10.38 mm. There was minimal activity of S13w and S17w on *P. aerugenosa* with inhibition diameters of only 3 mm. The sensitivity of *P. aerugenosa* on all the plant water extracts was the lowest except for S15w which had an inhibition diameter of 9 mm. *S. aureus* sensitive to S13w, S15w and S17w with the highest activity being exhibited by S13w inhibition diameter of 6.57 mm.

CONCLUSIONS

In comparison with the positive test, the efficacy of the five species used by traditional medicine men on all the bacteria tested was high. This explains that the Maasai traditional medicine men have effectively been controlling infectious diseases caused by these type of bacteria. It has also been established that the water extracts of these plants are equally efficacious thereby supporting the medicines men's prescriptions. It also worth noting that, *E. coli* showed higher sensitivity to the ethanol extract of *Myrica salicifolia* MSEe, *Erythrina abyssinica* (S7e), *Solanum aculeastrum* (S13e) and *Croton megalocarpus* (S15e). There was no significant difference in activity of the extracts from different extracting solvents.

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