PREVALENCE AND CHARACTERIZATION OF AVOCADO FRUIT FUNGAL DISEASES IN EMBU COUNTY, KENYA, AND THE EFFICACY OF *ALOE SECUNDIFLORA* CRUDE EXTRACTS IN THEIR CONTROL

Mwongeli M.F. Githae E.W and Mwangi J.M
Department of Biological Sciences, Chuka University, P. O. Box 109-60400, Chuka

franciscamwongeli30@gmail.com; egithae@chuka.ac.ke; jmmaina@chuka.ac.ke

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ABSTRACT
Avocado production faces various constraints that include common post-harvest fungal diseases. Most of these diseases are difficult to control using cultural measures while use of chemicals leads to pathogen reistance in addition to being harmful to human and environment. The objective of this study was therefore to determine the prevalence of the fungal avocado fruit diseases in Embu County, isolate and characterize the causal pathogens and test the efficacy of *Aloe secundiflora* extract in the diseases. Questionnaires were administered to a total of 120 respodents to gather additional information on avocado production, marketing and management. The stores and avocado bags were randomly identified and the fruits assessed for the prevalence of fungal avocado diseases. *Aloe* leaves were collected in the field and phytochemical analysis done using standard procedures. The efficacy of *Aloe secundiflora* extract against the avocado fungal diseases was done in the laboratory using a Completely Randomized Design. The extracts were then assessed for antimicrobial activity against *Colletotrichum gloeosporioides* (scab disease), *Sphaceloma perseae* (anthracnose) and *Cercospora purpurea* (Cercospora spot). In the results, chemicals were widely used to control the pathogens. The mean percentage prevalence of scab, cercospora spot and anthracnose diseases was 36.4%, 35.15% and 35.0%, respectively, among the markets, although anthracnose was highly prevalent within each market. Four avocado varieties were commonly sold with *Fuerte* and *Puebla* being the most susceptible to the fungal diseases. There were significant variations in the morphological characteristics of the pathogen isolates. The aloe extract revealed the presence of steroids, phenolics, flavonoids, saponins, terpenoids and phenolics. The extract prepared using ethanol had a high inhibitory activity against *C. gloeosporioides* (15.59mm) and

Cercospora purpurea (7.22mm), while hexane solvent extract had minimal antimicrobial activity (10.53 and 5.22mm respectively). Aloe crude extract is therefore a potential antifungal agent against *C. gloeosporioides* and *Cercospora purpurea*.
Keywords prevalence, avocado, fungal diseases, *Aloe secundiflora*

INTRODUCTION

Avocado (*Persea americana* Miller) is a significant fruit crop grown in tropical and subtropical regions (Selladurai & Awachare, 2020). The crop is gaining acceptance worldwide and has received extensive marketing due to its uses in pharmaceutical, cosmetic, oil and food industries (Kumar Gupta *et al.,* 2018). Avocado is grown in over three million hectares world wide. Mexico is the leading producer and exporter of the fruit worldwide with an average of 10.7 tonnes of avocado per hectare (FAOSTAT, 2020). This translates to about 33.9% of avocado harvested worldwide (Wasilwa *et al.,* 2018). The crop thrives well in subtropical climates such as Pacific America, South East Asia and Africa. Africa is one of the avocado fruit exporters mostly from Kenya and South Africa (Wolstenholme, 2008). Kenya has a conducive environment for avocado production. It produces approximately 13.22 tons of avocados per hectare for local use and for export (FAOSTAT, 2020) making it the sixth largest producer of avocado in the world and the leading in Africa (FAO, 2019). It produces 15.7 tons of avocados per hectare for fruits produced every year (Wasilwa *et al.,* 2018). About 70% of avocado is produced by small-scale growers mainly in Murang'a, Bungoma, Migori, Kiambu, Kisii, Nyamira, Tharaka Nithi and Embu counties of Kenya (Johnny *et al.,* 2019). Embu County accounts for approximately 30% of total avocado production in Kenya (Hortinews, 2015).

The popular avocado varieties grown in Kenya are *Hass*, *Fuerte*, *Puebla*, *Duke*, *G6* and *G7* (WAC, 2004). Avocado is a nutritious fruit rich in essential minerals such as manganese, phosphorus, iron and potassium. It is rich in vitamins such as vitamin A, B, E and β-carotene (FAO, 2018). It is a high source of protein and carbohydrate for populations in avocado producing and non-producing countries (Fan *et al*., 2017). Avocados can be consumed in various forms and do not require processing, preservatives or taste enhancers (Chaves & Borges, 2016). The fruit has various uses in pharmaceutical, cosmetic and oil industries (Kumar Gupta *et al*., 2018). In Kenya, production and marketing of avocadoes contributes to economic development and reduction of poverty because avocadoes are a source of income

and foreign exchange to the farmer, thus generating capital for economic development (Karingu *et al*., 2020). Avocado production and marketing has also contributed immensely to the growth of some rural areas in terms of job creation and income (Edna, 2019).

Avocado production in Kenya is faced by various biotic constraints for example diseases such as avocado anthracnose, scab disease, *Cercospora* spot, Verticillium wilt, Bacterial canker, among others. The fruit is also attacked by pests such as false codling moth, thrips, scales and fruit flies (Marais, 2016). Avocado fruit diseases of economic importance include anthracnose, scab and *Cercospora* spot that are caused by *Colletotrichum gloeosporioides*, *Sphaceloma perseae* and *Cercospora purpurea,* respectively (Marais, 2016). Characterizing these diseases is vital for developing and implementing effective control strategies.There are several post-harvest fungal pathogens that infect various avocado cultivars thus reducing the shelf life and marketability of the fruit. These post- harvest fungal diseases are estimated to cause about 83% losses in avocado production threatening the global avocado market (Fan *et al.,* 2017). Infection may go unnoticed during fruit development and appear at maturity in storage or while fruits are sold in the markets (Mekonnen *et al.,* 2015). This poses a challenge for their management because disease presence is identified when fruits begin to soften or rot, symptoms that usually occur at the market stage when it is not possible to control the problem (Sharma *et al.,* 2017).

Nevertheless, the awareness of farmers on protection of fruits from infection during growth in the field and postharvest care is little or lacking (Mekonnen *et al.,* 2015). Assessment of

the prevalence of these diseases is therefore important to map the geographic distribution and determine the status of the disease, in addition to providing baseline data to prioritize research problems (Eshte *et al.,* 2015). Post-harvest fungal diseases are mainly controlled using synthetic chemicals (Yoon *et al.,* 2013). However, the use of fungicides is becoming increasingly restricted particularly in many countries especially those applied after postharvest (Mekonnen *et al.,* 2015). For instance, regular copper fungicide sprays such as benomyl are applied in the postharvest control of avocado fruit diseases (Fesenko & Edwards, 2014). These chemicals contribute to toxicity in food and thus not suitable for human consumption, in addition to polluting the environment (Plant *et al.,* 2005). Excessive use of chemicals also contributes to microbial fungal resistance to fungicides, which complicates fungal disease managements in many countries (Roca *et al.,* 2015). Furthermore, losses due to fungal avocado diseases are still high about 83% despite the use of synthetic chemicals by avocado growers and traders (Fan *et al.,* 2017).

Cultural methods of controlling post-harvest fungal diseases include good sanitation procedures involving collection and disposal of diseased fruits (Agrios, 2005). Further, adequate postharvest handling practices are applied to prevent cuts and bruises to fruit surfaces (Wasilwa *et al.,* 2006). Fruits showing symptoms of postharvest diseases are not packed into cartons containing healthy fruits (Sarkhosh *et al.,* 2017). However, these methods are not effective, despite their use, market losses attributed to postharvest fungal diseases are still high (Agrios, 2005). It is therefore necessary to develop new and more antimicrobial substances against the pathogen for proper control and management (Carocho and Ferreira, 2013). Use of bio pesticide control agents is a promising approach for control of pathogens (Kesho, 2020) and plant extracts with secondary metabolites could be a good control measure against fungal pathogens.Natural plant extracts have been widely used in the control of plants and animals diseases (Savoia, 2012). Plant extracts are easily obtained, economical, non-toxic and do not interfere with the biological balance compared to synthetic chemicals (Proestos, 2020). The antimicrobial properties of most plant extracts are as a result of essential oils and other secondary metabolites in the plants such as terpenoids, flavonoids, saponins, alkaloids and tannins (Akinmoladun *et al.,* 2007).

Aloe plant is of economic importance in the control and management of diseases. It has been exploited since prehistoric times as a source medicine for the treatment of various diseases. This is because Aloe has antibacterial, antifungal, anticancer, antiviral and immunomodulatory properties (Bashir *et al.,* 2011). Despite the wide usage of Aloe, there is little literature on the type of the active ingredients within the plant's extracts (Bashir *et al.,* 2011). Aloe plant is tolerant to harsh environmental conditions and therefore able to reproduce continuously even during the dry season. As a way of survival, it produces some metabolites that enable them to survive. Many of these metabolites have antimicrobial activities and have been used to control some plant diseases such as *Fusarium oxysporum* that cause wilt in several plant species (Rachuonyo, 2016). Sixty-three species of Aloe occur in Kenya, of which around 50% are endemic (Bjorå *et al.,* 2015). They are widely distributed in the semi-arid areas and open grasslands of Kenya. Several of these species are threatened with extinction and knowledge about their use is important for their conservation strategies (Bjorå *et al.,* 2015). *Aloe secundiflora* Engl. is among the most important species. There is

limited information on the application of crude extracts of *Aloe secundiflora* in the control of post-harvest avocado fungal disease pathogens.

Statement of the Problem

Fungal avocado fruit diseases account for about 83% losses in avocado production hence threaten the avocado market worldwide. They infect all cultivars of avocado including the *Hass, Fuerte* and *Puebla* varieties grown in Kenya and hence reduce the shelf life and marketability of the fruit. Information on the prevalence of these diseases in market stores and packing houses before entering into global market is very limited and could be utilized as an early control and management measure. Cultural methods of controlling the

pathogens are ineffective while chemical methods are costly and unsafe to humans and environment. In addition, the continuous use of the chemicals has led to pathogen resistance hence creating a serious challenge in their management. Furthermore, despite their use, losses due to post- harvest avocado fungal infestation are still high. Natural plant extracts are an alternative and have been used widely to protect the plants and animals against disease causing pathogens. Among the plants is *Aloe secundiflora,* which has not been evaluated for its bioactivity against fungal avocado fruit diseases despite its antimicrobial activity against different fungal and bacterial pathogens. Objectives of the Study Broad Objective

To assess the prevalence of fungal diseases of avocado fruit in selected markets in Embu County, characterize the pathogens and test the efficacy of *Aloe secundiflora* crude extracts against the fungal pathogens.

Specific Objectives

i. To determine the prevalence of post-harvest fungal diseases affecting avocado fruits in selected markets in Embu County.
ii. To isolate and morphologically characterize postharvest fungal pathogens infecting avocado fruits.
iii. To evaluate the *in-vitro* efficacy of *Aloe secundiflora* crude extracts against isolates of fungal avocado fruit pathogens.

MATERIALS AND METHODS

The study was carried out in different markets in Embu County, Kenya. A descriptive cross-sectional survey design was adopted for this study. The survey was carried out in the six purposively selected avocado markets in the study area to assess the prevalence of fungal diseases of avocado fruit. A structured questionnaire was administered to avocado fruits sellers to gather information on challenges of avocado marketing, incidences of fungal diseases of avocado fruit, management strategies used and perceived success of such management options. In the laboratory, a 3×4 factorial experiment was laid out in a Completely Randomized Design. The isolates of the conidia were in three different levels and the concentrations of the plant extracts were at four different levels (0mg/ml, 2.5mg/ml, 5.0mg/ml, and 10.0mg/ml). Treatment with 0mg/ml concentration of Dimethylsulfoxide (DMSO) served as the negative control and Copper oxychloride was used as a positive control. The experiment was replicated three times.

Data Collection Procedures

Reconnaissance field visit was made to establish the administrative network and authority to conduct research, study the market and distribution of avocado in Embu County specifically in Runyenjes, Kiritiri, Siakago, Embu town, Mutunduri and Kianjokoma markets. Further studies were conducted to establish the nature and management of the fungal fruit diseases of avocadoes after harvesting. This was done through interaction with 100 vendors by administration of the questionnaire through face-face interviews and also discussions. The visit was also used to identify vendors that formed part of the sample used in the study of disease prevalence. Data on the prevalence of fungal avocado fruit diseases was obtained during market survey in avocado stores per the storage container i.e bags, crates, buckets. All avocado fruits in the storage containers, both infected and uninfected were counted and recorded to determine the prevalence as shown in equation 1 (Aslam *et al.,* 2015). Symptoms that were investigated included those related to fungal avocado fruit diseases such as dark brown lesions, grey spots on the epidermis of the fruit and rotten avocado fruits.

$$[1]\ \%\ \text{Prevalence} = \frac{\text{No.of fruits infected}}{\text{Total number of fruits assessed}} \times 100$$

Isolation of Avocado Fruit Fungal Pathogens

Infected avocado fruits were cleaned with distilled water to remove dust and dried using a soft cloth. The diseased fruits were surface sterilized with 70% ethanol using cotton wool. The scalpel was then flame sterilized. The diseased parts were removed from advanced margins of the lesion and carefully placed into a petri plate containing potato dextrose agar (PDA) media and another containing Corn meal agar. The petri plates were covered, sealed with parafilm and labeled with a permanent marker. They were then incubated at room temperature for 7 days in the laboratory. Colony morphology was observed and recorded. Isolation of the pure culture of the fungal pathogens was done by single spore isolation technique whereby an inoculating loop was sterilized and used to scrap a single colony from the isolated fungi and grown on PDA media.

Phenotypic Characterization of the Avocado Fruit Fungal Pathogens

Identification and characterization of the avocado fruit fungal pathogens was carried out according to Fawole & Oso (1995). A drop of Lactophenol cotton blue stain was placed on a clean microscopic slide. A small portion of the mycelium from each of the fungal cultures was picked using a sterile wire loop and placed in the drop of the stain. Two wire loops were used to spread the mycelium well on the slide and a cover slip gently placed over the slide. Microscopic examination was carried out under high power objective lens (×40). Phenotypic characteristics such as color, texture, shape of mycelia, hyphae septation colonies of the fungi were observed and recorded. The most prevalent fungal pathogen was also determined from the growth of colonies. One isolate from each species was further confirmed through Koch's postulate where the pure cultures of the isolates growing on PDA were inoculated to healthy avocado fruit at different surfaces. Pure cultures of fungal isolates grown on PDA for 10 days at room temperature were flooded with sterile distilled water and filtered through double layer cheese cloth to remove mycelia. The filtrate was collected in clean universal bottles and then diluted serially. Using a pipette, a drop of the filtrate was placed on a slide, covered with a cover slip and then placed on microscope stage. Lactophenol cotton blue stain was added to stain the spores to improve visibility. The shape of the spores was noted and the size (length and width) of the 3 spores per isolate was measured using calibrated ocular slide and stage micrometer.

Efficacy of *Aloe secundiflora* on Avocado Fruit

Fungal Pathogen Collection of *Aloe secundiflora*

Leaves and Preparation of Crude Extract

Mature Aloe leaves were collected from the field in Mbeere sub-county, a semi-arid region in Embu county. A knife was used to cut the Aloe leaves at the base. The leaves were then put in sterilized buckets and taken to the laboratory for analysis. In the laboratory the leaves were thoroughly washed with running water and rinsed with distilled water to remove dust particles and any other debris. They were then cut into small pieces and spread on a clean and sterilized bench to dry under shade for seven days. The dried Aloe leaves samples were ground into fine powder using a blender. Two samples each of 112g were then soaked in separate organic solvents; hexane and ethanol just enough to cover the mass of the samples. The samples were then left for 72hrs to allow enough time for absoprtion. Each sample was filtered using Whatmann filter papers and the filtrates poured into a round bottomed flask. The round bottomed flask with the contents was then connected to a rotary evaporator to evaporate the solvents under vacuum using a vacuum pump. This was carried out until maximum solvent had been removed. The extracts were then transferred into different beakers and kept into an oven and set at the respective boiling points of the solvents i.e for hexane 68 ºc and for ethanol 78 ºc to fully evaporate the remaining amount of the solvents in the extract to remain with solid phytochemicals. Different concentrations of 2.5mg/ml, 5.0mg/ml and 10.0mg/ml of the extracts were prepared.

For each concentration, a volume of 50ml was prepared for the separate extracts. To prepare 2.5mg/ml, 5.0mg/ml and 10.0mg/ml concentration, 0.125g, 0.25g and 0.50g of the plant extracts respectively were measured and transferred into a 50ml volumetric flask. Dimethylsulfoxide (DMSO) solvent was then added to dissolve the extract before topping it

to the mark. They were then transfered to reagent bottles ready for use.

Phytochemical Analysis of the Plant Extract

The phytocompounds in the *Aloe secundiflora* leaf extracts for the two solvents were examined using standard procedures according to Parekh and Chanda (2007). The antimicrobial test was done according to Assob *et al*. (2011). Fifty-four plates of Potato Dextrose Agar (PDA) media were prepared for the three most common pathogens: *C. gloeosporioides, Cercospora purpurea and Sphaceloma perseae*. The spore suspensions were cultured by transferring a lump of the picked fungal pathogen spores using the cork borer into sterile PDA medium on each plate. The antimicrobial test disc or the Whatman paper disc soaked with plant extract was then introduced on to the media at four different positions on the plate; the media was placed in an inverted position and incubated at 25 °C in an incubator

for one week in the Laboratory to allow for growth. The zones of inhibition in which no microbial growth had occurred were measured in millimetres. The negative control was done with water while the positive control was a fungicide (copper oxychloride).

Data Analysis

Descriptive statistics was used to summarize data into means and percentages. Data on prevalence of post harvest fungal avocado diseases recorded in percentages was subjected to one way analysis of variance using Microsoft excel software. Data obtained from efficacy of *A. secundiflora* crude extracts against the three most prevalent fungal avocado pathogens was subjected to analysis of variance using Scientific Analysis System (SAS) version 9.4 software. Significant means were separated using Least Significance Difference (LSD) $\alpha = 0.05$

RESULTS

Prevalence of Anthracnose, Cercospora spot and Scab Disease on Avocado Fruits

There was a statistically significant difference in the prevalence of the avocado fruit fungal diseases at 5% probability level.

Table 1: Percentage Mean Prevalence of anthracnose disease on avocado fruits

	Anthracnose	**Scab**	**Cercospora spot**
Markets	Mean estimate	Mean estimate	Mean estimate
RM	35.00a	32.75b	28.60b
KJM	34.00 b	35.85a	35.15a
SM	29.30bc	36.40a	26.23bc
KM	27.95bc	34.25ab	21.65cd
ETM	26.45c	28.70c	25.70bc
MM	21.30d	20.70d	18.95d
LSD	6.295	12.51	11.42
Mean	29.00	31.44	25.975
CV%	34.57	32.71	38.35

*****Means followed by the same letter are not significantly different. RM represents Runyenjes market, SM-Siakago market, KM- Kiritiri market, ETM-Embu town market, MM-Mutunduri market and KJM- Kianjokoma market.

Morphological Characterization of Fungal Avocado Fruit Pathogens

A total of 72 fungal isolates from diseased avocado fruits in all the markets were identified. A total of 40 isolates had whitish to greyish colour and cottony smooth mycelium on the top side and greyish cream colour as shown in figure 1 and on the bottom side similarly, the lower side of the sub-cultures had creamish grey colour. On re-isolation and inoculation into a healthy avocado fruit, a characteristic darkly coloured, rounded lesion on fruit epicarp and inside the fruit, mesocarp soft rot of light color was formed. A total of 18 isolates had yellow colonies. Cultures were white on the underside of the plates, and

mycelia had white and yellow layers. On re-isolation and inoculation into a healthy avocado fruit, a dark brown lesion was confirmed in the epidermis of the fruit. All the 18 isolates of *Sphaceloma perseae* typically formed a slow-growing mycelium, raised and gummy to mucoid colonies on PDA medium. The mycelial growth rate was very slow. *Sphaceloma perseae* isolates developed masses of conidiophores bearing hyaline conidia ovoid or elongated coloured conidia with septation and 5-8 x3-4 µm. The spores sizes of the isolates did not differ significantly ($P > 0.05$) in terms of width and length among the isolates

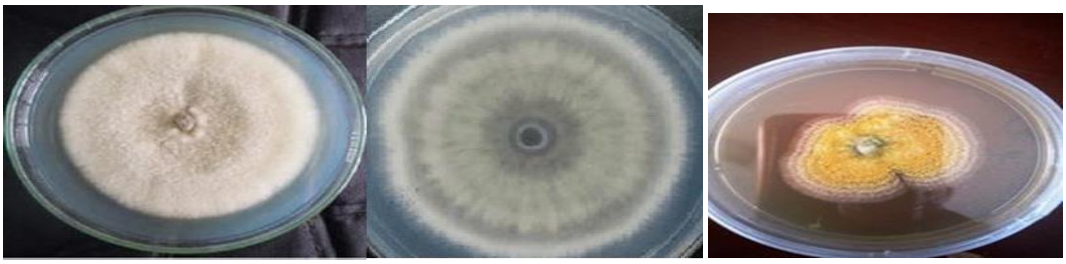

Figure 1: Pure Cultures Isolates of *C. gloeosporioides* upper side of the culture (a) and bottom side of the culture (b), and *S. perseae*(c)

The mycelial colour of the isolates varied from whitish grey, whitish cream and greyish on the upper side of the culture. Similarly, the lower side of the cultures was creamish grey in colour. In terms of mycelia structure, cottony was observed in 24 isolates as compared to velvety observed in 16 isolates. It had a fast growing mycelium that formed concentric zonations. All the *C. gloeosporioides* spores observed were cylindrical and straight with smooth round ends, of large masses that were scattered over the colony and lacked a perithecia with sizes ranging from 3.0-5.0 µm in width and 10.3 – 18.2 µm in length.

Morphological Characterization of *Cercospora purpurea*

Fourteen isolates had colonies which were white leathery with tuffs of grey-brown conidiomata on them, initially grey becoming brown with age (Figure 2). The 14 isolates of *Cercospora purpurea* formed a tufted, leather-like mycelium with a slow mycelial growing rate. *Cercospora purpurea* conidia were long rod shaped to cylindrical, with a blunt end, pale olive, 9 – 11 septate, straight or curved and 20 –34 x 2 – 5 µm were cylindrical in shape with a truncate base.

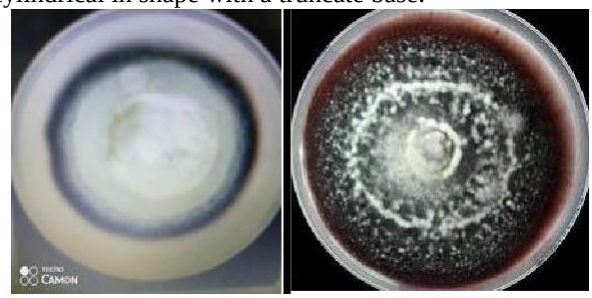

Figure2: A pure culture of *Cercospora purpurea* isolate, (a) for the upper side while (b) on the lower side

Qualitative Analysis of Phytochemicals in *Aloe Secundiflora* Crude Extract

A total of five phytocompounds were found using ethanol extract and four using hexane extract as shown in Table 2. Phenolics were only detected in ethanol solvent extract. Tannins and Alkaloids were not present in any of the crude extract.

Table 2: Results of phytochemical analysis of *Aloe secundiflora* extract

Phytochemical present	Plant crude Extract	
	Ethanolic solvent extract	Hexanic solvent extract

Saponins	+	+
Tannins	-	-
Alkaloids	-	-
Flavonoids	+	+
Terpenoids	+	+
Phenolics	+	-
Steroids	+	+

* (+) signifies presence of the phytochemical compound while (-) signifies the absence of the phytochemical compound.

Antimicrobial Activity of *Aloe secundiflora* Crude Extracts

The inhibitory activities of *A. secundiflora* on Avocado fruit fungal isolates were determined by measuring the diameter of zones of inhibition in millimetres. There were observable zones of inhibition for only *C. gloeosporioides* and *C. purpurea* in different concentrations. However, the extract did not inhibit *Sphaceloma perseae* in vitro. Various concentrations of plant extracts obtained using ethanol and hexane had inhibitory activities against *Colletotrichum gloeosporioides*. There was an increase in the diameter of zone of inhibition as the *Aloe secundiflora* extract concentration was increased. The effect of the different concentration of the plant extract was statistically significant ($P < 0.05$). The mean zone of inhibition for the ethanol extract ranged from 15.59 mm to 11.67 mm. *Aloe secundiflora* ethanol extract concentration of 10.0mg/ml had more microbial inhibitory activity on the *C. gloeosporioides* growth than the lower concentration of 5.0 mg/ml. The concentration of 2.5 mg/ml had the lowest zone of inhibition on the pathogen growth. However, the *Aloe secundiflora* hexane crude extract showed lower mean diameters of zones of

inhibition activity (10.59mm to 8.01mm) on *Colletotrichum gloeosporioides* than that of ethanol extract as shown in Table 3.

Figure 4: Zones of inhibition for *Colletotrichum gloeosporioides* isolates for *Aloe secundiflora* crude extract at 25mg/ml for (a) ethanol solvent extract and (b) for hexane.

Table 3: Mean Zones of inhibition recorded on Petri dishes treated with different concentrations of *A. secundiflora*
ethanol and hexane extract against *Colletotrichum gloeosporioides* isolates

	*ethanol	*hexane
Concentration(mg/ml)	Mean Zones of inhibition (mm)	Mean Zones of inhibition (mm)
10	15.59[a]	10.53a
5	13.20[b]	9.63b
2.5	11.67[c]	8.01c
LSD	0.49	0.21
Mean	13.50	9.36
CV	3.71	2.29

**Means followed by different letter are not significantly different at 5% probability level.

Values are means of three isolates each with three replicates.

Antimicrobial Activity against *Cercospora purpurea*

Increase in the concentration of *Aloe secundiflora* extract increased the inhibition of *C. purpurea*. The effect of the different concentration of the plant extract was statistically significant ($P < 0.05$) The Mean zone of inhibition for ethanolic extract ranged from 7.22 mm to 2.80 mm (table 4)The Mean zone of inhibition for hexane extract ranged from 5.27 mm to 3.38 mm as shown in table 10. The plant extract concentration of 10.0 mg/ml had more microbial inhibitory activity on the *Cercospora purpurea* growth compared to the concentration of 2.5 mg/ml which had the lowest zone of inhibition. The inhibitory activity against *Cercospora purpurea* increased linearly with an increase in the concentration of the *A. secundiflora* crude extract. The zone of inhibition on the positive control were significantly higher (21.67mm) than for the plant extract. The zone of inhibition for concentration 10.0 mg/ml was lower than the zone of inhibition of the positive control using copper oxychloride fungicide.

Table 4: Mean Zones of inhibition recorded on Petri dishes treated with different concentrations of *A. secundiflora*
ethanol and hexane extract against *Cercospora purpurea.*

	ethanol	hexane
Concentration(mg/ml)	Mean Zones of inhibition (mm)	Mean Zones of inhibition (mm)
10	7.22a	5.27[a]
5	4.31b	4.32[b]
2.5	2.80c	3.38[c]
LSD	0.37	0.15
Mean	4.77	4.32
CV	7.81	3.56

Means followed by the different letter are significantly different at 5% probability level.

Antimicrobial Activity against *Sphaceloma perseae*

There were observable zones of inhibition for copper oxychloride fungicide, positive control, against *Sphaceloma perseae* pathogen isolates with a mean diameter of 17.3mm. However, *A. secundiflora* crude extracts did not inhibit

the growth of the pure cultures of *S. perseae* at any concentration. There was no significant difference in the results between the negative control and all the concentrations of *A. secundiflora* crude extract.

DISCUSSION

The percentage mean prevalence of anthracnose disease of avocado caused by *C. gloeosporioides* was recorded in Runyenjes and Kianjokoma market stores as 41 percent and 36 percent respectively). These findings concur with Handiso *et al* (2019) who found that the highest and lowest anthracnose disease spread was observed in Alaba and Shashogo markets with cumulative prevalence of 41.88% and 36.81%, respectively. These results also concur with Mezgebe *et al*. (2016) findings on post -harvest losses of perishable fruits during harvesting, handling and storage in Ethiopia due to high prevalence of athracnose disease. The highest and lowest mean prevalence of infection by the avocado scab disease (*Sphaceloma perseae*) were recorded in Siakago market and Mitunduri market with a mean prevalence value of 35.16% and 16.37%, respectively. A study conducted by Trucks *et al* (2011) showed a high prevalence of *Sphaceloma perseae* pathogen of avocado which is carried to infection sites by wind, rain and insects. He did also report that injuries caused by thrips (insects) create entry wounds for *S. perseae* and greatly exacerbate scab development and its prevalence. This was also in agreement with findings by Omolo *et al*. 2011 and Dessalegn *et al*. (2016) on high prevalence of avocado scab whereby 100% avocado dark brown lesions and fruit rots on storage was recorded in on avocado fruits harvested by tree

shaking method.There was high prevalence of infection by the Cercospora spot (*Cercospora purpurea*) recorded in Kianjokoma market with and Mitunduri market with the lowest mean prevalence value among the selected markets. The same high prevalence of Cercospora spot was recorded by the findings of Darvas *et al* (1987) who reported that one of the most important pre and post-harvest fruit disease of avocado at Westfalia Estate was Cercospora spot, caused by *Cercospora purpurea*. The high percentage prevalence may have been due to presence of humid and rainy conditions in Kianjokoma region. Similarly, Kallideen, R. (2020) reported high prevalence of Cercospora spot disease in commonly avocado producing and marketing regions where warm, humid and rainy conditions persist in South Africa.

The fungal isolates from diseased avocado fruits showing symptoms of anthracnose collected from the study area varied in their morphological characteristics in terms of conidial lengths. A total of 40 isolates had whitish to greyish colour and cottony smooth mycelium on the top side and greyish cream colour and on the bottom side similarly, the lower side of the sub-cultures had creamish grey colour typical of *C. gloeosporioides*. These cultural characteristics were similar to those of *Colletotrichum gloeosporioides* isolates observed by Pallem *et al*. (2012) in avocado fruits with wide cultural variations among *C. gloeosporioides* isolates. The cultures produced spores were straight with rounded end, ranging within 3.0-5.1 micron in width and 10.3 – 18.2 micron in length characteristic of *C. gloeosporiodes* as reported by Kimaru (2020). The mycelial colour of the isolates observed was predominantly whitish grey compared to cream grey on the upper side of the culture. Creamish-grey however, was the most common colour on the lower side of the culture among isolates. The differences observed in cultural and morphological features of the fungus could be associated to their genetic variations and different conditions of growth in terms of temperature, light aspects and repeated laboratory sub culturing as also reported by Vidyalakshini and Divya (2013). A total of 18 isolates obtained from avocado fruits showing symptoms of avocado scab disease had white and yellow colonies. Cultures were white on the underside of the plates, and mycelia had white and yellow layers. *Sphaceloma perseae* isolates developed masses of conidiophores bearing hyaline conidia ovoid or elongated coloured conidia with septation and a range of 5-8 x3-4 µm. These findings were similar to those made by Fan *et al* (2018), who reported that the colour of the *Sphaceloma perseae* pathogen may be white to brown and also reported that the mycelia of the pathogen grows at a very slow rate wen grown on PDA media.

The remaining 14 isolates had colonies which were white leathery with tuffs of grey-brown conidiomata on them, initially grey becoming brown with age. These cultural characteristics were typical to those of *Cercospora purpurea* reported by Khallideen (2020) . Similar results were reported by Marais (2004) that once the pathogen is isolated it grows readily on ordinary nutrient media, producing a tufted leathery growth, which is first greyish in color, later becoming brown or blackish-brown. The conidia were long rod-shaped to cylindrical, with a blunt end, pale olive, 9 – 11 septate, straight or curved and 20 – 180 x 2 – 5 µm were cylindrical in shape with a truncate base. Similarly, Groenewald *et al* (2013) reported almost similar conidia characteristics with a wide range of the measurement of the length and width of the spores.

Efficacy of *Aloe secundiflora* Crude Extracts on the Fungal Avocado Pathogens

The study revealed the presence of various phytochemicals present within the *Aloe secundiflora* crude plant extracts prepared using Ethanol and hexane solvents. The two are organic solvents whereby ethanol is a polar solvent while hexane is non-polar and they are able to dissolve some phytochemicals (Gupta *et al.,* 2010). Different solvents are required in crude extraction and phytochemical analysis. The extraction yield and biological activity of the resulting extract is not only affected by the extraction technique but also by the extraction solvent (Ajana *et al.,* 2012). Many solvents, including methanol, ethanol, acetone, and water, have been used for extracting bioactive compounds from the plant material. Due to the variety of bioactive compounds contained in plant materials and their differing solubility properties in different solvents, the optimal solvent for extraction depends on the

particular plant materials, and the compounds that are to be isolated (Mahdi *et al.,* 2012). Therefore, recommendation of suitable extraction solvent for individual plant materials is generally difficult. *Aloe secundiflora* crude extract contains phytochemicals such as terpenoids, steroids, saponins, phenolics and flavonoids when extracted using ethanol solvent (Mariita, 2011). However, Kaingu *et al.,* (2013) revealed the absence of phenolics in hexane solvent extract of *A. secundiflora* when used to treat *Ascaridia galli* and various bacterial diseases. This difference in the phenolic content could be due to the solvent nature, method of extraction and duration of extraction having an effect on the concentrations of the phytochemicals.

Antimicrobial activity of *Aloe secundiflora* Crude Extracts

The mean diameter of zones of inhibition for *A. secundiflora* ethanol extract ranged from 15.59 mm to 11.67mm and for hexane extract ranged from10.59 mm to 8.01 mm. These findings were similar to the one reported by Micheni (2015) on the anti-fungal activity of *A. secundiflora* against *Pythium ultimum* that attacks potatoes and *Fusarium oxysporum* which is a pathogen that attacks bananas who reported the highest mean inhibition as 16.01mm for both organisms using ethanol solvent. Similar results were also reported by Itonga, (2011) who reported that the crude extract of *A. secundiflora* was effective against eight different fungi species. Al-Mujammaa'e, (2008) reported that *A. secundiflora* extracts induce the disruption in fungal cell metabolism, increased permeability of fungal plasma membrane and destruction of the conidial wall structure. The more the *A. secundiflora* extract is concentrated the higher the zone of inhibition level. The negative control plates treated with DMSO alone had no zone of inhibition which confirms that *A. secundiflora* extract has antifungal compounds that inhibited the growth of *C. gloeosporioides* .This could be attributed to the various phytochemical constituents such as; glucorunic acid, p- coumaric acid, ferulic acid, phenylpropanoids, dehydro α lapachone and lapachol, β-sitosterol, ferulic acid and iridoids that could be causing the zone of inhibition (Waithaka *et al.,* 2018). The study also revealed that as the concentration of *A. secundiflora* extract was increased, the zone of inhibition by the plant extract also increased .*Sphaceloma perseae* pathogen mycelial growth was not inhibited by crude extracts of *A. secundiflora.* There were observable zones of inhibition when treated with copper oxychloride fungicide as the positive control.

Antimicrobial Activity of *Aloe secundiflora* on the Fungal Avocado Fruit Pathogens

Al-Mujammaa'e, (2008) reported that *A.secundiflora* extracts induce the disruption in fungal cell metabolism, increased permeability of fungal plasma membrane and destruction of the conidial wall structure. Waithaka *et al.,* (2018), reported a similar finding that the antifungal activities of acetone extract of *A. secundiflora* were effective against various fungal pathogen. Moreover, he reported that the antimicrobial activity of the plant extract was due to the phytochemical constituents for instance terpenoids and other polyphenols present in the extracts. Rachuonyo *et al* (2016) also reported that *Aloe secundiflora* leaves extract contain glycosides, phenyl propanoid derivatives, and a eucommiol derivative that are toxic to plant fungal pathogen. He also reported that iridoid glycosides, phenyl propanoid derivatives from *Aloe secundiflora* leaves, is a volatile phyto-anticipin that has been shown to be responsible for the anti-microbial effects of species. Due to the lack of awareness from the consumer and the farmer, they are exposed to these synthetics when they consume food crops that pesticides are used in the management of the pest (Singh and Sharma, 2018).The plant extract had a variation in the zone of inhibition. The differences might be due to the difference in nature, quality and quantity of the inhibitory substances present in the botanicals (Ceylan & Fung, 2004). It was evident from results that the zones of inhibition of the *Colletotrichum gloeosporioides* and *Cercospora purpurea* pathogens by the plant extracts depends upon plant species and extracts concentration. Veresoglou *et al.,* (2013); Kumaran, (2003) did report comparable findings that the fungal susceptibility toward a plant extract was due to plant species, the solvent used for extraction and extract concentration, as well as the organism tested.

CONCLUSION

Most vendors in Embu County have avocado trees in their farms. Avocado fruits produced are consumed as a source of nutrients and sold as source of income both at local market. Marketing and production of avocado in the County is mainly by unskilled vendors who had little knowledge and awareness on proper fruits management. Most vendors did not use any strategy to manage the fungal avocado fruit diseases. However, few used fungicides such as Bayleton, Milraz and copper oxychloride to control anthracnose in avocado though not registered for use in avocado by the pesticide regulatory authority in Kenya, PCPB. Avocadoes have a high economic return in the county hence highly preferred by farmers and vendors. Anthracnose, Cercospora spot and Scab diseases of avocado cases were noted in all the area. *C. gloeosporioides*, *Cercospora purpurea* and *Sphaceloma perseae* were identified as the causal pathogens of anthracnose, avocado scab and Cercospora spot diseases through characterization. The growth of *C. gloeosporioides* and *Cercospora purpurea* isolates were inhibited by *Aloe secundiflora* crude extract *in vitro* at varying concentrations. Plant extracts used as an antimicrobial agent are relatively economical, safe and non-hazardous and show antimicrobial or antifungal activity against *C. gloeosporioides*, *C. purpurea* and other diseases caused by pathogenic fungal organisms. These plant extracts provide an effective measure for the management of the pathogens that cause avocado anthracnose and cercospora spots in avocadoes that may form an integral part of integrated management and it also has prospect as an alternative to reliance only on fungicides.

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