Phytochemistry and Utilization of *Vernonia glabra* (Steetz) Oliv. & Hiern.in Management of Food Spoilage and Poisoning Pathogens, in Kenya.

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Abstract
Food spoilage and poisoning pathogens lead to pre- and post-harvest losses of crop produce and poisoning of food and feed stuff; posing a great threat to food security and safety worldwide. This project aimed to investigate the pesticidal activity and presence of chemical compounds in Vernonia glabra; as an alternative control approach, to food crop protection. Organic extracts of leaves and flowers showed the highest activity against S. aureus (mean inhibition zones of 1.85 and 1.78 respectively), than the standard antibiotic (Streptomycin 1.30). Flavonoids were greatly present in all extracts screened. The results of this study justify the use of V. glabra in traditional herbal medicine, and suggest that the plant has ideal characteristics in the application as bio-pesticide control to crops and food stuff.

Key words: Nutritional value, Food quality, Post-harvest losses, Bio-pesticide.

1.0 Introduction

Food infection and intoxication are considered as the most common causes of food borne diseases worldwide (Lopez *et al*., 2003). According to FAO, (2005), nausea, diarrhoea and vomiting are the leading symptoms caused by ingestion of contaminated food with bacterial toxins, such as *Staphylococcus aureus* enterotoxin B and *Escherichia coli* shiga toxin. High concentrations of enterotoxins can result in multi-organ system failure (El Dairouty, n.d). *Aspergillus niger*, a filamentous fungus, causes pre-harvest losses and

post-harvest decay of fresh fruits including grapes, and contaminates legumes such as peanuts, by production of potent mycotoxins called Ochratoxin A, Fumonisin B_2, and aflatoxins which cause kidney necrosis (especially in pigs), kidney failure and death, liver cancer, immunosuppressant, and failure of nervous system (Gautam *et al.*, 2010). The contamination with the mycotoxins leads to discolouration of cereals, quality deterioration (loss of nutritional value), and reduction in commercial values (Gautam *et al.*, 2010). The food borne illnesses, spoilage & toxins contamination of foodstuff, pose a great threat to food security & safety in Kenya.

Prevention of food spoilage and poisoning pathogens in food and feed stuff is usually achieved by use of chemical preservatives, and chemical pesticides (*Lopez et al.*, 2003), which have negative impacts, including: health hazards associated with the application of chemicals, chemical residues in food & feed chains, environmental degradation, acquisition of disease & pests resistance to chemicals, emergence of secondary diseases & pests outbreaks and high cost to farmers (Peter, 2002; Yazdani *et al.*, 2011). Because of such concerns, there is a great need in applying nonchemical methods such as pesticidal plant extracts with antimicrobial properties in management of food spoilage and poisoning pathogens, which are safe, affordable, and easily degradable or environmentally friendly (Adejumo and Kamper, 2012). Plant extracts have long been used to control insects and preserve foodstuff. Increased use of spices and herbs used as seasoning agents in foods and beverages have been done extensively. Garlic onion, Cinnamon, and Clove are some of the subjects of these researches (Lopez *et al.*, 2003). However, use of pesticidal plants has not been accompanied by scientific evidence in the efficacy and safety to support its effective claims (WHO 2000-2005). Therefore, the aim of this project was to investigate the pesticidal activity (efficacy) and chemical compounds present in different parts of *V. glabra*; an herb utilized by traditional practitioners in Kenya to treat gastrointestinal problems, (Johns *et al.*, 1995) and snake bites, (Owour and Kisangau, 2006).

2.0 Material and Methods

2.1 Collection of Plant Material

The *V. glabra* was selected based on ethno-medicinal information from literature and collected from Kathiani village in Machakos County, Kenya in January 2010. The specimen was authenticated by a plant taxonomist in University of Nairobi and a voucher specimen (CK 2010/01) deposited at the University of Nairobi Herbarium.

2.2 Crude plant Extracts preparation

The flowers, leaves, stem, and roots were air-dried under the shade at room temperature, ground into powder and extracted using Dichloromethane/Methanol in the ratio 1:1, and water, according to standard extraction methods (Harborne, 1998).

2.3 Sources of Micro-organisms and Preparation of Standard inoculums

Pure cultures of bacteria; *Staphylococcus aureus* ATCC 259213, *Escherichia coli* NC 35218 (from School of Pharmacy, University of Nairobi) were maintained on nutrient broth slants at 4^oC, while the fungus, *Aspergillus niger* ATCC 16404 (a collection of the late Professor George M. Siboe, University of Nairobi) was maintained on Sabourauds' Dextrose agar at 4^oC. The standard inoculums suspensions were adjusted to turbidity equivalent to 0.5 McFarland standards to give a density of $1x10^8$ cells or spores/ml (Nostro *et al.*, 2000).

2.4 Anti-pesticidal Activity

Disc diffusion technique was used as the standard method for pesticidal activity and minimum inhibitory concentrations (MICs) for active extracts against *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus niger*. Stock solution at concentration 2000 mg/2 ml (1000 mg/ ml) was prepared for each plant part and entire plant used. Two fold serial dilutions were prepared from each stock solution. Readily manufactured sterile paper discs were used for each concentration prepared by pipetting 100µl onto individual paper discs (0.6cm) drop by drop using a micropipette. Paper discs with crude extracts were transferred onto plates inoculated with 1ml of standard inoculum for each test organism. The plates were labelled, and incubated at 37^{o}C for bacteria and 25^{o}C for fungus. Streptomycin (for bacteria) and Nystatin (for fungus) were used as standards, while discs with extraction solvents only were used as controls. These were done in duplicates under sterile conditions and results recorded after 24, 48, 72 and 96 hours. The pesticidal activity was determined by measuring clear inhibition zones diameters (including diameter of paper discs) formed using a transparent ruler (cm). Minimum inhibitory concentrations were determined by recording the lowest concentration of the active extracts that inhibited growth of the micro-organisms, Ochei and Kolhatkar, (2000).

2.5 Chemical Analysis of Selected Crude plant Extracts

The organic extracts that were active at low concentrations (≤ 25 mg/100 µl) were analysed for presence or absence of alkaloids, Sapogenins, terpenoids, quinones, and flavonoids using Thin Layer Chromatography (TLC) technique and the developed TLC plates were viewed under Ultra-Violet light and then sprayed with appropriate reagents for the detection of the chemical groups according to Harborne (1998).

2.6 Data Analysis

In order to analyse data, multiple way ANOVA was used to determine significant factors in production of inhibition zones, Tukey's Honest Significant Difference Test (THSDT) was used for means comparison within the significant factors (Brown, 2012).

3.0 Results

3.1 Pesticidal activity.

In this study, mean inhibition zones were used as the results for figures 1, 2 and 3 below. All the organic crude extracts of *V. glabra* parts used were active on at least one of the three test-organisms used (Fig. 1). It was observed that organic extracts of *V. glabra* leaf had the highest activity (inhibition zone of 1.85) against *S. aureus*, followed by organic extract of flower with inhibition zone of 1.78 against only *S. aureus*. The two extracts were significantly different in activity from streptomycin (inhibition zone of 1.30). Organic extract of leaf recorded high activity (inhibition zone of 1.43) against *A. niger*, slightly higher compared to nystatin (inhibition zone of 0.83), while the organic extract of whole plant (all parts mixed) showed significant activity (inhibition zone of 1.50) against *S. aureus* compared to streptomycin's (inhibition zone of 1.30) low activity. Aqueous extract of the whole plant of *V. glabra* was the only active aqueous extract against *S. aureus* (Fig.2).

(b) Extraction Solvent: The organic extracts of *V. glabra* were generally more active than the aqueous extracts most of which had lower activity or no activity against at least one of the three micro-organisms tested (Fig.3).

(c).Test-organism: *S. aureus* was the most susceptible to organic extracts (inhibition of 0.35) and was significantly different in susceptibility from *A. niger, and E. coli* which had inhibition zones of 0.05 and 0.01 respectively (Fig.3).

3.2 Minimum inhibitory concentration (MIC): MIC of each extract that was able to kill or inhibit the growth of at least one of the three micro-organisms tested in this study was exhibited by extracts that were active at concentration of 100mg/100µl to 0.02mg/100µl. The inverse of MICs was displayed on fig. 4 below. Organic extract of flower showed highest activity recording the lowest MIC of 1.5625 mg/100 µl against *S. aureus*, which was lower than the standard (streptomycin) with MIC of 6.25mg/100µl. The organic extracts of root, whole plant against *S. aureus*, and leaf against *A. niger and S. aureus* had low activity (Fig. 4). The organic extract of whole plant against *A. niger*, organic extract of stem against *A. niger*, and aqueous extract of whole plant against *S. aureus*, and organic extract of leaf against *E. coli* were less effective at higher concentrations (Fig. 4).

Note: Extracts/standards with high inverse of MICs indicate higher activity (MIC 0.64-0.98mg/100µl) at low concentrations; extracts/standards with inverse of MICs of 0.16-0.32mg/100µl indicate moderate activity, while the extracts with low inverse of MICs display low activity at higher concentrations.

3.3 Chemical Profile of five classes of Compounds identified in Different Parts of *V. glabra* Extracts

The crude extracts of *V. glabra* (flower, leaf, and root) were screened for alkaloids, sapogenins, terpenoids, quinones, and flavonoids. The presence of classes of compounds was displayed by simple scoring (Table 1). Flavonoids were present in all extracts in significant amounts. Sapogenins, terpenoids and quinones were present in moderate amounts, while alkaloids were present in only two extracts, including flower, and root extracts.

4.0 Discussion

The organic extracts of leaf (1.85) and flower (1.78) against *S. aureus*, had larger inhibition zones than the standard antibiotic (streptomycin 1.30). These results suggest that, these extracts could be used for management of foodborne illnesses caused by intoxication of *Staphylococcus aureus.* The organic extract of leaf (1.43 against *A. niger*) showed high activity compared to nystatin (inhibition zone of 0.83). This signifies that, this extract could be used to produce novel antifungal compounds, for management of pre-and post-harvest losses caused by *A. niger* and also prevent contamination of cereals by mycotoxins. The findings of this study are in agreement with the studies of Chika *et al*., 2007 that reported large inhibition zones of organic extracts of *Euphorbia hirta* leaf, against *S. aureus*, and justified its use by traditional medicinal practitioners for control of foodborne diseases and prevention of pre-harvest loses and post-harvest decays of cereals caused by *A. niger*. Therefore, *V. glabra* could be used as a potential bio-pesticide to ensure food security and safety in Kenya.

Low MIC in flower extract (1.5625 mg/100 µl) against *S. aureus* compared to streptomycin with MIC of 6.25 mg/100 µl suggest that it may elicit low chemical toxicity, and reduce resistance emergence in pest pathogens when used in small amounts (Mariita *et al*., 2010). The presence of chemical compounds may explain why the extracts exhibited significant pesticidal activity and that *V. glabra* may be a potential pesticidal plant that could possess novel pesticidal compounds for management of food spoilage and poisoning pathogens and reduce pre- and post-harvest losses and intoxication of humans and animals, hence improved food security and safety in Kenya.

5.0 Conclusions and Recommendations

The results of this study justify the use of *V. glabra* in traditional herbal medicine and suggest that the plant has ideal characteristics for utilization as a bio-pesticide in the control of food spoilage and poisoning pathogens, and reduction of pre- and post-harvest losses of field crops & toxins contamination on foodstuff. However, research on toxicology which is missing in this study is recommended for *V. glabra* in order to verify and document the safety of this pesticidal/ medicinal plant to the society.

6.0 Acknowledgement

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7.0 Figures and Tables

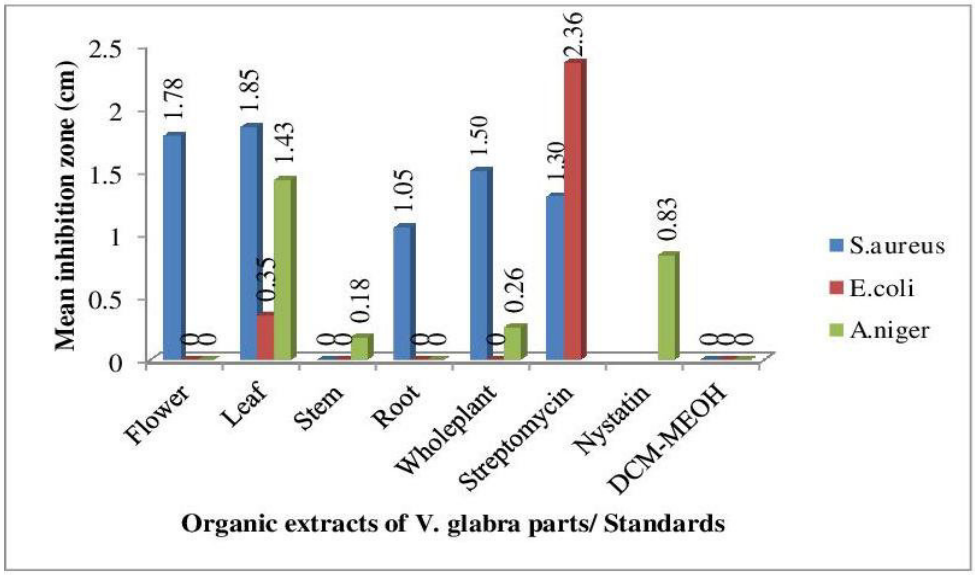

Fig.1 Antimicrobial activity of organic crude extracts of *V. glabra* parts compared to streptomycin and nystatin at 100 mg/100 µl.

Note: DCM-MeOH- Dichloromethane and Methanol in the ratio 1:1 (negative Control).

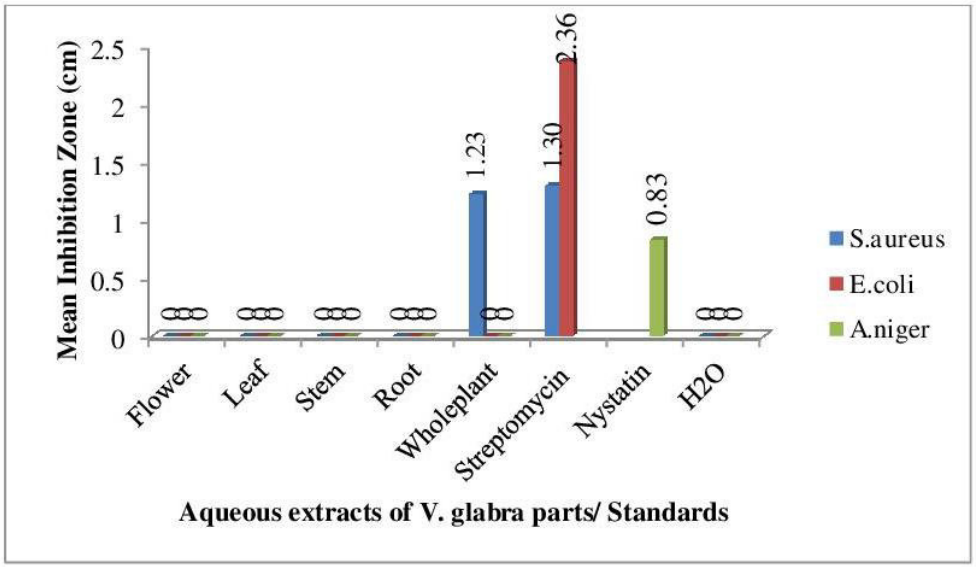

Fig.2. Antimicrobial activity of aqueous crude extracts of *V. glabra* parts compared to streptomycin and nystatin at 100 mg/100 µl.

The controls (DCM-Me-OH-Dichloromethane: Methanol 1:1 and H_2O-sterile distilled water) were not active against any test-organism.

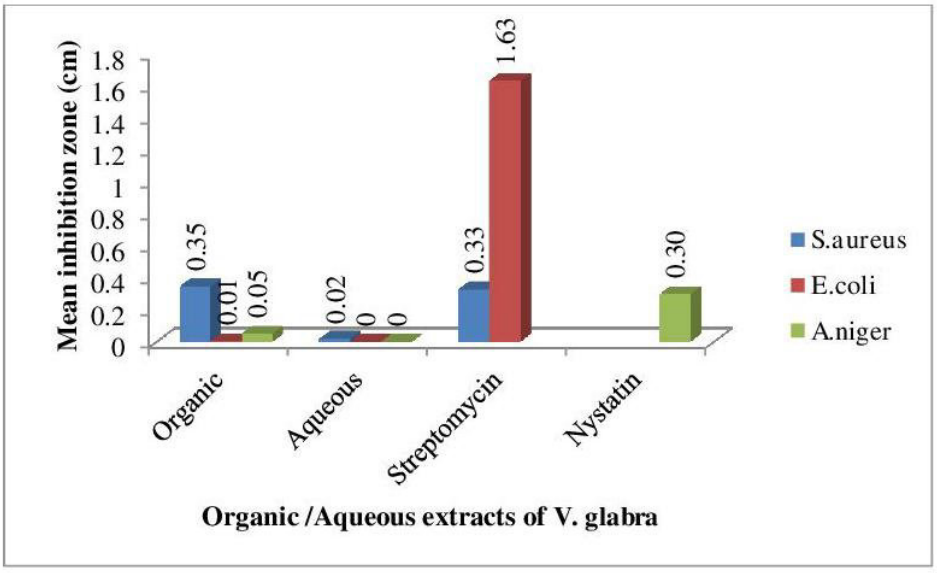

Fig.3. Organic versus aqueous crude extracts of *V. glabra* compared to streptomycin and nystatin at 100 mg/100 µl.

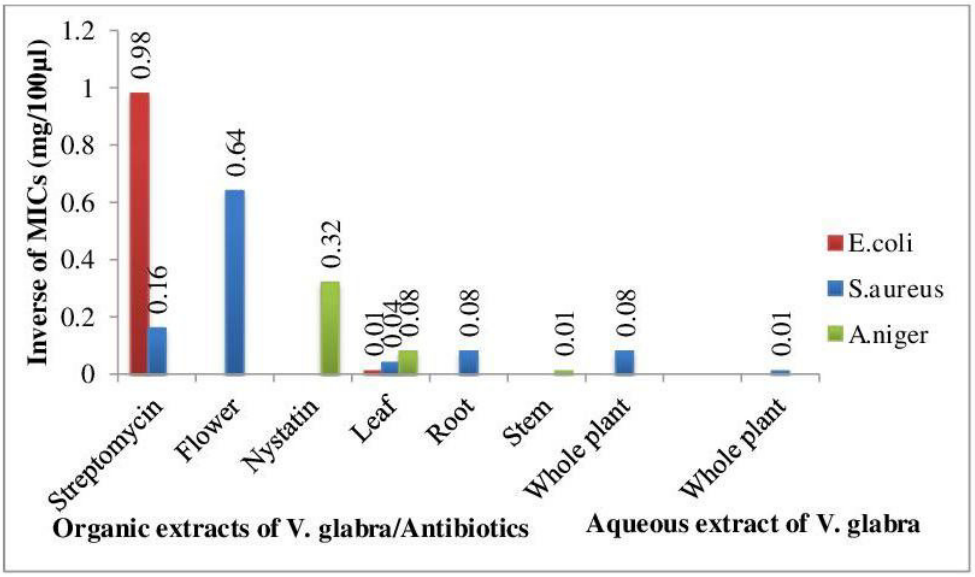

Fig. 4. MICs for organic and aqueous extracts of *V. glabra* compared to streptomycin and nystatin (standard antibiotics).

Table 1. Five classes of compounds screened present in *V. glabra* extracts (flower, leaf, and root)

	Five Classes of Compounds Screened Present				
Extracts	Alkaloids	Sapogenins	Terpenoids	Quinones	Flavonoids
Flower	+ + +	+	+ + +	+ +	+ + +
Leaf	_	+ + +	+ +	+ +	+ + +
Root	+ +	+ + +	+	+ + +	+ + +

Key: + + += high or greatly present; + + = moderately or fairly present; + = less present (trace amounts); - = Not present.

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