JOPAT Vol 22(1), 1035 - 1044, Jan. – June, 2023 Edition

https://dx.doi.org/10.4314/jopat.v22i1.1

Chemical Profile of Common Varieties of *Allium cepa L.* Sold in FCT-Abuja, Nigeria

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ABSTRACT

Onions *(Allium cepa L.)* belong to the family of vegetables and herbs with strong antioxidants properties that combats the formation of free radicals and other microbial based disease conditions. The study aimed at investigating chemical profile of common varieties of *Allium cepa L.* sold in FCT-Abuja, Nigeria. The samples were randomly purchased at point of sales, processed and analyzed for Phytochemical, antioxidant, mineral elements, microbial activities and GC-MS fingerprints using standard methods. Results indicated presence of alkaloid, flavonoids and tannin; antioxidant inhibitory concentrations (IC_{50}) range of 1.496 - 2.961 mg/mL and presence of Ca, Mg, Cu, Cr and Fe were observed. The GC-MS fingerprints identified 17 compounds with retention times ranging from 3.09 to 9.77 minutes. The presence of flavonoids, alkaloids and tannins corroborated report from the earlier studies of white onions. Inhibitory concentrations of the antioxidants obtained are lower than values reported from previous similar studies. The levels of the mineral contents of all the samples are within the safe limits in terms of mineral overloads and contamination, while the GC-MS fingerprints indicated nine (9), eight (8) and seven (7) compounds in the red, white and brown varieties respectively. The allyl group from organosulfur compounds is responsible for the antimicrobial activities. All the varieties of *Allium cepa L* analyzed contained alkaloid, flavonoids, free from heavy metals contamination and also had potent antimicrobial activities due to the presence of the 17 compounds revealed by the GC-MS fingerprints which was not previously reported. The findings of the present study has therefore contributed to additional knowledge on the existing database on Nigerian cultivars of *Allium cepa* .

Keywords: *Allium cepa L*, Phytochemicals, Minerals, Microbial Activities.

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INTRODUCTION

Onions *(Allium cepa L.)* is widely cultivated genus of *allium* family with over 700 members' with each having distinct tastes and colors, but close in phytochemicals and nutraceuticals contents [1]. The bulb is commonly used as food ingredient to enhance flavor and aroma.

It is an important source of phytonutrient such as flavonoids, fructooligosaccharides (FOS), thiosulfinates and other sulfur compounds with high level of phenolic compounds and strong antioxidant properties that are potent against several degenerative disease conditions such as cancer, cardiovascular, Parkinson's, stroke and neurological diseases, dysfunctions based on oxidative stress [2-3], the antioxidants and colorants properties of *Allium cepa L* prevents lipid peroxidation in food and help human body to reduce oxidative damages [4].The awareness of medicinal value of nutritional foods has been on increase in recent years due to increase in scientific researches on nutraceuticals and phytochemicals. Antioxidants plays a role of defending the body system against reactive oxygen species (ROS) or free radicals, which are harmful by-products generated during aerobic activity of normal cells [5]. Literatures reported onion as the richest source of flavonoids, vitamin-c and phytonutrient. The primary flavonoids found in onion are quercetin which acts as a strong reducing agent and kaempferol among others [6]. The previous study of Nigerian cultivars of *Allium cepa* reported basic information on their nutritive values such as proximate, and phytochemicals [7], while the current study had a wider coverage by focusing on GC-MS fingerprints, minerals, phytochemicals, antioxidants and their contribution to antimicrobial activities of the three varieties obtained within FCT.

The three common obtainable varieties of Onion (*Allium cepa*L.) widely circulating in the FCT-Abuja market are, the red, brown and the white varieties. The aim of the study is to investigate and compare the chemical profiles of the three varieties (Red, Brown and White) of Onion for antioxidant activity, phytochemicals and mineral content and microbial activities.

Figure 1a: Red Onions

Figure 2a: White Onions;

Figure 3b: Brown Onions.

MATERIALS AND METHODS

Materials and Reagents Used

All the chemicals and reagents used were of analar grade. Concentrated solutions of HNO_3 (Purity of 69 %, ACS reagent grade), Methanol, 0.1mM DPPH (2, 2-Diphenyl-1-picryl hydrazyl), Standard Garlic acid, water-bath (Karl Kolb, Model-D6072), Micropipette (Model: perfect choice, sizes 0-200µl and 0-1000µl), Plastic sample bottles ($100cm^3$) and Atomic Absorption Spectrometer (GBC Avanta version 2.0) and UV-Spectrophotometer (Jenway Model 6505).

Sample Collection

The fresh varieties of onion bulbs (Figure 1a: Red, Figure 2a: White and Figure 3a: Brown) were purchased in some market in FCT- Abuja and taken to the laboratory and kept at room temperature for further processing and analysis.

Sample preparation for microbial analysis

The fresh onion bulbs were chopped and blended using a sterile electric blender and filtered into a sterile plastic bottle as stock of the juice extract (100%) and kept at room temperature for subsequent analysis. A portion (50 µl) of the stock samples was dispensed into labeled 96 wells of plates in duplicates using sterile Mueller Hinton broth.

Positive control, "Ciprofloxacin" was prepared and dispensed into the 4thcolumn and diluted in the ratio of 1:2 for the samples and the control for the 1st to 11th wells and introduced 50 µl of the test microorganisms each into the 1stto 10th wells. The media sterility, onion sap and organism viability controls were also introduced into the 11th and 12th wells. The inoculated wells were incubated at 37°C for 24 hours. The tetrazolium solution was added to each of the 96 wells and incubated for 25 minutes and observed for color change where color change (disappearance of pink color) indicated absence of growth (activity) and retention of pink color indicated growth (No activity).

Sample preparation for GC-MS

The stock of the juice extract (100%) was extracted by partitioning the juice with equal volume of DCM and separated through a separating funnel and the fractions subjected to GC-MS analysis.

Gas Chromatography–Mass Spectrometry (GC-MS) analyses.

The DCM extracts were analyzed by GC-MS using Shimadzu QP-2010 GC [MSD, (electron energy=70 eV), scan range of 45-700 amu, at rate of 3.99 scans/sec]. The column was Optima-5 ms fused silica capillary with 5% phenyl-methylpoly siloxane stationary phase, length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 µm. The carrier gas was helium with flow rate of 1.61 mL/min. The oven temperature ranges were 60- 160°C at 15°C/min, and 160-280°C at 10°C/min. The injection port temperature was 250°C while detector temperature was 280°C. Sample flow-rate was1.0 µL was injected using autosampler and in the split mode with ratio of 20:80. The identified constituents' mass spectra were

compared with Mass Spectral of known compounds from NIST Library (NIST 11).The percentages of each component are reported as raw percentages based on the total ion current.

Phytochemical Analysis

The extract of the *Allium cepa*was screened for the presence of secondary metabolites using Trease and Evans [7] methods.

DPPH Free Radical Scavenging Activity

The free radical scavenging activity of each sample was determined based on the method adopted by Pérez-Gregorio *et al.,* [8]. The samples' extracts (50mg) and standard garlic acid (50mg) each were prepared within the concentration range of 0.1 – 5 mg/mL, incubated for 30 minutes and added 1.7mL of 0.1mM DPPH to 2mL of each and run on UV-visible spectrophotometer at 517nm where absorbance was obtained and the antioxidant activity calculated using the formulae:

$$\% \text{ Inhibition of DPPH} = \left\{ \frac{A_{\text{control}} - A_{\text{sample (t)}}}{A_{\text{control}}} \right\} x\ 100$$

A_{control} = Absorbance reading of control sample

$A_{\text{sample (t)}}$ = Absorbance reading of sample at t = 30 min

The free radical scavenging activity determined by DPPH was expressed as median inhibitory concentration (IC_{50}) value and calculated as mg of the extract per mL required to reduced 50% of the initial DPPH radical activity from the dose response curve (n = 3). Garlic acid was used as positive control, while the solvent (methanol) was used as negative control.

Sample Digestion for Elemental Analysis

Sample of 50mL juice extract was concentrated to 5mL and digested with concentrated Nitric acid (HNO_3), cooled, diluted, filtered and made-up to 50mL mark in volumetric flask with deionised water and subjected to the analysis.

Analytical Procedure for Heavy Metals Analysis

The digested sample solutions were analyzed for Cu, Ca, Cr, Mg, Fe, Zn, and Pb using Flame Atomic Absorption Spectrometer after optimization and calibration with standard solutions of the elements. The data obtained were processed by calculating the actual concentrations in the samples analyte using the relation:

$$\textbf{Metal (µg/mL)} = \frac{\textbf{C x } V_2 \textbf{ x D.f}}{\textbf{V}_1 \textbf{ (mL)}}$$

Where; C is the concentration of the sample solution in mg/L; V_2 is the volume of the diluted digested sample solutions in mL; V_1 is the volume of the stock sample solution and d.f is the dilution factor, if used.

RESULTS

This study analyzed 100% extracts of three varieties of Onion (*Allium cepa L.*) for phytochemicals, antioxidants activities, minerals contents, microbial activities and GC- MS fingerprint.

Table 1: Preliminary Phytochemical Contents

Samples' variety	Phytochemicals			
	Flavonoids	Alkaloid	Tannin	Saponin
Red Onion	+	++	+	-
Brown Onion	+	+	+	-
White Onion	+	+	-	-

Intense:++, Moderate: +, absent: -

Table 2. The DPPH scavenging effect of Standard and Onions (IC_{50})

S/N	**ANALYTE**	**IC_{50} value (mg/ml)**
1	Gallic Acid standard	0.254
2	White onion	1.496
3	Brown onion	1.520
4	Red onion	2.961

Table 3: Mineral Elements Content of the Onions

Mineral elements	Concentration (µg/g)		
	Red onion	Brown onion	White onion
Copper	0.67±0.00	0.54±0.02	0.57±0.00
Iron	3.52±0.02	1.31±0.04	3.57±0.11
Lead	0.06±0.00	ND	ND
Calcium	2.60±0.01	3.06±0.10	3.15±0.03
Magnesium	39.69±0.30	49.93±1.02	571.78±4.02
Chromium	0.73±0.03	1.24±0.00	1.08±0.03

Values expressed are mean ± SD, (n=3)
Abbreviation: ND: means not detectable

Table 4: Antimicrobial activity of brand of onion against *S. typhi*

Sample	Starting	MICROWELL										MIC	MBC
	Conc	1	2	3	4	5	6	7	8	9	10		
White	25%	-	-	-	+	+	+	+	+	+	+	6.30%	12.50%
Red	25%	-	+	+	+	+	+	+	+	+	+	25.00%	ND
Brown	25%	-	-	-	+	+	+	+	+	+	+	6.30%	12.50%
Cipro	80 µg	-	-	-	-	-	-	-	-	+	+	1.25µg	2.50µg

Active: (-), Not active: (+), Cipro: Ciprofloxacin, ND: not done,
MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration

Table 5: Antimicrobial activity of brand of onion against *Staphylococcus aureus*

Sample	Starting	MICROWELL										MIC	MBC
	Conc.	1	2	3	4	5	6	7	8	9	10		
White	25%	-	-	-	+	+	+	+	+	+	+	6.30%	12.50%
Red	25%	-	-	+	+	+	+	+	+	+	+	12.50%	25.00%
Brown	25%	-	-	+	+	+	+	+	+	+	+	12.50%	25.00%
Cipro	80 µg	-	-	-	-	-	-	-	+	+	+	1.25µg	2.50µg

Active: (-), Not active: (+), Cipro: Ciprofloxacin.
MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration

Table 6: Antimicrobial activity of brand of onion against *Pseudomonas aeruginosa*

Sample	Starting	MICROWELL	MIC	MBC

	Conc	1	2	3	4	5	6	7	8	9	10		
White	25%	-	-	-	+	+	+	+	+	+	+	6.30%	12.50%
Red	25%	+	+	+	+	+	+	+	+	+	+	R	ND
Brown	25%	+	+	+	+	+	+	+	+	+	+	R	ND
Cipro	80 µg	-	-	-	-	-	-	+	+	+	+	2.50µg	5.00µg

Active: (-), Not active: (+), Cipro: Ciprofloxacin, ND: not done,R: Resistance
MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration

Table 7: Antimicrobial activity of brand of onion against *Bacillus subtilis*

Sample	Starting	MICROWELL										MIC	MBC
	Conc	1	2	3	4	5	6	7	8	9	10		
White	25%	-	-	-	-	+	+	+	+	+	+	3.13%	6.30%
Red	25%	-	-	+	+	+	+	+	+	+	+	12.50%	25%
Brown	25%	-	-	-	-	+	+	+	+	+	+	3.13%	6.30%
Cipro	80 µg	-	-	-	-	-	-	-	+	+	+	1.25µg	2.50µg

Active: (-), Not active:(+), Cipro: Ciprofloxacin,
MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration

Table 8: Antimicrobial activity of brand of onion against *Escherichia coli*

Sample	Starting	MICROWELL										MIC	MBC
	Conc	1	2	3	4	5	6	7	8	9	10		
White	25%	-	-	-	+	+	+	+	+	+	+	6.30%	12.50%
Red	25%	-	+	+	+	+	+	+	+	+	+	25%	ND
Brown	25%	-	-	-	+	+	+	+	+	+	+	6.30%	12.50%
Cipro	80 µg	-	-	-	-	-	-	-	+	+	+	1.25µg	2.50µg

Active: (-), Not active :(+), Cipro: Ciprofloxacin, ND: not done,
MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration

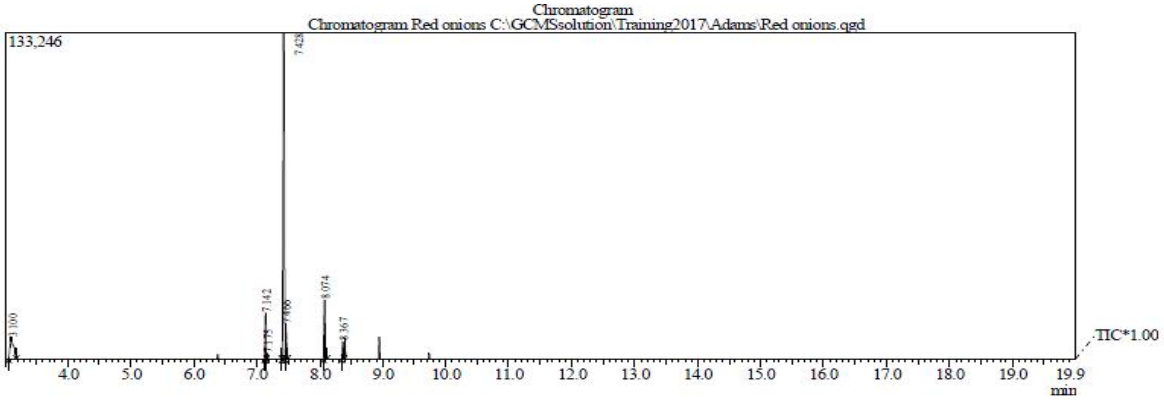

Figure 1b: GC-MS chromatogram of red onions DCM fraction

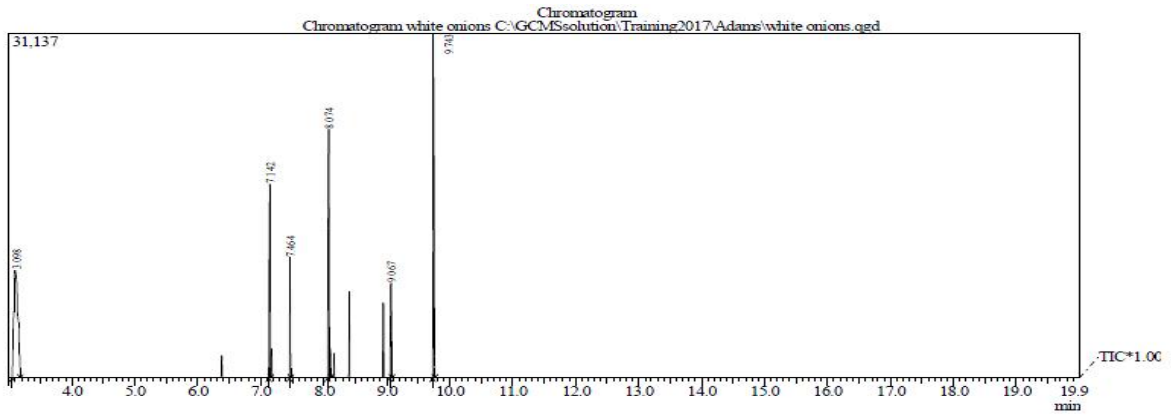

Figure 2b: GC-MS chromatogram of white onions DCM fraction

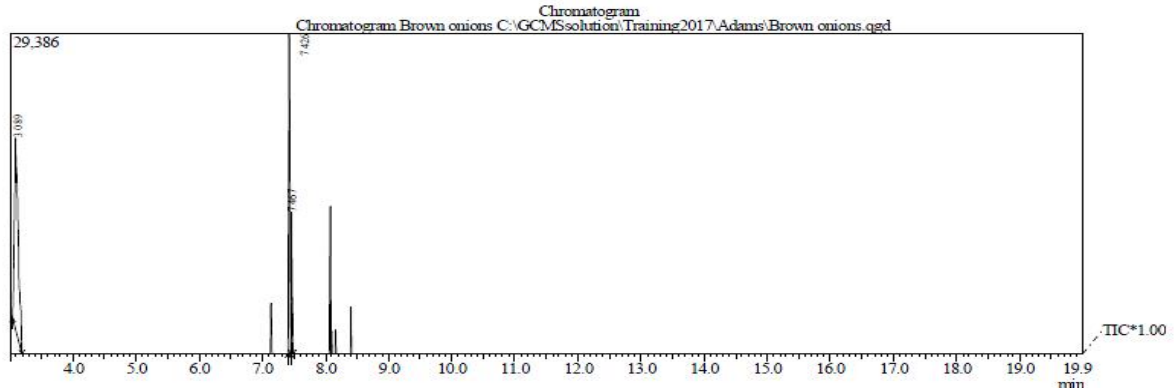

Figure 3b: GC-MS chromatogram of brown onions DCM fraction

DISCUSSION

The Phytochemicals of the varieties of onion screened in the present study indicated the presence of flavonoids, alkaloids and tannins (Table 1) which corroborated the earlier studies reported by Prakash *et al* [9]. These phytonutrient and others such as fructooligosaccharides (FOS), thiosulfinates and sulfur compounds are responsible for several medicinal uses apart its use as food ingredient[10].Recent studies reported that the antioxidant effect of plant products is attributed to phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes [11]. The antioxidant activities of the juice extracts of the three varieties' of the onions ranges from1.496 to 2.961 mg of extract/mL (Table 2), which was lower than results obtained from previous study of white onions [9-10].Several health benefits and effects of mineral elements has also been documented [12].The mineral content (Table 3) indicated more of magnesium in the white variety compared to other elements and in terms of mineral overloads/contamination with toxic element such as lead, all the samples are within the permissible limits [13] therefore they are safe for consumption.

Table 4 to 8 shows antimicrobial activity of the three varieties of the onion and

ciprofloxacin standard against *Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Escherichia coli* test microorganisms. For *S. typhi* (Table 4), the MIC concentrations at 6.30 % of White and Brown onions inhibited the growth followed by Red onion at the MBC concentration of 25%. For *S. aureus* (Table5), the MIC concentrations at12.50 % of Red and Brown followed by white onion at the MIC concentration of 6.30% inhibited the growth of *S. aureus*. The antimicrobial activity of White onion at MIC concentration of 6.30 % inhibited the growth of *P. aeruginosa* (Table 6) while no inhibition by Red and Brown onions. For *B. subtilis* (Table 7), the MIC concentrations at 3.13% of White and Brown onions followed by Red onion at concentration at MBC 12.50 % had activity. For *E. coli* (Table 8), the MIC concentrations of 6.30 % of White and Brown onions followed by Red onion at MBC concentration of 25% showed activity. Result of this study agreed with the reported findings of previous research on microbial activities of onion extracts [14-15].

The GC-MS finger prints of the juice extract of the three varieties indicated the presence of 17 compounds with retention times which ranges from 3.09 to 9.77 minutes as shown in chromatograms Figure 1b: (Red variety), Figure 2b: (White variety) and Figure 3b: (Brown variety) which indicated nine (9), seven (8) and eight (7) compounds respectively. The percentage of the major compounds identified in red variety are; 5-Methyl-2-octyl-3(2H)-furanone (53.85%), Sulfurous acid, butyl isohexyl ester (13.37 %), 1, 1, 3-Trimethylcyclopentane (9.45%), Isoheptane (6.80%), 6-Methyl-1-octanol (6.67%), Cyclobutanecarboxylic acid, 2-propenyl ester (6.01%), 2-(Methoxymethyl)-1-pyrrolidinecarbaldehyde (2.44%), Oxalic acid, allyl butyl ester (0.85%) and 2,3-Hexanediol (0.56%); the brown variety compounds are Sulfurous acid, butyl isohexyl ester (50.51%) 5-Methyl-2-octyl-3(2H)-furanone (23.56%), 2-Amino-3-methyl-4-pentynoic acid (9.74%), Ethyl formyldithiocarbamate (7.02%) and 6-Methyl-1-octanol (4.80%), Methyl N-(beta.-methylcrotonyl) glycine (3.16%) and 2-(Methoxymethyl)-1-pyrrolidinecarbaldehyde (1.21%) while the white variety compounds are Sulfurous acid, butyl isohexyl ester (32.39 %), Caprynic acid (24.86%), 1, 1, 3-Trimethylcyclopentane (15.28%), 6-Methyl-1-octanol (10.60%), Oxalic acid, cyclohexyl propyl ester (7.67%), Trifluoromethylthiocyanate (6.20%), Propargylaldehyde diethyl acetal (1.67 %) and Methyl N-(beta-methylcrotonyl) glycine (1.32 %).Previous study of *Allium cepa* extracts reported presence of polyphenols and organo-sulfur compounds which shown potent antimicrobials activities due to the allyl group[16-17]. Toxicological study confirmed that, the organo-sulphur compounds were safe for human consumption [18].

Conclusion

This study indicated the three varieties of the onions analyzed are rich source of phytonutrient required for optimal health and safe for human consumption.

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