



Determination of Antioxidant Value and Chemical Groups in Selected Medicinal Plants Used for Conditions Associated with Herpes Simplex and Herpes Zoster Infections in Kakamega County, Kenya

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Authors' contributions

This work was carried out in collaboration among all authors. Author AOR designed the study, wrote the protocol, identified the plant species, managed the data collection, analyses, literature search and wrote the first draft of the manuscript. Authors MK, AOM and FMT critically reviewed every step in the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To Determine antioxidant value and chemical groups in selected medicinal plants used for conditions associated with Herpes simplex and Herpes zoster infections in Mukhwa sub-location, Kakamega County, Kenya.

Study Design: A qualitative ethnobotanical survey for plant identification and chemical analysis for antioxidant assay and chemical group detection.

Place and Duration of Study: Plant samples were collected in Mukhwa sub-location in September 2014. Sample processing and chemical group detection was carried out at the Center of Traditional Medicine and Drug Research of Kenya Medical Research Institute. Antioxidant assay was carried out at the Department of Medical Laboratory Sciences of the Kenya Medical Training College.

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Methodology: All 12 Community Health Workers, comprising 7 females and 5 males, were interviewed for identification of plant species. Antioxidant assay was carried out using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) reduction assay and detection of flavonoids, terpenoids, alkanoids, saponins and phenols carried out using physico-chemical methods.

Results: *Caesalpinia decapetala*, *Garcinia buchananii* and *Entada abyssinica*, were the most potent sources of antioxidant with the concentration giving 50% DPPH reduction (RSa₅₀) of 50, 20 and 10 µg/ml, respectively. The most abundant chemical groups were; alkaloids in *Schkuhria pinnata*, terpenoids in *E. abyssinica*, flavonoids in *G. buchananii*, the latter also contained the highest amount of phenols.

Conclusion: The findings of antioxidant and chemical groups in selected medicinal plants support their use for HIV conditions.

Keywords: Antioxidant; chemical groups; medicinal plants; herpes simplex; herpes zoster.

1. INTRODUCTION

The practice of seeking health benefits from medicinal plants is a worldwide phenomenon [1]. Evidence of benefits is often anecdotal and when established for specific conditions, can hardly be attributed to one factor given that plants contain assorted phytochemicals known for such properties as antimicrobial, anticancer and those that are nutritionally active [2]. In Kakamega County, Kenya, the practice of using medicinal plants to treat people living with HIV was observed in Mukhwa sub-location. Medicines were mainly prescribed for opportunistic infections, and anecdotal claims of cure were reported among the local population. Those used against Herpes simplex infection were investigated by Radol et al. [3]. The anti-herpetic activity obtained in some of the investigated plant species could be attributed to phytochemicals acting singly or in co-operation with other chemical groups, while those not showing activity could have some indirect health promoting value such as antioxidant.

The relationship between progression of HIV to AIDS and oxidative stress was reported by Tang et al. [4] and Dhalwal et al. [5]. Individuals who have high level of free oxidative radicals tend to experience faster progression to AIDS [4]. Dhalwal et al. explained that microbial infections cause the release of highly oxidative molecules from cells due to enhanced metabolism of oxygen, leading to extensive damage to cells and tissues [5]. The cycle of increase in pro-oxidant molecules due to infection and progression of HIV because of an increase of pro-oxidants, indicate the central role of antioxidant in care and management of HIV. A part from the relationship between HIV and antioxidants, wide range of chronic diseases are known to be associated with oxidative stress [6]. Liu [7] cited plants as

important source of antioxidant and a cure of many chronic ailments including those associated with endogenous viruses. From the preceding observations, it is clear that the antioxidant factor of plants must be considered when relief from infectious conditions are attributed to medicinal plants. Such consideration is more relevant when viral infection is being evaluated, given that viability of a cell is central to infection resistance. The value of plants as antioxidant source, the health benefits reported by PLWHIV and the cell protection by some plant species against HSV-1 as reported in our previous work [3], was the central basis for this study. Thus the aim of this investigations was to determine the antioxidant and chemical groups in selected medicinal plants used for conditions associated with *Herpes simplex* and *Herpes zoster* infections in Mukhwa sub-location, Kakamega County, Kenya. Such investigation supports the World health organization (WHO) goals of harnessing potential contribution of tradition and complementary medicine (T& CM) to health, wellness and people centered care and Universal Health Coverage, and promoting safe and effective use of T&CM through regulation, research and integration of T& CM products, practices and practitioners in health systems.

2. MATERIALS AND METHODS

2.1 Study Design

A qualitative ethnobotanical survey design was employed to identify medicinal plants used for HIV conditions from Community Health Workers (CHWs) as respondents. Chemical analysis was used to quantify the antioxidant content of the plant species and detection of phytochemical groups present.

2.2 Study Site

The study was carried out at Mukhwa sub-location in Mumias sub-county, Kakamega County, Kenya (Map 1). The sub-locations were identified for study because of the high numbers of long term serving CHWs and high number of cumulative number of HIV clients registered under community care, as reflected in HIV programme reports from the year 2008 [8].

2.3 Sampling Criteria

Data was obtained from all 12 CHWs who were serving under the jurisdiction of Mukhwa. Each Community Health Unit (CHU) is equivalent to a sub-location and is the smallest health administration unit as per the Kenya's ministry of health (MOH) structure for community health strategy. Respondents comprised 7 females and 5 males.

2.4 Sampling of Plants

As reported by Radol *et al.*, plant species reported for Herpes simplex Virus (HSV), Herpes

zoster (HZ) or related infection symptoms such as genital, oral ulcer or skin lesion treatment were targeted for antioxidant determination to establish possible independent or synergistic benefits to users.

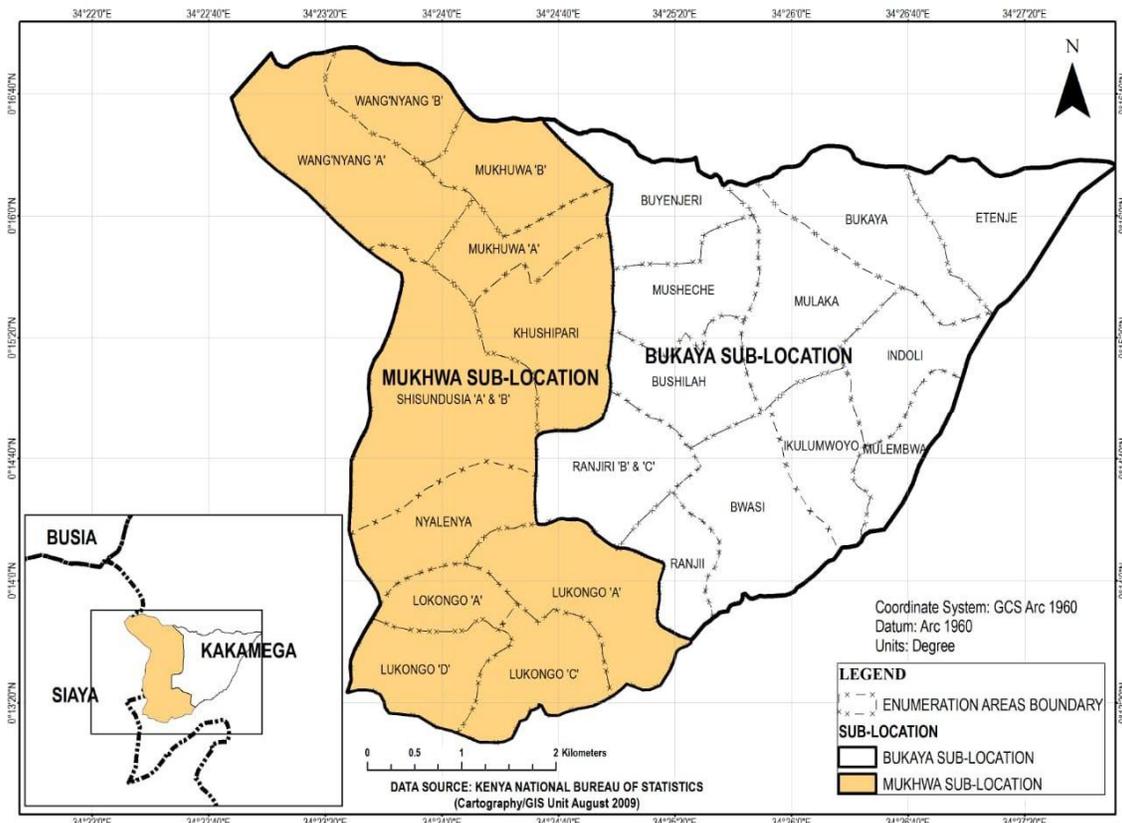
2.5 Data Collection Procedure

2.5.1 Key informant interviews

Key informant interview and sample collection was carried out in September 2014. A key informant interview guide was used to collect the data. Information was obtained on knowledge of conditions associated with HIV infection and the status of herbal medicine used by people living with HIV (PLWHIV). Further inquiry was about the herbal medicines used by their clients for care and management of HIV conditions, plant parts used, the method of preparation and application and the local names.

2.5.2 Collection of medicinal plants

The CHW were used as guides during field trips to collect samples for laboratory analysis and



Map 1. Mukhwa sub-location in Kakamega County, Kenya

voucher specimens. Plant collection and handling was according to Tolo et al. [9]. For scientific identification, the specimens comprising leaves, flowers and or seeds were taken and pressed between old newspapers and transported to the University of Nairobi herbarium within 36 hours. In the herbarium, the plants were identified in accordance with taxonomic practice. The same method of collection and handling was applied for laboratory samples. At least one kilogram of relevant parts of the plants was wrapped in old newspapers and packed in cartons. The samples were delivered to the laboratory drying room within 36 hours of collection. Further treatment before extraction was done according to Tolo et al. [9]. The medicinal part of the plant was dried at room temperature for 2 weeks before being powdered for hot water extraction. Using hot water was the method of choice by the community for phytochemical extraction.

2.5.3 Hot water extraction and preparation of the extract

The dried material was ground to form powder using an 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.

2.5.4 Antioxidant assay

The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) reduction assay as described by Igbinosa, was used with slight modification [10]. The DPPH reagent was prepared at a concentration of 0.116 mM in methanol. The samples were plant extracts prepared in duplicate concentrations ranging between 3.9 – 500 µg/ml in methanol, and reference standard of ascorbic (Howse and Mc George LTD) acid prepared in duplicate concentrations ranging between 8 – 0.25 µg/ml in methanol. The experiment was carried out by mixing 1 ml of the sample with 1 ml of DPPH reagent. A DPPH control was set up by mixing 1ml of DPPH reagent and 1 ml of methanol. The mixtures were vortexed thoroughly and incubated in the dark for 30 minutes at room temperature. The absorbance of the samples and the control were read spectrophotometrically at 518 nm and using the mean absorbance of each duplicate, the reduction power was obtained using the formula below;

$$\text{Reduction power (\%)} = \frac{[(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}})]}{[(\text{Absorbance}_{\text{Control}})]} \times 100$$

Where

Absorbance_{control} = Absorbance of control,

Absorbance_{sample} = Absorbance of reference standard or extract

A graph relating mean reduction power (%) of three different experiments in vertical axis and concentration of sample on the horizontal axis was used to estimate the concentration of sample reducing the absorbance of DPPH control by 50% (Rsa₅₀).

2.5.5 Chemical characterization

The extracts were tested for presence of Flavonoids, Terpenoids, Alkaloids, Saponins and Phenols. For each fraction, a 1 mg/ml concentration was prepared in methanol and used for chemical tests as explained below.

2.5.5.1 Flavonoids

To 1 ml of extract solution in methanol, 5 ml of dilute ammonia was added; the solution was examined for development of yellow colour upon addition of 1 ml of sulphuric acid. Further, the yellow colour was examined for disappearance on standing [11].

2.5.5.2 Terpenoids

To 5 ml of extract, 2 ml of chloroform and 3 ml of concentrated, H₂SO₄ was added and a layer of reddish brown coloration at the interface of the two solutions was examined for indication of the presence of terpenoids [12].

2.5.5.3 Alkaloids

To 5 ml of the extract, few drops of Mayer's reagent were added; formation of white precipitate was examined as indication of the presences of alkaloids [13].

2.5.5.4 Saponins

The extract was diluted with 10 ml of distilled water and shaken for 15 minutes. The solution was examined for formation of stable foam that indicates presence of saponins [12].

2.5.5.5 Phenols

To 5 ml of extract solution, drops of 10% Ferric chloride was added and examined for formation of intense blue colour indicating presence of phenols [14].

3. RESULTS

The responses shown in Table 1 show that CHW were knowledgeable on diseases associated with HIV. The use of herbal medicine was regarded positively by most of them as the responses from; KK/BKA/01, 02, 03, 04, 05, 07, 09, 10, 11, 12.

3.1 Plants Selected for Antioxidant Assay

Eight (8) plant species were identified based on prescription for Herpes simplex or Herpes zoster infection or infection symptoms such as genital, oral ulcers or skin lesions (Table 2).

3.2 Screening of Aqueous Extracts for Antioxidant Activity

At 500 µg/mL, *S. pinnata* (KK02) gave the highest DPPH reduction. The extract of *P. alba* (KK08) did not reduce DPPH (Table 3).

3.2.1 Sample concentration reducing DPPH by 50% (RSa₅₀)

Extracts giving more than 60% DPPH reduction were further investigated for the sample concentration giving 50% DPPH reduction (RSa₅₀). The RSa₅₀ were; *T. diversifolia* ~ 110 µg/ml, *S. pinnata* ~150 µg/ml, *E. abyssinica* ~20 µg/ml, *G. buchananii* ~ 10µg/ml, *V. adoensis* ~ 240 µg/ml and *C. decapetala* ~50 µg/ml (Fig. 1) and compared with ascorbic acid. The RSa₅₀ for positive control, the Ascorbic acid was 0.8 µg/ml (Fig. 2).

3.3 Phytochemical Screening of Extracts for Common Pharmacologically Active Principles

Alkaloids was detected in all the extracts while Saponins were absent by methods employed (Table 4).

Table 1. Responses to knowledge of HIV conditions and status of herbal medicine use in the community

Respondent code	HIV conditions	Status of herbal medicine use
KK/BKA/01	Diarrhea, skin problems, mouth infections	'The herbal medicines are useful to HIV patients'
KK/BKA/02	Stomach problems, Fever, poor appetite, rashes	'Those that work are used a lot'
KK/BKA/03	Herpes zoster, Headache, Syphilis, coughs	'Some people use them and get well, they can be given a trial'
KK/BKA/04	TB, Weight loss, rashes, diarrhea	'They are not effective for HIV management'
KK/BKA/05	Fever, coughs, skin problems, diarrhea	'It is not effective and cause more harm on the patients' health just because it doesn't have dosage
KK/BKA/06	Tiredness, pneumonia, TB, rashes	'I discourage herbal drugs to be used by HIV patients but instead use hospital drugs which are effective
KK/BKA/07	Wounds, stomachache, Malaria, mouth sores	'A lot of people use them'
KK/BKA/08	Anaemia, vomiting, TB, coughs, fever, diarrhea	'Some people combine ARVs and herbal medicine'
KK/BKA/09	Diarrhea, skin problems, genital ulcers, eye problems	'Herbal medicine can be a good alternative because may be you can take for some time and stop. Not like ARVs where you take for the rest of your life'
KK/BKA/10	Pneumonia, skin rashes, mouth ulcers, diarrhea	'They are useful for stomach problems'
KK/BKA/11	Skin problems, headache, stomach ache, fever	'I know of HIV patients who have been using herbal medicine for the last 5 years'
KK/BKA/12	Herpes zoster, mouth sores, diarrhea, fever	' People like them very much'

Table 2. Plants selected for antioxidant assay

Scientific name of plant species	Local name of the plant species	HIV condition cited for use	Village	Part used	Method of preparation	Method of application
<i>Caesalpinia decapetala</i>	Lunani	Genital ulcers Herpes simplex Poor appetite, Mouth sores	Wang nyang 'A'	Whole root	Boiling	Drinking
<i>Croton macrostachys</i>	Omutswitswi	Fever and skin conditions, Herpes simplex	Mukhwa 'A'	Stem bark	Boiling	Drinking
<i>Entada abyssinica</i>	Museembe	Skin ulcers, Herpes simplex lesions	Mukhwa 'A'	Stem bark	Boiling	Drinking
<i>Garcinia buchananii</i> <i>Plumeria alba</i>	Khumukhomeli Frangipani	Herpes zoster Herpes zoster	Mukhwa 'A' Mukhwa 'B'	Stem bark Leaves	Boiling Pounding Crushing) Cold infusion or boiling	Drinking Topical
<i>Schuhria pinnata</i>	Olwayi	Mouth ulcers, cold sores	Mukhwa 'B'	Leaves	Boiling	Topical
<i>Tithonia diversifolia</i>	Amabinzo	<i>Herpes zoster</i>	Wang Nyang 'A'	Whole root	Boiling	Drinking
<i>Vernonia adoensis</i>	Khumululusia kumuseja	Herpes simplex Genital ulcers, Herpes zoster	Lukongo 'C'	Whole root	Boiling	Drinking

Table 3. Antioxidant activity at 500 µg/mL

Plant species	Collection code	Part used	Percentage DPPH reduction
<i>T. diversifolia</i>	KK01	Whole root	86.9
<i>S. pinnata</i>	KK02	Leaves	97.3
<i>E. abyssinica</i>	KK03	Stem bark	96.1
<i>G.buchananii</i>	KK04	Stem bark	89.1
<i>C. macrostachys</i>	KK05	Stem bark	28.2
<i>V. adoensis</i>	KK07	Whole root	58.7
<i>P. alba</i>	KK08	Leaves	0
<i>C. decapetala</i>	KK09	Whole root	92.3

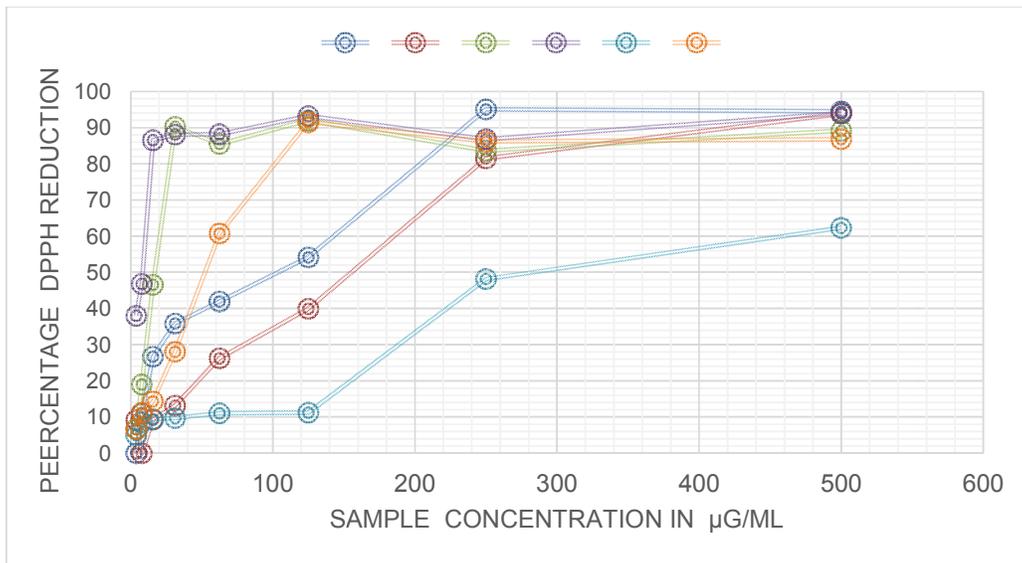


Fig. 1. RSa₅₀ of plant extracts

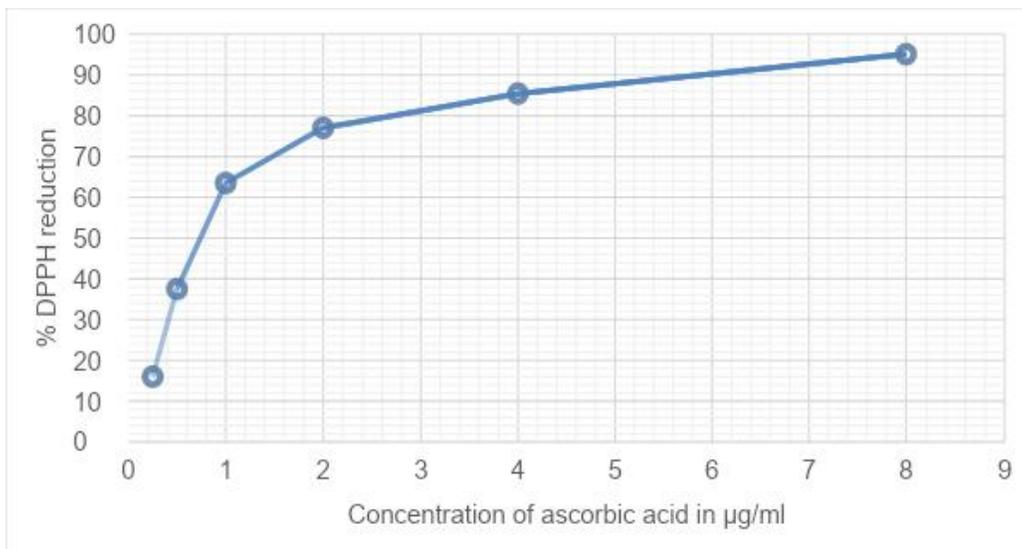


Fig. 2. RSa₅₀ of ascorbic acid reference standard

Table 4. Chemical characterization of sampled plant species

Sample	Alkaloids	Terpenoids	Saponins	Flavanoids	Phenols
<i>T. diversifolia</i>	+	+	-	+	+
<i>S. pinnata</i>	++	+	-	+	-
<i>E. abyssinica</i>	+	++	-	+	+
<i>G. buchananii</i>	+	+	-	++	++
<i>C. macrostachys</i>	+	-	-	+	-
<i>V. adoensis</i>	+	+	-	+	-
<i>P. alba</i>	+	-	-	+	-
<i>C. decapetala</i>	+	+	-	+	-

+ Chemical group present; ++ Chemical group is strongly present; - Chemical group absent

4. DISCUSSION

Prior to inquiry on plants used for management and care of HIV conditions, the study sought to assess the knowledge of CHW on HIV conditions and the status of herbal medicine use. The aim was to determine awareness of the relationship between HIV diseases and application of alternative medicine, existence of herbal medicine use in the community, the attitude about herbal medicine use, and observations on people using them.

Except syphilis, all other conditions are consistent with the WHO symptomatic staging of HIV disease (WHO, 2006). The knowledge of HIV conditions by CHW was not surprising, given that they are often the target of training programmes in HIV as part of community health strategy for primary health care (CHS) [15,16]. Further, the attitudes expressed by CHW show that medicinal plants are appreciated as optional treatment for HIV conditions.

The results show that *E. abyssinica*, *G. buchananii* and *C. decapetala* were the most potent source of antioxidant. These findings support results by previous studies [17-21] on antioxidant values of the plant species. The findings further support their medicinal use by PLWHIV, alleviating deficiency status associated with HIV as reported by Tang et al. [22]. Kamlesh et al. [23] explained that many microbial infections cause the cells to release the highly oxidative molecules due to enhanced metabolism of oxygen leading to extensive damage to cells and tissues. The anti HSV findings by Radol et al. [3] is consistent with the results on the basis of synergistic support provided by antioxidants in the *in vivo* mice experimental model.

The relatively more abundant chemical groups were; alkaloids in *S. pinnata*, Terpenoids in *E. abyssinica*, Flavanoids in *G. buchananii*, the latter also contained the highest amount of

Phenols. Flavanoids have been associated with antimicrobials, anti-inflammatory and antioxidant properties with Quercetin flavonoids being associated with anti HSV-1 activity [24,25]. Some of the alkaloids such as β -carbolines, furanoquinolines and camptothecin are reported to inactivate viruses by interacting with DNA [23]. Ferguson et al. [26] observed that compounds that are rich in phenols are most effective in antioxidant activity. The terpenoid groups are known for their interferon induction and some derivatives such as glyceric acid have been associated with HSV inhibition [25]. From these results it can be concluded that the high content of flavonoids and phenols in *G. buchananii* accounts for its antioxidant activity in the current report as well as anti HSV activity as reported by Radol et al. [3].

5. CONCLUSION

Three plant species; *E. abyssinica*, *G. buchananii* and *C. decapetala* have a potential for dual benefits to patients as an antioxidant source as well as anti-herpes. Phytochemicals known for antiviral activities are present in *S. pinnata*, *E. abyssinica*, and *G. buchananii*. The specific phytochemicals responsible for the present findings need to be isolated and characterized to determine and optimise their health benefits.

ETHICAL APPROVAL

Approval for the study was obtained from the Scientific and ethics 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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