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Systematic Implication of GC-MS Analysis of Secondary Metabolites in *Duranta erecta* L. Forms in Nigeria

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Abstract

Duranta erecta L. is one of the most important plants used in landscaping with many medicinal properties though not studied well. Several reports have been previously published on the variations in this species leading to various taxa names. The methanol extracts were analysed by GC-MS in order to identify specific compounds unique to the different forms that exists. Eighty-nine compounds were scored for the eight forms, carboxylic acids and alcohol occupied the most abundant compounds and ketone, aldehyde, alkane, alkene, alkyne, nitrile, ester, amide, epoxide and heterocyclic compounds were also detected. All the eight forms revealed similarity percentage value lower than 50% and the highest value for the compounds was observed between variegated yellow and variegated yellow double (VY X VYD 32.14%) and the lowest between variegated white and thorny green (VW X TG 14.08%). The Cluster Analysis (CA) revealed that all the variegated forms cluster together while the broad green, yellow bush and green bush form another cluster. The thorny green remains in isolation from other forms and standing as the major cluster two. On PC1 highest score was correlated with TG form while others had values with negative scores. In component 2 (PC2), highest score is correlated with YV, VW, VYD and VY which are morphologically similar while others had negative scores. GC-MS was successfully employed in this study to classify the forms therein proposing as hybrids using the multivariate analysis.

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1. Introduction

The genus *Duranta* belongs to the family Verbenaceae, which comprises 17 to 34 species (Troncoso, 1974; Sanders, 1984) of branched shrubs or little trees with a height of 1–3 m. They have several stems or drooping spiny branches. It is widely grown as an ornamental hedge plant for its bluish purple or white flowers, fleshy and bright yellow-orange fruits (Judd & Sanders, 1986). *Duranta* are commonly referred to as golden dewdrop, pigeon berry, angel whisper, or sky flower and it is one of the traditional medicinal plants (Subsongsang & Jiraungkoorskul, 2016). In Nigeria, the green foliage *Duranta* is commonly called green bush and the yellow called yellow bush. It was believed to have been introduced to Nigeria by the colonial master, who used it as a hedge plant to beautify their surroundings. The taxonomy and nomenclature of *Duranta* species are relatively complex as various variations exists in *Duranta erecta* as a result of horticultural practices due to human preference, for colour, taste, habit and forms. Sanders (2001) reported 20 synonymous intra and inter-generic names on *D. erecta* due to the gross variations (in habit, thorns, leaf, flower colour and shape, and margins of the leaves) that exists in this species.

Three varietal names have earlier been published by Bailey (1913) under *D. plumieri* which is synonymous to *D. erecta* namely var. *alba*, var. *ellisia*, and var.

normalis i.e., the normal or the typical variety. Munir (1990) reported that all these varieties are distinguished from each other primarily by the colour of their flowers. Under var. Bailey (1913) also recorded forma variegata in which the leaves are marked with irregular patches of different colours. All these infraspecific taxa are grown in Nigerian gardens and nurseries. These varieties are difficult to distinguish in dry state in various herbaria because the flower and leaf colour often completely fade. To date no recent published regional floras have recorded any naturalized variety for this species.

In order to properly assess the systematic status of a taxon and its phylogeny, studying of morphological characters is age long and alone is not sufficient. So, the other branches of study are also taken into account for the correct assessment of the systematic position of a taxon. Chemotaxonomy of plant involves the study of chemical variation in different plants and the use of this information in their classification. Presence or absence of phytochemical compounds regardless of their compositions provide a very valuable taxonomic character. *Duranta erecta* is known to produce many phytocompounds e.g., phenol, alkaloid, saponins, glycosides (Abou-Setta *et al.,* 2007; Sharma *et al.,* 2012; Manjunatha *et al.,* 2013). This work focuses on the taxonomy complexity of *Duranta erecta* using the differences in the chemical constituent. The species is not easily differentiated from closely related species due to the large variations in similarities range causing misidentification and misinterpretation of the components.

2. Materials and Methods

2.1 Plant Collection

The leaves of each form of *D. erecta* namely: (Green bush, yellow bush, variegated yellow, variegated white, plain yellow, variegated yellow double, broad green and thorny green) were collected from different parts of the country and maintained as living germplasm at the University of Ilorin Botanical Garden experimental site in 2018-2019 (Table 1), identified and voucher specimen of each taxon was deposited in the herbarium of the Department of Plant Biology in the University of Ilorin.

Table 1: Brief Descriptions and Coordinates of the *Duranta erecta* forms Studied

S/N	Forms of *D. erecta*	Sample Sources (States)	Geopolitical Zones	GPS COORDINATE	Brief Morphological Description of Samples at their Location
1	Green bush	Kwara	North Central	8º28’48.30672N 4º40’34.9824E	Erect stem with serrate to entire green leaves, branches long rarely with a single node with fascicle leaves poorly developed.
2	Yellow bush	Kwara	North Central	8º28’48.30672N 4º40’34.9824E	Branches composed of several nodes and internodes with fascicle serrated to entire yellow leaves well develop.
3	Variegated yellow	Kwara	North Central	8.28’48.30672N 4º40’34.9824E	Erect stem with serrate to dentate variegated yellow leaves, branches are a bit longer with decussate opposite thorn and leaves.
4	Variegated white	Kwara	North Central	8º28’48.30672N 4º40’34.9824E	Erect stem with serrate to dentate variegated white leaves, branches are a bit longer with decussate opposite leaves.
5	Thorny green	Kebbi	North West	12º27’16.22N 4º12’2.14E	Erect stem with fully serrated green leaves, branches are upright, armed with thorn on opposite sides.
6	Variegated yellow double	Kebbi	North West	12º27’16.22N 4º12’2.14E	Erect stem with serrate to dentate plane with variegated yellow leaves, branches are a bit longer with decussate opposite leaves.
7	Plain yellow	Sokoto	North West	13º1’37.77N 5º14’20.998E	Erect stem with serrate to dentate plain yellow leaves, branches are straight with decussate opposite thorn and leaves.
8	Broad green	Borno	North East	11º47’24N 13º10’12E	Widely spread branches with half serrated to entire glabrous leaves.

2.2 Preparation of samples for extraction

The plants were thoroughly washed with sterile distilled water in a tray. After removing adhering materials and cleaning, the plants were air dried at ambient temperature for two weeks and ground into a powder separately using an electric blender and finally stored in airtight bottles at room temperature.

Twenty-five grams of powder of each sample were extracted with 250 ml of methanol for 24 hours and filtered using Whatman filter paper No. 1. The extract was dried in a rotary evaporator for 30 min.
GC-MS (Gas Chromatography Mass Spectrophotometer) analysis was carried out by using 6850 Network GC system, Agilent Technologies with HP5MS capillary column (nominal length 30.0 m, nominal diameter 250.00µm and nominal film thickness 0.25µm). GC-MS analysis of the leaf samples were carried by 5975 C VLMSD with Triple Axis Detector, Agilent Technologies. Helium was used as carrier gas with a flow rate of 1.0ml/min on split ratio 10:1. The temperature was programmed from 60-250 ^{o}C; initially 60-180^{o}C at the rate of 2.50 ^{o}C and 180-250 ^{o}C at the rate of 5 ^{o}C. The injector temperature was 200 ^{o}C.

2.3 Identification of chemical constituents

The constituents of the extracts were identified using spectrometric electronic, National Institute of Standards in Technology (NIST11) library. The constituents with the highest S.I value were selected and reported as the identified compounds.
For proper identification, compounds were separated based on their mass to charge (M/Z) ratios in the mass spectrometer. Peak height represents the quantity of corresponding compound. All compounds were identified by making comparison of mass spectrum of known compound in computer libraries with mass spectrum of compounds from the extract.
Methanol extracts were used for GC-MS analysis in this study as it has been reported that the methanol extracts gave better results (Abarca-Vargas *et al*., 2016; Truong *et al*., 2019).

2.4 Data Analysis

The Constituents showing the highest similarity with that of the NIST mass spectra library were loaded as variables. For cluster analysis, Euclidean distance was selected for measuring the similarity among plant samples.
The percentage composition of the constituents of the crude extract was used to determine the relationship between the forms by Principal Component Analysis (PCA) and cluster analysis using Paleontological Statistical Software (PAST). While the percentage similarity between the forms was calculated using the formula.

Number of compounds in common/ total number of compounds in each sample (i.e., $a^i + a / A + B$)
Where
a^i = Number of compounds similar to sample B

a = Total number of compounds similar to sample A

A = Total number of compound present in sample A

B = Total number of compounds present in sample B

3. Results

3.1 Gas chromatography and mass spectrometer

The peaks indicated the presence of a compound and the height indicates the concentration of the compound in each extract. The numbering of the peaks also corresponds to the serial numbering of the compound on each table.
The GC- MS analysis of the eight *Duranta* forms revealed 89 compounds (Table 2) in total with thorny green presented the highest number of compounds (44); 24, 23, 26,27,28,30 and 31 compounds are present in green bush, yellow bush, variegated yellow, variegated white, broad green, variegated yellow double and plain yellow forms respectively. The retention time varied from 5.14 for benzaldehyde to 20.93 for 2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-. Nine compounds were common to all the eight forms which include Benzofuran, 2,3-dihydro-, trans-Cinnamic acid (which was detected twice at different retention time in green bush, yellow bush, variegated yellow, variegated white, variegated yellow double and broad green forms), Phytol, n-Hexadecenoic acid, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 2-Methoxy-4-vinylphenol, 2-Propenoic acid, 3-(4-methoxyphenyl)-, Diisooctyl phthalate and Cyclopenta[c]pyran-4-carboxylic acid, 7-methyl-, methyl ester. All the compounds present were grouped based on functional group. Carboxylic acid has the highest number of compounds in all the eight forms followed by alcohol, other functional groups include alkane, alkene, alkyne, ketone, ester and heterocyclic compound.

3.1.1 Green bush *Duranta*

Compounds reported are grouped into Carboxylic acid, ketone, alcohol, ester, heterocyclic, alkene and nitrile with the retention time ranged from 6.77- 21. 82 and the molecular weight of 120 - 390. The major bioactive metabolites among others include 1H-Tetrazole, 5-(3,4-dimethoxyphenyl)- with the highest concentration of 26.01%, trans-Cinnamic acid, Phytol, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-, Cyclopenta[c]pyran-4-carboxylic acid, 7-methyl-, methyl ester, n-Hexadecanoic acid, Benzofuran, 2,3-

dihydro-, 2-Amino-3-hydroxypyridine, 3-Deoxy-d-mannoic lactone, 2-Propenoic acid, 3-phenyl-, methyl ester.-*

3.1.2 Yellow bush *Duranta*

Among the active compounds include trans- cinnamic acid, 4,7- Dimethoxy-2-methylindan-1-one, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-, 2-Propenoic acid, 3-(4-methoxyphenyl)-, n-Hexadecanoic acid, Phytol,. The compound with the lowest concentration is 3-Allyloxy-1,2 propanediol (0.33%) while 4,7-Dimethoxy-2-methylindan-1-one has the highest concentration with (25.68%). In total, seven functional groups were detected in this plant. Among those, there were five Ketone compounds, five alcohol compounds, one ester, and one heterocyclic compound, two nitrile compounds and eight carboxylic acid compounds.

Table 2: Phyto compounds isolated and identified from the eight *Duranta erecta* forms

COMPOUND NAME	Molecular Formula	Molecular weight	GB	YB	VY	VW	VYD	YV	BG	TG
1,6-Anhydro-2,4-dideoxy-.beta.-D-ribo-hexopyranose	$C_6H_{10}O_3$	130	1	0	0	0	0	0	1	1
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	1	1	1	1	1	1	1	1
5-Hepten-2-ol, 6-methyl-	$C_8H_{16}O$	128	1	0	0	0	0	0	0	0
Benzofuran, 2,3-dihydro-	C_8H_8O	120	1	1	1	1	1	1	1	1
2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	1	1	1	1	1	1	1	1
trans-Cinnamic acid	$C_9H_8O_2$	148	1	1	1	1	1	1	1	1
2-Propenoic acid, 3-phenyl-, methyl ester	$C_{10}H_{10}O_2$	162	1	1	1	0	1	0	1	0
2,4-Decadien-1-ol, (E,E)-	$C_{10}H_{18}O$	154	1	0	0	0	0	0	0	0
5-(3,4-Dimethoxyphenylmethylidene)-2,2-dimethyl-1,3-dioxan-4	$C_{15}H_{16}O_6$	292	1	0	0	0	0	0	1	0
Cyclopenta[c]pyran-4-carboxylic acid, 7-methyl-, methyl ester	$C_{11}H_{10}O_3$	190	1	1	1	1	1	1	1	1
3-Deoxy-d-mannoic lactone	$C_6H_{10}O_5$	162	1	1	0	0	0	0	1	0
2-Propenoic acid, 3-(4-methoxyphenyl)-	$C_{10}H_{10}O_3$	178	1	1	1	1	1	1	1	1
2-Ethyl-3-oxo-4-pyrrolidin-2-ylidene-butyronitrile	$C_{10}H_{14}N_2O$	178	1	1	0	0	1	1	0	0
[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	322	1	0	0	0	0	0	0	0
1H-Tetrazole, 5-(3,4-dimethoxyphenyl)-	$C_9H_{10}N_4O_2$	206	1	0	1	1	1	0	0	0
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	1	1	1	1	1	1	1	1
Phytol	$C_{20}H_{40}O$	296	1	1	1	1	1	1	1	1
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	1	1	1	1	1	1	1	1
Octadecanoic acid	$C_{18}H_{36}O_2$	284	1	0	0	0	0	0	0	0
9-Octadecenoic acid (Z)-, phenylmethyl ester	$C_{25}H_{40}O_2$	372	1	0	0	0	0	0	0	0
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)	$C_{19}H_{38}O_4$	330	1	0	0	0	1	0	0	0
Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	1	0	0	0	0	0	0	0

2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144	0	1	0	1	1	0	1	0
3-Allyloxy-1,2 propanediol	$C_6H_{12}O_3$	132	0	1	0	1	0	1	0	0
2-Nonen-1-ol, (E)-	$C_9H_{18}O$	142	0	1	0	0	1	0	0	0
4,7-Dimethoxy-2-methylindan-1-one	$C_{12}H_{14}O_3$	206	0	1	0	0	0	1	1	1
2-Oxaadamantan-6-ol	$C_9H_{14}O_2$	154	0	1	0	0	0	0	0	0
2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1	$C_{15}H_{22}O$	218	0	1	0	0	0	0	0	0
1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10	$C_{33}H_{56}$	452	0	1	0	0	0	0	0	0
Diisooctyl phthalate	$C_{24}H_{38}O_4$	390	0	1	1	1	1	1	1	0
6-Methyl-2-heptyne	C_8H_{14}	110	0	0	1	0	0	0	1	0
5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	$C_{10}H_{16}O_2$	168	0	0	1	0	0	1	0	0
Methyl (Z)-dec-2-en-4,6-diynoate	$C_{11}H_{12}O_2$	176	0	0	1	0	1	0	1	0
Decahydroquinoline-10-ol	$C_9H_{17}NO$	155	0	0	1	0	1	0	1	0
3-Methoxycinnamic acid	$C_{10}H_{10}O_3$	178	0	0	1	1	1	0	0	1
2-Propenoic acid, 3-(4-methoxyphenyl)-, methyl ester, (E)-	$C_{11}H_{12}O_3$	192	0	0	1	1	1	1	0	0
2-Amino-3-hydroxypyridine	$C_5H_6N_2O$	110	0	0	1	1	0	0	1	0
2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester	$C_{11}H_{12}O_4$	208	0	0	1	1	0	0	0	0
9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0	0	1	1	1	1	0	0
9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	$C_{11}H_{16}O_4$	212	0	0	1	0	1	1	1	1
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	0	0	1	0	0	1	1	1
2-Decen-1-ol, (E)-	$C_{10}H_{20}O$	156	0	0	0	1	0	1	0	0
Bicyclo[4.2.0]octa-1,3,5-triene-7-carboxylic acid	$C_9H_8O_2$	148	0	0	0	1	0	1	0	0
3,7-Diazabicyclo[3.3.1]nonane, 9,9-dimethyl-	$C_9H_{18}N_2$	154	0	0	0	1	0	0	0	0
2,6-Dimethyl-4-(2-furyl)pyridine	$C_{11}H_{11}NO$	173	0	0	0	1	0	0	0	0
.delta.-Thionodecalactone	$C_{10}H_{18}OS$	186	0	0	0	1	0	0	1	0
6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)-	$C_{25}H_{36}O_2$	368	0	0	0	1	0	0	0	0
.alpha.-Tocopheryl acetate	$C_{31}H_{52}O_3$	472	0	0	0	1	0	0	0	0
Bicyclo[3.2.1]octan-3-one, 6-hydroxy-, exo-(.+-.)-	$C_8H_{12}O_2$	140	0	0	0	0	1	0	0	0
Acetic acid, (2-isopropenylcyclopentylidene)-, methyl ester	$C_{11}H_{16}O_2$	180	0	0	0	0	1	1	0	0

Benzo[4,5]imidazo[2,1-c][1,2,4]triazole-3-thione, 2,9-dihydro-	$C_8H_6N_4S$	190	0	0	0	0	1	1	0	0
Megastigmatrienone	$C_{13}H_{18}O$	190	0	0	0	0	1	0	0	1
3-Hydroxy-4-methoxycinnamic acid	$C_{10}H_{10}O_4$	194	0	0	0	0	1	0	0	0
2H-3,9a-Methano-1-benzoxepin, octahydro-2,2	$C_{15}H_{26}O$	222	0	0	0	0	1	0	0	0
2-Propyl-tetrahydropyran-3-ol	$C_8H_{16}0_2$	14	0	0	0	0	0	1	0	1
Cycloheptanone, oxime	$C_7H_{13}NO$	127	0	0	0	0	0	1	0	0
2-Propenoic acid, 3-phenyl-	$C_9H_8O_2$	148	0	0	0	0	0	1	0	1
1,3-Isobenzofurandione, 4,7-dimethyl-	$C_{10}H_8O_3$	176	0	0	0	0	0	1	0	0
Arecoline	$C_8H_{13}NO_2$	155	0	0	0	0	0	1	0	0
(E)-3-(2-Methoxyphenyl)-2-propenoic acid	$C_{10}H_{10}O_3$	178	0	0	0	0	0	1	0	0
4-Quinolinol, 2,8-dimethyl-	$C_{11}H_{11}NO$	173	0	0	0	0	0	1	0	0
Carbamic acid, 2-(dimethylamino)ethyl ester	$C_5H_{12}N_2O_2$	132	0	0	0	0	0	1	0	0
3(2H)-Furanone, dihydro-5-isopropyl-	$C_7H_{12}O_2$	128	0	0	0	0	0	0	1	0
2-Nitro-1-buten-3-ol	$C_4H_7NO_3$	117	0	0	0	0	0	0	1	0
9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimeth	$C_{27}H_{52}O_4Si_2$	496	0	1	0	0	0	0	1	0
Benzaldehyde	C_7H_6O	106	0	0	0	0	0	0	0	1
6-Acetyl-.beta.-d-mannose	$C_8H_{14}O_7$	222	0	0	0	0	0	0	0	1
Benzenemethanethiol, .alpha.-methyl-	$C_8H_{10}S$	138	0	0	0	0	0	0	0	1
1-Cyclohexyl-2,2-dimethyl-1-propanol	$C_{11}H_{22}O$	170	0	0	0	0	0	0	0	1
5H-1,4-Dioxepin, 2,3-dihydro-2,5-dimethyl-	$C_7H_{12}O_2$	128	0	0	0	0	0	0	0	1
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	$C_6H_8O_3$	128	0	0	0	0	0	0	0	1
Octanoic acid, 7-oxo-	$C_8H_{14}O_3$	158	0	0	0	0	0	0	0	1
Cyclohexane, 1,3-dimethoxy-5-methyl-, stereoisomer	$C_9H_{18}O_2$	158	0	0	0	0	0	0	0	1
2,6-Dihydroxybenzaldehyde, carbamoylhydrazone	$C_8H_9N_3O_3$	195	0	0	0	0	0	0	0	1
Indolizine	C_8H_7N	117	0	0	0	0	0	0	0	1
Cinnamamide, N-(2-thiazolyl)-	$C_{12}H_{10}N_2OS$	230	0	0	0	0	0	0	0	1
Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$	154	0	0	0	0	0	0	0	1
3-Acetylbicyclo[3.3.1]non-6-ene	$C_{11}H_{16}O$	164	0	0	0	0	0	0	0	1
Piperazine, 1-ethyl-4-phenyl-	$C_{12}H_{18}N_2$	190	0	0	0	0	0	0	0	1
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0	0	0	0	0	0	0	1
Tridecanal	$C_{13}H_{26}O$	198	0	0	0	0	0	0	0	1

2,5-Dimethoxycinnamic acid	$C_{11}H_{12}O_4$	208	0	0	0	0	0	0	0	1
.alpha.-D-Glucopyranoside, O-.alpha.-D-glucopyranosyl-(1.fwdar	$C_{18}H_{32}O_{16}$	504	0	0	0	0	0	0	0	1
cis-Z-.alpha.-Bisabolene epoxide	$C_{15}H_{24}O$	220	0	0	0	0	0	0	0	1
d-Mannitol, 1-decylsulfonyl-	$C_{16}H_{34}O_7S$	370	0	0	0	0	0	0	0	1
9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z	$C_{21}H_{36}O_4$	352	0	0	0	0	0	0	0	1
9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281	0	0	0	0	0	0	0	1
2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-	$C_{15}H_{26}O$	222	0	0	1	0	0	0	0	1
13-Oxabicyclo[9.3.1]pentadecane, 15-chloro-	$C_{14}H_{25}ClO$	244	1	0	0	0	0	0	0	0

Note: GB = Green bush; YB = Yellow bush; VY = Variegated yellow; VW = Variegated white; VYD = Variegated yellow double; YV = Plain yellow; BG = Broad green and TG = Thorny green

3.1.3 Variegated yellow *Duranta*

The most prominent compound is 1H-tetrazole, 5-(3,4-dimethoxyphenyl)- with 27.53% concentration, followed by 2-propenoic acid, 3-(4-methoxyphenyl)- (13.25%). The compound with the lowest concentration is 5-isopropenyl-2-methyl-7-oxabicyclo (4.1.0) and heptan-2-ol (0.28%). The compounds were separated and grouped into ketone, alkyne, heterocyclic, alcohol, carboxylic acid and ester.

3.1.4 Variegated white *Duranta*

A total of twenty-seven (27) compounds were grouped into 12 carboxylic, 4 compounds each for alcohol and heterocyclic, 3 ketones, 2 esters and 1 for alkane. The major compounds are 1H-Tetrazole, 5-(3,4-dimethoxyphenyl)- (25.4%), 2-propenoic acid, 3-(4-methoxyphenyl)- (15.24%) and 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (8.69%). The retention times varied from 6.47 to 20.67 and molecular weights from 110 to 390.

3.1.5 Variegated yellow double *Duranta*

GC-MS chromatograms of variegated yellow double *Duranta* revealed the presence of 30 compounds (Fig. 7 and Table 17). Twenty bioactive compounds were identified. In variegated yellow double, the retention time was between 142 – 390 that separated fourteen (14) carboxylic compounds, six (6) ketones, five (5) alcohols, one (1) nitrile and two (2) each for heterocyclic and esters. Major compounds include 1H-tetrazole, 5-(3,4-dimethoxyphenyl)- (23.19%) concentration, followed by 9,12,15-octadecatrienoic acid (2,2,2)- (10.93%) while the compound Bicyclo (3.2.1) octan-3-one, 56-hydroxy-exo (.+.-)- (0.24%) has the lowest concentration.

3.1.6 Plain yellow *Duranta*

Carboxylic compounds were the most prominent in this *Duranta* form with fifteen (15) compounds while trans-Cinnamic acid showed the widest area covered (37.03%) and 4,7-Dimethoxy-2-methylindan-1-one reported the highest concentration with (24.74%). The molecular weight ranged from 14 to 390 with seven (7) compounds of alcohol, six (6) ketones and one (1) each of heterocyclic, nitrile and ester.

3.1.7 Broad green *Duranta*

The major compounds detected in this form include 4,7-dimethoxy-2-methylindan-1-one (28.34%) followed by trans-cinnamic acid (13.45%) and phytol (9.2%). The lowest concentration was observed in 3(2H)-furanone, dihydro-5-isopropyl-(0.2%) and 2-Nitro-1-buten-3-ol (0.27%) respectively. The molecular weight ranged from 110 to 496 having ten (10) compounds of carboxylic, seven (7) ketone, four (4) alcohol, three (3) heterocyclic, 2 ester and on compound each for alkene and alkyne.

3.1.8 Thorny green *Duranta*

At retention time of 20.93min, 44 different compounds were detected with highest group of carboxylic compounds (12), followed by alcoholic compounds (10), ketones (6), heterocyclic and aldehyde group of four (4) compounds each and one compound each for thiol, ester, alkane, alkene, nitrile and epoxy while two compounds of amide group were also detected. The molecular weights for these compounds ranged from 117 to 504 and the compound 2-Propenoic acid, 3-phenyl- (15.63%) reported the highest concentration.

3.2 Similarity percentage between the eight forms of Duranta erecta

The number of compounds similar for all the eight forms of *Duranta* was determined for the leaf methanol extract (Fig. 1). All the eight forms revealed similarity percentage value lower than 50% and the highest value for the compounds was observed between variegated yellow and variegated yellow double (VY X VYD 32.14%) followed by variegated yellow and broad green forms (VY X BG 31.48%). The lowest similarity percentage was presented between variegated white and thorny green (VW X TG 14.08%). The figure made known that the similarity percentage between thorny green and other samples appeared very low while that of variegated yellow are very high. In addition, trans-cinnamic acid displayed the highest concentrations of chemical for all the forms except for variegated white and thorny green forms with the highest concentration in 2-Propenoic acid, 3-(4-methoxyphenyl)-. The presence of these chemicals (2-Propenoic acid and 3-(4-methoxyphenyl-) characterized these two forms from the others.

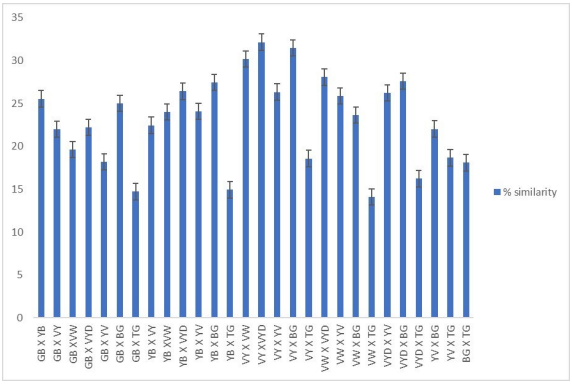

Fig. 1: Similarity percentage between the eight *Duranta erecta* forms.

Legend: GB= Green bush; YB=Yellow bush; VY= Variegated yellow; VW= Variegated white; VYD= Variegated yellow double; YV= Plain yellow; BG= Broad green; TG= Thorny green

3.3 Principal component analysis

The PC generated a heatmap dendrogram in which two main clusters were observed with other subclusters (cluster 1, cluster 2 and cluster 3) (Fig. 2). On inspection, the green bush stands as a sister out group to the broad green (BG) and yellow bush (YB) all been in the same cluster (subcluster 1), while the variegated forms established another subcluster 2 with plain yellow as sister group. The thorny green stands out of the other forms as major cluster II. It was observed on the dendrogram that all the variegated forms cluster together while the broad green and yellow bush form another cluster. The thorny green remains in isolation from other forms and standing as the major cluster two. The first three component accounted for 61.69% of the cumulative percentage variance of the original variables. Table 3 shows the Eigen value for the component variance of the eight forms studied and Fig. 3 revealed the closeness among the eight forms. The plot of component 1 versus component 2 separated the eight *Duranta* forms into three group with each group occupying different quadrant. On PC1, highest score was correlated with TG form while others had values with negative scores. In component 2 (PC2), highest score is correlated with YV, VW, VYD and VY which are morphologically similar while others had negative scores.

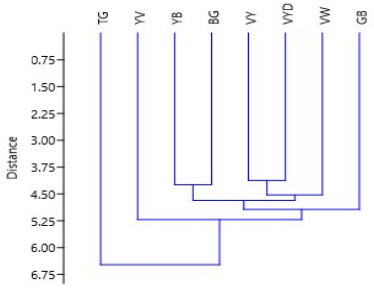

Fig. 2: Dendrogram by cluster analysis using UPGMA model for the eight forms of *Duranta erecta*

Legend: GB= Green bush; YB=Yellow bush; VY= Variegated yellow; VW= Variegated white; VYD= Variegated yellow double; YV= Plain yellow; BG= Broad green; TG= Thorny green

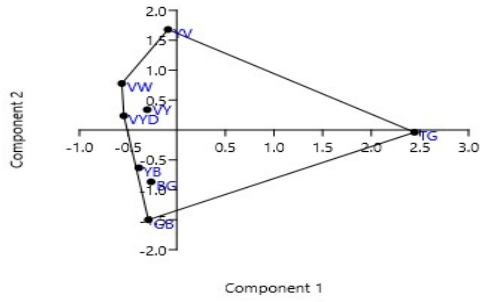

Fig. 3: PCA scattered plot of the eight forms of *Duranta erecta*

Legend: GB= Green bush; YB=Yellow bush; VY= Variegated yellow; VW= Variegated white; VYD= Variegated yellow double; YV= Plain yellow; BG= Broad green; TG= Thorny green

4. Discussion

For chemical analysis of the chemical data present in all the 8 forms, gas chromatography and mass spectrometry (GC-MS) was used as an analytical tool for the separation and identification of small and volatile molecules such as steroids, fatty acids and hormones. The major advantages of using this technique are its ability to separate complex mixtures and to quantify analytes (TFS, 2018).

Past studies on the GC-MS analysis on *Duranta erecta* were mostly on the essential oil of the plant and not on crude methanolic extracts. According to Hayes (2017), GC-MS analysis on essential oils would mostly yield light and volatile compounds while crude extracts would yield slightly heavier aromatic, steroids and fatty acids with important therapeutic properties. In GC-MS all compounds are eluted on the GC/MS chromatogram at different retention times (RT). The retention time of a compound is a measure of the time taken for a compound to be eluted from a chromatography column (TFS, 2018). It is usually calculated as the time from injection to elution off the peak. The retention time (RT) for the compounds present in the eight forms are not fixed, even when the same compounds are detected, many factors can influence it even if the same gas chromatography and column are used (TFS, 2018). The GC-MS analysis of the methanol extract revealed the presence of bioactive compounds with pharmaceutical, industrial as well as taxonomical importance.

Table 3: Eigenvalues and percentages of the Principal Coordinates Analysis for the compounds

PC	Eigenvalue	% Variance	Cumulative %
1	4.05527	28.819	28.82
2	2.60751	18.531	47.35
3	2.01753	14.338	61.69
4	1.70477	12.115	
5	1.51729	10.783	
6	1.32877	9.4431	
7	0.840283	5.9716	

The number of compounds that were identified from the leaves in the eight morphological forms confirm to the report of Gottlieb (1982) who revealed that number of isolated substances or the number of their occurrences could also be used to display similarities between groups of different plants. The number of compounds detected are very similar for all the forms except for that of the thorny green which has higher number of compounds.

The differences in the numbers of compounds could be due to the effect of the environment where the samples were collected and due to the variations in their genetical make up (Hegnauer, 1977). Variation in the secondary metabolite could be more of ecological than physiological. These metabolites are taxonomically useful at the generic level. Padalia *et al*. (2014) opined such observations could be as a result of expression of different genes at different developmental stages of the plant. The nine compounds common to all these forms could be the compounds relating to their taxonomical group linking them together in the same genus. Davis & Heywood (1973) also reported similar assertion that certain compounds and related substances may be characteristic of certain taxonomic groups. Pooja, (2012) also reported the presence of these compounds in *Duranta repens*.

The percentage similarity that was higher between VY X VYD, VY X BG, VW X VYD, VY X VW, VYD X TG and GB X YB conform to the report of Atal (1982) and Rasool *et al.* (2010) who established that chemotaxonomic classification relies on the chemical similarity of taxon. The cluster also revealed more information in that the thorny green which separated out of the other samples shows that it has accumulated more genetical information which may lead to speciation over time.

The PCA analysis could not separate clearly the eight forms of the studied *Duranta erecta*. The forms tend to form a morphological band instead of separating or clear the clustering base on their chemical composition. However, some morphologically similar forms (green bush and yellow bush), (the variegated types) located out of their mixed groups. Similar observation was noticed by Sander (2001) who reported morphological discrepancies in *Duranta* species.

The degree of similarities in percentage between the variegated yellow and others was higher and that of variegated white; this could possibly be due to the hybridization which had taken place for a long time, and this high intraspecific variation could not allow clear separation of the *Duranta* forms. Knop & Jensen (1980) also supported the statement that if hybridization has taken place for a long time, due to introgression, backcross or segregation products will often be quite similar to one of the parental taxa. Moreover, the expansion of the smaller leaves in green bush and yellow bush to the bigger leaves in the variegate forms support the hypothesis of hybridization between the forms. Therefore, the GC-MS employed for this study could be used successfully to classify the forms of *Duranta*.

5. Conclusion

In conclusion, the results of this work confirm the chemotaxonomic usefulness of chemical constituents of plant species particularly at the species level. The comparison of the percentage similarity, cluster and the principal component analysis data obtained in this research work suggest a close relationship between the eight *Duranta* forms. This provides further evidence for the utility of chemical compounds as a rapid, reliable, and inexpensive method to assess preliminary chemotaxonomic relationships. However, to make meaningful taxonomic conclusions, a wider sampling across different geographical locations will be needed in future investigation of the genus.

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AbdulRahaman A.A contributed to the manuscript development and supervised the work done by Sagaya, A. who did data acquisition, writing and laboratory work.

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