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Potentials of Underexploited Seed of *Trichosanthes cucumerina* Linn

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ABSTRACT: The cultivation and usage of *Trichosanthes cucumerina* have declined with time, especially in Nigeria. The study evaluated the nutritional and anti-nutritional composition of *T. cucumerina* seeds. Standard procedures were followed in the determination of the bioactive compounds (proximate composition, vitamins, amino acids and phytochemicals) and mineral elements. Waters 616/626 Liquid chromatography (HPLC) was used in quantifying some of the bioactive compounds while atomic absorption spectrophotometer (AAS). The proximate composition of *T. cucumerina* seed revealed thus: carbohydrate (37.08%), protein (32.80%), lipid (5.69%), crude fibre (5.42%), ash (7.27%) and moisture content (11.73%). The fat-soluble vitamins (60.37%) were more in the *T. cucumerina* seeds than water-soluble vitamins (39.63%). Twenty amino acids (essential and non-essential) were detected with threonine (13.09%) been the most abundant and tryptophane (1.32%) amino acids out of the total amino acid. The study showed that the seed contains phytochemicals (oxalate, tannin, saponin, phytate, trypsin-inhibitor, hydrogen cyanide, flavonoids, alkaloids, phenolics and glycosides) with varying concentrations. Also, *T. cucumerina* seed contains different proportions of mineral elements (Mg, Cu, Mn, K, Zn, Ca, Na, N, P, Sn, Pb, Cd, Se, Cr, Co, Ni, As, Hg, Ag and Fe). The study has shown that the underexploited or neglected seeds of *T. cucumerina* has nutritional and anti-nutritional properties. Hence, the cultivation of *T. cucmerina* should be encouraged and the potentials harness to tackle some of the health challenges faced by human, thereby preventing *T. cucumerina* from going into extinction.

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The plant, *Trichosanthes cucumerina* Linn. is commonly called “snake tomato, snake gourd, viper gourd or lone tomato” and belongs to the family Cucurbitaceae (Sandyha *et al.*, 2010). Robinson and Decker-Walters (1997) documented that the family Cucurbitaceae contains about 70 genera and over 700 species world-wide. The genus *Trichosanthes* comprises about 100 species with only few domesticated in Asia and snake tomato being the prominent one. Only landraces of *T. cucumerina* are used in West and Central Africa, while enhanced cultivars from India are grown in East Africa (Khare, 2007). It is herbaceous annual climber with perennial root stock, and grown mainly as ornamentals in relation to other edible cucurbits like pumpkin, melon, and cucumber. Snake tomato is propagated by seed and it produces tendril bearing vines that will sprawl if not supported. The seeds are thick, brownish and sculptured with sizes ranging from 1 – 1.5 cm long. It grows well in fertile soils that are rich in organic matter. In most western Africa, the young unripe fruits are eaten as vegetable while the matured ripe fruits are blended to make stew and broths after removing the seeds. The blended matured endocarp pulp usually deep red and has sweet aromatic taste, which does not sour easily compared to the solanaceous tomato paste. According to Adebooye *et al.* (2005), this later property made it a suitable replacement for solanaceous tomato.

Padulosi *et al.* (2013) observed and reported that most neglected underutilized species have started receiving attention due their abilities in modifying menace of farming production systems and dietary quality. However, *T. cucumerina* in Nigeria is fast going into extinction because most agriculturist have stopped the cultivation and rarely found in home garden. Although, the plant possesses several secondary metabolites and its use in alternative system of medicine ranged from its pharmacological potentials such antioxidant antidiabetic activities of fruits and seeds, hepatoprotective, anti-inflammatory, larvicidal effects, cytotoxicity against some cancer line (Kar *et al.*, 2003; Adebooye, 2008; Kongtun *et al.,* 2009; Sandhya *et al.,* 2010; Arawwawala *et al.*, 2013). Studies have shown that the seeds are used as purgative, astringent, abortifacient, aphrodisiac, and have anti-bacterial, insecticidal, and antispasmodic properties (Nadkani, 2002; Madhaya *et al.*, 2008). The study seeks to evaluate the potentials of the seeds of this neglected and underutilized species, *T.*

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cucumerina. These potentials ranged from amino acids, vitamins, proximate composition, phytochemicals, and minerals inherent in the seed of *T. cucumerina*.

MATERIALS AND METHODS

The matured ripe fruits were harvested from a home garden, the seeds separated from the endocarp pulp, and cleaned for analyses (Plates 1a-c). The matured fruits were identified and authenticated by the Taxonomist at the University of Port Harcourt Herbarium.

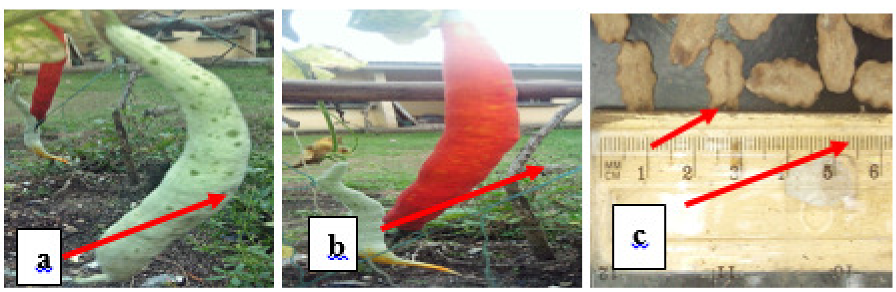
*Proximate Analysis***:** Proximate analysis (moisture, ash, protein, carbohydrate, lipid content and crude fibre) was determined using the standard method of Association of Analytical Chemists (AOAC, 1990).

Mineral elements: The sample was digested on a labcon digester at 300oC in a mixture of hydrogen peroxide, sulphuric acid, selenium and salicyclic acid (Okalebo *et al.*, 2002). The mineral contents (Mg, Cu, Mn, K, Zn, Ca, Na, N, P, Sn, Pb, Cd, Se, Cr, Co, Ni, As, Hg, Ag and Fe) of *C. moschata* were determined using Atomic Absorption Spectrophotometer (AAS).

Vitamins: The extraction and determination of vitamin A, B_2, B_6, B_{12}, and E were according to the method described by Okonwu *et al.* (2018a, 2018b) using 616/626 Water HPLC while vitamin C was determined using titrimetric method (Okwu, 2004).

Amino Acids: The amino acids preparation and determination using Waters 616/626 LC (HPLC) were carried out according to the method described by Okonwu *et al.* (2018a, 2018b).

Flavonoids and alkaloids (extraction and analyses): The flavonoids and alkaloids determination using Waters 616/626 LC (HPLC) were carried out in line with the method described by Okonwu *et al.* (2017).



Plates 1(a-c): *Trichosanthes cucumerina* (a) Young fruit (b) Ripe fruit (c) Seeds

Glycosides (extraction and analysis): 0.5g plant sample was weighed into a set of digestive tubes, 5ml of 0.1 M HCl was added, warmed gently for 15 minutes at 105°C and transferred into a 50ml volumetric flask. The procedure was repeated twice. Rinsed with two to three aliquot, allowed to filter completely and the filtrate volume was made up to 100ml with the extractant solution and mixed thoroughly. Then, 5ml of extract solution was taken from the 100ml flask and ran through a 2cm layer (resin is packed on a macro pipette tip) cation exchange resin (CEC). Glycosides compounds was eluted with 10ml of absolute ethanol. The ethanol was washed from the column with ultrapure water (10ml) and the supernatant was transferred to a sample vial and ran on water 616/626 HPLC HPLC. The conditions for the analysis of glycosides were as follows: (i) An autosampler (ii) An automated gradient controller (iii) Gradient elution HPLC pump (iv) Reverse-phase HPLC column, thermostatically heated in a temperature-controlled room. (v) Detector by fluorescence (vi) Carrier gas: Nitrogen gas at flow rate of 38ml/mins. (vii) Temperature: Detector- 167°C; Injector port- 183°C and Column- 130°C (viii) Computer facilities for storing data. (ix) Printer for results reporting.

Phenolics (extraction and analysis): 2g plant sample was weighed into a set of test tubes, 3ml of 70% acetone in water was added and the tube placed in an ultrasonic water bath at 10°C for 5 minutes. Stirred occasionally with a glass rod. Filtered through a 50-60µ Gooch crucible into a 50ml Erlenmeyer flask. The extraction was repeated 3 times by adding 3ml of 70% acetone in water and allowing it stand in the water bath at 10°C for 5 minutes. The test tube was rinsed with the final 3ml portion of 70% acetone in water and empty into the test tubes. Then 2ml of 0.1M yb-acetate and 15ml of 0.1M TEA reagent were added into the filtrate. The flask was closed with rubber stopper, swirled and shaked for 20 minutes after transferring the sample solutions to a set of plastic volumetric tubes. Allowed to settle for 4 hours and the supernatant was collected for analysis using HPLC. The conditions for the analysis of phenolics were as follows: (i) An autosampler (ii) An automated gradient controller (iii) Gradient elution HPLC pump (iv) Reverse-phase HPLC column, thermostatically heated in a temperature-controlled room. (v) Detector by fluorescence (vi) Carrier gas: Argon gas at flow rate of 60ml/mins. (vii) Temperature: Detector- 120ºC; Injector port- 155ºC and Column- 117ºC (viii) Computer facilities for storing data. (ix) Printer for results reporting.

RESULTS AND DISCUSSION

Proximate and mineral composition: The study showed that *T. cucumerina* seed contains the following percentage proximate composition: carbohydrate (37.08%), protein (32.80%), lipid (5.69%), crude fibre (5.42%), ash (7.27%) and moisture content (11.73%), respectively. The presence of macro and micro-elements was detected in the seed of *T. cucumerina* in varying proportion. These mineral elements and their concentration are thus: N (5.25%), K (3.042%), Ca (2.130%), P (0.649%), Mg (0.129%), Na (1.237 ppm), Fe (2.261 ppm), Mn (9.829 ppm), Cu (3.230 ppm), Zn (0.137 ppm), Sn (0.115 ppm), Pb (0.055 ppm), Cd (0.670 ppm), Se (0.039 ppm), Cr (0.341 ppm), Co (0.079 ppm), Ni (0.456 ppm), As (0.021 ppm), Hg (0.026 ppm), and Ag (0.102 ppm).

Vitamins: The percentage concentration of fat-soluble vitamins was 60.37% in *T. cucumerina* seed while the water-soluble vitamins recorded the least at 39.63% with respect to total vitamins (Figure 1). Vitamin A is the predominant vitamin in the *T. cucumerina* seed and its concentration exceeds the concentration of total water-soluble vitamins.

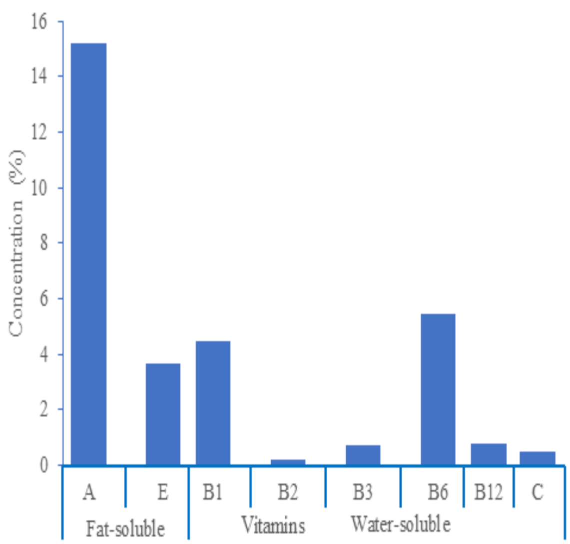


Fig 1: The vitamins composition of *T. cucumerina* seed

Table 1: Percentage composition of Amino acids in *Trichosanthes cucumerina*

Group of amino acids	Amino acids	Percentage (%)
Essential	Histidine	0.541
	Isoleucine	0.843
	Leucine	1.700
	Lysine	0.740
	Methionine	0.401
	Phenylamine	2.388
	Threonine	3.800
	Tryptophane	0.384
	Valine	1.616
Non-Essential	Alanine	0.801
	Arginine	0.892
	Asparagine	2.530
	Aspartic acid	1.544
	Cystine	0.739
	Glutamic acid	1.170
	Glutamine	1.589
	Glycine	0.629
	Proline	3.641
	Serine	1.080
	Tyrosine	1.986
	Total Amino Acid	**29.023**

Amino acids: The non-essential amino acid (16.61%) had a higher concentration than the essential amino acid content (12.41%) of *T. cucumerina* seed (Table 1). Threonine was the highest occurring amino acid in the *T. cucumerina* seed at 3.80% while tryptophane was the lowest occurring amino at 0.384%. Phytochemicals: *Trichosanthes cucumerina* seeds were found to contain different phytochemicals (Figure 2, Table 2).

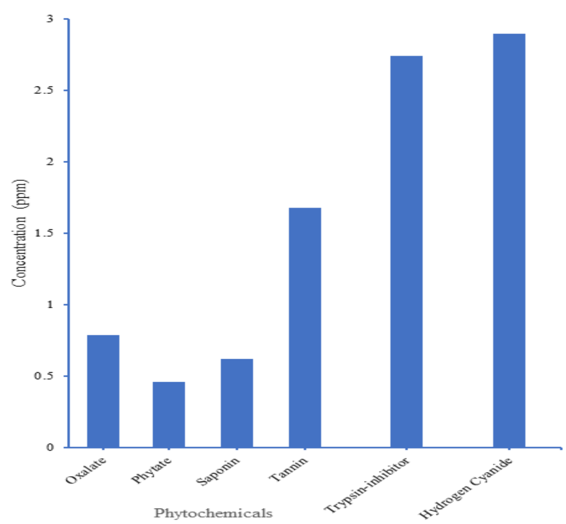


Fig 2: Phytochemical components of *T. cucumerina* seed

Table 2: Concentration of flavonoids and alkaloids in *T. cucumerina* seeds.

Class of Flavonoids	Flavonoids	Conc. (g/100)	Class of alkaloids	Alkaloids	Conc. (g/100g)
Flavones	Acacetin	0.704	Purine (Pseudo) alkaloids	Caffeine	0.492
	Apigenin	1.704		Nicotine	0.558
	Diosmin	0.463		Theobromine	1.179
	Luteolin	0.600		Theophylline	0.772
	Neodiosmin	1.760			
	Nobiletin	0.621	Proto alkaloids	Colchicine	0.255
	Rhoifolin	1.370		Ephedrine	0.080
	Tangeretin	1.065		Norpseudoephedrine	1.529
				Phenylethyllamine	1.492
Flavan-3-ols / flavanols	Catechin	0.165			
	Epicatechin	0.732	Piperidine	Conine	0.601
	Epicatechingallate	0.095		Lobeline	0.100
	Epigallotechin	0.350		Piperine	0.974
	Epigallocatechingallate	0.430			
	Proanthocyanidins	1.580	Pyridine	Nornicotine	2.510
	Taxifolin	0.362		Pyridine	0.517
	Theaflavins	0.790		Ricinine	0.286
	Thearubigins	0.407			
			Isoquinoline	Berberine	0.618
Isoflavones	Daidzein	0.495		Cephaline	0.602
	Genistein	0.710		Codeine	0.933
	Glycitein	0.325		Emetine	0.594
				Heroin	0.950
Flavonols	Isorharmnetin	0.060		Morphine	0.870
	Kaempterol	0.606		Narcotine	0.630
	Myricetrin	0.313		Papaverine	0.778
	Quercetin	2.943		Psychotrine	0.334
				Tubocurarine	0.360
Flavanone	Didymin	0.526			
	Eriocitrin	2.877	Acridine	Acridine	1.606
	Eriodictyol	0.864			
	Hesperetin	0.490	Indole/benzypyrole	B-carboline	1.973
	Hesperidin	0.530		Ergotamine	1.762
	Nanirutin	0.460		Eserine	0.495
	Naringin	0.381		Rauwolfia	0.764
	Naringinenin	0.639		Reserpine	0.754
	Neoeriocitin	1.099		Strychine	0.076
	Raxifolin	1.522		Vinblastine	0.661
	Poncirin	0.760		Vincristine	0.355
Anthocyanidine	Anthocyanine	1.667	Tropane	Atropine	0.720
				Apoatrophine	0.250
	Total Flavonoids	32.353		Hyoscine	0.589
			Quinolone	Apormorphine	0.657
				Cinchonidine	1.160
				Cinchonine	1.084
				Quinidine	0.682
				Quinine	1.359
				Quinoline	0.085
				Total Alkaloids	39.042

Phenolics and Glycosides: Phenolic compounds and glycosides in *T. cucumerina* seeds are presented in Table 3. The seed had varying concentrations of phenolic compounds and glycosides. Protocatechic acid was the most predominant phenolics with a concentration of 10.18 g/100g while mendelic acid had the least concentration (0.222 g/100g). The abundant glycosides were hydrochlorathiazide acid, atenonol acid and oleandrin acid with percentage occurrence in relation to the total glycosides as 11.76%, 11.63% and 10.39%, respectively. Proximate composition of *T. cucumerina* seeds showed that it is a rich source of nutrients. It revealed that lipid and crude fibre had the lowest proximate content at 5.69% and 5.42%, in that order. Moisture content (11.73%) and ash (7.27%) were a bit higher than the later while carbohydrate (37.08%) and protein (32.80%) were high and significant when compared to others. This suggest that *T. cucumerina* seed is a good source of carbohydrate and protein. The low percentage moisture content is an indication that the seed can be stored for a longer period under favourable condition due to the presence of strong seed coat. Ihekoronye and Ngoddy (1985) stated that high moisture content favours the spoilage of agricultural products by fungi. Chuku *et al* (2004) also reported that high moisture content led to greater fungal growth and thus low storability of the seeds of *Irvingia gabonensis*. Low lipid content makes *T. cucumerina* seeds very healthy for consumption. Ash on food determines largely the extent of mineral matters likely to be found on food substance (Davidson *et al.,* 1975). The predominant presence of vitamin A in *T. cucumerina* seed indicates its usefulness in promoting good vision especially in low light. Also, the presence of vitamin B complex (B_1, B_2, B_3, B_6 and B_{12}) suggests that they play a role in converting nutrients into energy, thereby supporting cellular metabolism. Studies had shown that Vitamin C, also known as ascorbic acid, promotes wound healing, strengthens resistance to infections by enhancing the immune system and improves absorption of Fe (Ojiako and Igwu, 2008; Gayatri *et al.,* 2013). In addition, vitamin E is use as an antioxidant in the humans and could also help in the proper functioning of the immune system including neurological functions and proper functioning of the cells.

Table 3: Concentration of different phenolics and glycosides found in *T. cucumerina* seed.

Phenolics	Conc. (g/100g)	Glycosides	Conc. (g/100g)
Aesculetin acid	1.358	18-beta-glycyrrhetinic acid	0.004
Astringin acid	0.384	Ameodipine acid	0.410
Benzoic acid	0.985	Atenonol acid	0.897
Cafein acid	0.740	Captopril acid	0.335
Caffaric acid	1.181	Digitoxin acid	0.425
Caffeic acid	0.953	Digoxin acid	0.348
Carreic acid	0.637	Enalapril acid	0.781
Castainol C4 acid	3.269	E-strophanthin acid	0.524
Castarinol C1 acid	1.740	Furosemide acid	0.523
Castarinol C2 acid	0.632	Glycyrhizic acid	0.511
Castarinol C3 acid	0.649	Glycyrrhetinic acid	0.053
Catechin acid	0.688	Hydrochlorathiazide acid	0.907
Cinnamic acid	1.710	Lisinopril acid	0.097
Contaric acid	1.213	Metoprolol acid	0.382
Coumaric acid	0.541	Nifedipine acid	0.050
Cutissin acid	0.878	OLeandrin acid	0.801
Cyanidin 30-glucoside	0.304	Propranolol acid	0.601
Cyanidin coumaroyl 30-glucoside	0.780	Varapamil acid	0.061
Ethy/ gallon acid	2.780		
Ethyl/caffeati acid	0.740	Total Glycosides	7.710
Ferteric acid	0.572		
Ferulic acid	0.923		
Galic acid	0.590		
Galic acid	0.739		
Genticitic acid	0.180		
Homogentisic acid	0.527		
Homovanilic acid	1.928		
Homovanillic acid	0.590		
Izoferulic acid	0.696		
Mendelic acid	0.222		
M-OH-benzoic acid	0.605		
P-cumaric acid	0.300		
Piperonic acid	0.430		
P-OH-benzoic acid	1.473		
P-OH-Phenyloacetic acid	0.455		
Protocatechic acid	10.18		
Pyrogallic acid	0.160		
Salicitic acid	1.008		
Salicylic acid	1.165		
Sinagic acid	3.758		
Sinamic acid	0.782		
Singlic acid	1.752		
Syringic acid	1.460		
Valnilic acid	0.740		
Veratoc acid	0.435		
Total Phenolics	53.83		

Other bioactive compounds such as phytochemicals which includes saponins, tannins, oxalate, phytate, trypsin inhibitor, hydrogen cyanide, alkaloids, phenolics and flavonoids were also present in the seeds of *T. cucumerina*. The detected phytochemicals are potent bioactive compounds which serve as precursors for the synthesis of useful drugs (Sofowora, 1993; Okigbo *et al*., 2009). Saponins and tannins were present in a low amount in *T. cucumerina* seed. According to Okwu and Emenike (2006), saponins are bitter phenolic compounds produced by plants which act as defensive mechanism to halt pathogenic attack, thus they are antimicrobials. They enhance the penetration of proteins through cell membranes (Sule *et al.,* 2011) and also lower cholesterol level, reduces the risk of high blood pressure, improves the immune function and prevents cancer. Okwu (2004) reported that the presence of saponins and tannin in the plant sample aids in the reduction of cancer and other degenerative disease. Tannins play a role in human body as antioxidants in preventing heart diseases, prevents cancer by preventing cellular damage. Most tannins fight cavities, as well as treatment of inflamed or ulcerated tissues (Enyinnaya, 2016) and diarrhea.

Some of the phytochemicals such as flavonoids, tannins, alkaloids, saponins and steroids have been found to possess a wide range of activities which may help in the protection against chronic diseases and responsible for the central nervous system (Hostettmann *et al.,* 2000). Alkaloids have been very useful in medicinal purposes as agents possessing analgesic, anti-malarial, antiseptic, anti-inflammatory, anti-carcinogenic and bacterial activities: therefore, the presence of alkaloid in *T. cucumerina* seeds suggest that it could be beneficial in addressing some of the health challenges. Total flavonoids content of the seeds of *T. cucumerina* obtained from the research was high. Flavonoids are known to be biologically active against liver toxins, tumors, viruses and other microbes, allergies and inflammation. It was also reported that they protect blood vessels especially tiny capillaries that carry oxygen and nutrients to cells (Okwu, 2004; Harisaranraj *et al.,* 2009). Phenolics were found abundant in the seeds of *T. cucumerina.* Phenolic compounds had been reported to possess the ability to perform anti-cancer activities as well as fight various diseases associated with oxidative stress (Arts and Hollman, 2005). Prior studies have demonstrated that the health beneficial effects of dietary phenols are due to their ability to exhibit antioxidant, anti-inflammatory and anti-clastogenic activities (Lambert *et al.,* 2015). Phenolics are also involved in tissue regeneration in wounds and burns by preventing loss of fluid (Okuda, 2005)

Conclusion: The study had shown that *T. cucumerina* seeds contain a large number of bioactive compounds ranging from phytochemicals to vitamins, minerals and amino acids. The presence of these bioactive compounds underscores the inherent potential in this underexploited seed. This suggests the need to promote the cultivation of *T. cucumerina,* thereby halting the downward trend toward extinction. It is worthy to mention that both the fruit and the seed are useful sources of nutrient and anti-nutrient. The study therefore recommends increase awareness of this underutilized plant, *T. cucumerina*, and so harness effectively the latent values.

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