Track 3: Traditional Medicine **Venue: KEMRI Conference Hall**

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Development of TMR5 as a management therapy for herpes infections: Results of preclinical evaluations

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Background: TMR5 is a product of a Kenyan medicinal plant, prepared as a lyophilized extract and a cream. The two products have been evaluated for preclinical safety and efficacy in suitable *in vitro* and *in vivo* systems of herpes infections. Herpes is a viral infection affecting over 60% of the sub-Saharan Africa young adult population. It is caused by two similar viruses, HSV-1 and HSV-2 which share 50% gene sequence homology. The infection in a major cause of genital ulcer disease, associated with increased risks of HIV acquisition and transmission.

Objective: To develop TMR5 as an alternative anti-herpes agent, this being necessitated by increased resistance to available drugs and the cost of the drug of choice, acyclovir.

Methodology: The trypan blue exclusion test, plaque inhibition and viral yield reduction assays were applied *in vitro* for assessment of cytotoxicity and EC_{50}. *In vivo*, Mice and guinea pig cutaneous and genital HSV infection models were used respectively to evaluate efficacy following oral and topical treatments.

Results: Cytotoxic concentrations of TMR5 in mammalian cell lines indicated a wide therapeutic index (CC_{50} $\geq$58.5$\pm$4.6µg/ml). An EC_{50} of $\leq$14.7$\pm$3.7µg/ml for both wild type and resistant strains of HSV was realized in plaque and viral yield assays. Oral (250 mg/kg) and topical (10% cream) administrations exhibited a significant delay in onset of infections, hindered progression of infection to lethal forms with increased mean survival times and low mortality. No acute toxicity has been realized at the therapeutic concentrations.

Conclusion/recommendations: TMR5 has demonstrated a great potential as an anti-herpes agent and arrangements are presently underway to evaluate its efficacy and safety in a higher mammalian model. A pilot production scheme is currently ongoing as one of the means of developing TMR5 as an alternative management therapy for herpes infections.

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In vitro effects of Warburgia ugandensis, Psiadia punctulata and Chasmanthera dependens on Leishmania major promastigotes

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Background: Leishmaniases are now endemic in 88 countries in Africa, Asia, Europe, North and South America. The global burden of leishmaniases caused a morbidity burden of 2.4 million disability adjusted life years (DALYs) and 59,000 deaths in 2001. Leishmaniases are endemic in Kenya. *Leishmania* parasite is transmitted by sand flies. The available chemotherapy is too toxic; show serious side effects, ineffective, expensive, unavailable while long term doses are faced with drug resistance and relapses. This work contains base line studies in which we investigated antileishmanial effects of extracts from *Warburgia ugandensis*, *Psiadia punctulata* and *Chasmanthera dependens*. These plants are used by communities in Baringo to treat kala-azar or splenomegaly related syndromes.

General Objective: To determine the chemotherapeutic, cytotoxic and immunostimulative effects of plant extracts from *W. ugandensis*, *P. punctulata* and *C. dependens***.**

Methodology: Plant water and alcohol extracts from *Warburgia ugandensis* Sprague (Family: Canellaceae), *Psiadia punctulata* Vatke (Family: Compositae) and *Chasmanthera dependens* Hoschst (Family: Menispermaceae) were tested for anti-leishmanial activity on *Leishmania major* promastigotes (Strain IDU/KE/83 = NLB-144), cytotoxic and immunomodulative effects on infected macrophages *in vitro*. Serial dilutions were made from each stock solution. Parasite viability was assessed before and after incubation with extracts by staining with Trypan blue and examining under a microscope. Control group was incubated in the absence of extracts. After establishing several inhibitory concentrations, the minimum inhibitory concentration that was able to eliminate 50% of the parasites was identified as IC_{50}. To determine levels of cytotoxicity, 10^5 macrophage live cells from clean BALB/c mice were introduced into reconstituted test compounds, negative control group in complete RPMI 1640 media and positive control in Pentostam. One hundred cells in each slide were counted; dead ones were ruptured or blue in colour while live ones were clear and cell membrane intact. For immunomodulation assays, adherent cultured macrophages were washed once and re-suspended in fresh medium, dead parasites removed from media by centrifugation, promastigotes added, washed again, incubated at 37^0C for 72 hrs, after which the supernatant were tested for nitrite content by Griess reaction.

Results: *Warburgia ugandensis* stem bark (*t*-value 12.6193, $p<0.05$), *Psiadia punctulata* (*t*-value 19.3624, $p<0.05$) and *Chasmanthera dependens* (*t*-value 13.3071, $p<0.05$) showed significant anti-leishmanial activity with an IC_{50} of 1.114 mg/ml, 2.216 mg/ml and 4.648 mg/ml respectively. The cytotoxicity of the plant extracts on BALB/c peritoneal macrophage cells was insignificant ($p<0.05$) as compared to the highly toxic current drug of choice Pentostam. *Warburgia ugandensis* (stem bark water extract), *Chasmanthera dependens* (stem bark water extract) and *Psiadia punctulata* (stem bark methanol extract) produced 112.3%, 94% and 88.5% more nitric oxide respectively, than negative control.

Conclusions: Plants crude extracts had significant ($p<0.05$) anti-leishmanial and immunomodulative effects but insignificant cytotoxic effects at 1mg/ml concentration. The killing of *L. major* promastigotes and immunomodulation by all the three plant species was dependent on part of plant, type of extract and its concentrations.

Recommendations: Bioassay guided fractionation and *in vivo* studies should be performed and synergistic/additive effects explored on *W. ugandensis* stem bark water and methanol extracts and *P. punctulata* stem bark methanol extract.

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Medicinal Properties of *Fuerstia africana* T.C.E. Friers (Lamiaceae)

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Background: *Fuerstia africana* is endemic in tropical East Africa. The leaves are used to treat malaria and mouth infections among other ailments. Due to the high morbidity and mortality associated with these conditions, and the high cost of conventional therapies, there is need to search for cheaper but safe and effective alternatives from plants.

Objective: The objective of this study was to establish the anti-malarial and anti-microbial activities and the cytotoxicity effects of *Fuerstia africana* extracts.

Methodology: Extracts from *Fuerstia africana* were prepared using organic solvents (petroleum ether, ethyl acetate and methanol) and tested for cytotoxicity on Vero cells. They were screened against *Plasmodium falciparum* (D6, chloroquine sensitive and W2, chloroquine resistant strains) and against *P. berghei* (strain ANKA) in mice. They were also screened for antimicrobial activities against five strains of bacteria and one strain of fungi using the diffusion method.

Results: The petroleum ether extracts of the aerial parts and roots of *Fuerstia africana* demonstrated the promising anti-plasmodial activity against the chloroquine sensitive plasmodial strain D6 (IC_{50} 1.5 & 4.6 µg/ml, respectively) and exhibited moderate cytotoxicity against Vero cells. The methanol extract of the root exhibited moderate anti-malarial activity in the in vivo bioassay. In the anti-microbial assays, all the extracts showed activity against the tested microbial strains and the methanolic extracts of the aerial parts had the best activity against *Klebsiella pneumoniae*, a gram-negative bacterial strain. Generally, methanolic extracts of the aerial parts showed the best activity against all the tested microbial strains with activity index values of 0.5 and above.

Conclusion: This study authenticates the traditional medicinal uses of *Fuerstia africana* by indigenous people for treatment of malaria, bacterial and fungal infections. Further studies to identify the bioactive molecules which can be used as markers for quality control are ongoing.

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Antimicrobial properties of *Plectranthus barbatus*: towards the development of herbal antimicrobial products in Kenya

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Introduction: *Plectranthus barbatus* (Lamiaceae) extracts are widely used in traditional medicine for treatment of a range of ailments including skin infections among others. In order to promote its wider utilization in the community, its extracts should be formulated into hygienic, appealing and yet effective and safe dosage forms such as antimicrobial soaps and body washes.

Objectives: To determine the antimicrobial activity of total organic (methanol and dichloromethane) and aqueous extracts of *Plectranthus barbatus* root-barks, stem-barks and leaves against some bacterial and fungal strains that are associated with skin infections.

Methodology: Organic and aqueous extracts were tested at a concentration of 100mg/ml against 3 bacterial strains (*Staphylococcus aureus*, Methycillin Resistant *Staphylococcus aureus* and Escherichia *coli*) and 2 strains of fungi (*Candida albicans* and *Trichophyton mentagrophytes*) using the disc-diffusion bioassay. Gentamycin and amphotericin-B were used as antibacterial and antifungal positive controls respectively.

Results: Antimicrobial activity was only demonstrated by organic extracts against *Stapylococcus aureus* (ATCC 25922). Dichloromethane extracts of the root-barks collected from Mbeere District showed the highest antibacterial activity with a zone of inhibition measuring 15.3 mm compared to the positive control at 35mm.

Conclusion: This study demonstrated modest antibacterial activity of *Plectranthus barbatus* extracts against *Staphylococcus aureus*. Further phytochemical and safety studies of the bioactive extracts are on- going.

3.305 Antimicrobial and toxicity studies of *Fuerstia africana* T.C.E. Fries (Labiatae).

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Background: Opportunistic microbial infections cause high morbidity and mortality among the immunocompromised patients. Antimicrobial agents currently in use have many limitations such as unwanted side effects, rapid development of resistance and high cost. This necessitates the search for new antimicrobials. Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. *Fuerstia africana*, a herb traditionally used in treating stomach ulcers, tongue infection, conjuctivities, malaria, gonorrhoea, skin complaints, and diarrhea, was screened for antibacterial and antifungal activity.
Objective: The aim of the study was to determine the antimicrobial activity and toxicity of *F. africana* extracts.
Methodology: Plants were collected, authenticated, dried, pulverized and extracted using both organic and aqueous solvents. Antimicrobial activity was determined against five bacterial strains and six fungal strains using the disc diffusion assay. *In vitro* cytotoxicity was determined using Vero E6 cell lines while *in vivo* acute toxicity using Swiss albino mice.
Results: The dichloromethane extract was the most active with MIC of 31.25µg/disc against both *Staphylococcus aureas* and Methicilin Resistant *Staphylococcus aurea*s, but the extract was quite cytotoxic (IC_{50} 7.91µg/ml). However, the extract did not exhibit any toxicity *in vivo* when tested at a dose of 5000mg/kgbw. Extracts of *F. africana* were not active against fungal strains tested.
Conclusions: The results supports to a certain degree the traditional medicinal use of *F. africana* Further investigations are needed to determine the compound(s) responsible for antibacterial activity.

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Antimalarial activity of *Hugonia castaneifolia* and *Turraea mombassana*

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Background: Despite intensive efforts to control malaria, the disease persists as one of the greatest health problems facing Africa. It causes over 1 million deaths, mostly in children younger than 5years, and 300-500 million episodes of acute illness each year. In Africa, the malaria pandemic is aggravated by an increasing prevalence of multi-drug resistant strains of the parasite which reduces the efficacy of anti-malarial drugs in use. Recent reports have indicated a decline in efficacy of artesunate-mefloquine along the Thai-Cambodia border. This highlights the need for the development of new anti-malarial drugs.
Objective: To evaluate anti-malarial activity and safety of *Hugonia castaneifolia* and *Turraea mombassana*.
Methodology: Plant materials were collected, cleaned, dried, pulverized and extracted using methanol and water. The extracts were evaluated for *in vitro* antiplasmodial activity against a chloroquine sensitive (D6) *Plasmodium falciparum* strain and i*n vivo*, against a *Plasmodium berghei* ANKA strain in mice. Cytotoxicity in Vero cell lines and acute toxicity in mice were also determined.
Results: Methanol extracts of *Turraea mombassana,* and *Hugonia casteinofolia* were

highly active *in vitro* with IC_{50} of 6.1 µg/ml and 8.86 µg/ml while the water extracts were moderately active with IC_{50} of 33.07 µg/ml and 23.92 µg/ml respectively. Aqueous extract of *H. casteinofolia* was weakly cytotoxic CC_{50} 22.00 µg/ml. Methanolic extract of *T. mombassana* had a chemosuppression of 52.86% while that of *H. casteinofolia* was 46.76%. No toxic effect or mortality was observed in mice treated orally with any of the extracts as a single dose of 5000mg/kg body weight.

Conclusion: The preliminary results demonstrate the antimalarial potential and safety of *T. mombassana,* and *H. casteinofolia* medicinal plants used traditionally for the treatment of malaria. Taking the moderate cytotoxicity of *H. castaneiofolia in vitro* into account, further work is needed to clarify the effective potential of the plant in clinical use.

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Antimicrobial Properties of *Maytenus heterophylla*

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Maytenus heterophylla are used in traditional medicine to treat several diseases in Kenya The aim of the study was to investigate antimicrobial activity of different parts of *Maytenus heterophylla* used in traditional medicine. The stem barks and root barks were extracted by various solvents, and tested against different bacteria; *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Eschericia coli* (ATCC 25922). The fungal strains used in this study were *Candida albicans* (ATCC 90028), clinical isolates of *Candida krusei*, *Cryptococcus neoformans, Microsporum gypseum* and *Trichophyton mentagrophytes* were also tested. Minimal Inhibitory Concentration (MIC) values of each active extracts were determined. The results obtained show strong activity of the methanol extract of against bacteria and fungi used as test organisms. This supports the use of this plant in traditional medicine to treat microbial infections.

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Antifungal activity of warbugia ugandensis extract and its potential for the treatment of fungal infections

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Introduction: The increase in the number of immunocompromised cases due to the Acquired immunodeficiency syndrome has led to increased number of patients susceptible to many opportunistic infections such as Candidiasis, dermatophytosis and Cryptococcosis. Fungal management challenges are the cost of antifungal drugs, toxicity and drug resistance. Traditional remedies are an option for healthcare however; few studies have been carried out to establish their therapeutic efficacy. The present study aimed at testing selected parts of *Warbugia ugandensis* extract for antifungal activity against both yeast and moulds. The test strains included *Candida albicans*, *Cryptococcus neoformans* and *Microsporum gypseum* and the media used was Sabourauds dextrose agar.

Results: *Warbugia ugandensis* was active against the tests yeast and moulds. The leave, stem and roots extract of elicited bioactivity against *C. albicans, C. neoformans*, and *M. gypseum* at various concentration.

Conclusions/ Recommendation: We confirm the claimed antifungal efficacy of *W. ugandensis* which could lead to development of pharmaceutical products for the management of yeast and moulds infection. Traditional medicine is preferred to modern medicine attributed to good accessibility, affordability local knowledge and expertise among local communities. However threat to sustainable use of medicinal plants in Kenya include high population growth and competing land uses, environmental degradation loss of indigenous knowledge, increased

commercialization of traditional medicine. Deliberate effort to conserve and sustain use of *Warbugia ugandensis* is recommended .