



## **Cytotoxicity and Anti - Herpes Activity of Selected Medicinal Plants Cited for Management of HIV Conditions in Kakamega County – Kenya**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author AOR designed the study, wrote the protocol, identified the species of plants, managed the experimental process, analyses the literature searches and wrote the first draft of the manuscript. Authors MK, AOM and FMT critically reviewed the study at all stages. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aim:** To determine safety and anti-herpes activity of selected medicinal plants cited by Community Health Workers in Mukhwa sub-location, Bukaya location in Kakamega County, Kenya.

**Study Design:** Ethno-medical interview for selection of medicinal plants and *In-vitro* experiment for determination of safety and anti-herpes activity.

**Methodology:** Eight medicinal plants were selected for safety and determination of anti-herpes activity of water extracts using Vero cell and Human herpes Virus type 1. The metabolism of 3 – (4, 5-Dimethylthiazole -2-y) -2, 5-diphenyltetra-zolium bromide (MTT) was used for cytotoxicity and different levels of extract antiviral experiments. End point titration technique (EPTT) was used for virus quantification and antiviral screening test.

**Place and Duration of the Study:** Plant samples were collected in September 2013 in Mukhwa

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sub-location, the processing and biological experiments were carried out between March 2014 and October 2015 at the center of traditional medicine and drug research of Kenya Medical Research Institute, Nairobi.

**Results:** Safety profile: *Tithonia diversifolia* (Whole root) gave maximum nontoxic extract concentration (MNC) at 20 µg/mL, extract concentration killing 50% of cells (CC<sub>50</sub>) was 460 µg/mL. *Schkuhria pinnata* (Leaves); MNC <20 µg/mL, CC<sub>50</sub> 90 µg/mL. *Entada abyssinica* (Stem bark); MNC 20 µg/mL, CC<sub>50</sub> > 500 µg/mL. *Garcinia buchanii* (Stem bark); MNC 40 µg/mL, CC<sub>50</sub> >500 µg/mL. *Croton macrostachyus* (Stem bark); MNC 40 µg/mL, CC<sub>50</sub> >500 µg/mL. *Vernonia adoensis* (Whole root); MNC 20 µg/mL, CC<sub>50</sub> 470 µg/mL. *Plumeria alba* (Leaves); MNC <20 µg/mL, CC<sub>50</sub> 120 µg/mL. *Caesalpinia decapetala* (Whole root); MNC 20 µg/mL, CC<sub>50</sub> 500 µg/mL. Anti-herpes activity: The best anti-herpes activity was obtained from *G. buchanii* (Stem bark), giving an extract concentration inhibiting 50% of virus activity (IC<sub>50</sub>) at 20 µg/mL) and *C. decapetala* (Whole root) giving IC<sub>50</sub> at 80 µg/mL. Therapeutic index of *G. buchanii* was > 25 and that of *C. decapetala* was > 6.

**Conclusion:** Majority of the medicinal plants selected for anti-herpes activity have narrow non-toxic limits. Of all the selected medicinal plants, *G. buchanii* and *C. decapetala* are the most promising for selective anti-herpes activity.

**Keywords:** Cytotoxicity; anti-herpes; medicinal plants.

## 1. INTRODUCTION AND BACKGROUND INFORMATION

Kakamega County is one of the 47 counties in the Republic of Kenya. It is located to the western side of the country, bordering Vihiga, Siaya, Bungoma, and Nandi counties. It covers an area of 3050.3 km<sup>2</sup> and has an altitude between 1,240 meters and 2000meters above sea level [1]. According to Kenya National AIDS Control program [2], the HIV prevalence estimate was 5.9% by 2012, being generally the same as the national average of 6%. The cited priority service areas on HIV included strengthening of linkage to care for individuals testing positive, thus addressing the statistic showing that only 37.4% were linked to the services [3]. Further, the report indicated that the proportion of eligible clients started on ART was 81%, meaning that about 19% were either seeking care to non-reporting facilities or using self-administered care, with possibility of taking traditional medicine. Although statistics on HIV deaths for the county was not indicated, the national statistic of 5% [4] points to gaps in HIV care. Previous studies elsewhere have indicated a need for improved care for People living with HIV (PLWHIV), with reports indicating emergence of resistant HIV strains [5], unsustainable donor driven free public sector provision of Antiretroviral treatments (ART), inhibited access to treatment due to factors such as lack of confidentiality, lack of transport to hospitals, shortage of health workers, long queues, and lack of adherence due to adverse drug reactions [6,7]. Some HIV patients resort to use of ethno-medicinal plants as an alternative or concomitant

use with ARTs in an attempt to overcome these challenges [8].

Medicinal plants have been cited as key ingredient of medication therapy in traditional medicine [9]. In its report, the International Bioethics Committee (IBC) of UNESCO highlights issues of uncertain benefits and risks associated with the use. On the other hand, studies on medicinal plants selected from the community have shown that the materials have potential to solve human health challenges. For instance, HSV has been shown to respond very well to plant species such as *Euphorbia continifolia*, *Euphorbia tirucali* [10] and *Carrisa edulis* [11]. However, the health value of the majority of the medicines are still mainly anecdotal and the utilization seem to be increasing and dynamic, fueled by problems of HIV and other chronic conditions [6,8].

The group of viruses referred to as Herpes belong to the family, *Herpesviridae*. They are morphologically identical and biologically closely similar. Their replication in the nucleus results in inflammatory response and destruction of host cells [12]. The agents are host specific and establish lifelong latent infection, with occasional reactivation. Infection sometimes results in malignant forms of host cells [13]. Viruses within the family includes Varicella zoster Virus (VZV), Epstein - Barr virus (EBV), Human cytomegalovirus (HCMV), HSV and Human herpes viruses [14]. Herpes Simplex Viruses are classified in the sub family *Alphaherpesvirinae* together with VZV. The subfamily characteristics include relative rapid replication cycle *In vitro*,

ability to establish lifelong infection in the sensory ganglia of the host and variable host range. Herpes Simplex virus type 1 and 2 vary in their clinical manifestation, but in both cases, symptomatic infections are characterized by painful vesicular or ulcerative lesions at various mucocutaneous sites [15]. Immunocompromised patients and infected neonates suffer severe complications due to extensive local disease, visceral involvement, general dissemination and or encephalitis [12]. Most immunocompetent individuals experience subclinical infections with either HSV-1 or HSV-2, mainly following reactivation of latent virus and subsequent mucocutaneous replication [14]. Infections may sometimes occur without symptoms and this usually lead to asymptomatic virus shedding, which is significant for oral HSV-1 transmission among children, sexually transmitted HSV in adults and mother to child transmission during birth [16]. Acyclovir resistant strains are much less virulent in immune-competent individuals but cause serious and persistent symptoms in the immunocompromised [14]. Pharmaceutical studies have confirmed existence of active principles against several viruses in plants [17] but it is expected that further studies will discover more compounds since majority of plants have not been fully evaluated.

Infection with Herpes viruses have long been associated with increasing the likelihood of HIV infection [18,19] and is recognized as one of the HIV defining illness [20]. The most widely used anti-herpes treatment are the nucleoside analogs, most of which the viruses are resistant to due to development of thymidine deficient strains. Medicinal plants seem to be a possible source of alternative compounds with different mode of action. This study sought to select and evaluate medicinal plants, claimed to be useful for conditions resembling herpes lesions. The medicines were cited by Community Health Workers (CHW) providing care to PLWHIV in Mukhwa sub-location, Bukaya location, Kakamega County in Kenya. The objectives of the study were to determine the safe limits of extract use and to detect their antiviral activity against Human herpes virus type 1.

## 2. MATERIALS AND METHODS

### 2.1 Plant Selection, Collection and Authentication

The plants were cited by community health workers providing care to people living with HIV

in Mukhwa sub-location Bukaya location, Kakamega County in Western Kenya. Field assistance for identification and collection was provided by the CHW citing the plants. The taxonomic identification and classification was carried out by comparing plant sample with herbarium specimen through the assistance of Patrick Mutiso of the University of Nairobi herbarium. The voucher specimens were deposited with collection number being the identifying codes. The plant names and codes were recorded with name(s) of specimen author(s). Plants for anti-herpes investigation were selected on the basis of conditions for which the medicines were prescribed such as skin, mouth and genitals sores. Communication with the CHW was through English, Kiswahili and the local language of Wangi. The Community health extension worker (CHEW) acted as a translator.

### 2.2 Preparation of Aqueous Extracts

Plant materials were harvested and dried at room temperature for 2 weeks. The dried material was grinded to form powder in electric grinding machine. Fifty grams of powdered material was soaked in 500 ml of distilled water and heated to 80°C for one hour. The extract was cooled to room temperature and filtered using cotton wool. The filtrate was then frozen using dry ice in acetone, freeze dried and kept at -20°C until required for use. For experiments, the freeze dried material was dissolved in phosphate buffered saline to make a stock concentration of 1000 µg/ml. Working concentrations were made from the stock.

### 2.3 Preparation of Cell and Virus Culture

A cryovial of Vero cell line, ATCC CCL 81 was obtained from viral hemorrhagic fever (VHF) research laboratory, of the Kenya Medical Research Institute (KEMRI). The vial was placed in a water bath at 37°C to thaw. The content was transferred into a 75 cm<sup>2</sup> cell culture flask containing 25 ml of Eagles minimum essential growth media (MEM) supplemented with 2 mM L-glutamine, 10% (v/v) fetal calf serum, 2.5% (v/v) of 7.5% (W/V) Sodium bicarbonate and 1% (v/v) of pen strep (10,000 I.U/ml penicillin combined with 10,000 µg/ml streptomycin). The seeded culture flask was incubated in a humidified environment at 37°C in a 5% CO<sub>2</sub> incubator until cell culture was about 90% confluent. The 75 cm<sup>2</sup> cell culture flask was used to prepare virus stock of herpes simplex type 1 (ATCC® VR -

1789<sup>TM</sup>) purchased from American Type culture collection (ATCC) as explained below;

The flask was emptied of growth media and 1 mL vial of herpes simplex virus type 1, HSV 1 ATCC<sup>®</sup> VR -1789<sup>TM</sup> emptied into the flask. Virus inoculum was adsorbed on to the cell monolayer for 1 hour in 5% CO<sub>2</sub> incubator at 37°C. The inoculum was removed and 25 ml of maintenance media made of the same chemically defined constituents as for growth media except fetal calf serum, used at 2.5%. The virus culture was incubated at 37°C in 5% CO<sub>2</sub> incubator and the virus stock harvested after 48 hours when about 90% cytopathic effect (CPE) was observed on the monolayer.

The virus was harvested by 3 times freezing in -80°C freezer and thawing in 37°C water bath. The maintenance media containing the virus was then centrifuged at 3,000 rpm for 10 minutes and the supernatant aliquoted in 1 ml cryovials and kept at -80°C. The stock titer was determined by endpoint titration technique (EPTT) as previously described by [10].

One cryovial of the stock virus was removed from the freezer and immediately thawed in a water bath at 37°C. The EPTT was carried out in a 24 hour growth culture of Vero cells in a 96- well plate, previously seeded with 100 µl of 2 x 10<sup>4</sup> cells /ml. The growth media was removed from the wells and the cells infected in quadruplicates with 100 µl of each tenfold dilution of the virus suspension from 10<sup>-1</sup>- 10<sup>-12</sup>. The plate was incubated for 48 hours in 5% CO<sub>2</sub> at 37°C. The virus titer was calculated using spearman-karba formula [10], based on the proportion of replicate virus dilutions showing above 75% cytopathic effect on the cells. The stock virus titer was found to be 10<sup>-7</sup> (The dilution of virus required to cause lysis of 50% of the inoculated cultures; TCID<sub>50(0.1ml)</sub>).

## 2.4 Cytotoxicity Test

Cytotoxicity was tested by determining viability of Vero cells after exposure to graded concentration of extracts. The methods previously used by Bentancur et al. [10] and Jaime et al. [21] was used. The method is based on the metabolism of 3 - (4, 5-Dimethylthiazole -2-y) -2, 5-diphenyltetra-zolium bromide (MTT), converting the yellow solution of the dye to purple colour. The intensity of the purple colour is directly proportional to the viability of the cells and is determined using a spectrophotometer. Three

different MTT experiments in triplicate wells of 96 well plate was carried out using Vero cells (ATCC CCL 81). Each 96 well plate was designed for 3 extracts. Vero cells were seeded at 2 x 10<sup>4</sup> cells per well in columns 1,2,4,5,7,8,10 and 11 in EMEM supplemented with 10% fetal calf serum, 1% penicillin/ Streptomycin, 1% glutamine and 2.5% of 7.5% (W/V) sodium bicarbonate solution. Wells in row 3, 6, 9 and 12 received only growth media to serve as test extract blank (tb) and cell control blank (ccb). The cells were grown in 5% Carbon dioxide at 37°C for 24 hours. The growth media was removed and replaced with 100 µl of maintenance media containing all the ingredients similar to growth media except fetal calf serum at 2.5%. A 100 µl of 1000 µg/ml of Plant extract was added to row H of 96 well plate and serial doubling dilutions carried out up to row C, representing 500, 250, 125, 62.5, 31.25, 15.625 µg/mL, leaving row B and A to serve as cell control (CC). Cells were incubated at 37°C for 48 hours. The wells were emptied of the media and 10 µl of MTT prepared at 5mg/ml in phosphate buffered saline added to all the wells, and incubated for 4 hours. The MTT was removed and 100 µL of dimethyl Sulphoxide (DMSO) added. The Optical density of the wells were read at 562 nm and the cell toxicity expressed as percentage cell viability at each extract concentration calculated as follows;

$$\text{Percentage cell viability} = \frac{[\text{ODt} - \text{ODtb}]}{[\text{ODcc} - \text{ODccb}] \times 100}$$

Where

ODt = Optical density of test extract  
 ODtb = Optical density of test extract blank  
 OD cc = Optical density of cells control  
 OD ccb = Optical density cell control blank

The cytotoxic concentration reducing the viability of cells by 50% was obtained by plotting a graph relating mean percentage of cell viability of 3 different experiments to respective extract concentration.

## 2.5 Anti-herpes Activity Screening

### 2.5.1 End point titration (EPTT)

End point titration technique (EPTT) as previously used by Betancur et al. [10] was carried out to detect anti- Herpes activity at 100 µgm/ml. The 96 well plate was seeded with the same Vero cell strain (ATCC CCL 81) at same

density as for cytotoxicity test and incubated in the same chemically defined growth media and condition for 24 hours.

A 100 µl of 100 µg/mL of extract in maintenance media was added to quadruplicate cell wells 1 hour before a tenfold dilutions of previously titrated virus was added at 100 µl per well. The 96 well plate virus culture was incubated for 48 hours in a humidified atmosphere at 37°C in a 5% CO<sub>2</sub>. Virus control (VC) consisting of maintenance media added 1 hour in quadruplicate wells before a tenfold dilution of previously titrated virus was added at 100 µl per well. Drug control (DC) was set up in the same way as extracts except that a 100 µL of acyclovir solution prepared at 5 µg/mL in phosphate buffered saline from the tablet was used instead of the extract. Cell control (CC) and extract/acyclovir cytotoxicity (CTC) controls were included in respective experiments, consisting of non-infected cells without extract/acyclovir and cells with extract/acyclovir respectively. The virus titer was calculated using spearman-karba formula as for the determination of stock virus titer. The antiviral activity was expressed as titer reduction factor (RF) by calculating the ratio of the VC titer over virus titer in the presence of the extracts. The extracts were then evaluated for antiviral activity by MTT colorimetric technique at 100 µg/mL and 25 µg/mL to confirm antiviral activity and screen for graded response.

### **2.5.2 MTT Confirmation of antiviral response and screening for graded response**

The method by Jaime et al. [21] was adopted. The selected extracts were screened for graded response at 100 µg/mL and 25 µg/mL using control virus (TCID<sub>50</sub> (0.1ml) = 1 x10<sup>7</sup>) by MTT colorimetric assay. The 96- well plate was seeded as for EPTT assay and cells grown for 24 hours. The growth media was removed and cells pre-incubated in triplicates with 100 µl of extract at 100 µg/ml and 25 µg/ml for 1 hour. The extracts were replaced with 90 µl of control virus followed by 10 µl of extract concentration at 1000 and 250 µg/ml to make 100 µg/ml and 25 µg/ml respectively. Each test concentration had its own cytotoxicity control (CTC) containing test extract without the virus. Virus control (VC) and cell control (CC) was included in the experiments. The VC contained the virus without test extract while CC contained maintenance media without the virus or extract. Acyclovir at 5 µg/ml was used as reference drug and treated the same

way as test extract. For each of the wells containing cells; test extract at 25 µg/mL, test extract at 100 µg/mL, CC and VC, a blank well containing respective solutions without cells were included. The wells were treated for spectrophotometric analysis by MTT procedure as explained for the cytotoxicity test. The antiviral activity was expressed in terms of percentage cell protection and calculated using the formula below;

$$\% \text{ Cell protection} = \frac{(\text{ODt} - \text{ODtb}) - (\text{ODvc} - \text{ODvcb})}{(\text{ODcc} - \text{ODccb}) - (\text{ODvc} - \text{ODvcb})} \times 100$$

ODt = Optical density of test extract, ODtb = Optical density of test blank, ODvc = Optical density of virus control, ODvcb = Optical density of virus control blank, ODcc = Optical density of cell control and ODccb= Optical density of cell control blank.

### **2.5.3 Determination of Inhibitory concentration 50 (IC<sub>50</sub>) and therapeutic index**

For extract giving more than 50% cell protection at both 25 and 100 µg/ml, inhibitory concentration 50 was determined. Growth media was removed from a 24 hour monolayer of Vero cells and replaced with extracts in duplicates at a final concentration of 500, 250, 125, 62.5, 31.25, 15.625 µg/mL with their respective CTC, blanks, CC and VC included and treated as explained above. The respective % cell protection was calculated and the IC<sub>50</sub> obtained by plotting a graph relating mean percentage protection of 3 different experiments and extract concentration. Therapeutic index (ie selective index) was expressed as the ratio of CC<sub>50</sub>/IC<sub>50</sub>.

## **3. RESULTS AND DISCUSSION**

Table 1 indicates medicinal plants investigated for activity against herpes simplex type 1. The table shows that majority of the plants possess secondary metabolites known to be pharmacologically active. Furthermore, many of these plants have shown activity against infectious agents and therefore have potential for human health benefits. However, none of the plants have previously been investigated for activity against Herpes simplex type 1. This is the first time the findings on anti-herpes study are being reported.

Table 1. Ethno-pharmacological and chemical data of the medicinal plants selected

Collection number	Family	Scientific name	Part used and condition(s) described for use by Mukhwa community	Method of preparation and application	Chemical composition	Pharmacological activity
KK01	Compositae	<i>Tithonia diversifolia</i> (Hemsl.) Gray [Anmabinzo]	Whole root. Herpes zoster	Decoction and drinking	Alkaloids, Saponin, Saponin glycosides, Tannins, Balsam, Cardiac glycosides and volatile oils [22]	Antimicrobial activity against <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> [22]
KK02	Compositae	<i>Schkuhria pinnata</i> (Lam) O.Ktze [Olwayi]	Leaves. Mouth ulcers, cold sores	Cold infusion and topical application		Ethno - medical use for malaria [23], Diabetes [24], <i>In vitro</i> antibacterial activity against <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> and <i>Escherichia coli</i> , and <i>In vitro</i> inhibition of Inflammatory enzymes [25]
KK03	Mimosaceae	<i>Entada abyssinica</i> (A. Rich) [Musembe]	Stem Bark. Skin ulcers, cold sores	Decoction and drinking	Alkaloids, flavonoids, Tannins, Saponins and cardiac glycosides; (5S,6R,8AR) -5-(carboxymethyl) - 3,4,4a,5,6,7,8,8a -octahydro - 5,6,8a - trimethylnaphalene-carboxylic acid ; methyl 3,4,5 - trihydroxybenzoate(methyl galate) ; benzene - 1,2,3 - triol (Pyrogallol); and 2,3 - dihydroxypropyltriacontanoate. Lipids; Hexadecanoic acid, 9-Octadecenoic acid and Octadecanoic acid [21,22]	<i>In vitro</i> antibacterial activity against <i>Klebsiella pneumonia</i> , <i>Pseudomonas aeruginosa</i> , <i>Proteus mirabilis</i> , <i>Shigella flexneri</i> , <i>Salmonella typhi</i> , <i>Escherichia coli</i> , <i>Enterococcus faecalis</i> , <i>Staphylococcus aureus</i> , Some strains of <i>Candida</i> species. High <i>In vitro</i> antioxidant activity [26,27]
KK04	Clusiaceae	<i>Garcinia buchanii</i> (Baker) [Khumukhomeli]	Stem Bark. Herpes zoster	Decoction and drinking	Isomanniflavanone; an ent-eriodictyol-(3a→6)-dihydroquercetin-linked biflavanone, 1,5-dimethoxyjacareubin; depsidone garcinisidone -G; (2''R,3''R)-preussiannon; euxanthone; 2-isoprenyl-1,3,5,6-tetrahydroxyxanthone; jacareubin; isogarcinol and garcinol [28]	<i>In vitro</i> inhibition of gastrointestinal motility [29]
Kk05	Euphorbiaceae	<i>Croton macrostachyus</i> (Hochst) [Omutswitswi]	Stem Bark. Fever and skin conditions, cold sores	Decoction and drinking	Benzylbenzoate, Linalool, gama-murolene, alpha-farnesene, delta-cadinene and alpha curcumene [30]	<i>In vitro</i> antibacterial activity against wide range of gram positive and gram negative bacteria [31,32] Anti.leishmanial activity, moderate toxicity to human monocytic leukemia cell line and erythrocytes from sheep [30] Ethnobotanical use for sore throat, TB, Uvula, heamostatics, stomachache, measles, STD, dysentery, diarrhea and malaria [33], <i>In vivo</i>

KK07	Compositae	<i>Vernonia adoensis</i> Walp [Khumululusia kumuseja]	Whole roots. Genital ulcers, <i>Herpes zoster</i>	Decoction and Drinking	Phenols, Saponins, Tannins, Flavonoids [35]	anti-Nociceptive and Anti-Inflammatory effect [34] <i>In vitro</i> inhibition of Inflammatory enzymes [25] Antibacterial activity against cariogenic bacteria [35]
KK08	Apocynaceae	<i>Plumeria alba</i> L. [Frangipani]	Leaves. <i>Herpes zoster</i>	Crush and apply on the sores	Leaves; Terpenoids, Flavonoids, Alkaloids, Glycosides and Phytosteroids [36], Flowers; Steroids, Flavonoids and Alkaloids [37].	<i>In vivo</i> antimalarial activity [38], <i>In vitro</i> antifungal activity against <i>Candida albicans</i> , <i>Aspergillus niger</i> and <i>Penicillium chrysogenum</i> [39], <i>In vitro</i> antibacterial activity against <i>Escherichia coli</i> , <i>Staphylococcus saprophyticus</i> , <i>Proteus vulgaris</i> and <i>Serratia marcescens</i> [37], <i>In vitro</i> and <i>In vivo</i> anti- cancer activity [40] Anticancer and antioxidant activity [43]
KK09	Caesalpinaceae	<i>Caesalpinia decapetala</i> (Roth.I.) Alston [Lunani]	Whole Root. Genital ulcers, Cold sores	Decoction and Drinking	6'-hydroxy-epoxy-propane)-2',3'-(1''β-hydroxy-2-carbonyl-cyclobutane)-1,1'-diphenyl; Octacosyl 3,5-dihydroxycinnamate; 2',4,4'-trihydroxychalcone; bonducellin; 7,3',5'-trihydroxyflavanone, daucosterin and β-sitosterol [41], Cassane diterpenoid (spathulenol; 4,5-epoxy-8(14)-caryophyllene,; squalene; lupeol; trans-resveratrol; quercetin; astragalin and stigmaterol [42], lupeol acetate; lupeol; oleanoic acid; pentacosanoic acid 2,3-dihydroxypropylester; 1-(26-hydroxyhexacosanoyl)-glycerol; stigmaterol; beta-sitosterol [43]	

Table 2. Cell viability response to extracts of selected plant species

Extract concentration in µg/mL	% Mean vero cell viability response to plant species ± sd of 3 different experiments							
	<i>T. diversifolia</i> (Whole root)	<i>S. pinnata</i> (Leaves)	<i>E. abyssinica</i> (Stem bark)	<i>G. buchani</i> (Stem bark)	<i>C. macrostachyus</i> (Stem bark)	<i>V. adoensis</i> (Whole root)	<i>P. alba</i> (Leaves)	<i>C. decapetala</i> (Whole root)
500	44.5000±.95394	6.0000±.91652	58.8000±3.01993	57.0000±2.00000	40.4000±2.07846	46.5000±1.30000	2.6±.62450	67.6667±1.52753
250	82.8000±1.01489	14.2000±.55678	72.5000±2.78388	68.0000±2.00000	78.0000±1.73205	81.2000±1.85203	13.4±.87178	76.5000±1.34536
125	85.7000±.91652	17.4000±1.40000	80.0000±3.46410	92.6000±1.50997	86.0000±1.73205	83.6000±1.32288	51±2.64575	78.5000±2.72213
62.5	86.2000±1.11355	82.0000±4.35890	94.0000±2.64575	98.0000±.43589	87.0000±1.00000	88.5000±.81854	62±3	92.6000±1.70587
31.25	96.0000±1.40000	95.3000±.70000	97.0000±1.20000	98.4000±.52915	88.0000±3.21403	93.3000±.65574	68±2.64575	91.0000±2.64575
15.625	100.0000±.00000	97.0000±1.11355	97.8000±.52915	98.6000±.26458	92.0000±1.73205	98.2000±.60000	70±5.29150	98.1333±1.20554
0	100.0000±.00000	100.0000±.00000	100.0000±.00000	100.0000±.00000	100.0000±.00000	100.0000±.00000	100.0000±.00000	100.0000±.00000

Cell viability experiments was aimed at detecting the limits beyond which the materials could cause harm to people using them, and also to detect the concentration that would allow the study of antiviral effect while minimizing toxicity. Except for *S. pinnata*, Table 2 indicates that most of the extracts preserved the viability of cells above 50% at 125 µg/ml but most of them had very limited or no none toxic range, thus Figs. 1-3 shows none toxic range at ~ 20, <20, 20, 40, 40, 20, <20 and 20 µg/ml for *T. diversifolia* Whole root), *S. pinnata* (Leaves), *E. abyssinica*

(Stem bark), *G. buchananii* (Stem bark), *C. macrostachyus* (Stem bark), *V. adoensis* (Whole root), *P. alba* (Leaves), *C. decapetala* (Whole root) respectively.

The extract concentrations reducing viability of cells by 50%, representing cytotoxic concentration 50 (CC<sub>50</sub>) shows values above 100 µg/mL except *S. pinnata*. Thus selecting 100 µg/mL concentration for EPTT antiviral screening would not have severely compromised cell viability, hence allowing viral cytopathic effect (CPE) assessment.

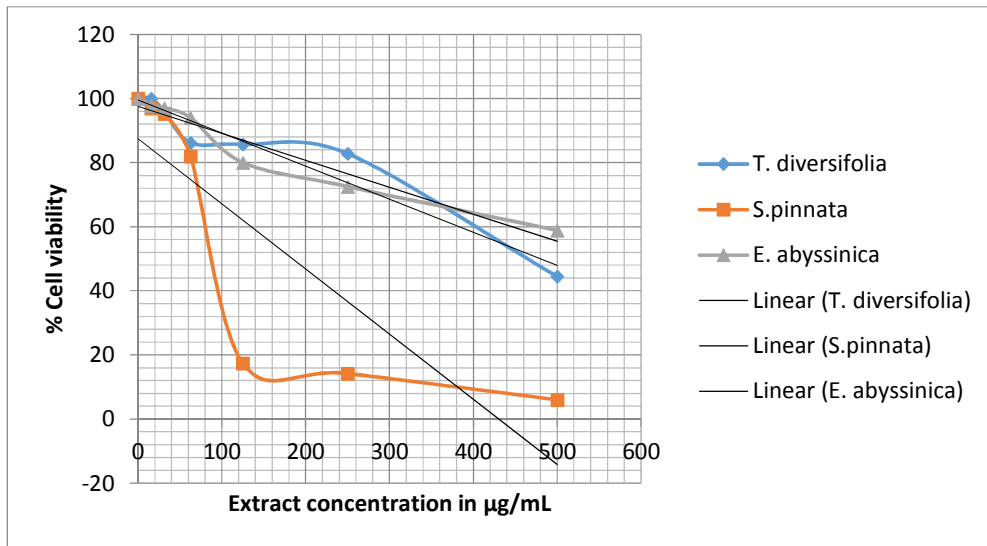


Fig. 1. Showing CC<sub>50</sub> of *T. diversifolia*, *S. pinnata*, *E. abyssinica* as; 460, 90 and >500 µg/ml respectively

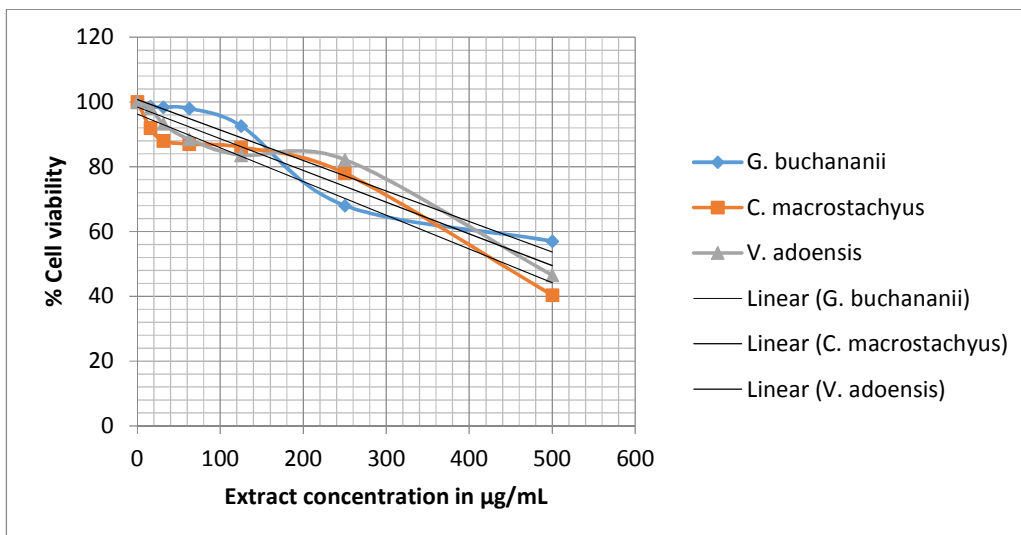


Fig. 2. Showing CC<sub>50</sub> of *G. buchananii*, *C. macrostachyus*, *V. adoensis* as; >500, >500 and 470 µg/mL respectively



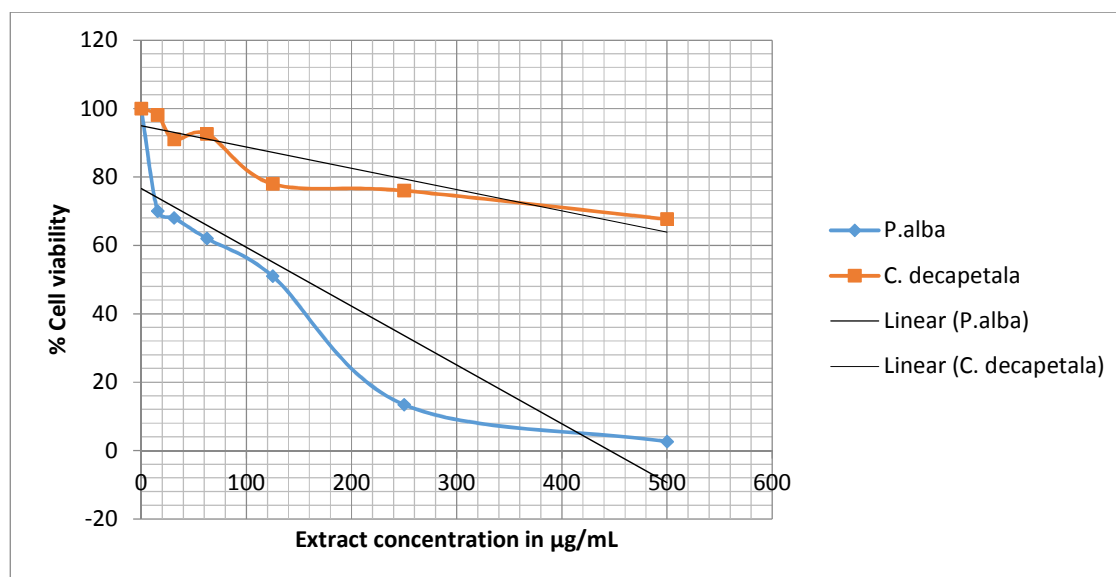


Fig. 3. Showing  $CC_{50}$  of *P. alba* and *C. decapetala* as; 120 and >500 g/mL respectively

The results of EPTT screening (Table 3) indicate that extract from *S. pinata*, *E. Abyssinica*, *P. alba* and *C. decapetala* provided at least  $10^1$  fold protection of cells against viral infection.

### 3.1 *In vitro* Antiviral Activity by EPTT

However, further screening test by MTT at 100 and 25 µg/mL (Table 4) indicated that only *G. b Buchananii*, *C. decapetala* and *E. Abyssinica* provided above 10% cell protection with *G. b Buchananii* showing the best response. The low percentage protection observed in *S. pinata* by MTT at 1.85 and 4.85% compared to above  $10^1$  fold RF by EPTT indicates that the antiviral activity observed by EPTT could be due to its toxicity or the extract contains both toxic and antiviral principles. The toxicity affected MTT metabolism without causing immediate death or change in morphology as based on EPTT determination. The same explanation applies to *P. alba*, showing above  $10^1$  RF and only 5 and 6.9% cell protection at 25 and 100 µg/mL extract concentration respectively. Both *V. adoensis* and *G. b Buchananii* gave a near  $10^1$  RF at 100 µg/mL showing  $10^{0.86}$  and  $10^{0.71}$  for *V. adoensis* and *G. b Buchananii* respectively. However, *G. b Buchananii* gave strong cell protection value by MTT showing that there might be a different factor contributing to cell recovery from effect of virus activity. Since Vero cells are known to be alpha and beta interferon deficient [44], factors involved in cell health such as antioxidant property of plant material might have played

synergistic role augmenting the little antiviral property of the extract.

Table 3. Virus titer reduction factor at 100 µg/ml (RF)

Plant	Part used	RF
<i>T. diversifolia</i>	Whole root	0.375
<i>S. piñata</i>	Leaves	1
<i>E. abyssinica</i>	Stem bark	1.16
<i>Garcinia b Buchananii</i>	Stem bark	0.71
<i>C. macrostachys</i>	Stem bark	0.17
<i>V. adoensis</i>	Whole root	0.86
<i>P. alba</i>	Leaves	1.25
<i>C. decapetala</i>	Whole root	1.26

Based on high percentage protection shown by *G. b Buchananii* and *C. decapetala* extracts, further determination was carried out at 7 different values. Fig. 3 for *G. b Buchananii* and Fig. 5 for *C. decapetala* shows percentage protection values in the table and comparison between protection and cell viability in the figures.

### 3.2 *G. b Buchananii*

For *G. b Buchananii*, maximum cell protection concentration (MCPC) obtained was at approximately 60 µg/ml and maximum non-toxic concentration (MNC) was approximately 40 µg/ml. Although the results show that maximum cell protection is higher than maximum non-toxic concentration, the maximum cell protection values does not severely reduce cell viability,

showing that there is good cell resistance to the effect of the extract. This phenomenon is indicated in therapeutic index (TI) showing  $> 25$ . The TI compares well with the TI value established in acyclovir [45], indicating that the extract could provide useful compounds, if fractionated and identified by bioactivity guided assays. Phytochemical studies has previously reported the presence of; Isomanniflavanone; an ent-eriodictyol-(3a→6)-dihydroquercetin-linked biflavanone, 1,5-dimethoxyjacareubin; depsidone garcinisidone -G; (2''R,3''R)-preussiannon; euxanthone; 2-isoprenyl-1,3,5,6-tetrahydroxyxanthone; jacareubin; isogarcinol and garcinol in ethanolic-aqueous extract of *G. buchananii* [28]. None of these compounds have previously been investigated for antiviral activity, but it is worth noting that phenolic groups comprising a significant proportion of ethanolic-aqueous extract, such as caffeic acid, chrysoplenols, leuteolin have previously been associated with anti HSV activity [46]. Strong antioxidant activity has been demonstrated and associated with the reported compounds in a bio-guided assay [28], and this could possibly contribute to enhanced cell health and therefore better cell preservation.

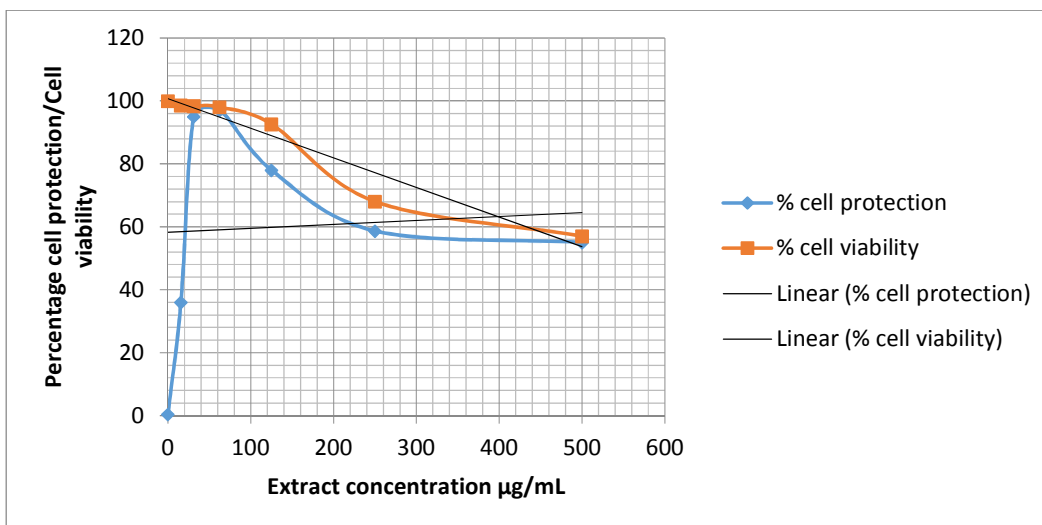
### 3.3 C. decapetala

For *C. decapetala*, the maximum cell protection concentration was approximately 120  $\mu\text{g/ml}$  and maximum non-toxic concentration of approximately 20  $\mu\text{g/ml}$ . The TI was  $>6$  and like

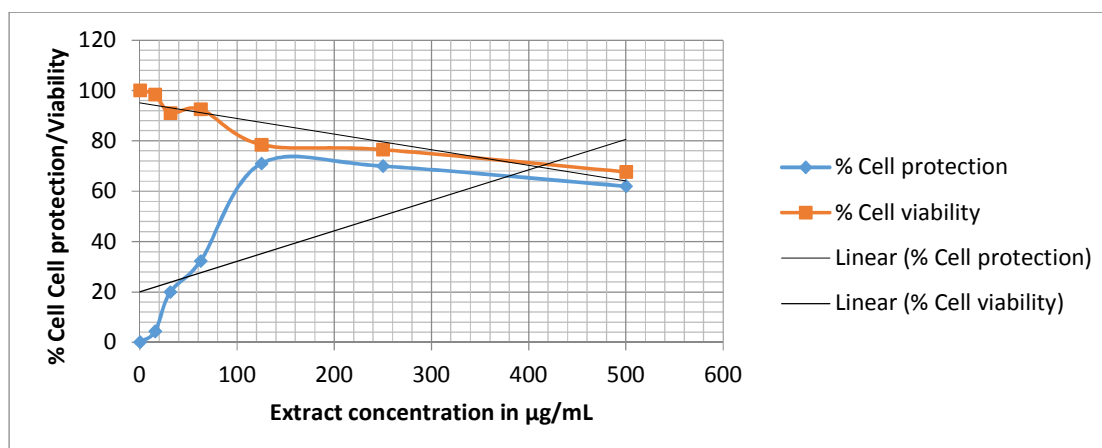
*G. buchananii*, there was good cell tolerance as well as favorable comparison with TI of acyclovir [45]. Previous studies have shown that the main constituents of *C. decapetala* belong to terpenoid and flavonoids [42]. The terpenoid compound previously associated with anti HSV activity was tetrahydro-cannabinol and as with *G. buchananii*, chrysoplenols and leuteolin have shown activity against HSV [46]. Again as in the case of *G. buchananii*, *C. decapetala*, has high antioxidant activity [45] which could partly explain the enhanced cell viability.

**Table 4. Percentage Cell protection of plant extracts against HSV 1 at 25  $\mu\text{g/mL}$  and 100  $\mu\text{g/mL}$**

Plant	Part used	Percentage cell protection	
		25 $\mu\text{g/mL}$	100 $\mu\text{g/mL}$
<i>T. diversifolia</i>	Whole root	0.02%	4.9%
<i>S. pinnata</i>	Leaves	1.85%	4.85%
<i>E. abyssinia</i>	Stem bark	13.7%	21%
<i>G. buchananii</i>	Stem bark	91.4%	100%
<i>C. macrostachyus</i>	Stem bark	0%	0%
<i>V. adoensis</i>	Whole root	0%	0%
<i>P. alba</i>	Leaves	5%	6.9%
<i>C. decapetala</i>	Whole root	17.1%	68.5%



**Fig. 4. Comparison of cell viability and cell protection against virus infection of *G. buchananii*. Maximum cell protection concentration obtained was at approximately 60  $\mu\text{g/ml}$  and maximum non-toxic concentration was approximately 40  $\mu\text{g/mL}$  and therapeutic index ( $\text{CC}_{50}>500 \mu\text{g/mL}/\text{IC}_{50}\sim 20 \mu\text{g/mL}$ )  $> 25$**



**Fig. 5. Comparison of cell viability and protection against virus infection of *C. decapetala*. Maximum cell protection concentration obtained was approximately 120 µg/mL and maximum non-toxic concentration was approximately 20 µg/mL and therapeutic index ( $CC_{50}>500$  µg/mL/ $IC_{50}\sim 80$  µg/mL) > 6**

#### 4. CONCLUSION

Majority of the medicinal plants selected for anti-herpes activity have narrow none-toxic limits. *G. b Buchananii* and *C. decapetala* have the most promising potential for selective anti-herpes activity.

#### CONSENT

Consent for the study was obtained from the Government of Kenya (GOK), Ministry of Health and the Community Health Workers involved as informants in the study.

#### ETHICAL APPROVAL

The study was approved by the Scientific and Ethics Review Unit (SERU) of the Kenya Medical Research Institute (KEMRI), approval number SSC 2285.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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