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Implications of Some Foliar Organic Compounds on the Taxonomy of *Capsicum* L. Species

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ABSTRACT

Capsicum L. varieties and species are closely related so much that there have been some confusions among different taxonomists on their taxonomic status. This study aimed to determine the taxonomic standing of *Capsicum* L. genus members in Nigeria to assess genetic divergences and similarities among them to provide some insight into their identification and the infrageneric classification (INC) of the genus. Seeds of five cultivars of *Capsicum* spp., collected from various sources and authenticated, were regenerated, and nurtured to fruit. Variations in their foliar organic compounds were identified quantitatively, using Gas Chromatography Mass Spectroscopy (GCMS). A total of 17 organic chemical characters (12 Esters, 2 Alkanols 1 each of Alkanoate, Alkanoic acid, and Alkane) were detected. The percentage peak area values obtained include 1.75 to 21.88 of esters, 2.24 to 11.99 of alkanols, 5.8 of alkanoate, 17.48 to 55.15 of alkanoic acid, and 4.9 of alkane. The cultivars of each genus were hierarchically clustered as operational taxonomic units (OTUs) using squared Euclidean distance computed through PASTatistics software (Ward's method). Artificial keys were also constructed for the identification of the species in the genus. The categories of chemical characters adopted gave useful insights into the INC of the genus, as their combination were sufficiently diagnostic of the species as evidenced by the artificial keys. The taxonomic status of Nigerian representatives of the genus *Capsicum* L. was successfully determined in connection to the distribution of their fruit capsaicin concentration (FCC), similar to those that was previously reported of morphology and phytochemicals in *Capsicum*. The challenge of vague infrageneric boundaries has also been partially resolved in the Nigerian *Capsicum* spp. studied.

Keywords: Nigeria, *Capsicum*, Capsaicin, GCMS, Taxonomic Key, Nigeria.

1. INTRODUCTION

The earliest and most popular method for classifying plants is likely data grouping based on morphological similarities; nevertheless, numerous alternative methods for taxonomic handling of plants have developed over time (Yagi et al., 2012). Recently, taxonomists have involved the use several techniques to solve the taxonomic confusion which are unresolved by the use of morphological markers. One example of such modern markers is the use of various computer softwares for the better understanding of phylogeny of Angiosperms (Baker et al., 2022). The study of systematics includes a much broader aspect that includes not only the

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earlier techniques of morphology and anatomy but also modern ones such as genetics, molecular biology, chemotaxonomy and evolutionary biology. According to Jain (2016), the 'new' systematics may be divided into the following branches: Numerical systematics, Biochemical systematics and Experimental systematics.

The flowering plant family of Solanaceae is home to some valuable agricultural crops in addition to several poisonous species. The family has been described by Friedman and McDonald (1997) as an important source of about 300 kinds of alkaloids, solanine, scopolamine, altropine and hyoscamine being the key alkaloids in this family (Stanker et al., 1994). Presence of the alkaloids makes this family medicinally important. The members of the family had been used for medicinal purposes since as early as 37 A.D. The people of that age were so much aware of the narcotic effects of *Datura stramonium* L and *Hyoscyamus niger* L. (Yousaf, 2007).

Capsaicin, the molecule that gives peppers their burning sensation, exclusively affects mammals and has no effect on birds. When the seeds are old enough to germinate, their fruit turns vividly colored, drawing the interest of the birds that disperse them. Pepper spray, a useful deterrent against aggressive mammals, is made from capsaicin extract. (Grubben and El Tahir, 2004). By using induced mutagenesis, it has been possible to create genetically enhanced cultivars of *Capsicum* (Daskalov, 1986; Daskalov and Baralieva, 1992; Devi and Mullainathan, 2011). Additionally, molecular marker-based reconstructed linkage maps have been created through the same method (Ben-Chaim et al., 2001). Furthermore, efforts have been made to construct complementary DNA (cDNA) libraries in order to produce expressed sequence tags (EST) with the purpose of creating novel *Capsicum* genes (Lee et al., 2002). The problems that are being addressed in this investigation into Nigerian *Capsicum* relate to species misidentification due to insufficient taxonomical markers; and that of poorly defined species boundaries.

The genus *Capsicum* in Nigeria has not been thoroughly revised, classified and identified. it has been observed that authors of some publications have misrepresented some Nigerian species of *Capsicum* because of lack of proper identification e.g. the local name 'shombo' (Yoruba name for a cultivar of *C. fructescens*) was assigned to *C. annuum* var. *grossum* (L.) Sendtn. and the local name 'atarugu' (hausa name for *C. chinense*) was assigned to *C. annuum* var. *grossum* (L.) Sendtn. in Aziagba et al. (2014). Also, 'tatashe' (local name for *C. annuum* var. *grossum* (L.) Sendtn.) was regarded as a variety of *C. frutescens* in Edeoga et al. (2010). These are clear cases of misidentification due to scanty nature of information on the species and varieties of *Capsicum* in Nigeria, which needs to be resolved by providing markers for their proper identification.

This study was aimed to re-examine the taxonomic status of the *Capsicum* species in Nigeria to determine any genetic relationships between the species and to find GC-MS analysis data markers that could provide a taxonomic fingerprint for the species and varieties.

2. MATERIALS AND METHODS

2.1. Collection and Regeneration of Germplasm for the Work

Seed samples of the species of the genus *Capsicum* whose fruits are displayed in Plate 1, were obtained from various locations in Nigeria as enumerated in table 1. The seeds collected were grown at the Botanical Gardens, Ladoke Akintola University of Technology, Ogbomoso, Nigeria, under the same natural environmental conditions, and to produce the various plant parts that are needed for analyses. The plants were authenticated at Obafemi Awolowo University Herbarium (OAUH) and they were later documented at Lautech Herbarium, Ogbomoso (LHO).

Plate 1. Images of ripe fruits of the cultivars of Capsicum spp studied (Adepoju et al., 2021). ***Note:*** *[ANN] = C. annuum var. grossum (L.) Sendtn., [BAW] = C. annuum var annuum L., [CHI] = C. chinense, [IJO] = C. fructescens var. fructescens and [SOM] = C. fructescens L. var. baccatum (L.) Irish.*

Table 1. List of the species/cultivars of Capsicum whose seeds were collected for the study in Nigeria.

S. No	***Species name***	***Cultivar/Local (Yoruba) name***	***Place of collection***	***GPS location***
1	*C. fructescens* var. *fructescens* L.	*Ijosi* (IJO)	Ilorin	8.5° N; 4.55° E
2	*C. fructescens* var. *baccatum* (L.) Irish.	*Sombo* (SOM)	Ilorin	8.5° N; 4.55° E
3	*C. annuum var annuum* L.	*Bawa* (BAW)	Ogbomoso	7.9° N; 4.32° E
4	*C. annuum* var. *grossum* (L.) Sendtn.	*Tatashe* (ANN)	NACGRAB hq	7.39° N; 3.9° E
5	*C. chinense* Jacq.	*Rodo* (CHI)	Inisa	7.85° N; 4.33° E

2.2. Analysis of the Foliar Organic Compounds in the Extracts.

With an injection volume of 1μl and a continuous flow rate of 1.5 ml/min (split ratio of 50:1), the carrier gas used was helium gas (99.999%). The injector had an average velocity of 45.67

cm/sec and a temperature of 300^{o}C. The oven was set to start at 100°C (isothermal for 4 minutes) and grow by 4°C per minute until it reached 240°C (film thickness of 0.25µm and length of 30m x 250µm). To determine GC-MS profile, an electron ionization system with ionization energy of 70eV was used. With an injection volume of 1µl and a continuous flow rate of 1.5 ml/min (split ratio of 50:1), the carrier gas used was helium gas (99.999%). The injector had an average velocity of 45.67 cm/sec and a temperature of 300^{o}C. The oven was set to start at 100°C (isothermal for 4 minutes) and grow by 4°C per minute until it reached 240°C. The GC took 49 minutes to complete in its entirety. By comparing the average peak area of each component with the total areas, the relative percentage amount was determined. The chromatogram and mass spectra are handled using the Turbomass program. For the detection, the NIST ver. 2.0 library (Paranthaman et al., 2012) was used. After the GCMS was conducted, the components that were found were identified using their spectra.

2.3. Identification of Constituents

The National Institute of Standard and Technology (NIST) database, which has more than 62,000 spectral patterns, was used to interpret the mass spectrum of the GC-MS. The NIST library's standard component spectra and the mass spectra of the unknown components were compared. Using the fragmentation patterns the test materials showed and the data in the library, the names, structures, and molecular weights of their constituent parts were also determined.

2.4. Taxonomic Treatments of Nigerian Species of *Capsicum*

The taxonomic treatments of the two genera studied were carried out as highlighted in sections 2.5 and 2.6. These included drawing out sizable number of taxonomic characters from different sources and clustering the species of each genus to show their phenetic relationships. Also using the diagnostic characters, from each source among the species in the two genera, a taxonomic key was constructed for species identification. On the other hand, the results of elemental and proximate analyses were discussed in relation to the nutritional and medicinal value of the plants.

2.5. Collation of Taxonomic Characters and Cluster Analyses

Merged qualitative and quantitative taxonomic characters (peak area values) were compiled from the species of *Capsicum* studied. The qualitative characters were first quantified by scoring presence as “1” and absence as”0”. Thereafter, the scores of both qualitative and quantitative characters were used as characters to perform a cluster analysis on the species; each of which was taken as an operational taxonomic unit (OTU). Dendrograms were obtained by adopting a hierarchical cluster analysis applying *squared Euclidean Distance* (*Ward’s* *method*). The software package used for this purpose was Palynology Statistical Software, (PAST) 4.0 (Hammer et al., 2001).

2.6. Construction of Artificial Keys

Using both the qualitative and quantitative characters from this study, a dichotomous key was constructed for the purpose of diagnosing the species in the genus. To achieve this, both the qualitative and quantitative characters were directly used as observed, after the means of quantitative characters have first been subjected to statistical significance across the species in the genus, to determine which ones were truly diagnostic.

3. RESULTS

3.1. Determination of Foliar Organic Compounds using GCMS.

GCMS information gathered from the leaves of the researched varieties of *Capsicum* are contained in table 2. A total of seventeen (17) various organic compounds were detected (Figs 1-5; Table 2) and these can be grouped into five, based on their functional groups, including alkanoate, alkanol, alkanoic acid, and alkane. 12 chemical compounds were esters, while the other groups have one compound each (Table 2).

Table 2. The percentage peak area value of some organic compounds detected in the leaves of Capsicum cultivars from Nigeria.

Compound	***Class***	***%age peak area values***				
		[IJO]	***[SOM]***	***[BAW]***	***[ANN]***	***[CHI]***
UAMME	Ester	19.08	0	1.75	0	10.33
HEAME	Ester	5.80	5.81	5.78	5.82	5.88
811- OCME	Ester	6.57	0	0	0	0
710-OCME	Ester	0	0	2.81	0	3.94
912-OCME	Ester	0	0	0	8.43	0
912-OCPE	Ester	17.40	0	0	0	0
912-OCRME	Ester	0	0	0	14.12	0
912-OCIME	Ester	0	3.55	0	0	0
13-OCME	Ester	0	0	13.06	0	29.88
9-OCME	Ester	0	21.77	0	0	0
HH-12-EE	Ester	0	3.50	0	0	0
H-16-MME	Ester	0	0	0	2.07	0
METED	Alkanoate	5.80	0	0	0	0
DODEA	Alkanoic acid	0	17.48	55.15	38.78	0
ET-2-OCD	Alkanol	0	11.99	0	0	0
PHYTOL	Alkane	0	0	0	4.90	0
1-HTC	Alkanol	0	0	0	0	2.24

Note: [ANN] = *C. annuum* var. *grossum* (L.) Sendtn., [BAW] = *C. annuum var annuum L.*, [CHI] = *C. chinense,* [IJO] = *C. fructescens* var. *fructescens* and [SOM] = *C. fructescens L. var. baccatum (L.)* Irish.

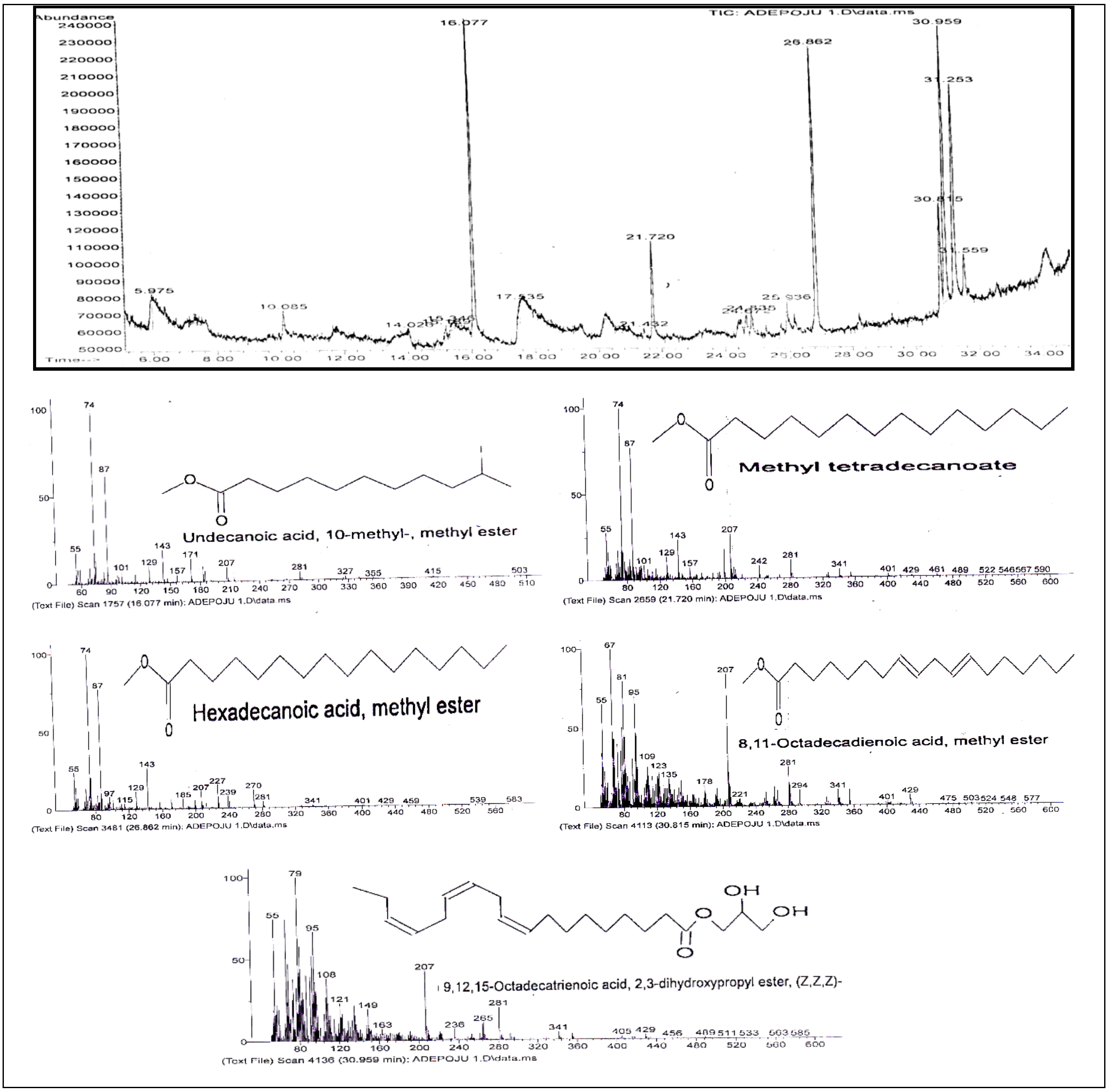

Figure 1. GC-MS spectrum and phytoconstituents in C. fructescens var. fructescens.

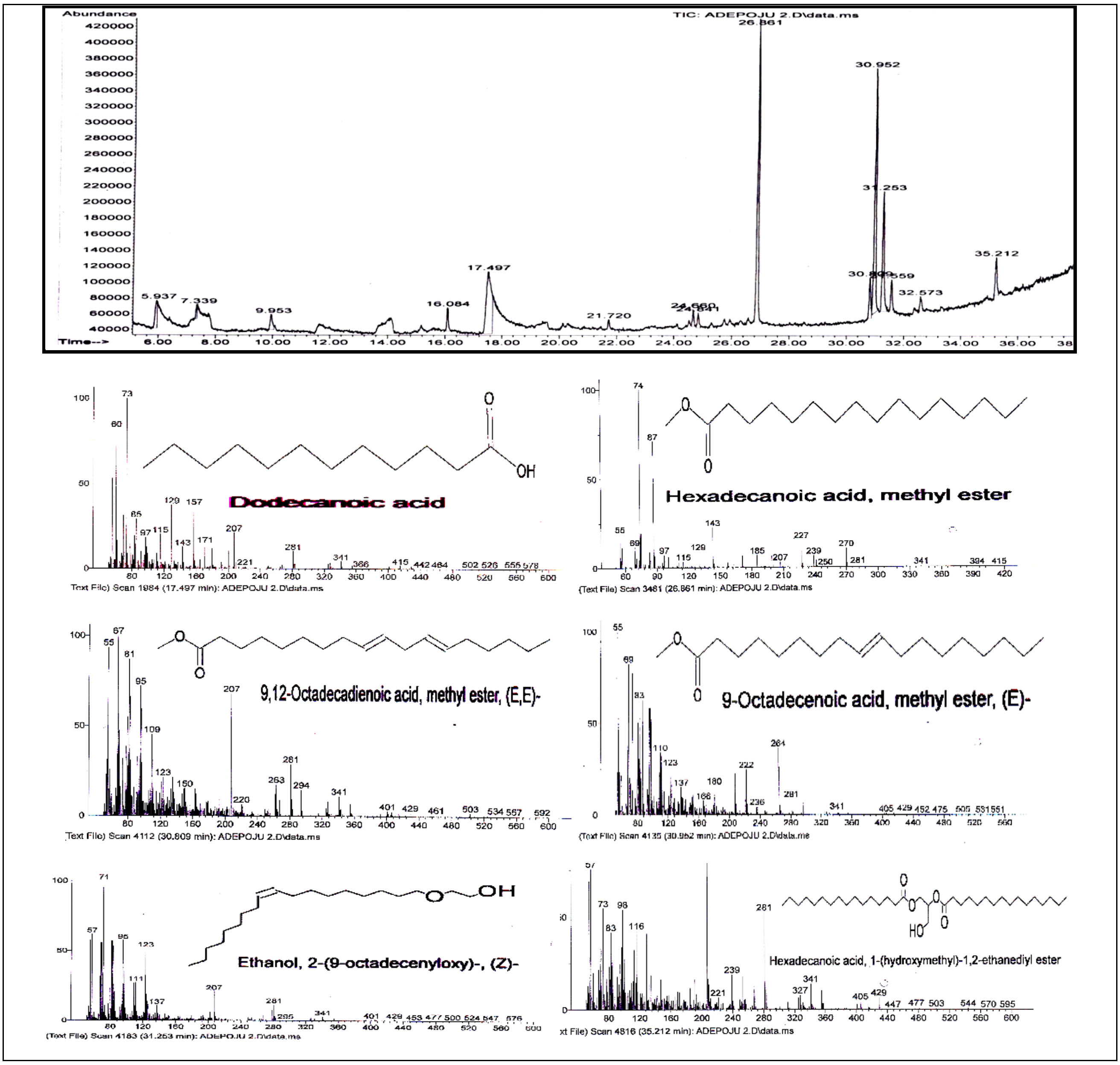

Figure 2. Phytoconstituents in C. fructescens L. var. baccatum Irish. and their GC-MS spectra.

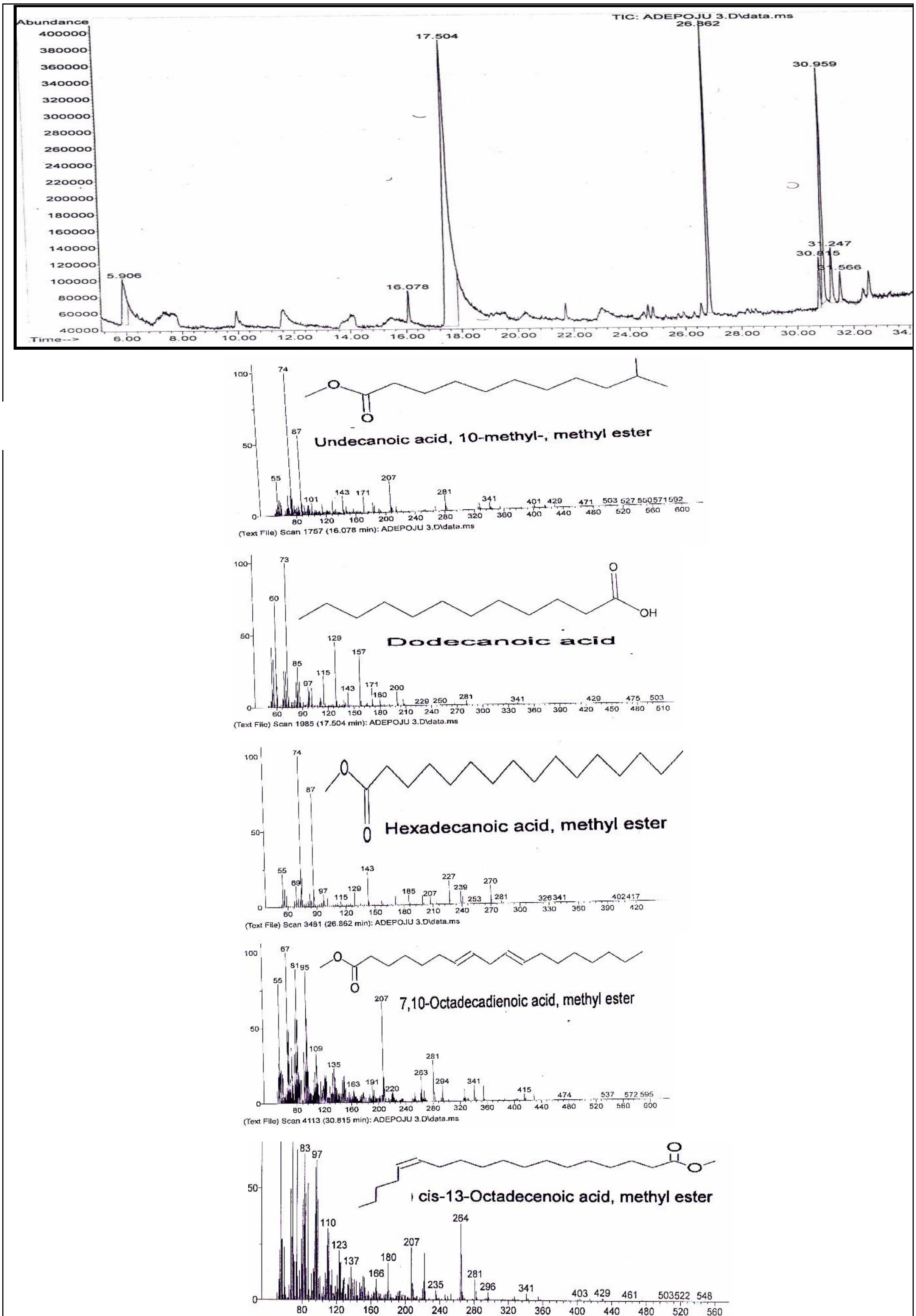

Figure 3. Phytoconstituents in C. annuum var annuum L. and their GCMS spectra.

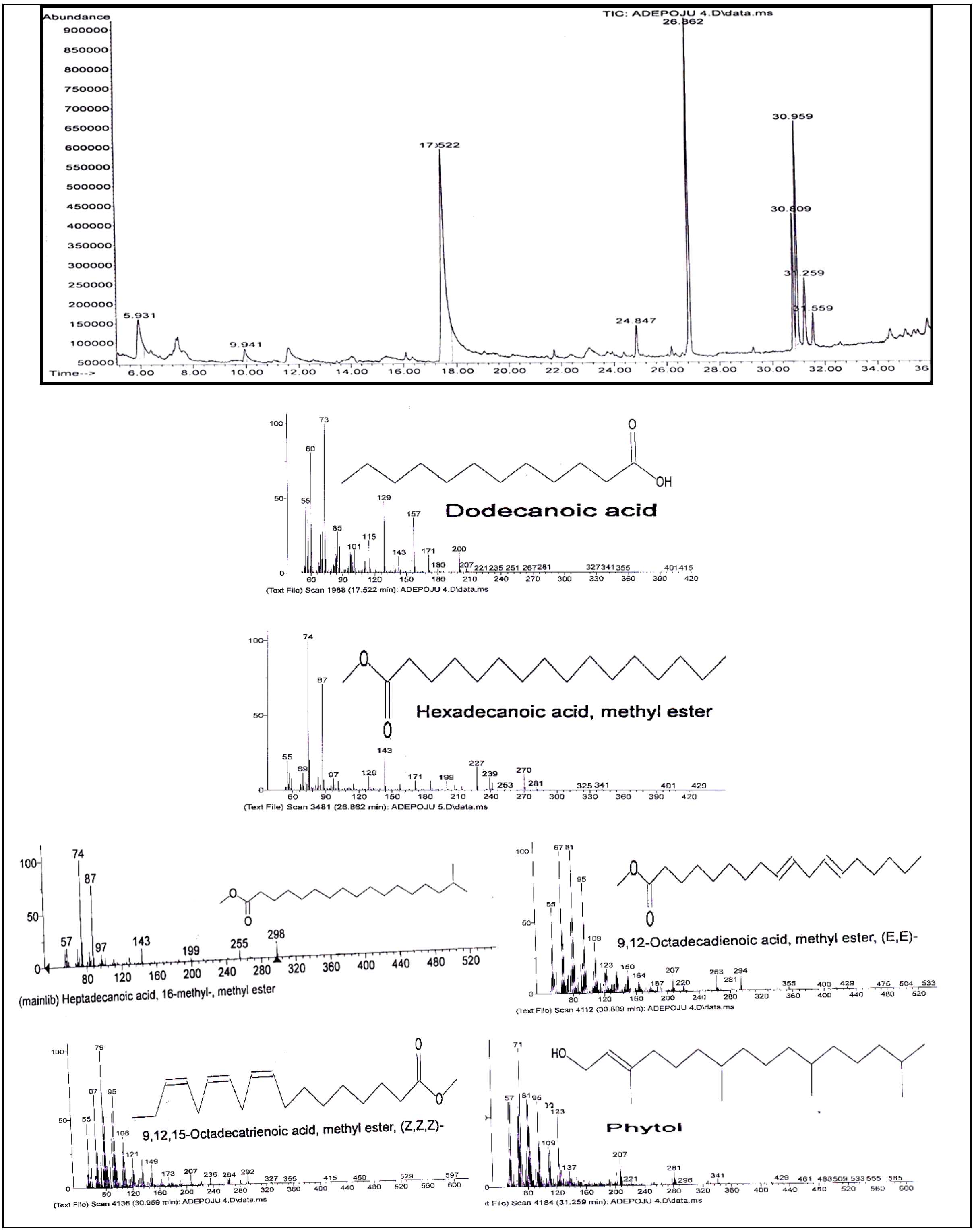

Figure 4. Phytoconstituents and GCMS spectrum of C. annuum var. grossum (L.) Sendtn.

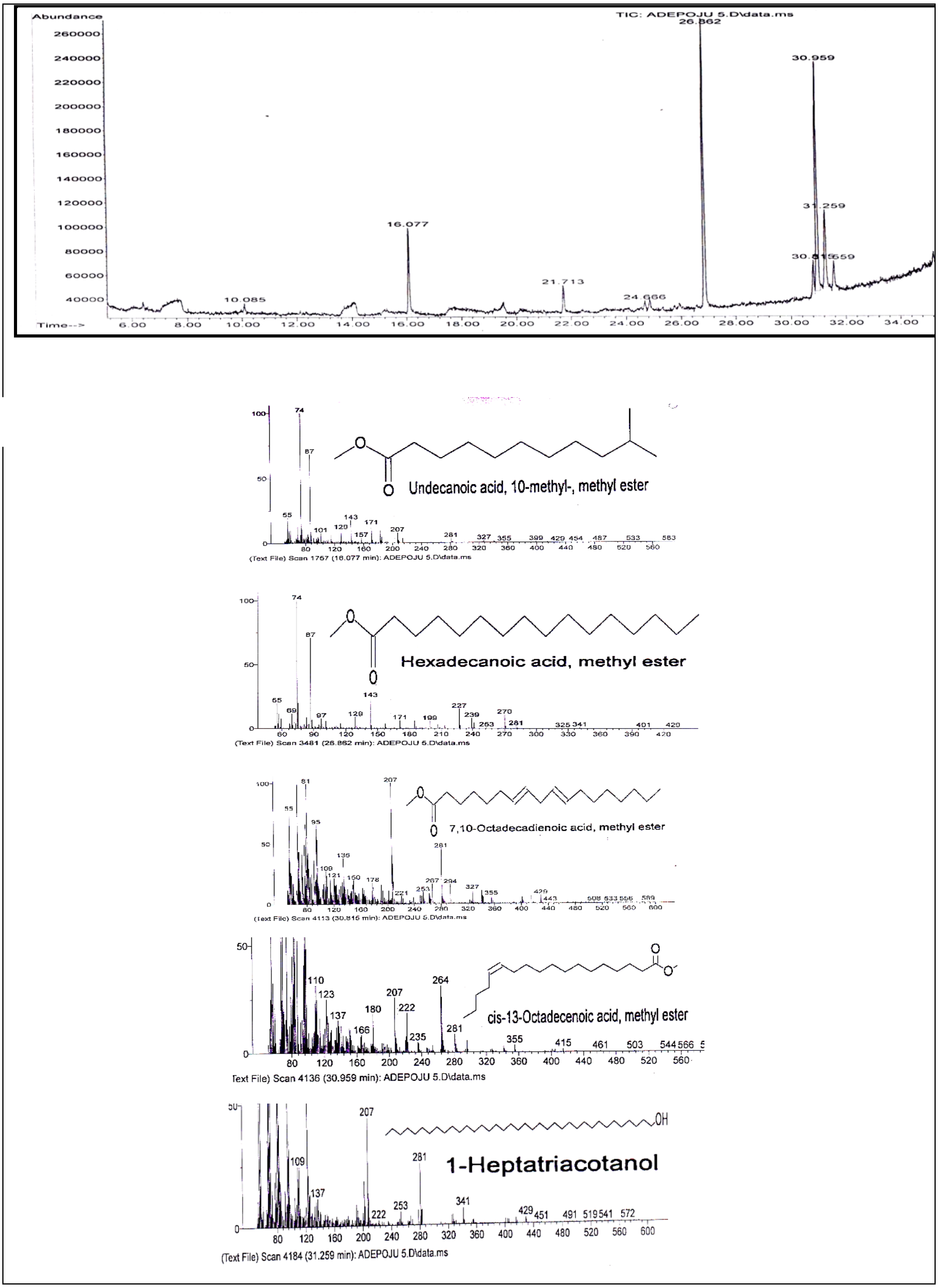

Figure 5. Chemical content of C. chinense revealed by GCMS.

3.1.1. 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-octadecatienoic acid, 2,3-dihydroxy propyl ester (Z,Z,Z); 912-OCRME=9,12,15-Octadecatrienoic acid, methyl ester, (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)-1,2-ethanediyl ester; H-16-MME=Heptadecanoic acid, 16-methyl-, methyl ester; METED=Methyl tetradecanoate; DODEA=Dodecanoic acid; ET-2-OCD=Ethanol, 2-(9-octadecenyloxy)- Z; and PHYTOL=Phytol, 1HTC= 1-Heptatriacotanol.

The largest number of organic compound content was six, and it was detected in *C. fructescens L.* var. *baccatum* (L.). Irish Next to this was the presence of five compounds which was detected in both *C. fructescens var. fructescens L.* and *C. annuum* var. *grossum* (L.) Sendtn. There were four in *C. annuum var annuum L.*, while *C. chinense* had only two compounds. Representatives of all the five classes of chemicals were found to occur among *C. annuum* var. *grossum* (L.) Sendtn. and the three varieties of *C. fructescens*. However, *C. chinense* contained only esters, and it completely lacked the organic compounds belonging to the other four classes.

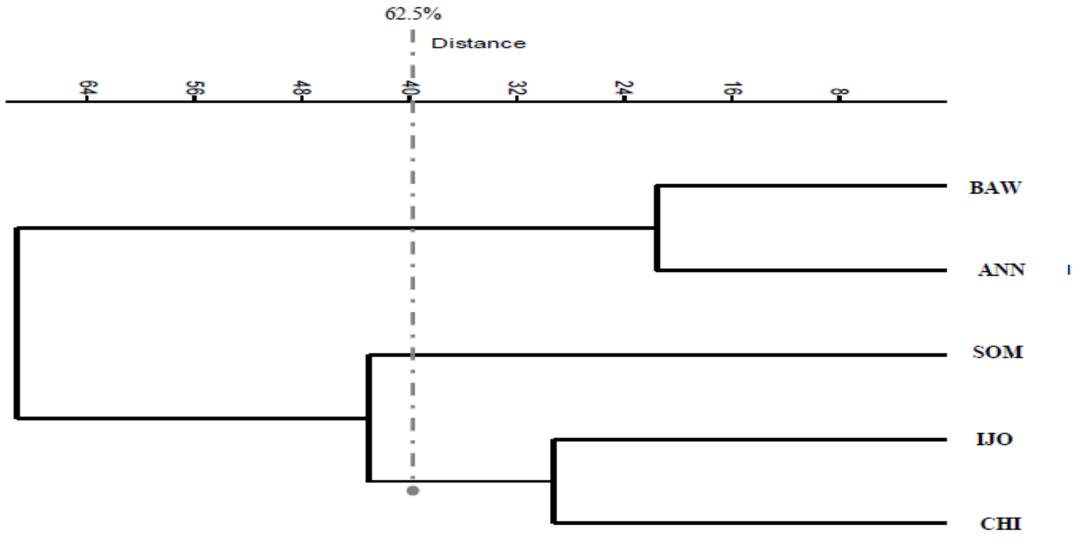

Figure 6. Dendrogram based on cluster analysis of peak area values of certain chemical components in the leaves of five researched Nigerian cultivars of Capsicum. ***Note:*** [ANN] = *C. annuum* var. *grossum* (L.) Sendtn., [BAW] = *C. annuum var annuum L.*, [CHI] = *C. chinense*, [IJO] = *C. fructescens* var. *fructescens* and [SOM] = *C. fructescens L. var. baccatum (L.)* Irish.

Figure 6 is the dendrogram obtained when the peak area values of the organic compounds detected among the plants studied were subjected to cluster analysis. The three groups observable at about 50% distance on this dendrogram are as follows: *C. annuum* var *annuum* L. and *C. annuum* var. *grossum* (L.) Sendtn. on the first, *C. fructescens var. fructescens L.* and *C. chinense* on the second, while the third had only *C. fructescens L.* var. *baccatum* (L.) Irish as a cluster.

4. DISCUSSION

A look at the dendrogram in figure 6 shows a cluster of *C. annuum* var *annuum* L. and *C. annuum* var. *grossum* (L.) Sendtn. as a group at 62.5% distance. These two taxa have earlier been pointed out to have the lowest capsaicin content among the five cultivars studied (Nwokem et al., 2010; Zeid et al., 2011). The second major cluster displayed the other three cultivars with higher capsaicin contents at that same distance. A comparison of this with the earlier dendrograms shows some similarities especially in the consistency of *C. annuum* var. *grossum* (L.) Sendtn. and *C. fructescens* as a distinct group.

A further scrutiny of figure 6 shows that it is similar to the results obtained from morphological and seed protein data of the same varieties in all respects (Adepoju et al., 2019; 2021). This indicates a concordance in the classificatory value of both wood anatomical and leaf organic chemical characters in *Capsicum*. In summary, it can be said that foliar organic compounds have a taxonomic value, that which is consistent with those of morphological, leaf epidermal, wood anatomical and seed protein characters.

4.1. A Dichotomous Key for the Classification of the Nigerian Cultivars of Capsicum based on their Foliar Organic Compound Content.

1a. 7, 10- octadecanoic acid, methyl ester (E, E) detected; Cis-13-octadecenoic acid methyl ester present in leaf extract; Undecanoic acid, 10-methyl, methyl ester occurring, but less than 11% peak area ……………….……………………………………………………….....2.

2a. Dodecanoic acid detectable; Cis-13-octadecanoic acid, methyl ester present but less than 14% ..………………………………………………………………...............BAW

2b. Dodecanoic acid not detectable; Cis-13-octadecanoic acid, methyl ester occurring with greater than 29% peak area ....CHI

1b. 7, 10- octadecanoic acid, methyl ester (E,E) not detectable; Cis-13-octadecenoic acid methyl ester absent in leaf extract; Undecanoic acid, 10-methyl, methyl ester either not detectable or if occurring, it is higher than 18% peak area. ……………………………..…...3.

3a. Dodecanoic acid occurring with less than 40% peak area, the following foliar organic compounds being absent: 9,12,15-octadecatienoic acid, 2,3-dihydroxy propyl ester (Z,Z,Z); 8,11-octadecanoic acid, methyl ester; methyl tetradecanoate; Undecanoic acid, 10-methyl, methyl ester ……………………………………...4.

4a. Dodecanoic acid occurred with greater than 35% peak area; 9, 12- octadecanoic acid methyl ester (E,E); Heptadecanoic acid, 16-methyl-, methyl ester; Phytol and 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) also detectable while the following three compounds are absent: 9-octadecenoic acid methyl ester; Ethanol, 2-(9-octadecenyloxy)- Z and Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester …………………….……………..…..ANN.

4b. Dodecanoic acid occurred but with less than 18% peak area; the following three compounds were also detected: 9-octadecenoic acid methyl ester; Ethanol, 2-(9-octadecenyloxy)- Z and Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, whereas the following four are not detectable: Heptadecanoic acid, 16-methyl-, methyl ester; Phytol; 9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z) and 9,12- octadecanoic acid methyl ester (E,E) ……………….....SOM

3b. Dodecanoic acid occurring with greater than 54% peak area; the following four organic compounds being present in the leaf exracts: 9,12,15-octadecatrienoic acid, 2,3-dihydroxy propyl ester (Z,Z,Z); 8,11-octadecanoic acid, methyl ester; methyl tetradecanoate and undecanoic acid, 10-methyl, methyl ester ..…………………BAW

[ANN] = *C. annuum* var. *grossum* (L.) Sendtn., [BAW] = *C. annuum var annuum L.*,
[CHI] = *C. chinense*, [IJO] = *C. fructescens* var. *fructescens* and
[SOM] = *C. fructescens L. var. baccatum (L.)* Irish.

That the detected organic compounds are diagnostic of the five cultivars of *Capsicum* studied is undisputable as demonstrated in the taxonomic key which is displayed above. It was observed that *C. fructescens var. fructescens L.* was the only taxon among the five studied that had the alkanoate (i.e. methyl tetradecanoate), and four other esters namely undecanoic acid, 10-methyl, methyl ester; hexadecanoic acid methyl ester; 8, 11- octadecanoic acid methyl ester; and 9, 12, 15-octadecatienoic acid, 2,3-dihydroxy propyl ester (Z, Z, Z). The uniqueness of *C. fructescens L.* var. *baccatum* (L.) Irish is also evident in that it is the only taxon with three esters (9, 12-Octadecadienoic acid, methyl ester; 9-octadecenoic acid methyl ester and Hexadecanoic acid, 1-(hydroxymethyl)-1, 2-ethanediyl ester) and it is the only plant with Ethanol, 2-/9-octadecenyloxy- Z content. The presence of two methyl esters (9, 12-

Octadecadienoic acid, methyl ester and Heptadecanoic acid, 16-methyl-, methyl ester) is diagnostic of *C. annuum* var. *grossum* (L.) Sendtn. Although *C. annuum* var *annuum* L. and C *chinense* were similar in many respects with regards to their organic compounds, the former is distinguishable from the latter by the presence of the alkanoic acid (dodecanoic acid). This cultivar also stood out distinctly among the plants studied by its possession of the highest peak area value (55.15%) of the alkanoic acid.

Ahmad et al. (2022) evaluated the quality variation for fruit (CF) samples of twenty-seven *Capsicum annuum* varieties, in terms of their volatile oil composition and biological activities. The GCMS analysis revealed the presence of seventy-one chemical compounds from different chemical classes with an average (%) composition of: 26.13 (alcohols) > 18.82 (hydrocarbons) > 14.97 (esters) > 3.08 (ketones) > 1.14 (others) > 1.07 (acids) > 0.72 (sugar) > 0.42 (aldehydes) > 0.15 (amino compounds). Alcohols and hydrocarbons were the most abundant in these CF samples with 1-Decanol, 2-octyl- and docosanoic acid, docosyl ester as the major components, respectively. Even though the authors did not name the varieties, it was revealed that the 27-CF samples were effectively distinguished based on quality variation, through the GC-MS profiles.

Reale et al. (2021) also investigated ten samples each of three italian Bell pepper varieties using headspace solid-phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS). The investigation of their volatile profile has led to the identification of 59 analytes based on sixteen diverse compounds which characterized the different bell pepper spices.

From the results of the present study, it can be stated that occurrence and abundance of esters, alkanoates, alkanoic acids, alkanols and alkanes are useful for resolving controversial taxonomic boundaries in *Capsicum*. In addition, these data have sufficient merit in diagnosing the five Nigerian cultivars of the genus as shown in section 4.1.

5. CONCLUSION

Results obtained from the chemotaxonomic examination on the five Nigerian *Capsicum* varieties have shown the presence of 17 foliar organic compounds. Generally, and considering the available data in this study, it can be concluded that apart from the fact that the problem of identification of Nigerian species of *Capsicum* have been overcome, the challenge of infrageneric groupings had also been partially resolved in the Nigerian *Capsicum* genus. Variations in chemotaxonomic data on Nigerian species of *Capsicum* have been documented in form of unambiguous artificial keys for proper identification of the species.

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7. CONFLICT OF INTEREST

The authors hereby declare that there are no conflicts of interest.

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