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POTENTIAL TOXICITY OF SOME TRADITIONAL LEAFY VEGETABLES CONSUMED IN NYANG'OMA DIVISION, WESTERN KENYA

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ABSTRACT

Traditional leafy vegetables are those plants whose leaves or aerial parts have been integrated in a community's culture for use as food over a long span of time. These vegetables are highly recommended due to their relatively high nutritional value compared to the introduced varieties, and are also important in food security. Qualitative phytochemical screening, using standard laboratory procedure, was carried out for alkaloids, saponins, cardenolides, flavonoids and polyphenols on traditional leafy vegetables consumed amongst the Luo, an agro-pastoral community living along the shores of lake Victoria, Western Kenya. The vegetables were: *Amaranthus hybridus* L. (subsp.*hybridus*), *Asystasia mysorensis* T. Anderson, *Coccinia grandis* (L) Voigt, *Crotalaria ochroleuca* (Kotschy) Polhill, *Cucurbita maxima* Duchesne ex Lam, *Portulaca quadrifida* L., *Sesamum calycimum* Welw. var. *angustifolium* (Oliv.) Ihlenf. and Siedenst., *Senna occidentalis* L. and *Sida acuta* Burm. F. All the vegetables were found to contain polyphenols and flavanoids while other classes of phytochemicals varied from species to species Brine shrimp lethality tests revealed that *S. calycimum* var. *angustifolium* (LC_{50} 84.8 μg/ml), *S. occidentalis* (LC_{50} 99.5 μg/ml), *S. acuta* (LC_{50} 99.4 μg/ml), *C. grandis* (LC_{50} 100.6 μg/ml) and *A. mysorensis* (LC_{50} 207.7 μg/ml) exhibited marked levels of toxicity. *C. ochroleuca* (Sunnhemp) contained all the five classes of phytochemicals, but proved less toxic (LC_{50} 4511.3 μg/ml). This vegetable is highly utilized in Nyang'oma, and seventy per cent of the respondents consume this species. *A. hybridus* (African spinach, or Amaranth) was found to be the least toxic (LC_{50} 6233.6 μg/ml) and this vegetable is recommended for consumption. From the results, five vegetables contain possible agents that can cause acute or chronic toxicities when consumed in large quantities or over a long period of time. Hence some vegetables should be consumed with great care. Though further studies are required to determine which of the phytochemicals are lethal to mammals.

Key words: Traditional vegetables, phytochemicals, toxicity, Luo, Nyang'oma

FRENCH

RÉSUMÉ

Les légumes verts traditionnels sont des plantes à feuilles ou des parties aériennes qui ont été intégrées dans la culture d'une communauté pour les utiliser comme des aliments pendant une longue période de temps. Ces légumes sont fort recommandés à cause de leur valeur nutritive relativement élevée par rapport aux variétés introduites de l'extérieur, et ils sont également importants en matière de sécurité alimentaire. Le dépistage phytochimique qualitatif, en utilisant la procédure standard de laboratoire, a été effectué pour détecter des alkaloïdes, des saponines, des cardenolides, des flavonoïdes et des polyphénoles sur les légumes verts traditionnels consommés chez les Luo, une communauté agro-pastorale qui vit le long des rives du

Lac Victoria, Province de l'Ouest du Kenya (Western Kenya). Ces légumes sont notamment: Amaranthus hybridus L. (subsp.hybridus), Asystasia mysorensis T. Anderson, Coccinia grandis (L) Voigt, Crotalaria ochroleuca (Kotschy) Polhill, Cucurbita maxima Duchesne ex Lam, Portulaca quadrifida L., Sesamum calycimum Welw. var. angustifolium (Oliv.) Ihlenf. et Siedenst., Senna occidentalis L. et Sida acuta Burm. F. Il a été prouvé que tous ces légumes contenaient des polyphénoles et des flavanoïdes tandis que d'autres classes de phytochimiques variaient d'une espèce à une autre. Des tests de létalité appliqués sur des crevettes grises de mer ont révélé que S. calycimum var. angustifolium (LC_{50} 84.8 μg/ml), S. occidentalis (LC_{50} 99.5 μg/ml), S. acuta (LC_{50} 99.4 μg/ml), C. grandis (LC_{50} 100.6 μg/ml) et A. mysorensis (LC_{50} 207.7 μg/ml) indiquaient des niveaux marqués de toxicité. Les C. ochroleuca (Sunnhemp) contenaient toutes les cinq classes de phytochimiques, mais ils ont prouvé moins de toxicité (LC_{50} 4511.3 μg/ml). Ce légume est fort utilisé à Nyang'oma, et soixante-dix pour cent des personnes interrogées consomment cette espèce. Bien que le test ait montré que A. hybridus (épinard africain, ou amarante) est le moins toxique (LC_{50} 6233.6 μg/ml), la consommation de ce légume est recommandé. A partir des résultats, cinq légumes contiennent des agents possibles qui peuvent causer des toxicités aiguës ou chroniques quand ils sont consommés en grandes quantités ou pendant une longue période de temps. Ainsi donc, certains légumes devraient être consommés avec beaucoup de précaution. Des études plus approfondies sont recommandées pour déterminer lequel des phytochimiques est mortel pour les mammifères, plus particulièrement pour les êtres humains.

Mots-clés: Légumes traditionnels, phytochimiques, toxicité, Luo, Nyang'oma

INTRODUCTION

Traditional vegetables from the wild or home gardens are mutually important for humans both in rural and urban set ups in Kenya [1,2,3]. Traditional leafy vegetables (TLVs) are those plants whose leaves or aerial parts have been integrated in a community's culture for use as food over a large span of time [4]. Since TLVs are highly recommended because they have a relatively high nutritional value compared to the introduced varieties, their consumption gives diversity to daily food intake, adding flavour and zest to the diet [5]. These vegetables are rich in vitamins, minerals, trace elements, dietary fibre and proteins [6,7,8,9]. Effectively, the vegetables are important in food security, during times of drought or poor harvest and are also vital for income generation. Withstanding their value as food, the vegetables also serve as a source of medicines, hence important in their ecological, agronomic and cultural values [10,11, 12].

Despite their advantages, several studies have established that some vegetable species are potentially toxic to humans and animals. Plant chemical compounds, toxic to humans and livestock, are produced as part of the plant's defence against being eaten by pests and herbivores or to gain an advantage over competing plants [13]. Plant poisons are highly active substances that may cause acute effects when ingested in high concentrations and chronic effects when accumulated [14,15]. Under stress conditions, brought on by food shortage, consumption of large amounts of vegetable toxins by animals can have negative consequences [16]. In many cases of poisoning resulting from consumption of endogenous toxicants such as those in toxic vegetables, death or prolonged and serious disabilities are reported. Most traditional vegetables are relatively unpalatable and their digestibility may be limited hence toxic. Usually unpalatibility comes from allelochemicals in plants and these chemicals may be toxic. In addition, traditional medicines prepared from medicinal plants and sometimes from food plants are not always safe.

Poisoning or toxic principles as relates to vegetables generally fall into various phytochemical groups, which include alkaloids, glycosides, oxalates, phytotoxins (toxalbumins), resins, essential oils, amino acids, furanocoumarins, polyacetylenes, protein, peptides, coumarins, flavonoids and glycosides [15,17,18,19, 20,21]. Others are minerals and photosensitizing compounds. For instance, *Lycopersicon esculentum* leaves and stems contain the toxic solanidan alkaloids; μ-solanine and demissine, and their aglycones [22]. The toxic pyrrolizidine alkaloids are a large group of related compounds that occur in plants, mainly in species of *Crotalaria*, *Senecio*, *Heliotropium, Trichodesma, Symphytum* and *Echium* and are poisonous.

Toxicity is a relative concept that must be considered in relation to the context in which these plants are used either as food or medicine. Since about 60 TLVs are commonly consumed in Nyangoma division and Kenya at large, a phytochemical screening of the vegetables for alkaloids, saponins, cardenolides, flavonoids and polyphenols need to be carried out and especially to determine the toxicity tests. The toxicity results should be used to create awareness as to which vegetables are safe for consumption as food and medicines. In the present study, a qualitative phytochemical screening was conducted on nine vegetables mostly consumed and sold in the local market during drought by the Luo of Nyango'ma division. The vegetables together with their common names are: *Amaranthus hybridus* L. (subsp.*hybridus;* Amaranth, or African spinach), *Asystasia mysorensis* T. Anderson, *Coccinia grandis* (L) Voigt, *Crotalaria ochroleuca* (Kotschy) Polhill,(Sunnhemp) *Cucurbita maxima* Duchesne ex Lam, (Pumpkin) *Portulaca quadrifida* L. (Purselane), *Sesamum calycimum* Welw. var. *angustifolium* (Oliv.) Ihlenf. (Onyulo) and Siedenst., *Senna occidentalis* L. (Cassia) and *Sida acuta* Burm.(Sida). However, research into the toxicity of the cooked vegetables of the same species is underway.

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METHODOLOGY

All Solvents and reagents used were purchased locally and were of analytical grade.

Sampling and identification of TLVs

Plant samples of TLVs were sampled in the field and collected from different habitats of Nyang'oma division, Western Kenya, between 29^{o} and 35^{o}E (Latitude and Longitude) of prime meridian. Information about the use of the vegetables and cooking methods were obtained by interviewing knowledgeable persons on the traditional vegetable species. The respondents were asked questions about the vernacular names, ecology, distribution, management, season, status and use of the TLVs.
Finally, identification of the TLVs followed the taxonomy of Flora of Tropical East Africa (FTEA) [22,23]. Voucher specimens were deposited at the JKUAT Botany Herbarium

Sample pre-treatment

The leaves and shoots of the TLVs were macerated using scissors, and dried under shade. The dried samples were separately ground into fine powder using a motor laboratory grinding mill (Christy and Norris Ltd. Chemsford-England).

Extraction

Ground leaf powder (200.0 g) of each vegetable was soaked in a mixture of methanol and chloroform (1:1, 24 hrs) and subsequently in methanol (100%, 24 hrs). The crude extracts were concentrated *in vacuo* and each concentrated extract was separately soaked in activated charcoal (15 minutes), in order to remove chlorophyll, stirred thoroughly and sieved using filter paper (595 Rundfilter, 270 mm). The filtrates were further concentrated *in vacuo* and stored in labelled sample bottles. Each extract (2.0 g) was used in the screening tests.

Screening for phytochemicals

Screening was done according to Chhabra, 1984 and Harbone, 1973, [24,25].

Alkaloids

Each extract was boiled (15 minutes) in HCl (25.0 ml, 1%). Equal volumes of the resulting suspension were filtered into two test tubes (**A** and **B**). To **A**, 5 drops of freshly prepared Dragendorrf's reagent were added. Formation of a precipitate indicated the presence of alkaloids. To confirm the results, **B** was treated with saturated sodium carbonate solution until a drop of the solution turned the Universal Indicator paper blue, (pH 8-9). The resulting solution was dissolved in $CHCl_3$ (4 ml) and allowed to stand. The aqueous layer was collected and acetic acid added to it dropwise, until the solution turned Universal Indicator paper yellow-brown (pH 5).

Cardenolides

The vegetable extracts were thoroughly mixed with distilled water (20.0 ml) and kept at room temperature (2 hrs). The suspension was filtered into two separate test tubes (**A** and **B**). To **A**, 4 drops of Kedde's reagent was added. The appearance of a blue violet colour indicated the presence of cardenolides. Test tube **B** was used to monitor and compare colour change.

Saponins

Each vegetable extract was added to water (15.0 ml) and warmed on a water bath (15 minutes). The resulting solution was filtered and left to cool to room temperature and was transferred (10.0 ml) in a test tube. This was shaken thoroughly for ten seconds and the height of the persistent (5-10 minutes) honeycomb froth measured. Honeycomb froth higher than 1 cm confirmed the presence of saponins.

Polyphenols

Ethanol (10.0 ml) was added to each extracts and the resulting solution (3.0 ml) was transferred in test tubes and warmed in a water bath (15 minutes). Three drops of freshly prepared ferric cyanide solution were added to the extract solution. Formation of a blue green colour indicated the presence of polyphenols.

Flavonoids

The vegetable extracts were added to water (10.0 ml) and methanol (5.0 ml). A few magnesium turnings were added to this mixture (3.0 ml) and followed by drop wise addition of conc. HCl (cyaniding). Development of either, orange, red and pink colours indicated presence of flavonoids.

Brine shrimp toxicity bioassay

The brine shrimp (*Artemia salina*) toxicity bioassay test was conducted according to McLaughlin *et al*, 1991 [26]. Artificial seawater was prepared by dissolving sea salt (38.0 g) in distilled water (1 L). The seawater was put in a small tank and a teaspoon of brine shrimp eggs added to one side of the divided tank, which was covered. The other side was not covered so as to allow light that would attract the hatched shrimps. The tank containing the brine shrimp eggs was left at room temperature for 48 hours to allow the eggs to hatch.

The test tubes used were washed and sterilized in an autoclave machine. Different concentrations of vegetable extracts were prepared, using dimethyl sulfoxide (DMSO, 1.0 ml) in triplicates (1000, 100, 80, 60, 40 20 μg/ml). Brine shrimp larvae (nauplii, 10) were added to each test tube. All test tubes were covered at room temperature (24 hrs). After this period, the number of the dead and the surviving brine shrimps was recorded.

The counting was again done after another day. The obtained data was subjected to Probit Analysis, using Statistical Analysis Systems (SAS) computer program, and the lethal concentration values that killed fifty percent of the shrimps (LC_{50}) were determined for each vegetable.

RESULTS

The traditional leafy vegetables collected in Nyang'oma area exhibited diverse habitats and most species were collected mainly from the wild.

Results of Qualitative screening

The screening of nine traditional leafy vegetables that serve as buffer during periods of relish shortage was conducted using standard screening methods of Harborne, 1973 and Chhabra *et al*., 1984 [24,25]. The vegetables screened were: *A. hybridus*, *A. mysorensis* , *C. grandis*, *C. ochroleuca*, *C. maxima*, *P. quadrifida*, *S. occidentalis*, *S. calycimun* var.*angustifolium*, and *S. acuta*. These vegetables belong to eight different families that occur in diverse ecological locations and soil types. However, these vegetables are wide spread in the UIC soil type, which is stony, sandy, shallow and dry. The screened phytochemicals included alkaloids, saponins, cardeneloids, polyphenols and flavonoids. Table **1** gives a summary of the results of phytochemical screening of the nine vegetables.

Results of Brine shrimp toxicity bioassay

The brine shrimp lethality test was carried out on the nine vegetables screened for phytochemicals. The number of the dead in various individual crude extract concentration determined the mortality, hence the toxicity levels of the traditional vegetables. Five vegetables exhibited toxicity levels of between 20 μg/ml and 1000 μg/ml, and were classified as the most toxic. These were *A. mysorensis, C. grandis, S. occidentalis, S. angustifolium* and *S. acuta.* Table **2** is a summary of LC_{50} (lethal concentration that is capable of killing half of the brine shrimps) values and associated statistics for brine shrimp toxicity tests on these five TLVs' extracts showing higher toxicity at 95% confidence intervals.

S. calycimum var. *angustifolium* is the most toxic vegetable, with LC_{50} value of 84.4 μg/ml, yet this vegetable is commonly consumed in many households and is also readily sold in the local markets.
The other four species of vegetables showed activity between 1500 μg/ml and 12,500 μg/ml and were classified as the least toxic vegetables. Table **3** gives a summary of LC_{50} values and the associated statistics for brine shrimp toxicity tests of the four TLVs' extracts showing lower toxicity at 95% confidence intervals.

DISCUSSION

Qualitative screening

From Table **1**, the nine vegetables contain different phytochemicals. These observations reveal that TLVs constitute a rich, but still largely untapped pool of natural products. All TLVs tested positive for polyphenols. *C. ochroleuca* tested positive for all classes of phytochemicals. The vegetables *A. hybridus, A. mysorensis, C. ochroleuca, P. quadrifida, S. occidentalis* and *S. acuta* gave a positive results for alkaloids while *A. mysorensis* tested negative for saponins and cardenoloids. However, *C. grandis, C. ochroleuca, C. maxima, P. quadrifida, S. occidentalis, S. calycimum* var. *angustifolium* and *S. acuta* gave positive results for saponins and cardenolides. All vegetables tested positive for polyphenols and flavonoids.

Toxicity of extracts to brine shrimps

The number of dead shrimps determined the toxicity of individual vegetable extract concentration. The TLVs that showed least toxicity in the first experiment were eventually subjected to a second experiment where they were administered in higher concentrations.

From the range of concentrations initially tested, five vegetable species namely, *A. mysorensis, C. grandis, S. occidentalis, S. angustifolium* and *S. acuta* exhibited toxicity of between 20 μg/ml and 1,000 μg/ml (most toxic, Table **2**). The other four species namely, *A. hybridus, C. ochroleuca, C. maxima* and *P. quadrifida* showed no activity upto 1,000 μg/ml hence were administered in higher concentrations of between 1,500 μg/ml and 12,500 μg/ml, Table **3.**

The vegetable extract with the lowest LC_{50} value is the most toxic while that with highest LC_{50} value is the least toxic. *S. calycimum* var. *angustifolium* is the most toxic vegetable followed by *S. acuta, S. occidentalis, C. grandis* and *A. mysorensis* respectively. There is little variation between the LC_{50} of *S. occidentalis, S. acuta* and *C. grandis,* probably because the extracts from these species tested positive for almost the same phytochemicals.

S. calycimum var. *angustifolium* tested positive, only, for saponins, cardenolides, flavonoids and polyphenols, Table **1**. Its high toxicity could be due to the quantities and nature of the compounds present in the extract of the vegetable. The variation in phytochemical composition in plants may be influenced by plant factors (species and stage of growth) or environmental factors (season, weather and soil).

There is a major variation in the LC_{50} of *A. mysorensis* compared to the other vegetables. This may be explained by the fact that *A. mysorensis* only screened positive for alkaloids, polyphenols and flavonoids. However, the variation on the slope for the five vegetables may be due to their phytochemicals' composition rather than variations in brine shrimp responses to the different treatments, since the shrimps had the same conditions in terms of age and nutrient consumption. The slope of *A. mysorensis* is typically low while for the other four (*S. acuta, S. occidentalis, C. grandis* and *S. calycimum* var. *angustifolium*), are generally higher. The values of the χ^2, which test for the homogeneity or linearity, of brine shrimp response to the various treatment of the vegetable extracts, are not significantly different for *S. occidentalis, S. acuta* and *C. grandis* but are significantly different for *A. mysorensis* and *S. calycimum* var. *angustifolium.*

From the results, the five vegetables contain possible agents that can cause acute or chronic toxicities when consumed in large quantities or over a long period of time. Therefore, preparation of these vegetables for consumption should be done with caution. Luo women know that vegetable species, such as *C. grandis,* and *S. occidentalis*, have contra-indications and are therefore prepared using traditional cooking methods to make consumption of the vegetables safe.

From Table **1**, *C. ochroleuca* and *A. hybridus* tested positive for all the classes of compounds, but when subjected to the brine shrimp lethality bioassay (Table **3**), proved less toxic. This implied that the type of compounds present in these vegetables, by nature, have no toxic effects to the brine shrimps. *C. ochroleuca* is highly utilized in Nyang'oma, and seventy per cent of the respondents consume this species. The vegetable has a mild taste and is morphologically broad-leafed.

CONCLUSIONS

The results obtained from the study show that five of the TLVs investigated contain a range of phytochemical that may be toxic to humans. There is need to develop and apply standard scientific methods to evaluate the nutritional and toxic properties of the traditional leafy vegetables, to educate the public on the nutritive and possible toxicity of the TLVs. This will promote user confidence and increase vegetable consumption.

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TABLES

Table 1:

Results of phytochemical screening

TLV	Alkaloids	Saponins	Cardenolides	Flavonoids	*Polyphenols*
A. hybridus	+	+	+	+	+
A. mysorensis	+	-	-	+	+
C. grandis	-	+	+	+	+
C. ochroleuca	+	+	+	+	+
C. maxima	-	+	+	+	+
P. quadrifida	+	+	+	+	+
S. occidentalis	+	+	+	+	+
S. calycimun var.*angustifolium*	-	+	+	+	+
S. acuta	+	+	+	+	+

$^{+}$present; $^{-}$absent

Table 2:

Summary of LC_{50} values and associated statistics for brine shrimps toxicity tests on five TLVs extracts showing higher toxicity

Vegetable	LC_{50} (μg/ml)	Slope	Lower limit	Upper limit	Intercept	χ^2/df
A. mysorensis	207.7	4.3	171.1	266.7	-9.94	144.1/1
C. grandis	100.6	277.9	-	-	556.55	0.0/1
S. occidentalis	99.5	287.5	-	-	-574.4	0.0/1
S. calycimum var. *angustifolium*	84.8	30.9	82.5	87.3	-59.7	69.78/1
S. acuta	99.4	285.0	-	-	-569.3	0.0/1

$^{-}$ no fiducial limit

Table 3:

Summary of LC_{50} values and the associated statistics for brine shrimp toxicity tests on four TLVs extracts showing lower toxicity

Vegetable	LC_{50} (μg/ml)	Slope	Lower limit	Upper limit	Intercept	χ^2/df
A. hybridus	6233.6	50.6	6154.1	6313.1	-191.99	146.9/1
C.ochroleuca	4511.3	39.33	44.34.3	4588.8	-143.8	128.4/1
C. maxima	4311.0	20.3	4195.0	4432.0	-73.7	156.5/1
P. quadrifida	3103.0	6.2	2848.0	3480.0	-21.9	59.7/1

A. hybridus is the least toxic vegetable (LC_{50} 6233.6 μg/ml), this species of vegetable grows mainly in the UIC and Ulrl soil types around Nyango'ma.

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