

*Full Length Research Paper*

# **Comparative studies on the antimicrobial, chemical and biochemical contents of the foliar extracts of *Capsicum frutescens* L. varieties**

**Adepoju Adeyinka Olufemi<sup>1</sup>, Omotoso Ifeoluwa Omotola<sup>2</sup>, Femi-Adepoju Abiola Grace<sup>3\*</sup> and Karim Abdul Babatunde<sup>1</sup>**

<sup>1</sup>Department of Biological Sciences, Fourah Bay College, University of Sierra Leone, Freetown, Sierra Leone.

<sup>2</sup>Department of Microbiology, University of Ilorin, Ilorin, Nigeria.

<sup>3</sup>Department of Plant and Environmental sciences, Kwara State University, Malete, Nigeria.

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***Capsicum frutescens* is a variety of *Capsicum* with much nutritional and therapeutic usefulness. This study is aimed at investigating the antimicrobial, chemical and biochemical properties of *C. frutescens*. The biochemical, mineral and phytochemical analyses of the extract were carried out using standard methods. The organic contents of the extracts were determined by GCMS to identify its bioactive constituents which were tested for antibacterial and antifungal activities against 7 bacterial and 3 fungal isolates using disc-diffusion method. The biochemical analysis showed that the leaves were high in protein, fibre, carbohydrate and fat. Different mineral elements were detected in the leaves and they include Magnesium, Calcium, Iron, Sodium, Copper, Potassium, Zinc and Manganese. GC-MS revealed 13 different organic compounds belonging to four groups of chemicals namely alkanol, alkanolic acid, alkanolate and ester. The methanolic extracts of *C. frutescens* leaves at a dose range of 5 and 25 mg/ml showed significant antibacterial and antifungal activity on some test organisms. The presence of great quantity of dodecanoic acid among other compounds in the extract of bawa suggested the reason for its profound anti-staphylococcal and anti-candidal activities. This study concluded that *C. frutescens* foliar extract is rich in important chemical and biochemical metabolites which have shown some therapeutic properties. An advocacy in the higher consumption of these peppers among folks is hereby recommended.**

**Key words:** *Capsicum frutescens* extracts, chemicals and biochemical composition, antimicrobial activity.

## **INTRODUCTION**

Plants including cryptogams have shown to be rich in bioactive constituents (Femi-Adepoju et al., 2018). *Capsicum* species with several bioactive compounds are

increasingly gaining interest due to their effectiveness in improving nutrition and human health (Unuofin et al., 2017).

\*Corresponding author. E-mail: [grace.femiadepoju@kwasu.edu.ng](mailto:grace.femiadepoju@kwasu.edu.ng).

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The origin of the name *Capsicum* is most likely from the Latin word, "capsa" meaning to bite, in allusion to the hot pungent properties of the fruit and seeds with several universal English names, include chili, sweet pepper, hot pepper and bell pepper, belongs to the family Solanaceae (Adepoju et al., 2019). Their fruit becomes brightly colored once its seeds are mature enough to germinate thereby attracting the attention of birds that then distribute the seeds. In West Africa, the genus *Capsicum* is represented by two cultivated species, namely, *Capsicum annum* and *Capsicum frutescens*, with numerous varieties. They are the third in Nigeria among the cultivated vegetables being utilized in the dry state as spice due to their capsaicin content. Peppers generally have been found to contain essential vitamins, minerals, and nutrients in various quantity which serves great usefulness for human health. They also contain a number of phytochemicals such as carotenoids, capsaicinoids, flavonoids, ascorbic acid, and tocopherols which have been reported to prevent inflammatory diseases associated with oxidative damages and maintain optimum health (Kim et al., 2019). Many reports have been given and published on the comparative analysis of metabolite compositions in *Capsicum* spp. and *C. frutescens* has not been left out (Kantar et al., 2016; Sarpras et al., 2016).

*Capsicum* belongs to a group of crops that are widely cultivated for its spicy nature and nutritional value. Five species (*C. annum* var. *annuum*, *Capsicum chinense*, *Capsicum frutescens*, *Capsicum baccatum* varieties *pendulum* and *umbilicatum*, and *Capsicum pubescens*) were domesticated by American natives. After Columbus, they became widely exploited in tropical to temperate regions because of their fruits, which have high nutritional contents, especially in vitamins. Peppers are constituents of the human diet, the pungent cultivars are used as spice ("ajies," "paprika," "chilies," "hot peppers") and the sweet types as vegetables ("sweet pepper," "bell pepper," "pimiento"). Hot red peppers consist of spicy compounds called capsaicinoids which include capsaicin, dihydrocapsaicin, nordihydrocapsaicin and other compounds (Ludy et al., 2012). Capsaicin, water-insoluble derivative of homovanillic acid and the main active ingredient in capsicum fruits, is responsible for hot sensation to the tongue (Papoiu and Yosipovitch, 2010). *C. chinense* species have been reported traditionally to contain the hottest cultivars (Canton-Flick et al., 2008). According to González et al. (2004), the seed and placenta tissues of *C. chinense* are reported to contain most of the capsaicin with 37 and 62%, respectively.

The crop is employed both as condiment and food; the thick sweet fleshy or non-pungent varieties are used in salads or stuffed with meat and cooked (Adepoju et al., 2019). In addition to the use of capsicum fruits in traditional medicine and food additives, it has been reported to be useful in the treatment of sore throat, cough, healing wound, toothache, parasitic infections and

rheumatism (Singletary, 2011), it has also been utilized as an antiseptic (Pawar et al., 2011), immunomodulator and antioxidant (Otinola et al., 2017; Maji and Banerji, 2016), to protect against gastrointestinal ailments (Low Dog, 2006) including dyspepsia, loss of appetite, gastroesophageal reflux disease and gastric ulcer (Maji and Banerji, 2016). Other useful effects of Capsicums include antibacterial (Neelam et al., 2016) and anticancer (Pawar et al., 2011).

The fruits of pepper contain a range of bioactive phytochemicals including flavonoids, carotenoids, phenolics, and other antioxidant compounds (Alvarez-Parrilla et al., 2011). Several classes of plant chemicals including phenolic compounds and antioxidants are sufficiently available in high quantity in vegetables and fruits; thereby forming an important part of human consumption. Since numerous studies have suggested that eating foods rich in phytochemicals reduces risk of certain forms of cancer, cardiovascular diseases and stroke, much attention has been drawn to natural foods especially spices and vegetables rich in these compounds (Kaur and Kapoor, 2001; Prior and Cao, 2000). Takahashi et al. (2018) reported high antioxidant properties of the fruit extracts of *C. frutescens* from green to red stages based on the oxygen radical absorbance capacity (ORAC) and DPPH tests (Takahashi et al., 2018). This work focused on investigating the antibacterial and antifungal efficacies vis-avis biochemical, mineral, phytochemical and organic chemical contents of three varieties of *C. frutescens*, with a view to establish any relationships between them.

## MATERIALS AND METHODS

### Collection and identification of plant samples

Fresh leaves of three different *C. frutescens* varieties ijosi, bawa and sombo were collected into a sterile polythene bag. The leaves were identified at the Biology Laboratory Complex, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The harvested leaves were cleaned using clean drinkable water and air-dried for four weeks in the general biology laboratory complex, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The dried leaves were crushed using porcelain mortar and pestle and the resulting powders were kept in air-tight containers placed in cool, dry environment.

### Preparation of extracts

Extractions were carried out by soaking 20 g of each of the powders in 200 ml of absolute methanol in well-labelled clean conical flasks and corked. After seven days of extraction, the soaked leaf powder were removed and the decanted solutions were concentrated on a rotary evaporator. The dried extracts were kept in a refrigerator in readiness for analyses. Enough quantity of powder was kept for various analyses for which they were needed. The analyses carried out on the dried leaf samples included those of biochemical and mineral compositions, while phytochemicals and organic chemical compositions by GC-MS analysis were carried out

on the leaf extracts, which were also used for the antimicrobial testing (Sukhdev et al., 2008).

#### Collection, growth and maintenance of test organism

Test organisms used for this study were obtained from the microbial gene bank of Pure and Applied Biology Department, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. These include bacteria cultures of different strains, such as *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Staphylococcus aureus* and *Klebsiella pneumoniae* maintained at 37°C; then, fungal culture of *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* maintained at 28°C.

#### Inoculum preparation

For bacteria inoculums, two loopful of overnight grown and morphologically similar colony of bacteria was inoculated into 5 ml of sterile nutrient broth and incubated for 2 h at 37°C when the turbidity was equivalent to a 0.5 BaSO<sub>4</sub> standard. For fungi inoculums, 0.2 g of yeast extract was mixed with 1 g of sucrose in 100 ml of distilled water. 5 ml was pipette in test tubes and sterilized for 15 min at 121°C. After cooling, two loopful of test fungi was inoculated into the medium and incubated for 2 h after which the suspensions were maintained at 4°C for further use.

#### Biochemical analysis

The biochemical compositions of the dried leaf samples were determined using standard analytical methods. All measurements were done in duplicates and their values are presented in percentages. Moisture, ash, crude fibre, crude protein, fat and carbohydrate in the leaves of all the species studied were determined in accordance with the following procedures: moisture content (AOAC, 1995); crude fibre (James, 1995); protein (Pearson, 1976); fat (Onwuka, 2005); carbohydrate (Arithmetic Difference Method, that is, %CHO = 100 - (% fat + % ash + % fiber + % protein).

#### Mineral content analysis

Estimation of mineral substances in dried grinded leaves was performed by using a NOVA400 atomic absorption spectrometer (ANALYTIK JENA AG, Jena, Germany) hollow cathode lamps and acetylene/air flame to measure absorbance. By using slits, wavelengths and lamp current; sodium (Na), potassium (K), manganese (Mn), magnesium (Mg), zinc (Zn), phosphorus (P), copper (Cu) and iron (Fe) were calculated. The analyzed results for Na, Mg, Ca, K, Zn, Cu and Fe contents were expressed in ppm.

#### Qualitative and quantitative phytochemical analysis of plant extracts

Chemotaxonomic studies on *C. frutescens* varieties ijosi, bawa and sombo focused on qualitative and quantitative analyses for phlobatannins, alkaloids, tannins, cardiac glycosides, saponins, flavonoids, terpenoids and phenols. The qualitative analysis of the bio-constituents present in the plant extracts was performed using the methods of Trease and Evans (1989).

#### Quantitative phytochemical screening

Determination of saponin content was done by the

spectrophotometric method described by Brunner (1984), flavonoids was determined according to the method outlined by Harborne (1984). The quantity of alkaloids, tannins, total phenols and cardiac glycosides were determined by the alkaline precipitation gravimetric method described by Harborne (1984), spectrophotometric method of Makkar et al. (1993), the method described by Mahadevan and Sridhar (1982) and the use of Buljet's reagent as described by El-Olemy et al. (1994), respectively.

#### Analysis and identification of organic compounds in the plant extracts

The GC-MS analysis of the leaf extracts was carried out at the department of Chemical Engineering, University of Ilorin on Agilent 19091S Gas chromatograph (GC) interfaced to a mass spectrometer 433HP-5MS instrument employing the following conditions: silica capillary column fused with 100% phenyl methyl silox, (length; 30 m × 250 µm; film thickness 0.25 µm). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1.5 ml/min and an injection volume of 1 µl was employed (Split ratio of 50:1) injector temperature-300°C; average velocity of 45.67 cm/s. The oven temperature was programmed from 100°C (Isothermal for 4 min) with an increase of 4°C min<sup>-1</sup> to 240°C. Total GC running time was 49 min. The relative percentage amount of each component was calculated, by comparing its average peak area to the total areas. The software adopted to handle mass spectra and chromatogram was a turbomass. The detection employed the NIST Ver. 2.0 year 2009 library<sup>29</sup>. After the performance of the GCMS, was the identification of the components detected using their spectra.

#### Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of Standard and Technology (NIST) which contains more than 62,000 patterns. The mass spectra of the unknown components were compared with the spectrum of the known components contained in the NIST library. The name, molecular weight and structure of the components of the test materials were also ascertained using the fragmentation patterns they exhibited and the information available in the library.

#### Antimicrobial assay of plant extracts on test organisms

The antibacterial and antifungal activities of the plant extracts were studied using the disc-diffusion method. Petri plates were prepared with 20 ml of sterile nutrient agar for bacteria and potato dextrose broth for fungi. The bottom of each plate was divided into segment 5 and 25 mgL<sup>-1</sup>, respectively with the control at the centre which each name of the organisms boldly written on each plate. The test organisms were swabbed on the solidified sterile media and the perforated filter paper (4 mm) each was put into the extract at varied concentration and was put into the marked area that match the disc. Methanol was used as control. Plates were incubated at 37°C for 24 h and 25°C for 48 h for bacteria and fungi, respectively. Positive control was prepared using broad spectrum antibiotic entamicin and augmentin as the case may be. The diameters of zone of inhibition (clearance) were recorded in millimeter.

#### Statistical analysis

Data obtained were analyzed with IBM SPSS version 20 software and subjected to one-way ANOVA to assess significant difference

**Table 1.** Percentage biochemical composition of three species of *Capsicum* studied.

Parameter	Fibre	CHO	Dry matter	Fat	Moisture	Protein	total ash
IJO	16.01 <sup>b</sup> ± 0.10	7.88 <sup>b</sup> ± 0.12	2.40 <sup>b</sup> ± 0.02	12.303 <sup>b</sup> ± 0.23	37.700 <sup>a</sup> ± 0.23	33.100 <sup>b</sup> ± 0.22	15.730 <sup>a</sup> ± 0.11
SOM	16.21 <sup>a</sup> ± 0.21	6.30 <sup>c</sup> ± 0.11	2.36 <sup>b</sup> ± 0.01	12.100 <sup>c</sup> ± 0.22	36.100 <sup>b</sup> ± 0.23	33.250 <sup>a</sup> ± 0.27	15.300 <sup>b</sup> ± 0.23
BAW	14.00 <sup>c</sup> ± 0.22	12.06 <sup>a</sup> ± 0.21	5.63 <sup>a</sup> ± 0.03	17.106 <sup>a</sup> ± 0.21	31.624 <sup>c</sup> ± 0.23	30.123 <sup>c</sup> ± 0.35	10.906 <sup>c</sup> ± 0.12

Mean values in columns with different superscripts of alphabets are significantly different at  $P \leq 0.05$  while those with the same alphabets are not significantly different at  $P \leq 0.05$ . IJO= *C. frutescens* var. *ijosi*, SOM= *C. frutescens* var. *sombo*, BAW= *C. frutescens* var. *bawa*.

**Table 2.** Mineral element composition (ppm) in the three species of *Capsicum* studied.

Parameter	Mn	Zn	Cu	Na	K	Mg	Ca	Fe	P
IJO	0.24 <sup>b</sup>	0.53 <sup>c</sup>	0.45 <sup>a</sup>	1.52 <sup>c</sup>	27.010 <sup>a</sup>	13.00 <sup>a</sup>	27.231 <sup>b</sup>	0.346 <sup>a</sup>	0.323 <sup>b</sup>
SOM	0.20 <sup>c</sup>	0.55 <sup>b</sup>	0.41 <sup>b</sup>	1.62 <sup>b</sup>	27.010 <sup>a</sup>	13.01 <sup>a</sup>	7.244 <sup>a</sup>	0.345 <sup>a</sup>	0.322 <sup>b</sup>
BAW	0.32 <sup>a</sup>	0.61 <sup>a</sup>	0.42 <sup>b</sup>	1.72 <sup>a</sup>	27.001 <sup>b</sup>	13.01 <sup>a</sup>	26.009 <sup>c</sup>	0.306 <sup>b</sup>	0.421 <sup>a</sup>

Mean values in columns with different superscripts of alphabets are significantly different at  $P \leq 0.05$  while those with the same alphabets are not significantly different at  $P \leq 0.05$ . (IJO= *C. frutescens* var. *ijosi*, SOM= *C. frutescens* var. *sombo*, BAW= *C. frutescens* var. *bawa*).

between groups followed by Tukey post-hoc test at 95% significance level and expressed in mean ± standard deviation (SDEV) using Microsoft office Excel version 2007.

## RESULTS AND DISCUSSION

The result of the biochemical composition of the leaves studied is shown in Table 1 while that of the mineral elements is presented in Table 2. The leaves of all the plants were significantly rich in important nutritional factors such as crude fibre, carbohydrate, fat and protein. Of all the nutrients detected, the leaves were particularly high (30-33%) in protein, but low (2.1-2.6%) in dry matter content (Table 1). The quantities of the proximate contents (%) of the *Capsicum* spp. studied can be listed from the highest to the lowest as moisture>protein> crude-fibre>total ash> crude

fat>carbohydrate>dry matter.

The mineral elements detected in the leaves analyzed were Calcium, Magnesium, Iron, Sodium, Potassium, Copper, Zinc and Manganese. The leaves were particularly high in Calcium (26.0-27.3%), Potassium (27-27.7%) and Magnesium (13%), but low as expected of trace elements, in Manganese, Zinc and Copper (Table 2). On the whole, the quantities of the mineral elements in the plants studied can be enumerated from the highest to the lowest as Potassium>Calcium>Magnesium>Sodium>Zinc>Copper>Phosphorus>Iron and Manganese.

Crude fibre reportedly increases stool mass and hastens digestion. It does not get digested in humans and animals but it aids the normal functioning of the intestinal tract. According to Bello et al. (2008), fibre assists in maintaining human health and it has been reported to function

in the reduction of cholesterol levels of the body (Bello et al., 2008). Diets with low fibre contents have been associated with increased risks of heart malfunctions, cancer of the colon and rectum cells, varicose veins, phlebitis, obesity, appendicitis, diabetes and even constipation (Ibanga and Okon, 2009; Lajide et al., 2008; Saldanha, 1995) established that minerals are essential in human nutrition while O'Dell (1979) stated that minerals from plants are less readily available than those from animals. From a recent research conducted by Kim et al. (2019), the results of percentage variability for proximate analysis of some *Capsicum* spp. extract, it was reported that, the quantity of ash, crude fiber, moisture, and crude fat were significantly affected by year and location (Kim et al., 2019). The levels of each mineral across four environments showed a high variation, which indicates that these

**Table 3.** Quantitative phytochemical contents of the *Capsicum* plants studied (mg/g).

Parameter	Alkaloid	Saponin	Phenol	Tannin	Flavonoid	Terpenoid	Card. Glycosid	Phlobatannin
IJO	0.65 <sup>b</sup> ± 0.001	0.60 <sup>b</sup> ± 0.003	0.13 <sup>b</sup> ± 0.009	0.33 <sup>b</sup> ± 0.019	0.56 <sup>b</sup> ± 0.002	0.64 <sup>c</sup> ± 0.012	0.94 <sup>a</sup> ± 0.002	0.32 <sup>b</sup> ± 0.03
SOM	0.61 <sup>b</sup> ± 0.012	0.73 <sup>a</sup> ± 0.002	0.14 <sup>b</sup> ± 0.02	0.41 <sup>a</sup> ± 0.018	0.53 <sup>b</sup> ± 0.001	0.77 <sup>a</sup> ± 0.009	0.95 <sup>a</sup> ± 0.030	0.33 <sup>b</sup> ± 0.004
BAW	0.92 <sup>a</sup> ± 0.001	0.61 <sup>b</sup> ± 0.01	0.31 <sup>a</sup> ± 0.001	0.40 <sup>a</sup> ± 0.003	0.72 <sup>a</sup> ± 0.02	0.70 <sup>b</sup> ± 0.021	0.82 <sup>b</sup> ± 0.001	0.40 <sup>a</sup> ± 0.111

Mean values in columns with different superscripts of alphabets are significantly different at  $P \leq 0.05$  while those without alphabets are not significantly different at  $P \leq 0.05$ . IJO= *C. frutescens* var. *ijosii*, SOM= *C. frutescens* var. *sombo*, BAW= *C. frutescens* var. *bawa*.

compounds are strongly influenced by environmental factors. However, previous studies have shown that mineral contents in peppers depend on a variety of factors (Sarpras et al., 2016). Four varieties belonging to the *Capsicum* spp. were characterized by their nutritional constituents, antioxidant vitamins and capsaicin contents in another research conducted by Olatunji and Afolayan (2020). They found out that, there was variability in nutritional, vitamins and capsaicin contents among varieties and that higher levels of vitamins could be found in fresh than in dry samples. Reports have also shown that varieties of *Capsicum* spp. contain important micro- and macro-elements with antioxidative vitamins, in varying quantities which can provide significant proportions of the recommended daily intake and help improve overall health of humans (Olatunji and Afolayan, 2020)

Minerals are vital for the overall physical and mental healthiness of man as they are important constituent of bones, muscles, teeth, tissues, nerve and blood cells (Soetan et al., 2010). Calcium and phosphorus are jointly essential for growth and maintenance of muscles, bones and teeth (Okaka et al., 2006; Ladan et al., 1996). Magnesium, according to Borgert and Briggs (1975) is a component of chlorophyll and it is important for calcium metabolism in bones whose deficiency can lead to ischemic heart disease

(Elegbede, 1998). Zinc is involved in normal functioning of immune system and is associated with protein metabolism. Iron is an essential trace element for the formation of haemoglobin and also for the normal functioning of central nervous system (Asaolu et al., 1997). The deficiency of these nutrients and minerals are known to adversely affect health in animals.

### Phytochemicals in the leaves of Nigerian cultivars of *Capsicum*

Total of eight secondary metabolites were detected in the leaves of the three Nigerian species of *Capsicum* studied. These included alkaloids, saponin, phenols, tannins, flavonoids, terpenoids, cardiac glycosides and phlobatannins (Table 3). Of these eight, the quantities of only two, that is, cardiac glycosides and phlobatannins did not show significant differences among the taxa studied.

The presence of phytochemicals in the foliar extracts of the *Capsicum* cultivars studied, suggests possible medicinal properties in them (AOAC, 1995). Saponins, phlobatannins and steroidal glycosides were detected in all the cultivars. Saponins have also been reported in *Senna alata* and *Cajanus cajan* by Lawal et al. (2014) and also in *Lophira lanceolata* seeds by Lohlum et al. (2010). According to Harborne (1984), saponins

possess anti-hypercholesterol, anti-inflammatory, cardiac health enhancement properties and also appear to mortalise or inhibit growth of cancer cells without adversely affecting the normal body cells (Okwu, 2001). Phlobatannins were reported by Asquith and Butter (1986) as inhibitors of growth in many microorganisms like bacteria, fungi and viruses. According to De-Bruyne et al. (1997), tannins are plant polyphenols which can form complexes with metals ions and with macromolecules such as polysaccharides and proteins. Enujiugha and Agbede (2000) established that tannins usually form insoluble complexes with proteins, thereby interfering with their bioavailability.

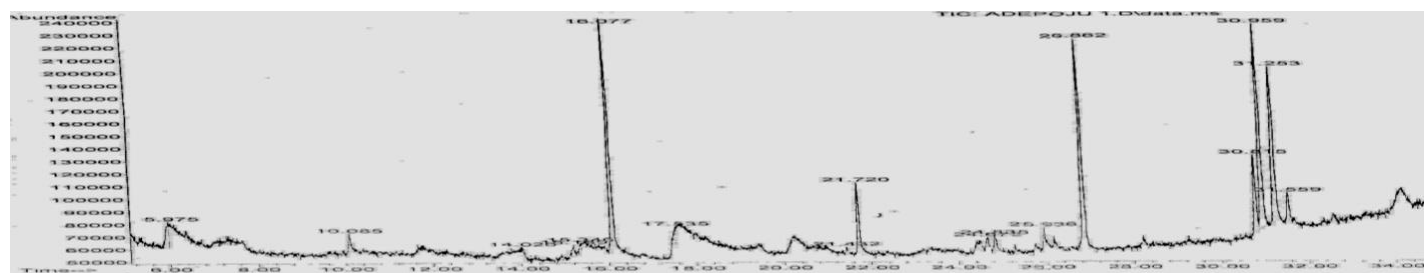
### The phytochemicals

UAMME=Undecanoic acid, 10-methyl, methyl ester; HEAME=Hexadecanoic acid methyl ester; 811- OCME=8,11- octadecanoic acid methyl ester; 710-OCME=7,10- octadecanoic acid methyl ester; 912-OCME=9,12- octadecanoic acid methyl ester (E,E); 912-OCPE=9,12,15-octadecanoic acid, 2,3-dihydroxy propyl ester (Z,Z,Z); (Z,Z,Z); 912-OCIME=9,12-Octadecadienoic acid, methyl ester; 13-OCME=Cis-13-octadecenoic acid methyl ester; 9-OCME=9-octadecenoic acid methyl ester; HH-12-EE=Hexadecanoic acid, 1-(hydroxymethyl)-

**Table 4.** The percentage peak area value of some organic compounds quantified in the leaves of three Nigerian cultivars of *Capsicum*.

Compound	Class	Percentage peak area values		
		[IJO]	[SOM]	[BAW]
UAMME	Ester	19.08	0	1.75
HEAME	Ester	5.80	0	0
811- OCME	Ester	6.57	0	0
710-OCME	Ester	0	0	2.81
912-OCME	Ester	0	0	0
912-OCPE	Ester	17.40	0	0
912-OCIME	Ester	0	3.55	0
13-OCME	Ester	0	0	13.06
9-OCME	Ester	0	21.77	0
HH-12-EE	Ester	0	3.50	0
METED	Alkanoate	5.80	0	0
DODEA	Alkanoic acid	0	17.48	55.15
ET-2-OCD	Alkanol	0	11.99	0

[IJO]= *C. frutescens* var. *ijosi*, [SOM]= *C. frutescens* var. *sombo*, [BAW]= *C. frutescens* var. *bawa*.



**Figure 1.** GC-MS Spectrum and some phytoconstituents in *C. frutescens* var. *ijosi*.

1,2-ethanediyl ester; METED=Methyl tetradecanoate; DODEA=Dodecanoic acid; and ET-2-OCD=Ethanol, 2-(9-octadecenyl)- Z.

### The foliar organic compounds in Nigerian species of *Capsicum* via Gas Chromatography Mass Spectroscopy (GCMS)

The entries in Table 4 are the results of GC-MS analysis conducted on the leaves of the cultivars of *Capsicum* studied. A total of 13 different organic compounds were detected and these belong to four different groups of chemicals namely ester, alkanoate, alkanolic acid and alkanol. While 10 of the organic compounds were esters, only one belonged to each of the other groups (Table 4). Each of *C. frutescens* var *sombo* and *C. frutescens* var *ijosi* had five, the highest number of organic compounds detected by GCMS analysis, while *C. frutescens* var. *bawa* had four. Figures 1 to 3 present the GC-MS spectra and phytoconstituents in *C. frutescens* varieties. Neelam

et al. (2016) reported that the GC-MS analysis of *n*-hexane and chloroform extracts of the seeds of *C. frutescens* revealed the presence of a total of 29 compounds from different classes. The major components found include Octadecadienal (*Z*), 3-Carene, Hexadecanoic acid, Tetracosane, Heptadec-8-ene-2,4-dione, 2(3H)-Furanone,dihydro-5-(2-octenyl)-, (*Z*) and Hexadec-8-ene-2,4-dione, in the *n*-hexane extract, while in the chloroform extract, Hexadecanoic acid, 9,12-Octadeca dienoic acid, 1-Hexadecene and 5-Eicosene, (*E*) were found. Octadecane, Eicosane, Docosane, 9,12-Octadecadienoic acid, methyl ester and Hexadecanoic acid, were found to be common in both the extracts (Neelam et al., 2016). Some of the compounds reported by Neelam et al. (2016) are also discovered by this study.

### Antimicrobial activities of methanolic extracts of *C. frutescens* varieties on test organisms

The antimicrobial activity of methanolic extracts of

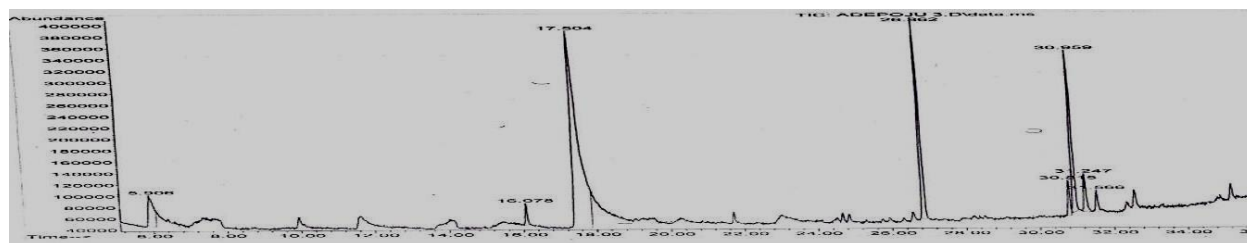


Figure 2. GC-MS Spectrum and some phytoconstituents in *C. frutescens* var *sombo*.

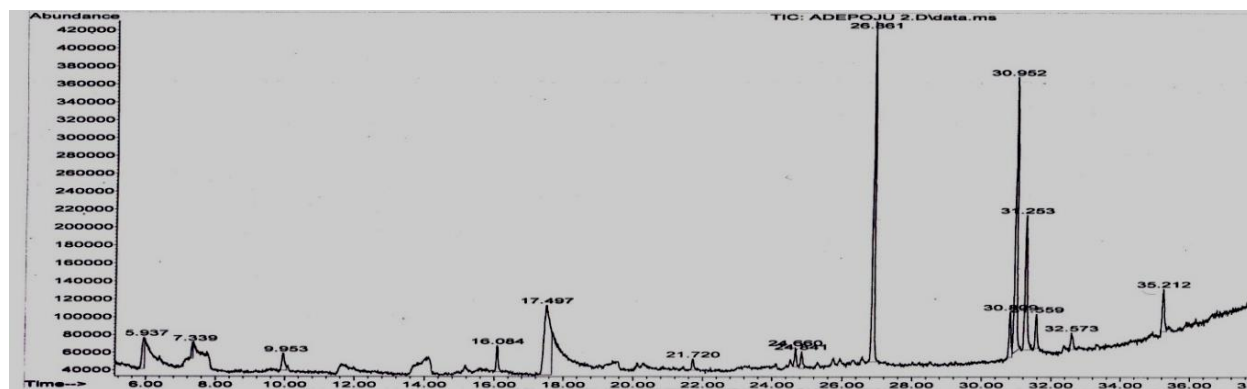


Figure 3. GC-MS Spectrum and some phytoconstituents in *C. frutescens* var *bawa*.

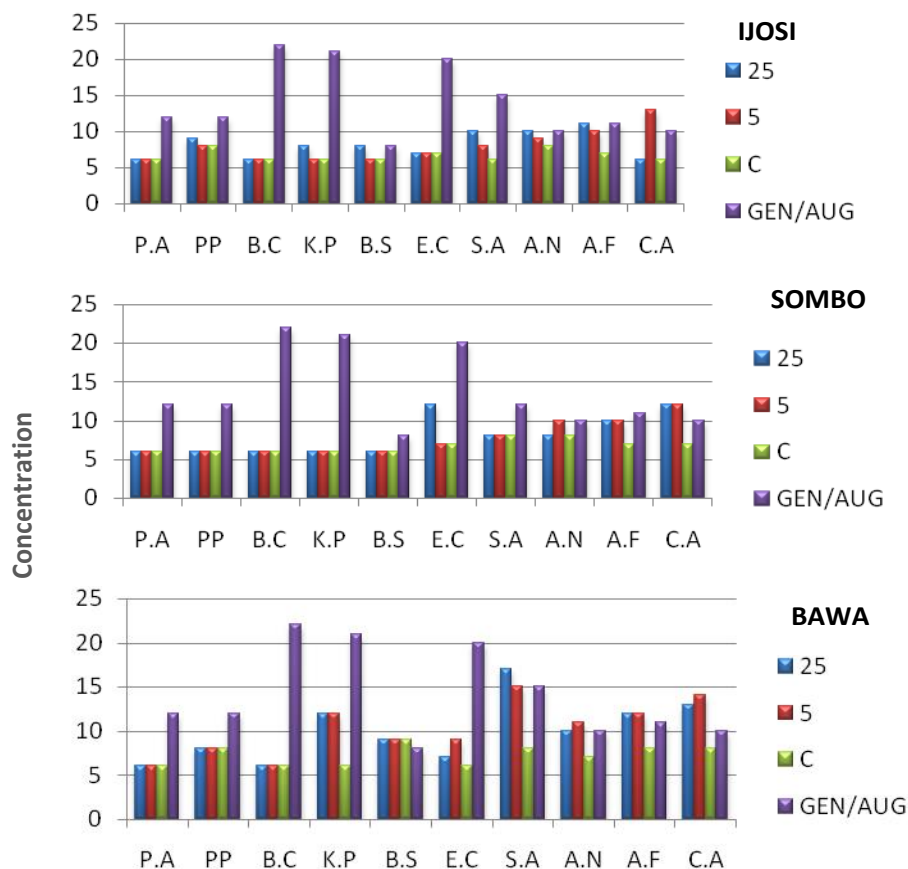
*Capsicum* leaf were investigated against the selected pathogens *P. aeruginosa*, *P. putida*, *B. cereus*, *K. pneumonia*, *B. subtilis*, *E. coli*, *S. aureus*, *A. niger*, *A. flavus* and *C. albicans* by disc diffusion method. The antimicrobial effects of leaves extracts of *C. frutescens* as shown by zone of inhibition in millimeters (mm) on selected bacteria and fungi are presented in Figure 4. The antibacterial activity of the extracts was found to be the highest at 5 mg/ml against *C. albicans* which produced an inhibition zone of 13 mm for ijosi; 5 and 25 mg/ml produced the highest zone of inhibition of 12 mm for sombo and 17/15 mm for bawa against *C. albicans*. *S. aureus* was found to be susceptible most to the foliar extract of bawa.

#### Significance of organic, proximate, mineral and phytochemical contents on antimicrobial efficacy of the extracts

In the present study, most of the identified volatile compounds belong to the class ester, alkanol, alkanic acid and alkanolate. These compounds have been reported to be pharmacologically active. For example, hexadecanoic acid is known to have potential antibacterial and antifungal activities (McGraw et al., 2002); unsaturated fatty acids are also suggested to be responsible for the anti-inflammatory activity (Li et al.,

2004); long-chain unsaturated fatty acids, such as linoleic acid, also show antibacterial activity and are the key ingredients of antimicrobial food additives and some antibacterial herbs (Chang et al., 2005), and hexadecanoic acid, methyl ester and 9,12-octadecadienoic acid (*Z,Z*-), methyl ester have shown antioxidant and anticancer properties, respectively (Wei et al., 2011).

According to Ishida et al. (2000), crude fibers in the diet are necessary for digestion and effective to eliminate the risk of coronary heart disease, constipation, hypertension, colon, and diabetes and breast cancer (Ishida et al., 2000). Thus, these medicinal plants are regarded as a valuable source of dietary fiber in human nutrition. There is a strong correlation between fibre and moisture contents, as the fibre are easily digested and disintegrated which could be of interest to human health (Hussain et al., 2010). Minerals are required for normal growth, activities of muscles and skeletal development, copper and iron are responsible for cellular activity and oxygen transport, respectively, magnesium for chemical reaction in the body and intestinal absorption, sodium and potassium used in fluid balance and nerve transmission. Manganese plays a major role in production of energy and in supporting the immune system (Muhammad et al., 2011). Deficiency of these nutrients and minerals are known to affect the performance and health in both humans (MERCK, 2005).



**Figure 4.** The antibacterial and antifungal potentials of leaf extracts of *C. frutescens* on test organisms at varying concentrations in mg/ml. P.A= *P. aeruginosa*; P.P= *P. putida*; B.C= *B. cereus*; K.P= *K. pneumonia*; B.S= *B. subtilis*; E.C= *E. coli*; S.A= *S. aureus*; A.N= *A. niger*; A.F= *A. flavus* C.A= *C. albicans*; C = Control.

In earlier studies, the antimicrobial activity of ethanolic, methanolic and aqueous extracts of *C. frutescens* has already been reported against a number of microorganisms (KoffiNevry et al., 2012; Shariati et al., 2010). In a study by Vinayaka et al. (2010), even the foliar methanolic extract of *C. frutescens* showed dose-dependent antibacterial activity against *S. aureus*, *K. pneumoniae* and *P. aeruginosa*. Aqueous extracts of the leaf and fruit of *C. frutescens* have also exhibited potentials to prevent growth of seed-borne fungi (Soumya and Nair, 2012). In the study carried out by Neelam et al. (2016), broad spectrum activity was shown to be exhibited by low polar *n*-hexane and chloroform extracts of *C. frutescens*. This significant antimicrobial activity could be attributed to the compounds identified in the GC-MS spectrum. Isolation and proper identification of these antimicrobial agents from capsicum can lead to an important improvement in the area of food safety and can be used in the prevention of certain human diseases (Neelam et al., 2016). Also, the antimicrobial property of silver nanoparticles fabricated from the extract of *C. frutescens* was higher than that of the other two spices

(Otunola et al., 2017).

Flavonoids have been studied to be very useful as an antimicrobial agent, inhibitor of mitochondrial adhesion, as an anticancer agent and antiulcer agent (Biju et al., 2014). It has been confirmed that consumption of food and beverages rich in phenol prevent diseases, such as cancer (Chalise et al., 2010). In this study, the presence of flavonoid and phenolic compounds in *C. frutescens* var. leaf extracts confer health benefits associated with it. The significant antimicrobial activity of methanolic extracts of *C. frutescens* var. might be due to the synergistic effects of compounds identified in the proximate, organic, mineral and phytochemical analyses of the plant part. Something striking to note is that bawa extract was found to be most active against *S. aureus* and *C. albicans* with a zone of inhibition that suggests a better performance than the test drugs.

## Conclusion

The leaves of the Nigerian species of *Capsicum* studied



are rich in important mineral elements such as calcium, magnesium, iron, sodium, potassium, copper, zinc and manganese; and are also sufficiently rich in some notable nutritional factors such as protein, crude fibre, total ash, crude fat and carbohydrates. The data obtained from the antimicrobial tests, as well as those of phytochemical constituents and organic compounds in the leaf extracts have revealed that the extracts contain important bioactive compounds. The significance of this is that an advocacy for more consumption of these peppers among folks can deal with infections associated with these pathogens. They can also be useful in the formulation of disinfectants and antiseptics as well as in chemotherapy. Relative abundance of the foliar nutrients in the species of the genus studied has far reaching implications in an effort to diagnose the species as potential leafy vegetables and as components of herbal formulations.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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