Potential Toxicity of Some Traditional Leafy Vegetables Consumed in Nyang'oma Division, Western Kenya

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Abstract

Traditional leafy vegetables have higher nutritional value compared to the introduced vegetable varieties, are important for food security, and they are vital for income generation. Furthermore the vegetables are important for their medicinal, ecological, agronomic and cultural values. Despite this, several studies have established that some vegetable species are potentially toxic to humans and animals since they contain a wide range of phytochemicals that are toxic. Hence there is need for extensive phytochemical studies of the vegetables commonly consumed in order to educate the communities on the possible side effects for those vegetables that may contain toxins.

Qualitative phytochemical screening from traditional leafy vegetables commonly consumed amongst the Luo, an agro-pastoral community living along the shores of Lake Victoria, Western Kenya revealed that all the vegetables contain polyphenols, while other classes of phytochemicals varied from species to species. *Sesamum calycimum* Welw. var. *angustifolium* (Oliv.) Ihlenf. and Siedenst (Pedaliaceae; LC_{50} 84.8 μg/ml), exhibited marked levels of toxicity. *C. ochroleuca* (Sunnhemp) contained all classes of phytochemicals, but proved less toxic (LC_{50} 4511.3 μg/ml), though *A. hybridus* (African spinach, or Amaranth) was found to be the least toxic (LC_{50} 6233.6 μg/ml).

The methanol: chloroform (1:1) crude extract of the leaves of *S. calycimum* var. *angustifolium* was subjected to chromatographic separation, to obtain 3,4', 5,7-tetrahydroxyflavone (Kaempferol) and β-(3', 4-dihydroxyphenyl-O-α-L-rhamnopyranosyl (1-3)-β-D- (4-O-caffeoyl) glucopyranoside (Verbascoside).

Key words: Traditional vegetables, toxicity, verbascocide, kaempferol

1. 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, and are 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). The screening was followed by brine shrimps toxicity bioassay and isolation, purification and characterization of compounds, from the traditional leafy vegetable that exhibited highest toxicity against brine shrimps.

2. RESULTS AND DISCUSSION

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

2.1 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 Chhabra *et al*., 1984, and Harborne, 1973 [23,24]. 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. Table **1** gives a summary of the results of phytochemical screening.

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

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.

2.2 Results of Brine shrimp toxicity bioassay

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} values and associated statistics for brine shrimp toxicity tests on these five TLVs' extracts showing higher toxicity at 95% confidence intervals.

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

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.

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. 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, 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.

2.3 Structure elucidation of compounds extracted from *S. calycimum* var. *angustifolium*

Two compounds were isolated, using chromatographic techniques, from the traditional leafy vegetable that exhibited highest toxicity against the brine shrimps. The structures of the compounds were determined by spectroscopic analysis and comparison with literature data.

2.3.1 β-(3', 4'-dihyroxyphenyl-O-α-L-rhamnopyranosyl (1-3)-β-D- (4-O-caffeoyl) glucopyranoside **(**Verbascoside, **1)**

The 'H nmr spectral data for **1** is summarised in Table **4**

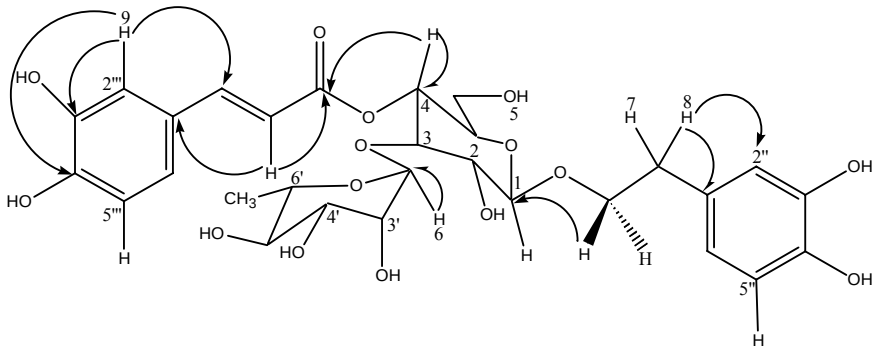

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Table 4: ^{1}Hnmr data of **1** in CD_3OD.

Glucosyl		**Rhamnosyl**		**Phenylethlyl**		***E*-Caffeoyl**	
Position	**(δ)ppm**	**Position**	**(δ)ppm**	**Position**	**(δ)ppm**	**Position**	**(δ)ppm**
1	4.45 (*d,* 7.7)	**1'**	5.30 (*br.s*)	**2"**	6.78 (*d,* 1.9)	**2"'**	7.20 (*d,* 2.1)
2	3.55 (*m*)	**2'**	3.68 *m*)	**5"**	6,79 (*d,* 7.9	**5"'**	6.88 (*d,* 8.1)
3	3.90 (*m)*	**3'**	3.98	**6"**	6.58 (*dd,* 2.1, 8.1)	**6"'**	7.04 (*dd,* 1.9, 8.3)
4	4.98 (*t,* 9.4)	**4'**	3.40	**α 1**	3.70 (*dt*)	**α**	6.32 (*d,* 16.0
5	4.00 (*m*)	**5'**	3.65	**α 2**	4.02 (*dt*)	**β**	7.61 (*d,* 15.8)
6	3.5, 3.60	**6'**	1.12 (*d,* 6.0)	**β**	2.77 (*t,* 7.5)		

The 'H nmr spectrum of **1** exhibited characteristic signals belonging to an *E*-caffeoyl unit, showing three aromatic protons as an ABX system [δ6.88, (*d, J*=8.1Hz. H-5'"), δ7.04 (*dd, J*=1.9.8.3 Hz, H-6'")]. There were two *trans* olefinic protons at 6.32 (*d, J*=16.0Hz, H-α) and 7.61 (*d,* 15.8 Hz, H-β). Signals belonging to the 3,4 dihydroxyphenylethanol moiety (the aglycone) included a set of aromatic protons displaying an ABX system [6.58 (*dd, J*= 2.1,8.1Hz, H-6), 6.74 (*d, J*=7.9Hz, H-5) and 6.78 (*d, J*=1.9Hz, H-2)] and two multiplets of coupled methylenes (α and β). The chiral centre at C-1 is responsible for the observed non-equivalency of the α-methylene protons of the aglycone, which was found resonating at δ3.70 (*d, t*) and δ4.02 (*d, t*). The benzylic or β-methylene protons resonated at δ2.77 (*t J*=7.5Hz). The β-glucopyranosyl unit showed the anomeric proton at δ4.45 (*t,*

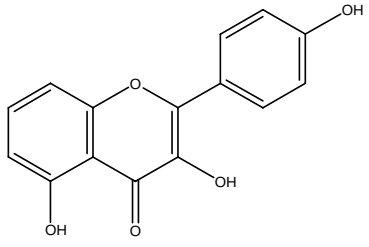
J=7.7Hz). The configuration of the anomeric centres of both glucosyl and rhamnopyranosyl moieties were confirmed by coupling constant and chemical shifts. Linkage of the rhamnosyl unit to the central glucosyl unit is defined by the downfield value observed for H-3 (δ3.90). The *E*-caffeoyl unit was located at C-4 of glucose as concluded from the more downfield shift of H-4 at δ 4.98 as a triplet (9.42 Hz). The ^{13}C nmr of **1** is summarized in Table **5.**

Table 5: Summary of ^{13}C nmr data for **1** in CD_3OD.

Glucosyl		**Rhamnosyl**		**Phenylethyl**		***t*-Caffeoyl**	
Position	**(δ)ppm**	**Position**	**(δ)ppm**	**Position**	**(δ)ppm**	**Position**	**(δ)ppm**
1.	103.2	**1'**	101.6	**α**	71.1	**C=O**	166.9
2.	75.9	**2'**	71.7	**β**	35.7	**α**	144.52
3.	79.8	**3'**	71.5	**1'**	130.7	**β**	146.6
4.	69.2	**4'**	73.1	**2'**	116.5	**1'**	127.0
5.	75.3	**5'**	69.2	**3'**	143.8	**2'**	144.8
6.	61.8	**6'**	17.8	**4'**	145.2	**3'**	145.8
				5'	116.0	**4'**	148.6
				6'	120.2	**5'**	116.0

The ^{13}C-nmr data showed a total of 29 carbons, which included 23 oxygenated and six sp^2 quaternary carbons. The hydrogen bearing aromatic carbon signals were observed at δ116.5, 116.0, 120.2 and showed HMQC correlative with H-2", H-5" and H-6" respectively on the aromatic ring of the aglycone. The oxygenated aromatic signal at δ143.8 and 145.2 were on the basis of key HMBC correlation assigned at C-3" and C-4", respectively. The methylene carbons were revealed by DEPT. These resonated at δ 35.7 for β-C and, more downfield, at δ 71.1 for the oxygenated α-carbon of the aglycone, and 61.9 for C-6 glucose. A similar procedure led to the assignment of signals at δ114.8 (C-2'"), 116.0 (C-5'"), 122.5 (C-6'"), 45.8 (C-3'") and δ 148.6 (C-4'") and the subsequent correlation of these signals to the caffeoyl unit. The other carbons linked to the *E*-caffeoyl unit include the α, β-unsaturated carbonyl resonating at δ 166.9 and the two olefinic carbons at δ114.5 (α-C) and δ 146.6 (β-C). The anomeric carbon and methyl carbon of the rhamnosyl unit were found to resonate at δ101.6 (C-1') and δ17.8 (C-6'). The anomeric carbon of glucose moiety was at δ103.2 (C-1') while C-3' the point of linkage for the rhamnosyl unit was slightly deshielded at δ79.8.

Verbascoside, **1,** has been isolated from over 60 plant species pertaining to 14 families [25]. The family Pedaliaceae does not contain cyanogens, saponins and proanthocyanidins but iridoids have been detected and, **1,** has been isolated from three of the 13 genera in Pedaliacaeae namely, *Harpagophytum, Rogeria* and *Sesamum* [25]. However, this is the first time, **1** has been isolated from the species *S. calycimum* var. *angustifolium.* Verbascoside has hepatoprotective, sedative, cytotoxic, immunosuppresive and analgesic properties [26], and exhibits antihepatotoxic, hypertensive, and antiinflammatory activities [22].
Similarly, 3,4', 5,7-tetrahydroxyflavone (Kaempferol, **2**) was isolated and characterized.



2

Kaempferol is reported to be a potent antioxidant [27] with direct cytotoxicity [28] and has also been isolated from the leaves of other plant species such as pumpkin, lettuce, tobacco, faba bean, cacao, peach, pepper and onions [29]. However, this is the first time that the flavone, **2,** has been isolated from *S. calycimum* var. *angustifolium.*

3. EXPERIMENTAL

3.1. Plant materials

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) [30]. Voucher specimens were deposited at the JKUAT Botany Herbarium

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).

3.2. General experimental procedure

The ^{1}H nmr spectra was recorded on Bruker AMX-400 spectrometers using the UNIX data systems at 400 MHz while the Carbon 13 Nuclear Magnetic Resonance (^{13}C-NMR), Proton Correlation Spectroscopy (^{1}H-COSY), ^{1}H-^{13}C Proton Homonuclear Quantum Correlation (HMQC) and Heteronuclear Multiple Bond Correlation (HMBC) spectra were recorded on Bruker AMX-400 at 400 MHz respectively and the coupling constant (*J*) measured in Hz. The spectra were recorded in deuteriated methanol (CD_3OD) solvent system and the chemical shifts (δ) reported in parts per million (μg/ml). The multiplicities were recorded as *d* doublets, *dd* double of doublets, *s* singlet, *brs* broad singlet, *t* triplet, and *q* quartet. The MS was performed on Finnigan MAT SSQ 7000 Single stage quadrupole analyzer at 70 ev in CD_3OD and the Ultra Violet spectroscopy performed on UV Shimadzu UV-210PC Spectrometer. The Infrared spectroscopy was performed on IR Perkin-Elmer 2000-FT-IR Spectrometer.

3.3. Extraction and isolation

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.

Ground leaf material (1000 g) of *S. calycimum* var. *angustifolium* was soaked in MeOH: $CHCl_3$ (1:1, for 24 h) and subsequently in MeOH (100%, 5 L) for another 24 hours respectively then filtered using filter paper (595 Rundfilter, θ 270 mm) and concentrated *in vacuo* (40˚ C). Analytical silica gel G (Merck, 0.25 cm) pre-coated plates were used for Thin-Layer chromatography (TLC) and the spots on TLC plates visualised under ultra violet (UV) light (λ=366 nm and λ=254 nm) and by spraying with vanillin spray. Column chromatography (CC) was carried out using Sephadex-L_{20} and silica gel 60 $HF_{254+366}$ while preparative chromatography (PC) was carried out using silica gel (Merck, 60 (0.040:0.063 mm). All the solvents used for chromatography were of analytical grade.

3.4. Screening for phytochemicals

Screening was done according to Chhabra, 1984 and Harbone, 1973, [23, 24].

3.4.1 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).

3.4.2 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.

3.4.3 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.

3.4.4 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.

3.4.5 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.

3.5 Brine shrimp toxicity bioassay

The brine shrimp (*Artemia salina*) toxicity bioassay test was conducted according to McLaughlin *et al*, 1991 [32]**.** 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.

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