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(RESEARCH ARTICLE)

Qualitative phytochemical and GC-MS analysis of some commonly consumed vegetables

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Abstract

The aim of this research work is to unveil the phytoconstituents of some commonly consumed leafy vegetables which are *Talinum triangulare* (water leaf), *Telfairia occidentalis* (fluted pumpkin) and *Occimum gratissimum* (scent leaf). Freshly harvested vegetables were separately processed into fine powder. 15 g, each of the vegetable powder samples was extracted with methanol. Phytochemical and GC-MS analysis was carried out on the samples using standard procedures. The analysis showed that methanolic leaf extract of fluted pumpkin contains tannins, saponins, phenolics, proteins, anthraquinone and alkaloids with the presence of tannins and saponins being more abundant. However, enquiry into the phytoconstituents of *Talinum triangulare* leaf indicated that all phytochemicals investigated were reportedly present with saponins and alkaloids being more abundant. For *O. gratissimum* leaf, tannins, saponins, flavonoids, steroids, phenolics, proteins, anthraquinone and alkaloids were reportedly present. However, like *T. occidentalis*, tannins and saponins were more abundant. GC-MS analysis carried out on the methanolic leaf extract of *T. occidentalis* revealed that nine compounds were present. The five most abundant of the nine compounds include n-Hexanoic acid (35.23%), 9, 12, 15-octadecatrienal (12.4%), imidazolidinedione-5-methyl (10.96%), L-Proline, 5-oxo-, methyl ester (9.76%) and 3- [Prop-2-enoyloxy] tetradecane (8.96%). Meanwhile, *O. gratissimum* showed the presence of only phenol-2-methyl-5-(1-methylethyl) (90.73%) as the major constituent, while eight (8) compounds were found present in the methanolic leaf extract of *T. triangulare* (water leaf) and the five more abundant of the eight include pentanoic acid (28.28%), DL-proline,5-oxo-methyl-buthylester (24.89%), 3 pyridinecarboxyaldehyde, O-cetyloxine (E) (21%), Z-dodecanol (12.68%) and 4-pyridinol (4.61%). Findings from this research further support the claim on vegetables as repositories of nutraceuticals.

Keywords: Vegetable; Phytochemical; Nutraceutical; Disease

1. Introduction

Vegetables are herbaceous plants or a portion of a plant that can be consumed whole or in part, either raw or cooked in order to obtain potentially health aiding compounds called nutraceuticals for effective growth and protection of the body against diseases [1]. The fact that vegetables constitute the major proportion of the diet of the populace in many parts of the world strongly correlates with the increasing public awareness on their health benefits. In fact, research has linked consumption of vegetable rich diets to lowered incidence of cardiovascular diseases, while intake of vegetable deficient diets has been implicated in 31% of ischaemic heart disease and 11% of stroke cases [2].

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Owing to urbanization, arable lands have been converted to industrial layouts which translate to a monumental decrease in agricultural productions, while population growth sustains a persistent rise, an imbalance that culminates to food insecurity and consequently, reduced intake of certain nutrients with therapeutic and prophylactic potentials. Therefore, in order to strike a balance between nutrition and health among the populace, the need to x-ray potential sources, notably leafy vegetables for subsequent extraction and identification of inherent phytoconstituents for food bio-fortification purposes becomes an imperative measure towards tackling nutrition and health related urbanization problems.

Talinum triangulare (water leaf)a cosmopolitan weed and a member of the family Portlacaceae, *Telfairia occidentalis* (fluted pumpkin) a Curcurbitaceous family member and native to the West African high rainfall forest belt and *Occimum gratissimum* (scent leaf) a perennial aromatic herb belonging to the family Labiatae are the most commonly consumed leafy vegetables by the people of different ethnic background not only in Nigeria but in the entirety of the African continent [3] who have reposed their confidence in these plants for their prophylactic and therapeutic strength against some notable diseases such as measles, anemia and cholera respectively etc [4], a critical factor that stimulates the need for this research which aims at unveiling the phytoconstituents in the aforementioned vegetables to further deepen the understanding of their therapeutic values.

2. Material and methods

2.1. Collection and processing of vegetables

Fresh leaves of *Talinum triangulare* (water leaf), *Telfairia occidentalis* (fluted pumpkin) and *Occimum gratissimum* (scent leaf) harvested from the university farm were subsequently identified at the herbarium unit of the Department of Forestry, Micheal Okpara University of Agriculture Umudike, Abia State, Nigeria. The leaves were washed with tap water and shade dried at room temperature (28 ±2 ˚C) for seven (7) days. The dried leaves of each of the vegetables were separately ground with the aid of an electric blender and sieved to fine powder. 15 g each of the vegetable powder samples was extracted with methanol according to the method described by [5]. Extract obtained was subjected to qualitative phytochemical and GC-MS analysis.

2.2. Qualitative phytochemical analysis

2.2.1. Tests for reducing sugar by Fehling's test

About 0.5 g of each vegetable samples extract was dissolved in distilled water and filtered. The filtrate was heated with 5 ml of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars [6].

2.2.2. Test for protein by xanthoproteic test

Few drops of nitric acid was added to 1 ml of extract of each of the samples by the sides of the test tube and observed for formation of yellow color [7].

2.2.3. Test for tannins

About 0.5 g each of vegetable samples extract was stirred in 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. Appearance of a blue-black, green or blue green precipitate indicates the presence of tannins [8].

2.2.4. Test for saponins

1 g of each of the samples extract was boiled with 5 ml of distilled water, filtered. To the filtrate, about 3 ml of distilled water was further added and shaken vigorously for about 5 minutes. Frothing which persisted on warming was indicative of the presence of saponins [6].

2.2.5. Test for alkaloids by Mayer's test

Exactly 50 mg of extract devoid of solvent is stirred in 2 ml of dilute hydrochloric acid (HCl) and filtered. To the filtrate, few drops of Mayer's reagent were added by the side of the test tube. Formation of white or creamy precipitate indicated the presence of alkaloids [9].

2.2.6. Test for flavonoids by Shinoda's test

About 0.5 g of each of the samples extract was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips were then introduced into the filtrate. This was followed by the addition of few drops of conc. HCl. Appearance of pink, orange, or red to purple colour showed that flavonoids were present [8].

2.2.7. Test for terpenoids by Salkowski test

Exactly 2 ml of chloroform was added to 0.5 g of the extract. This was followed by the addition of 3 ml conc. H2SO4 to form a layer. The emergence of a reddish brown colour at the interface was indicative of the presence of terpenoids [9].

2.2.8. Test for phenols by ferric chloride test

Few drops of neutral 5% ferric chloride solution was added to 50 mg each of samples extract dissolved in 5 ml of distilled water. A dark green colour indicated the presence of phenolic compounds [10].

2.2.9. Test for steroids

Precisely 5 ml of distilled water was introduced into a test tube holding 0.5 g of extracts and the mixture shaken vigorously and observed for a stable persistent froth. The resulting froth was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for formation of an emulsion [7].

Tests for cardiac glycosides by Keller Killiani's

Precisely 100 mg each of samples extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under layered with 1 ml of concentrated H_2SO_4 acid. A brown ring obtained at the interface indicated the presence of de-oxysugar characteristic of cardiac glycosides [7].

Test for free anthraquinones

About 2 mg each of samples extract was placed in a dry test tube after which 5 ml of chloroform was added and shaken for at least 5 mins. This was filtered and the filtrate was added into an equal volume of 10% ammonia solution, and was shaken again. The presence of bright pink colouration in the aqueous upper layer was an indicator of the presence of free anthraquinones [11].

2.3. GC-MS of vegetable samples

An agilent 7890B Gas Chromatography (GC) system fitted with a 30 m× 250 µm × 0.25 µm Rtx-5MS capillary column coupled to Agilent 5977A Mass Spectrometric (MS) was used at a temperature of 325 ^{o}C. Ultra-high purity helium (99.99%) formed the mobile phase at constant flow rate of 1.0 cm^3/min. The injector, transfer line and ion source temperature were set at 290 ^{o}C. The ionizing energy was 70ev. Electron multiplier voltage was obtained from auto tune. The oven temperature was programmed from 60 ^{o}C for 2 mins, then 10 ^{o}C /min to 110 ^{o}C/min and then 280 ^{o}C at the rate of 5 ^{o}C/min. The sample fractions were diluted with appropriate acetone (1/100 v/v), filtered and 1 µL was injected into the inlet. All data were obtained by collecting the total ions currents (TIC). The percentage composition was determined from calibration curve (0-0.9g/cm^3). The sample and the standard were prepared in like manner. The standard was processed separately prior to being spiked into the sample and signal of the sample was obtained from the difference of the spiked sample and that of the standard. The experiment was repeated more than six times. As a quality control, percentage relative standard (%RSD) was estimated by comparing coefficient of determination (R2) values of calibration curves using both standard signal and spiked sample signal [12].

3. Results

Table 1: Qualitative phytochemical constituents of some commonly consumed vegetables

	Vegetables		
Phytochemicals	**Pumpkin Leaf**	**Water leaf**	**Scent leaf**
Tannins	++	+	++
Saponins	++	++	++
Flavonoids	+	+	+
Steroids	_	+	+
Terpenoids	_	+	_
Cardiac glycosides	_	+	_
Phenolics	+	+	+
Proteins	+	+	+
Reducing sugars	_	+	_
Anthraquinnones	+	+	+
Alkaloids	+	++	+

+: abundant, ++: more abundant, -: absent

Table 2: Gas Chromatography-Mass Spectrometric analysis of *T. occidentalis* (fluted Pumpkin) leaf

RT	**Name of Compound**	**Molecular Formula**	**MW**	**Peak Area %**
6.264	2,4-Imidazolidinedione, 5-methyl	$C_4H_6N_2O_2$	114	10.96
7.088	L-Proline, 5-oxo-, methyl ester	$C_6H_9O_3$	143	9.76
9.739	n-Amylcyclohexane	$C_{11}H_{22}$	154	7.99
9.840	Hexahydroindole	$C_8H_{13}N$	123	4.72
14.604	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	35.23
16.899	6- Octen-1-ol, 3,7-dimethyl (±)	$C_{10}H_{20}O$	156	4.57
17.142	E,E-1,9,17- Docasatriene	$C_{22}H_{40}$	304	5.57
17.247	9,12,15- Octadecatrienal	$C_{18}H_{30}O$	262	12.24
17.551	3- [Prop-2-enoyloxy]tetradecane	$C_{17}H_{32}O_2$	268	8.96

RT= Retention Time; **MW**= molecular weight

Table 3: Gas Chromatography-Mass Spectrometric Analysis of *O. gratissimum* (Scent) leaf

RT	**Name of Compound**	**Molecular Formula**	**MW**	**Peak Area %**
5.934	Phenol, 2-methyl-5-(1-methylethyl)	C10H14O	150	90.73

RT= Retention Time; **MW**= molecular weight

Table 4: Gas Chromatography-Mass Spectrometric Analysis of *T. triangulare* (water) leaf

RT	Name of Compound	Molecular Formula	MW	Peak Area %
7.074	DL-Proline, 5-oxo-, methyl-, buthyl ester	$C_6H_9NO_3$	143	24.89
7.951	1-Pentanamine, N-pentyl	$C_{10}H_{23}N$	157	2.73
9.454	4- Fluorohistamine	$C_5H_8FN_3$	129	2.16
9.833	4- Pyridinol	C_5H_5NO	95	4.61
11.793	Adenosine 3',5'- Cyclic monophosphate	$C_{10}H_{12}N_5O_6P$	298	3.22
12.981	Z-2-Dodecanol	$C_{12}H_{24}O$	184	12.68
13.064	3- Pyridinecarboxaldehyde, O-acetyloxime, (E)	$C_8H_8N_2O_2$	164	21.43
14.132	Pentanoic acid, 2-methyl, butyl ester	$C_{10}H_{20}O_2$	172	28.28

RT= Retention Time; **MW**= molecular weight

4. Discussion

Phytochemicals are biologically active chemical compounds in plants which wield both medicinal and nutritional potentials [13].This is evident by the fact that n-hexadecanoic acid, one of the major compounds in the methanolic leaf extract of *T. occidentalis* (fluted pumpkin) has been described as a potent antioxidant as well as a dependable anti-cancer agent [14]. Similarly, 6-octen-1-ol, 3,7dimethyl also in pumpkin leaf has demonstrated antimicrobial activity [15]. Other compounds such as pyridinol a pyridine derivative and Z-2-Dodecanol derived from the methanolic leaf extract of *T. triangulare* (water leaf) have shown exciting antibacterial activity especially against gram negative bacteria such as *E. coli* and *S. albus* [16]. Phytochemicals are known to impact on plant's colour, aroma, flavor and shield plants against both biotic and abiotic stresses ([17]; [18]). Figure 1.0 shows the results obtained from the qualitative phytochemical analysis performed on three commonly consumed vegetables in Nigeria which are *Talinum triangulare* (water leaf), *Telfairia occidentalis* (fluted pumpkin) and *Occimum gratissimum* (scent leaf). The analysis revealed the presence of tannins, saponins, phenolics, proteins, anthraquinones and alkaloids in the methanolic leaf extract of fluted pumpkin with the presence of tannins and saponins being more abundant. This may be attributed to the solubility characteristics of the phytochemicals in the solvent (methanol) used for extraction. These findings are consistent with the work of Ogbonnaya and Uadia [19] which reported similar degree of presence (++) for tannins and saponins in the aqueous root extract of *Telfairia occidentalis*. However, analysis on the phytochemical constituents of *Talinum triangulare* leaf revealed that all phytochemicals screened for were reportedly present with saponins and alkaloids being more abundant. These findings are consistent with the work of Abideen *et al*. [20] which reported more presence of saponins and alkaloids in the ethanolic leaf extract of *T. triangulare*. In the case of *O. gratissimum* leaf, tannins, saponins, flavonoids, steroids, phenolics, proteins, anthraquinones and alkaloids were reportedly present. However, like the *T. occidentalis*, tannins and saponins were more abundant. This may be linked to the solubility characteristics of the phytochemicals. These findings are in tandem with the work of Priscillia [21] which reported the presence of tannins and saponins in aqueous leaf extract of *O. gratissimum.* Interpretation on mass spectrum GC-MS was performed with the aid of the National Institute Standard and Technology (NIST) database which has over 62,000 patterns. Spectrum generated on unknown compounds was compared with the spectrum of known compounds deposited in the NIST library. The name, molecular weight and structure of the compounds were determined. GC-MS analysis performed on the methanolic extract of *T. occidentalis* revealed that nine compounds were present as shown in Table 2.0. The five most abundant of the nine compounds include n-Hexdecanoic acid (35.23%), 9, 12, 15-octadecatrienal (12.4%), imidazolidinedione-5-methyl (10.96%), L-Proline, 5-oxo-methyl ester (9.76%) and 3- [Prop-2-enoyloxy]tetradecane (8.96%). Table 3.0, shows the outcome of the GC-MS analysis performed on *O. gratissimum* revealing only the major compound phenol, 2-methyl-5-(1-methylethyl) (90.73%). For *T. triangulare* however, eight (8) compounds were reportedly present and the most abundant five include pentanoic acid (28.28%), DL-proline-5-oxo-methyl-buthylester (24.89%), 3-pyridinecarboxyaldehyde, O-cetyloxine (E) (21%), Z-dodecanol (12.68%) and 4-pyridinol (4.61%).

5. Conclusion

Findings from this research clearly justify the known fact that vegetables are rich in phytochemicals including tannins and saponins known for their characteristic therapeutic and prophylactic potentials. GC-MS analysis performed on the methanolic extract of the aforementioned vegetables further revealed some compounds with known therapeutic values and many candidate compounds for drug development.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors wish to state that there is no conflict of interest on this work.

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