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Evaluation of Phytochemicals and Lead Ions Concentration in *Amaranthus palmeri* and *Brassica oleraceae Acephala* Plants Extracts

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Background: In recent times there has been a pronounced interest in plants and their derived phytochemicals as food source for many population in the world. *Amaranthus palmeri* and *Brassica olearaceae Acephale* are some such commonly consumed vegetable by many households in Kenya. They are known to possess potent bioactive components, but there consumption has remained a preservative for low income earners in rural areas as opposed to urban population. In some areas their leaves are often neglected or discarded and used as fodder.
Aim: The aim of the research was to analyze the presence of different phytochemicals and the concentration of lead metal ions of hexane, ethyl acetate, dichloromethane and methanol leave extracts of *Amaranthus palmeri* and *Brassica oleraceae Acephala*.
Methodology: Inductive Coupled Plasma (ICPE 9000) was used to determine the levels of lead metals. Phytochemical screening was done using a standard procedure.
Results: Phytochemical screening revealed the commonly encountered phytochemical constituents in the leaf extracts of the *Amaranthus palmeri* and *Brassica olearaceae acephale* species which included flavonoids, alkaloids, terpenoids and tannins. Lead concentration ranged from 13.00 ± 2.00 mg/kg to 52.33± 1.76 mg/kg in the roots, 12.33 ± 1.80 mg/kg to 49.33 ± 1.22 mg/kg in the stem and 45.33 ± 1.76 mg/kg to 71.67 ± 1.86 mg/kg in the leaves.

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Conclusion: Since the heavy metal analyzed was above the permissible levels, there is a n indication of heavy metal contamination of this plants which are used as vegetables in Kericho. This may pose a serious health hazard to the consumers of these vegetables in the County. Therefore, policy makers and health practioners should provide legislation on the use of chemicals that releases heavy metals into the environment. Public awareness and education should also be carried out with the aim of reducing exposure of heavy metals to the vegetables and soils to avoid health hazards that may arise from contamination.

Keywords: Amaranthus palmeri; Brassica oleraceae Acephala; phytochemicals; lead.

ABBREVIATIONS

WHO: World Health Organization; ICP-ES: Inductive Coupled Plasms Emission Spectrometer; FAO: Food Agricultural Organization; DCM: Dichloromethane; EtoA: Ethyl-acetate.

1. INTRODUCTION

The development in science and technology has led to the exploration of the possibilities of improved medicine with high efficacies. This has resulted to a conclusion that food can be technologically designed as medicine that can help us stay away from degenerative diseases. Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties [1]. Use of Medicinal plants has played a central role not only as traditional medicines but also as commercial commodities meeting the demand of distant markets especially the traditional herbalist. To compete with the increasing market and demand, there is need to expeditiously exploit and scientifically validate more medicinally useful plants which are common amongst many communities in the world. Because of the appearance of drug resistance to antimicrobial agents, more effort is being made to find substitute antimicrobial components from plants. It had been suggested that natural products are a preferable option to synthetic ones. Qualitative analysis as well as quantification of the phytochemicals of a medicinal plant is regarded as vital step in any kind of medicinal plant research [2]. Phytochemicals are structurally diverse secondary metabolites synthesized by plants and also by non-pathogenic endophytic microorganisms living within plants. They help plants to survive environmental stresses, protect plants from microbial infections and environmental pollutants, provide them with a defense from herbivorous organisms as well as lure pollinators and other symbiotes of these plants. In addition, many phytochemicals can extend longevity in heterotrophic organisms across phyla via evolutionarily conserved mechanisms [3]. All plants produce these compounds as part of their normal metabolic activities. They are classified as primary metabolites such as sugar and fats, which are found in all plants and secondary metabolites compounds which are found in a smaller range of plants, serving a more specific function. For example, some secondary metabolites are toxins used to deter predation and others are pheromones used to attract insects for pollination [4]. Secondary metabolites play an important role in the treatment of diseases and in the ecological interaction of plants with other organisms. Each plant species, family or genus produces a characteristic mixture of these secondary metabolites [5]. Thousands of phytochemicals have been discovered in several major classes but the health properties of only a few have been investigated [6]. Many of the known phytochemicals that belong to several chemical classes have been reported to have inhibitory effect on a wide range of microorganisms [7,8]. They have been used as medicines, flavouring and colouring agents. Some of the well-known phytochemicals are: Alkaloids, flavonoids, saponins, tannins polyphenols and terpenoids. Alkaloids are known to possess many pharmacological properties like analgesic (morphine), stimulants (caffeine), antitumor (vinblastine) antimalarial (quinine), antibacterial (berberine) and amoebicide (emetine) [5]. Flavonoids and Flavanols are polyphenolic compounds with strong antioxidant activity. Biological activities ascribed in this class include: antifungal, antiviral, antibacterial, anti-inflammatory and ant-carcinogenic activity [9,10]. Saponins are known to be immune boosters. They are also known to demonstrate anti-inflammatory, anti-haemolytic, cholesterol

lowering and anticancer properties [10,11]. Tannins display good antimicrobial and antiviral activity and play a role in inhibiting the growth of bacteria by reacting with protein on the cell wall [12]. Terpenes are an abundant class of natural products that are responsible for many fragrances. They are made up of C5 isoprene units and possess strong antimicrobial properties [7]. *Brassicas* are known to possess antioxidant activity [13]. The *Brassica palmeri* vegetables and *Amaranthus oleraceae* serves as an excellent source of antioxidants. Their consumption in large amounts as a regular vegetable worldwide are added advantages. Beneficial health properties are due to the presence of health-promoting compounds such as vitamins, carotenoids, phenols, flavonoids, minerals, and glucosinolates [14]. The content of these compounds in *Brassica* vegetables varies significantly depending on the genotypes of cultivars, the specific plant tissue, fertilization, growing season, and several other environmental factors [15]. Recent reports suggests that cruciferous vegetables act as a good source of natural antioxidants and strong epidemiological evidence shows that presence of such active compounds prevents human body against damage by reactive oxygen species. In recent past there has been an increasing interest in edible plants especially those that are rich in secondary metabolites commonly called as phytochemicals. However the plants *Amaranthus palmeri* and *Brassica oleraceae Acephala* which are used as vegetables in Kericho have little information on the bioactive compounds present. In view of these perspectives this research was aimed at analyzing and identification of the bioactive compounds present their inorganic crude extracts.

2. MATERIALS AND METHODS

2.1 Sample Collection

The plants samples were randomly collected in triplicates from each of the study sites located in Kericho West Sub-County. Vegetable roots, leaves and stems of *Brassica oleracea Acephala* and *Amaranthus palmeri* were collected, packed in well labelled plastic bags and transported to University of Kabianga chemistry laboratory. The samples were washed with distilled water, chopped into small pieces and air dried. The samples were finally ground, labelled and stored awaiting digestion.

2.2 Sample Preparation

The ground samples were sieved using 2 mm sieve. About 0.25 g of finely ground samples were weighed using electronic analytical balance (AAA Model from Adam Equipment Company limited). The weighed samples were transferred to the tubes. They were ashed using Fisher Scientific Isotemp Programmable muffle furnace for four and half hours at 450℃ and allowed to cool. The acid mixture of concentrated HCl and HNO_3 in the ratio 1:1 and 20% H_2O_2 was then added. The mixture was heated gently on a hot mantle at 70℃ until brown fumes disappeared. It was then re - dissolved using 0.5 N of HCl, cocked in the specimen tube and allowed to stand for at least 5 hours for re extraction. This was done in a fume chamber. The contents were transferred into a 50 mL volumetric flask and made up to the mark with deionized water. The calibration standards were run followed by the sample for quantification of the analyst of interest. The samples were aspirated to the instrument for analysis. All the samples were analyzed using ICP-ES.

2.3 Preparation of Solvent Extracts

Separately, powdered 500 g of each of the 3 plant species were sequentially extracted with 2 L of distilled hexane, ethyl acetate, dichloromethane and methanol for 2 days in each of the solvent in order of polarity as hexane < ethyl acetate < dichloromethane < methanol. The mixtures of each solvent were then filtered through active charcoal and the filtrates were dried *in vacuo* using a rotatory evaporator. The filtrate of each solvent was evaporated to a residue in a drying cabinet.

2.4 Phytochemical Analysis

The collected extract fractions of *A. palmeri,* and *B. oleracea Acephala* were tested for the presence of phytochemical constituents. These were identified by characteristic color changes using standard procedures by Evans [16]. The tests were based on the visual observations of color change or formation of a precipitate after addition of specific reagent.

2.4.1 Flavones

Flavones were tested according the method described by Geissman [17]. This was achieved by adding 5 mL of ammonium solution to 1 mL of aqueous filtrate of the plant extract followed by

addition of 2 mL sulphuric acid. A yellow coloration indicated the presence of flavones.

2.4.2 Flavonoids

The test for flavonoids was carried out according to the method described by Geissman [17], whereby 5 mL of dilute ammonia solution was added to a portion of the aqueous filtrate of the leaves powder extracts. This was followed by addition of concentrated sulphuric acid. Yellow coloration indicated the presence of flavonoids.

2.4.3 Alkaloids

Alkaloids were tested as described by Evans, [16]. This was achieved by mixing 5 g of the powder sample extract with 25 mL of 1% sulphuric acid. It was then allowed to stand and then filtered. About 10 mL of the filtrate was shaken and Meyer's reagent added. Formation of a white precipitate indicated the presence of alkaloids.

2.4.4 Saponins

Tests for saponins was carried out according to the standard method as described by Evans, [16]. Whereby 5 g of the leaves powder extracts were boiled in 50 mL of distilled water on a water bath and then filtered. About 12 mL of this filtrate was then mixed with 6 mL of distilled water and shaken vigorously to give a stable persistent froth. After the mixture has settled three drops of olive oil were then added and the mixture shaken vigorously, then observed for the formation of emulsion.

2.4.5 Carotenoids

Test for carotenoids followed a procedure described by Evans [16]. The plants extracts were also tested for carotenoids. About 25 g of each plant extract was boiled in 100 mL of clean water. The mixture was then concentrated with concentrated sulphuric acid. Blue color indicated the presence of carotenoids.

2.4.6 Terpenoids

Test for terpenoids was carried out by adding about 5 mL of chloroform to the 10 mL of the each crude extract. The equal volume of concentrated sulphuric acid was added. Formation of the bluish red coloration indicated presence of terpenoids. This procedure was as described by Evans [16].

2.4.7 Tannins

Tannins were tested following the procedure described by Okwu and Josiah [18]. This was performed by mixing 1.5 g of the sample with 30 mL of water in a beaker. It was then followed by addition of 4 mL of 0.1 M $FeCl_3$ was added. Formation of blue black coloration indicated presence of tannins.

3. RESULTS AND DISCUSSION

3.1 Phytochemicals Present

The phytochemicals screening revealed that terpenoids, alkaloids, tannins, flavonoids, flavones and carotenoids were present in most extracts from *Amaranthus palmeri* and *Brassica oleraceae Acephala* (Tables 1 and 2). There were negative test for carotenoids in Hexane and DCM leaves extracts of *Amaranthus palmeri*, whereas there were high concentration of alkaloids, terpenoids, tannins and flavonoids in ethyl acetate and methanol leaf extracts of both plants extracts. This could be due to high polarity of this solvents. The extracts from the plants have also been reported to have antimicrobial activities, this could be due to the presence of such bioactive compounds [18]. Tannins for instance have been shown to form irreversible complexes with procline rich proteins which could lead to inhibition of cell wall proteins synthesis, a property that may explain the mode of action of this chewing stick extracts. They also have evident ability to suppress bacterial cell proliferation by blocking essential enzymes of microbial metabolism such as the proteolytic macerating enzymes. Saponins act by altering the permeability of cell walls and hence exert toxicity on all organized tissues. They exert some antibacterial activity by combining with cell membranes to elicit changes in cell morphology leading to cell lysis. The activity could also be due to the ability of the solvents to extract some of these active components which are reported to be antimicrobial [18]. This justifies the traditional use of the juice of the leaves of this plants against antimicrobial infections like skin infections, dandruff treatment, sore throat treatment, eye infections, colic and urinary tract infections and its use as a natural preservative [18]. Terpenoids on the other hand have been demonstrated to be active against bacteria, fungi, viruses and protozoa [19], which has enabled food scientists to use terpenoids present in essential oils of plants to control *Listeria monocytogenes* [20]. The mechanism of action of

Table 1. Phytochemicals present in stems and leaves of *Amaranthus palmeri*

	Stems				Leaves			
	Hexane	**DCM**	**EtoA**	**Methanol**	**Hexane**	**DCM**	**EtoA**	**Methanol**
Alkaloids	+	+	+	+	+	+	++	++
Terpenoids	+	+	+	+	+	+	++	++
Tannins	+	+	+	+	+	+	++	++
Flavonoids	+	+	+	+	+	+	++	++
Flavones	+	+	+	+	+	+	+	+
Carotenoids	+	+	+	+	-	-	+	+

Key: *+ denotes present, ++ denotes highly present, - denotes absent*

Table 2. Phytochemicals present in stems and leaves of *Brassica oleraceae Acephala*

	Stems				Leaves			
	Hexane	**DCM**	**EtoA**	**Methanol**	**Hexane**	**DCM**	**EtoA**	**Methanol**
Alkaloids	-	-	-	-	+	+	+	+
Terpenoids	++	+	++	++	++	++	++	++
Tannins	++	++	++	++	++	++	++	++
Flavonoids	++	++	++	++	++	++	++	++
Flavones	+	+	+	+	+	++	++	++
Carotenoids	+	+	+	+	-	+	+	+

Key: *++ denotes highly present, + denotes present, - denotes absent*

Table 3. The concentration of lead in the vegetables

	Plant part	**Concentration of Pb (mg/kg) n=3, (ẍ ±SE)**		
		Kabianga	**Sosiot**	**Kiptere**
Amaranthus palmeri	Roots	39.00 ± 1.53	35.33 ± 2.08	41.33 ± 2.08
	Stems	32.67 ± 1.76	44.00 ± 1.73	49.33± 1.22
	Leaves	61.33 ± 1.67	61.33 ± 1.76	63.67 ± 1.86
Brassica oleracea	Roots	15.33 ± 2.87	20.33 ± 1.86	52.33 ± 1.76
Acephala	Stems	41.67 ± 1.03	30.67 ± 1.76	40.00 ± 1.73
	Leaves	66.67 ± 1.96	71.67 ± 1.83	56.67 ± 1.86
	Safe limit = <0.3 mg/kg}, 2.5 mg/kg: WHO/FAO (2007), 0.30 mg/kg.			

Source: European Union [23]

terpenes is by lipophilic membrane disruption. Indeed, Mendoza [21] found that increasing the hydrophilicity of kaurene diterpenoids by addition of a methyl group drastically reduces their antimicrobial activity. Flavanoids on the other hand are known to be produced in plants in response to microbial infections.

3.2 Lead Metal Ion Concentration

From the results in Table 3, Lead concentration ranges from 13.00 ± 2.00 mg/kg to 52.33± 1.76 mg/kg in the roots, 12.33 ± 1.80 mg/kg to 49.33 ± 1.22 mg/kg in the stem and 45.33 ± 1.76 mg/kg to 71.67 ± 1.86 mg/kg in the leaves. It can be noted that the concentration of Pb increases from roots to leaves in all the vegetables. This showed that vegetables have high affinity of accumulation of Pb^{2+} ions in their leaves. From all the areas, the vegetables had a high concentration of Pb^{2+} ions as compared to the recommended permissible levels in vegetables. In comparison with the safe limits, the concentration of Pb^{2+} ions in all the vegetables were above the recommended safe limit (0.3 mg/kg) set by FAO/WHO [22]. The phytochemicals are useful to plants as they protect them against microbial infections this means the plant should be free of any contamination [24]. In all the selected areas of Kericho West Sub-County. This may pose a serious health hazard to the consumers of vegetables in the Sub- County. Use of organ chlorinated pesticides has been pointed out as a major contribution of heavy metal contamination of the soil [25-27], hence farming practices in this areas should also be monitored to evaluate the types and amounts of fertilizers used.

4. CONCLUSION

There is a growing interest in the recent times, in plant and plant derived phytochemical as food source because of its divergent nutritional, functional, antioxidant and other therapeutic properties. The present research revealed the presence of commonly encountered phytochemical constituents in the leaf and stem extracts of the *Amaranthus palmeri* and *Brassica olearaceae acephale* species which included flavonoids, alkaloids, terpenoids and tannins. Hence the study suggests that *Amaranthus palmeri* and *Brassica olearaceae acephale* can be strongly recommended in developing potential health and food products for safe consumption due to its promising bioactive compounds. Lead concentration ranged from 13.00 ± 2.00 mg/kg to 52.33± 1.76 mg/kg in the roots, 12.33 ± 1.80 mg/kg to 49.33 ± 1.22 mg/kg in the stem and 45.33 ± 1.76 mg/kg to 71.67 ± 1.86 mg/kg in the leaves. This values were higher than the recommended safe limit by WHO, thus efforts should be made to ascertain the sources of this lead concentration and advice the users on the dangers they pose.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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