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Phytochemical Profiling of Six Morphologically Identical Species of Mimoisoideae

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Abstract

The use of morphological characters in authenticating *Parkia biglobosa* (Jacq.), *Tetrapleura tetraptera* (Schum. &Thonn.), *Albizia adianthifolia* (Schum.), *Pentaclethra macrophylla* (Benth.), *Leucaena leucocephala* (Lam.), and *Prosopis africana* (Guill. &Perr.) has been most challenging due to their morphological similarities. This study explored the principle that plant species are reservoirs of chemical compounds, hence could yield discriminatory results sufficient enough for their authentication. The samples were subjected to standard methods of GC MS screening and characterization aided by NIST library. The results showed the presence of flavonoid compounds in all species, alkaloids in all except *P. biglobosa* and *A. adiantifolia*, esters in *P. macrophylla* only, organic acids and fatty acids in all except *T. tetraptera*, *A. adaintifolia* and *P. africana*, terpenes in all except *A. adiantifolia* and *P. macrophylla*, alkanes and glycosides in all except *A. adiantifolia* and *P africana*, phenolics and alcohols in *P.biglobosa* and *T. tetraptera* respectively, amine in all except *T. tetraptera* and *P. africana* and Vitamin E in all except *A. adiantifolia*. Differentially, the results showed the unique presence of Oxirane, tetradecyl- in *P. biglobosa*, Cyclohexaneethanol in *T. tetraptera*, benzenamine, 2,6-bis(1,1-dimethylethyl)-4-nitro- (an amine) compound in *A. adianthifolia*, Pentanoic acid, 2-methyl- in *P. macrophylla*, 1,4-Hexadiene, 3,3,5-trimethyl- in *L. leucocephala*, Squalene in *Prosopis africana*.. A taxonomic key meant to be used as standard for authenticating these morphologically identical species was constructed for each phytochemical group. The study concludes by recommending same approach to the authentication of other morphologically identical taxa.

Key Words: Profilling, Mimosoidea, Phytochemical, Authentication,

Introduction

Huge reliance on indigenous and ecosystem services of plant species is placing unparalleled burden on the classical organoleptic and micro/macro characters methods of authentication. The burden is further compounded especially in the tropics with high rate of speciation. The dearth of well trained field taxonomists and grossly inadequate funding in African countries where huge biodiversity resources occur, implies that a plethora of flora species exist that are unknown to the scientific world. Environmental factors have long been recognized as causal agents for species mimicry. In Africa, geographical barriers and habitat fragmentation occasioned mainly by anthropogenic actions has often resulted in reproductive isolation creating strains and in the long run, evolution of new species. The absence of chemical and/or genetic banks for most of these morphologically indistinct plant taxa is a stressor factor even for well trained and versatile taxonomists.

Chemo-taxonomy has often been applied for species delimitation across all taxonomic ranks (Dewick, 2002; Baranska *et al.*, 2005; Middleton 2008; Ayan *et al.*, 2009; Rasool *et al.*, 2010; Wink, 2008, 2010a,b; Singh, 2016). These reports were largely limited to the qualitative representations of one or few chemical ingredients present in the taxa under investigations. Although, quantitative representations of active plant chemical ingredients in a taxon are often poor discriminatory character due to variations mediated by environmental, species life stage/metabolic processes and period of harvesting, its usefulness in delimitating taxa based on range in percentage value of component chemical compounds present has been poorly and scantily applied (Demirci, *et al.*, 2004; Ebuehi and Okorie, 2009; Chen, *et al.*, 2012; Chuang, *et al.*, 2013; Hussain, et al., 2017; Butnariu, 2016; Syahidah, *et al*. 2017) in species authentication. Other quantitative reports of chemical compounds present in a given species concerned themselves largely to the therapeutic influences of few secondary metabolic groups of interest (Akiyama, *et al.*, 2001; Kimura *et al.*, 2001; Hanus *et al.*, 2005; Al-Daihan, *et al.*, 2013; Ahmad *et al.*, 2010b; Singh *et al.*, 2011; Cuthbertson *et al.*, 2013). In all, a holistic and quantitative profiling and characterization of all chemical compounds present in any one plant taxon without recourse to its medicinal applications is almost nonexistent. Where one exists, it is likely being held in the plant chemical compounds bank of most first class herbaria and hence not easily accessible by the larger scientific world. In Nigeria as in most third world countries, the near absence of functional analytical equipment has made research in chemical compound profiling and characterization all the more challenging. This research is therefore conceived to use GC-MS to quantitatively characterize, identify, delimit and document all the active chemical ingredients present in the six morphologically identical members (*Parkia biglobosa* (Jacq.), *Tetrapleura tetraptera* (Schum. &Thonn.), *Albizia adianthifolia* (Schum.), *Pentaclethra* *macrophylla* (Benth.), *Leucaena leucocephala* (Lam.), and *Prosopis africana* (Guill. &Perr.) of Mimosoideae.

Materials and Methods

Each species was collected in triplicate across three different ecological zones of Cross River Nigeria. The leaves of the candidate plant samples were washed under running tap to remove impurities and air-dried at room temperature (25°C). In order to rupture the cells and cause them to release active ingredients in them, the dried sample leaves were ground to uniform powder using an electric blender (Kumar & Matthew, 2014). The fine powders were then packed separately in ziplock bags to avoid the effect of humidity and then stored at room temperature (Yusuf *et al*., 2014). Plate A-F is a pictorial representation of the species.

Plate A: *Pentaclethra macrophylla* Benth

Plate B: *Tetrapleura tetraptera* (Schum & Thonn) Taub

Plate C: *Leucaena leucocephala* (Lam) DE WIT. Plate D: *Albizia adianthifolia* (Schum.) W.F. Wit

Plate E: *Parkia biglobosa* (Jacq.)G. Don Plate F: *Prosopis africana* (Guill & Perr.) Taub

Plate A - F : Images of investigated species

GCMS Analysis

An agilent 5890N gas chromatography equipped with an auto sampler connected to an agilent Mass Spectrophotometric Detector was used.1microlitre of sample was injected in the pulsed spitless mode onto a 30m x 0.25mm id DB 5MS coated fused silica column with a film thickness of 0.15micrometer. Helium gas was used as carrier gas and the column head pressure was maintained at 20psi to give a constant of 1ml/min. Other operating conditions were present. the column temperature was initially held at 55^{o}C for 0.4min, increased to 200^{o}C at a rate of 25^{o}C/mins, then to 280^{o}C at a rate of 8^{o}C/mins and to final temperature of 300^{o}C at a rate of 25^{o}C/mins, held for 2mins.The identification time was based on retention time since each of the active ingredients has its separate retention time in the column. Those components with lower retention time were eluted before the ones with high retention time.

Study Period

This study was conducted between October 2016 and June 2018.

Results

The result of GCMS analysis was recorded. Fig. 2 – 7 shows the chromatograms indicating the retention times (min.) and the abundance of each component, while Table 2 shows the correspondent compounds identified and the percentage composition of each component in the sample.

Parkia biglobosa

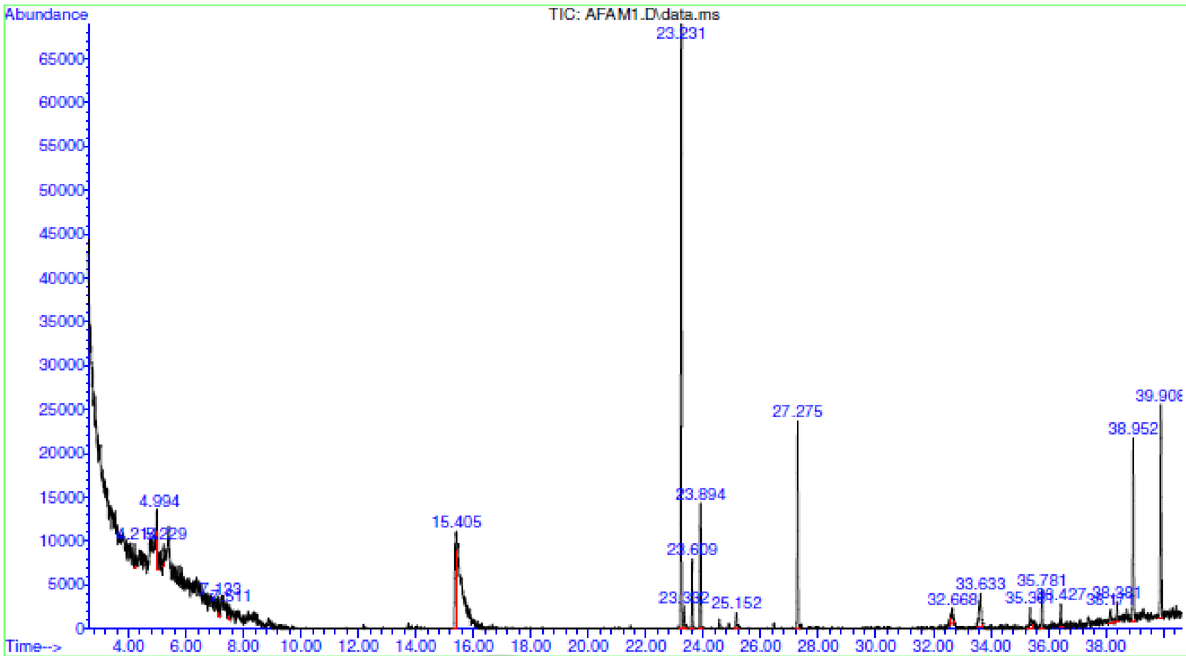

Fig 2: GCMS chromatogram of *P. biglobosa*.

Tetrapleura tetraptera

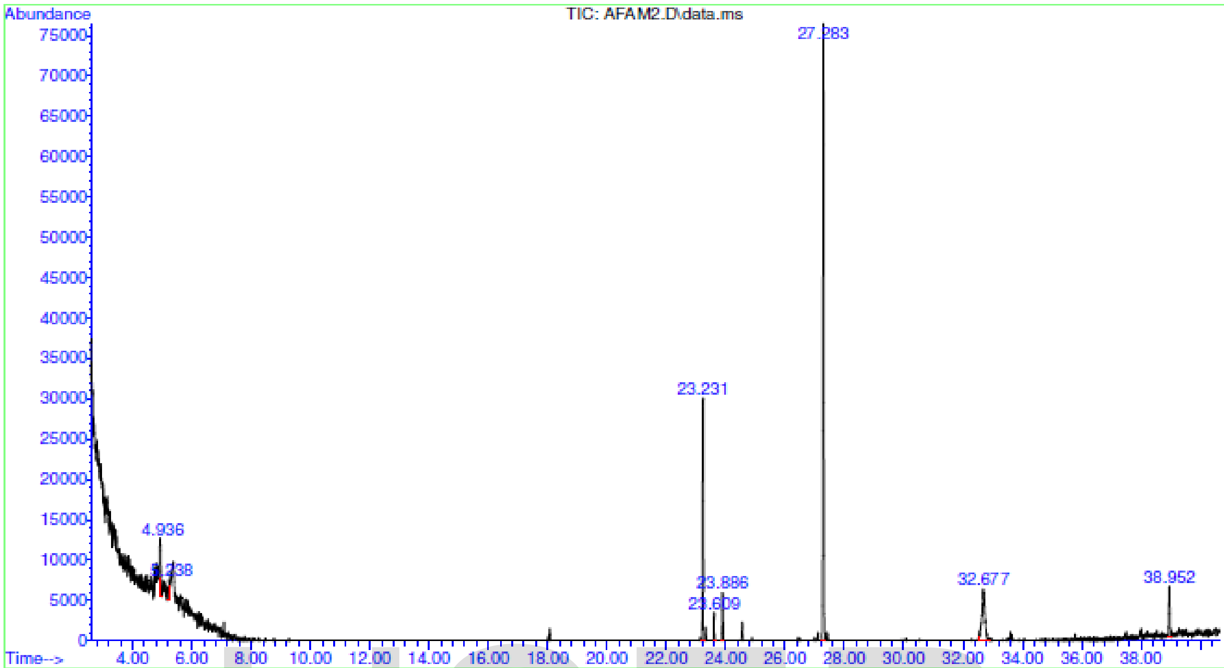

Figure 3: GCMS chromatogram of *T. tetraptera.*

Albizia adianthifolia

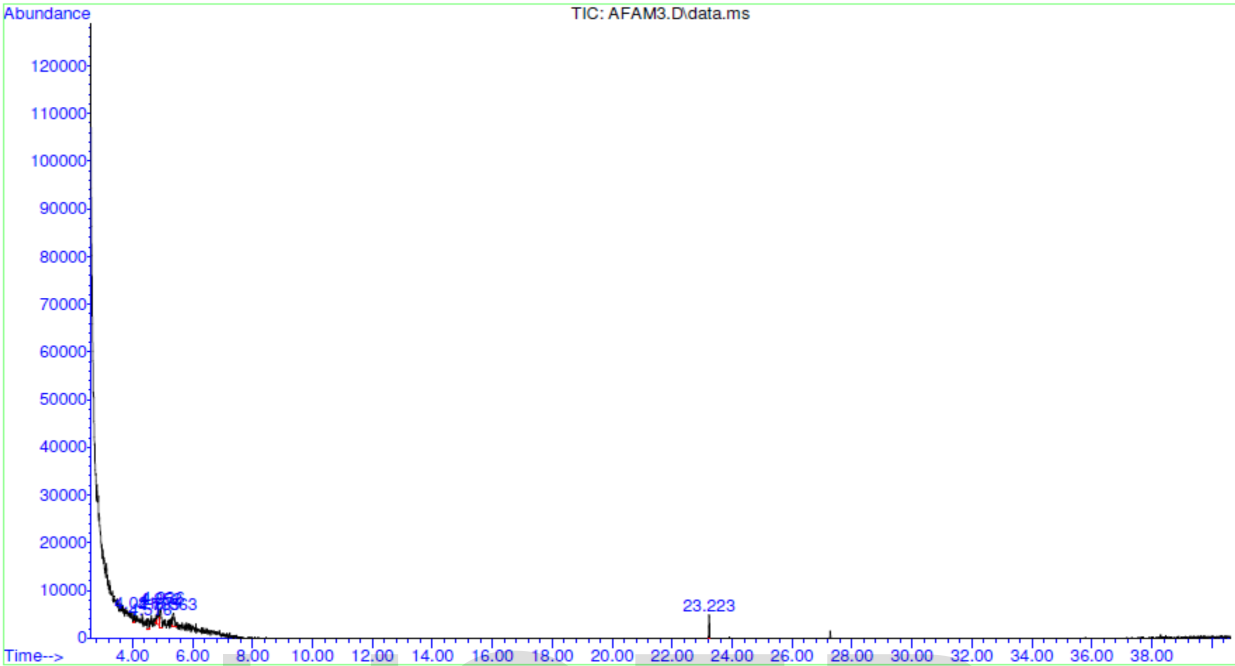

Figure 4: GCMS chromatogram of *A. adianthifolia.*

Pentaclethra macrophylla

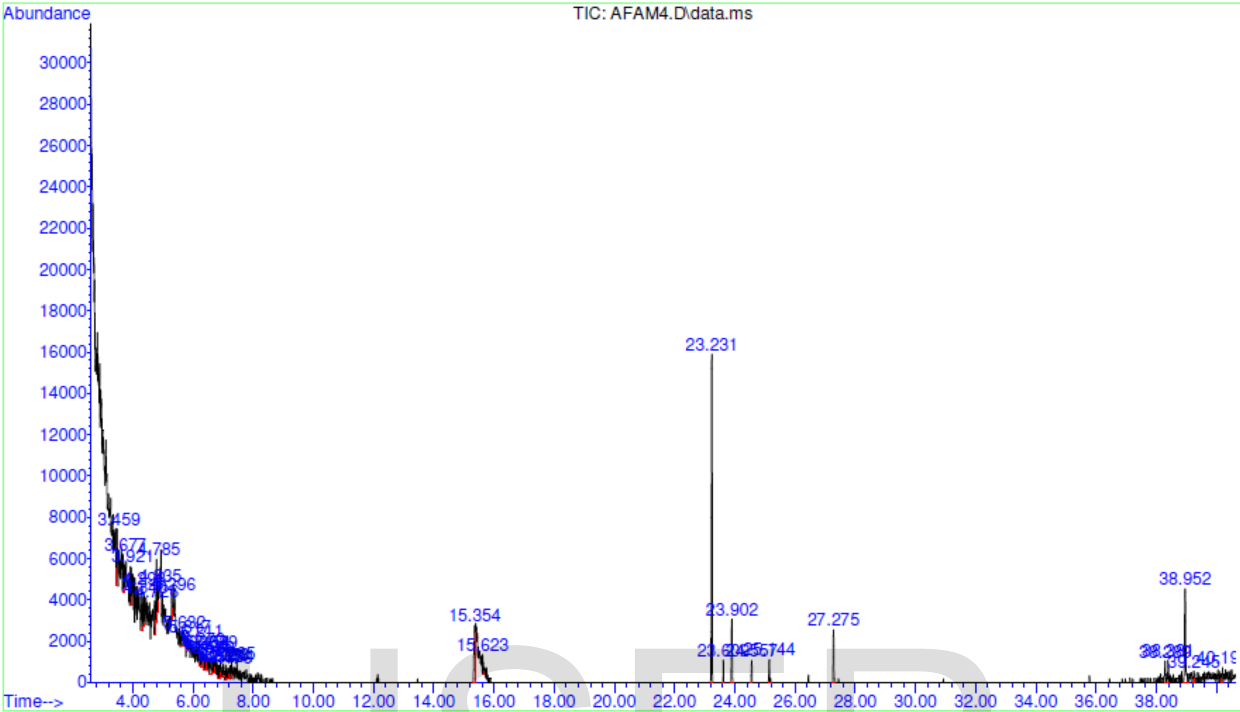

Figure 5: GCMS chromatogram of *P. macrophylla.*

Leucaena leucocephala

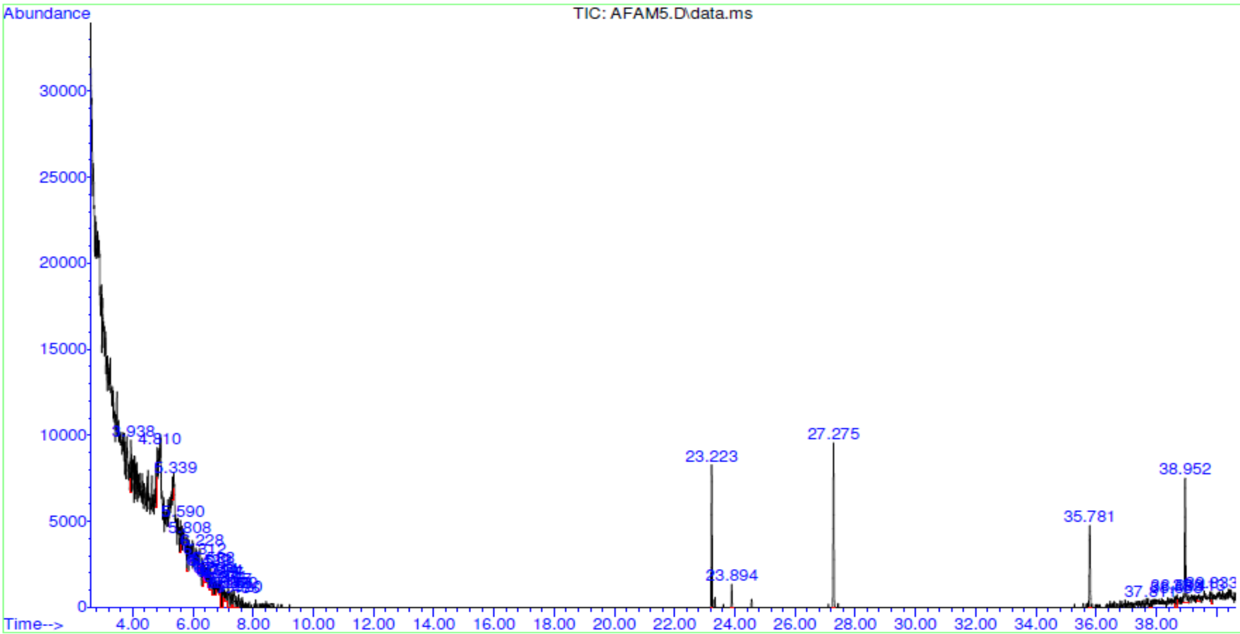

Figure 6: GCMS chromatogram of *L. leucocephala.*

Prosopis africana

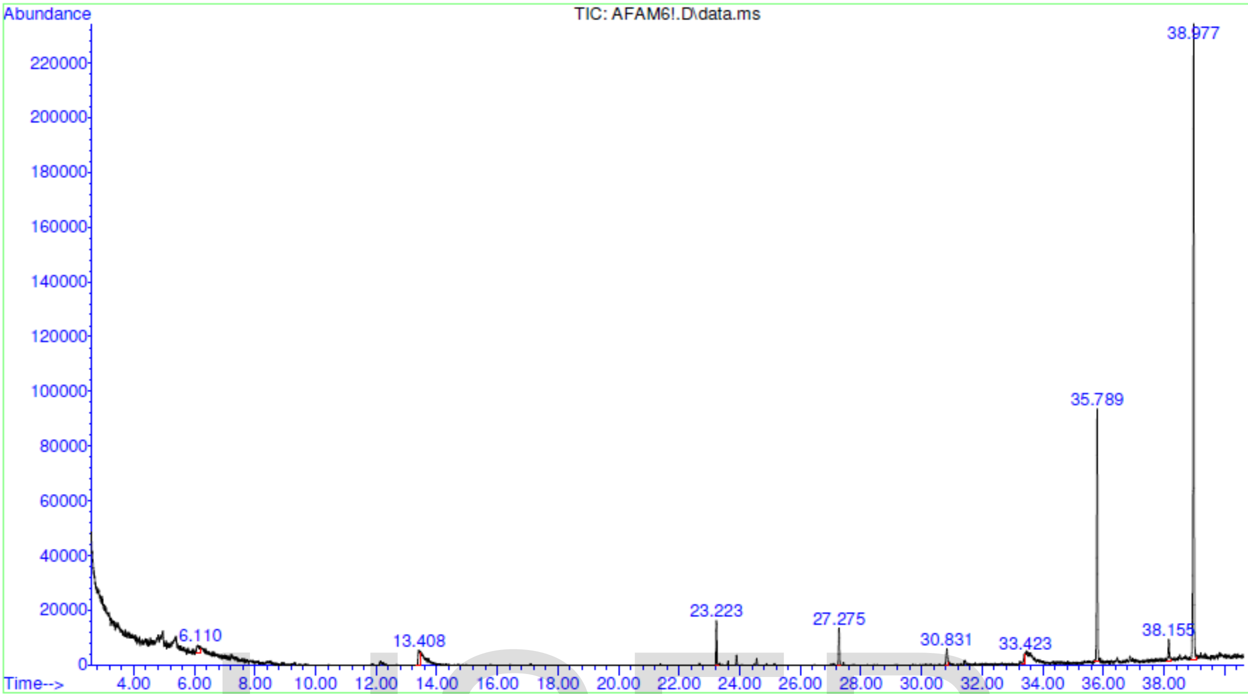

Figure 7: GCMS chromatogram of *P. africana.*

The presence and relative contribution of each chemical group per species is provided in Table 2.

Table 2: GC MS Characterization of Chemical Compounds

Compounds		Mol. formula	*P.B(%)*	*T.T(%)*	*A.A(%)*	*P.M(%)*	*L.L(%)*	*P.A(%)*
Flavonoids	1,2,3-Benzenetriol	$C_6H_6O_3$	12.730	-	-	1.293	-	-
	1,2,4-Benzenetriol	$C_6H_6O_3$	-	-	-	6.030	-	-
	1,10-Decanediol	$C_{10}H_{22}O_2$	3.121	-	-	-	12.124	-
	1-Butanol, 4-butoxy-	$C_8H_{18}O_2$	0.866	-	-	-	-	-
	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	5.515	47.457	-	-	13.301	-
	1,4-Naphthoquinone, 6-ethyl-2,3,5,7-tetrahydroxy-	$C_{12}H_{10}O_6$	0.944	-	7.218	-	-	-
	3-Nonen-1-ol, (Z)-	$C_9H_{18}O$	-	3.207	-	4.642	-	-
	3-Benzyloxy-1,2-dihydro-2-oxoquinoxaline	$C_{15}H_{12}N_2O_2$	-	-	9.372	-	-	-
	Phthalazin-1(2H)-one, 4-methyl-2-(4-methylphenyl)-	$C_{16}H_{14}N_2O$	-	-	26.748	-	-	-
	3-Buten-2-one, 4-(4,7-dimethoxy-1,3-benzodioxol-5-yl)-	$C_{13}H_{14}O_5$	-	-	13.538	-	-	-
	1-Methyl-2,5-dichloro-1,6-diazaphenalene	$C_{12}H_8Cl_2N_2$	-	-	-	1.847	-	-
	4,6-Octadiyn-3-one, 2-methyl-	$C_9H_{10}O$	-	-	-	5.607	-	-
	Phthalazine-1,4(2H,3H)-dione, 2-(2-methylphenyl)-	$C_{15}H_{12}N_2O_2$	-	-	-	1.430	-	-
	2,3-Dihydro-2-methyl-4-(4-methylphenyl)-1H-1,5-benzodiazepine	$C_{17}H_{18}N_2$	-	-	-	1.349	-	-
	2-Hydroxy-3-(thiophen-2-yl)methyl-5-methoxy-1,4-benzoquinone	$C_{12}H_{10}O_4S$	-	-	-	3.507	-	-
	4-Iodothioanisole	$C_7H_{17}S$	-	-	-	1.717	-	-
	2,4,5-Trichlorophenyl propenoate	$C_9H_5Cl_3O_2$	-	-	-	1.630	1.014	2.068
	trans-2-Undecen-1-ol	$C_{11}H_{22}O$	-	-	-	24.794	-	-
	Pent-1-yn-3-one	C_5H_6O	-	-	-	0.977	-	-

	4-Methyl-2,4-bis(p-hydroxyphenyl) pent-1-ene, 2TMS derivative	$C_{24}H_{36}O_2Si_2$	-	-	-	1.644	2.791	-
	6-Octen-2-one	$C_8H_{14}O$	-	-	-	0.938	-	-
	1,1'-Biphenyl, 2,4-dichloro-2',5'-dimethyl-	$C_{14}H_{12}Cl_2$	-	-	-	-	4.379	-
	Ethanone, 1-(3-amino-4-methoxymethyl-6-methylthieno[2,3-b]pyrid-2-yl)-	$C_{12}H_{14}N_2O_2S$	-	-	-	-	1.120	-
	3,4-Dimethylcyclohexanol	$C_8H_{16}O$	-	-	-	-	1.248	-
	3,5-Dihydroxybiphenyl	$C_{12}H_{10}O_2$	-	-	-	-	-	2.313
Alkaloids	Pyrazole-3-carboxylic acid, 4-iodo-1-methyl-	$C_5H_5IN_2O_2$	-	-	-	2.488	-	-
	Pyrimidine, 5-bromo-2,4-bis(methylthio)-	$C_6H_7BrN_2S_2$	-	-	-	1.068	8.382	-
	1-Benzyl-3-phenyl-1H-1,2,4-triazol-4-oxide	$C_{15}H_{13}N_3O$	-	6.516	-	-	-	-
	10-Dodecenol	$C_{12}H_{24}O$	-	-	14.957	-	-	-
	Pyrimidine, 5-bromo-4,6-dimethoxy-	$C_6H_7BrN_2O_2$	-	-	-	-	3.410	-
	4,4-Dimethyl-5-methylene-2-benzylimino-1,3-thiazolidine	$C_{13}H_{16}N_2S$	-	-	-	-	7.447	-
	Pyrrolo[3,4-c]pyridine-1,3-dione, 2-phenethyl-	$C_{15}H_{12}N_2O_2$	-	-	-	-	1.879	-
	Methaqualone	$C_{16}H_{14}N_2O$	-	-	-	7.529	-	-
	Z-2-Dodecenol	$C_{12}H_{24}O$	-	-	-	3.592	-	-
	2,4(1H,3H)-Pyrimidinedione, 6-iodo-5-methyl-	$C_5H_5IN_2O_2$	-	-	-	1.835	1.620	-
	2-Pyrrolidinemethanamine, N-methyl-, (S)-	$C_6H_{14}N_2$	-	-	-	-	3.896	-
	1,3,4-Thiadiazole, 2,5-bis(4-aminofurazan-5-yl)-	$C_6H_4N_8O_2S$	-	-	-	0.859	1.083	-

	1-Methyl-5-iodouracil	$C_5H_5IN_2O_2$	-	-	-	-	1.201	-
	Resorcinol	$C_6H_6O_2$	-	-	-	-	-	2.433
Esters	Ether, 3-butenyl propyl	$C_7H_{14}O$	-	-	-	1.681	-	-
Organic acids, Fatty acids	2,2'-Bifuran]-5,5'-dicarboxylic acid, dimethyl ester	$C_{12}H_{10}O_6$	1.132	-	-	-	-	-
	Hexanoic acid	$C_6H_{12}O_2$	1.168	-	-	-	-	-
	Sulfurous acid, nonyl 2-propyl ester	$C_{12}H_{26}O_3S$	1.296	-	-	-	-	-
	Pentanoic acid, 2-methyl-	$C_6H_{12}O_2$	-	-	-	1.235	-	-
	1H-Indole-2-carboxylic acid, 4-bromo-3-methyl-5-(phenylmethoxy)-	$C_{17}H_{14}BrNO_3$	-	-	-	1.645	-	-
	Acetic acid, cyano-, 2-methoxyethyl ester	$C_6H_9NO_3$	-	-	-	0.812	-	-
	Cyclopropanecarboxylic acid, 1-amino-	$C_4H_7NO_2$	-	-	-	-	2.240	-
	Carbamic acid, N,N-dimethyl-, 4-isopropylphenyl ester	$C_{12}H17NO_2$	-	-	-	-	1.200	-
	Benzoic acid, 4-methyl-2-trimethylsilyloxy-, trimethylsilyl ester	$C_{14}H_{24}O_3Si_2$	-	-	-	-	0.944	-
	l-Leucine, N-butoxycarbonyl-, undec-10-enyl ester	$C_{22}H_{41}NO_4$	-	-	-	-	-	2.024
Terpenes	Neophytadiene	$C_{20}H_{38}$	35.314	19.288	-	-	-	7.468
	Trifluoroacetyl-lavandulol	$C_{12}H_{17}F_3O_2$	2.110	-	-	-	-	-
	1,4-Hexadiene, 3,3,5-trimethyl-	C_9H_{16}	-	-	-	-	8.042	-
	Squalene	$C_{30}H_{50}$	-	-	-	-	-	22.303
Alkanes and Glycosides	Cyclopropane, 1,1-dimethyl-2-(1-methyl-2-propenyl)	C_9H_{16}	0.989	-	-	-	-	-
	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	$C_{15}H_{26}O$	4.078	-	-	-	-	-

	1,4,7,10-Cyclododecatetraene	$C_{12}H_{16}$	-	1.495	-	-	-	-
	2,4,6-Trimethyl-1-nonene	$C_{12}H_{24}$	1.223	-	-	-	-	-
	1-Formyl-2,2-dimethyl-3-trans-(3-methyl-but-2-enyl)-6-methylidene-cyclohexane	$C_{15}H_{24}O$	-	15.136	-	-	-	-
	Pentane, 1,2-dichloro-	$C_5H_{10}Cl_2$	-	-	-	1.189	-	-
	1-Cycloocten-5-yne, (Z)-	C_8H_{10}	2.99	-	-	-	-	-
	Oxirane, tetradecyl-	$C_{16}H_{32}O$	12.642	-	-	-	-	-
	(3-Aminopropyl) dipropylborane	$C_9H_{22}BN$	1.012	-	-	-	-	-
	2,4,6,8-Tetramethyl-1-undecene	$C_{15}H_{30}$	1.198	-	-	-	-	-
	9,9-Dichloro-9-silafluorene	$C_{12}H_8Cl_2Si$	-	-	-	0.913	4.37	-
	β-D-Glucopyranose, 1,6-anhydro-	$C_6H_{10}O_5$	-	-	-	1.334	-	-
	Nortricyclyl bromide	C_7H_9Br	-	-	-	1.578	-	-
	Glucitol, O-2,3,4,6-tetra-O-methyl-_-D-galactopyranosyl-(1.fwdarw.3)-O-2,4,6-tri-O-methyl-_-D-galactopyranosy	$C_{30}H_{58}O_{16}$	-	-	-	-	3.029	-
Phenolics	4-tert-Octylphenol, TMS derivative	$C_{17}H_{30}OSi$	1.010	-	-	-	-	-
Alcohol	Cyclohexaneethanol	$C_8H_{16}O$	-	1.740	-	-	-	-
Amines	benzenamine, 2,6-bis (1,1-dimethylethyl)-4-nitro-	$C_{14}H_{22}N_2O_2$	-	-	9.672	-	-	-
	4-Pyridinamine, 3,5-dibromo-	$C_5H_4Br_2N_2$	-	-	-	3.622	-	-
	Propanenitrile, 3-[4-diethylamino-1-methyl-1-(1-methylethyl)-2-butynyloxy]-	$C_{15}H_{26}N_2O$	0.988	-	-	-	-	-
	2-Amino-6-benzyl-4-hydroxypteridine	$C_{13}H_{11}N_5O$	-	-	-	-	0.820	-
	5-Amino-2-isopropyl-1-phenyl-2,3(1H)-dihydro-pyrrole-3,3,4-tricarbonitrile	$C_{16}H_{15}N_5$	-	-	18.495	-	-	-

	Cyclobutaneacetonitrile, 1-methyl-2-(1-methylethenyl)-	$C_{10}H_{15}N$	-	-	-	-	1.022	-
Vitamin E	α –Tocopherol	$C_{29}H_{50}O_2$	9.745	-	-	10.366	13.434	58.724
	β – Tocopherols	$C_{29}H_{50}O_2$	-	5.100	-	-	-	-
	γ-Tocopherol	$C_{28}H_{48}O_2$	-	-	-	-	-	2.667

NB: *PB= P. biglobosa* *TT= T. tetraptera* *AA= A. adianthifolia* *PM= P. macrophylla* *LL= L. leucocephala* *PA= P. africana*

Discussion

The results indicated differencies in chemical compounds present and percentage concentrations. These variations in chemical composition have been shown to depend on either extrinsic factors, such as solvent composition, time of extraction, temperature, pH, polarities and particle size, or variations in phytochemical composition of the study plant species (Amusa *et al.*, 2014; Igwenyi *et al.*, 2015). Also, the retention time of study species suggest that *P. africana* contains mainly lower molecular weight compounds as against *A. adianthifolia* with higher molecular weight compounds. Retention time has also been directly correlated to species compound polarity (Lytovchenko *et al.*, 2009; Cuthbertson *et al.*, 2013). The compounds in each species revealed different phytochemical groups ranging from alkaloids, hydrocarbons, fatty acids, phenolics, sterols, terpenes, esters, fatty alcohol and ketones

Flavonoids: A total of thirty three (33) active compounds belonging to the flavonoid phytochemical group were identified. No one specie was observed to possess all the flavonoid bioactive ingredients but each specie contained at least one compound. The presence of at least a flavonoid compound in each of the species could account for homologies in color, aroma of flowers, fruit, pollinators and fruit dispersion. These plants have also shown similar seed types, similar spore germination, similar growth and development of seedling (Samanta et al 2011). These compounds could also have accounted for the protection of these species against biotic and abiotic stresses (Samanta *et al.*, 2011; Mierziark *et al*., 2014), their roles in UV-filter and signal functioning (Tevini and Teramura, 1989; Samanta *et al.*, 2011) Plants possessing flavonoids have also be shown exhibiting allelopathic effects and expressing phytoalexins effects. Their detoxifying roles and antimicrobial defensive properties has also been reported (Iwashina, 2003; McNally *et al.*, 2003). These plants may have roles against frost hardiness, drought resistance and may play a functional role in plant heat acclimation and freezing tolerance (Treutter, 2008; Palma-Tenango *et al.*, 2017). A taxonomic key capable of authenticating these species based on flavonoid content alone is constructed.

Figure 8: Taxonomic key for six mimoisoideae species based on flavonoid content:

1a Plants containing 3,7,11,15-Tetramethyl-2-hexadecen-1-ol

(*P. biglobosa*, *T.tetraptera*, *L. leucocephala*)

2a Plant containing 1-Butanol, 4-butoxy-

P. biglobosa **1**

2b Plants without 1-Butanol, 4-butoxy-

(*T.tetraptera*, *L. leucocephala*)

3a Plant containing 3-Nonen-1-ol, (Z)-

T. tetraptera **2**

3b Plant without 3-Nonen-1-ol, (Z)-, but 1,1'-Biphenyl, 2,4-dichloro-2',5'-dimethyl-

L. leucocephala **3**

1b Plants without 3,7,11,15-Tetramethyl-2-hexadecen-1-ol

(*A. adianthifolia*, *P. macrophylla*, *P. africana*)

4a Plant containing 2,4,5-Trichlorophenyl propenoate

(*P. macrophylla*, *P. africana*)

5a Plant containing 4-Iodothioanisole

P. macrophylla **4**

5b Plant without 4-Iodothioanisole, but 3,5-Dihydroxybiphenyl

P. africana **5**

4b Plant without 2,4,5-Trichlorophenyl propenoate, but Phthalazin-1(2H)-one, 4-methyl-2-(4-methylphenyl)-

A. adianthifolia **6**

Alkaloids: All species except *P. biglobosa* showed the presence of alkaloids. *P. macrophylla* and *L. leucocephala* were the only species observed to have Pyrimidine, 5-bromo-2,4-bis (methylthio, 2,4(1H,3H)-Pyrimidinedione, 6-iodo-5-methyl and 1,3,4-Thiadiazole, 2,5-bis(4-aminofurazan-5-yl) in common. All other 14 alkaloids were found unique to each species. The anti oxidant, anti viral and anti bacterial properties of these two species (Akah *et al.*, 1999; Ajayi *et al.*, 2010; Iwu *et al.*, 2016; Zayed and Samling, 2016; Umaru *et al.*, 2018) could be owed partly to the occurence of the pyrimidine moiety while the efficacy of the two plants against fungal pathogens (Aderibigbe *et al.*, 2011; Zayed and Samling, 2016; Zarin *et al.*, 2016) could be owed to the actions of the Thiadiazole compound. Huge diversity of the six species in our environment could be owed much to these alkaloid compounds they contain and serve as anti - herbivory functions (Hussain et al 2017). A taxonomic key to be adopted for authenticating the six species based on alkaloid content is shown in Fig 9.

Figure 9: Taxonomic key for six mimoisoidea species based on alkaloid content:

1a Plants containing alkaloids

(*T. tetraptera, A. adianthifolia, P. macrophylla, L. leucocephala, P. africana*)

2a Plants containing Pyrimidine, 5-bromo-2,4-bis(methylthio)-

(*P. macrophylla, L. leucocephala*)

3a Plant containing Pyrimidine, 5-bromo-4,6-dimethoxy-

L. leucocephala **. 1**

3b Plant without Pyrimidine, 5-bromo-4,6-dimethoxy-, but Pyrazole-3-carboxylic acid, 4-iodo-1-methyl-

P. macrophylla **. 2**

2b Plants without Pyrimidine, 5-bromo-2,4-bis(methylthio)-

(*T. tetraptera, A. adianthifolia, P. africana*)

4a Plant containing 1-Benzyl-3-phenyl-1H-1,2,4-triazol-4-oxide

T. tetraptera **. 3**

4b Plants without 1-Benzyl-3-phenyl-1H-1,2,4-triazol-4-oxide

(*A. adianthifolia, P. africana*)

5a Plant containing 10-Dodecenol

A. adianthifolia **. 4**

5b Plant without 10-Dodecenol, but Resorcinol

P. africana **. 5**

1b Plant without alkaloids

P. biglobosa **. 6**

Ester: *P. macrophylla* was observed as species among the investigated taxa possessing an ester group. The aroma of the leaves and fruits of *P. macrophylla* could be owed to Ether, 3-butenyl propyl it contains. More importantly, this bioactive ingredient is discriminatory enough as a measure of authenticating this species.

Organic acids and fatty acids: No two species exhibited the occurrence of any of the ten (10) organic and fatty acids compounds observed, implying compound specificity for each taxon. *P. biglobosa*, *P. macrophylla* and *L. leucocephala* had three organic/fatty acids each as against *P. africana* with one. No organic/fatty acid was recorded for *A. adianthifolia* and *T. tetraptera*. Species exhibiting similar phytochemical groups are closely related (Harborne, 1973). Plants possessing organic acids have proved useful as chelating agents (Li *et al.*, 2008; Koelmel *et al.*, 2016; Speight, 2017) and soil phosphate fixers (Akintokun *et al.*, 2007; Mahdi *et al.*, 2012; Ch'ng *et al.*, 2014).
A taxonomic key to be adopted for authenticating the six species based on organic acid /fatty acids contents is shown in Fig. 10.

Figure 10: Taxonomic key for six mimoisoidea species based on organic acid/fatty acids content:

1a Plants containing organic acids and fatty acids

(*P. biglobosa, P. macrophylla, L. leucocephala, P. africana*)

2a Plant containing Hexanoic acid

P. biglobosa **1**

2b Plants without Hexanoic acid

(*P. macrophylla, L. leucocephala, P. africana*)

3a Plant containing Pentanoic acid, 2-methyl-

P. macrophylla **2**

3b Plants without Pentanoic acid, 2-methyl-

(*L. leucocephala, P. africana*)

4a Plant containing Cyclopropanecarboxylic acid, 1-amino-

L. leucocephala **3**

4b Plants without Cyclopropanecarboxylic acid, 1-amino-, but l-Leucine, N-butoxycarbonyl-, undec-10-enyl ester

P. africana **4**

1b Plants without organic acids and fatty acids

A. adianthifolia* and *T. tetraptera

Terpenes: As shown in Table 2, terpene compounds were detected in four (4) of the six (6) plant species under study but with different concentrations of 8.042% in *L. leucocephala*, 19.288% in *T. tetraptera*, 29.771% in *P. africana* and 37.424% in *P. biglobosa*. Terpenoid compounds were absent in *A. adianthifolia* and *P. macrophylla*. Neophytadiene, the dominant terpene in *P. biglobosa* and *T tetraptera* is known for its organoleptic odour and flavour properties (Akubor, 2007; Liman *et al.*, 2010). This compound may be the overarching factor for the pungent odour in these two plants (Kemigisha *et al.*, 2018). The weak musty odour for *P. africana* when compared to the pungent smell for *P. bigobosa* and *T. tetraptera* could further attests to Neophytadiene as an odour controlling compound. The reduced concentration of Neophytadiene in *P africana and its* dominant concentration in *P. bigobosa* and *T. tetraptera* observed in this study could have accounted for the odour degrees in the three species where neophytadiene were present. The unique occurrence of Trifluoroacetyl-lavandulol, 1,4-Hexadiene, 3,3,5-trimethyl and Squalene in *P. biglobosa*, *L. leucocephala* and *Prosopsis africana* respectively is discriminatory enough for species authentication as shown in the terpenoid taxonomic key for the six taxa under investigation.

Figure 11: **Terpenoid taxonomic key for the six species:**

1a Plants containing terpenes

(*P. biglobosa, T. tetraptera, L. leucocephala, P. africana*)

2a Plants containing Neophytadiene

(*P. biglobosa*, *T. tetraptera*, *P. africana*)

3a Plant containing Trifluoroacetyl-lavandulol

P. biglobosa **. 1**

3b Plants without Trifluoroacetyl-lavandulol

(*T. tetraptera*, *P. africana*)

4a Plant containing Squalene

P. africana **. 2**

4b Plant without Squalene, but neophytadiene only

T. tetraptera **. 3**

2b Plants without Neophytadiene

L. leucocephala **. 4**

1b Plants without terpenes

A. adianthifolia* and *P. macrophylla

Alkanes and Glycosides: With the exception of *Albizia adiantifolia* and *Prosopis africana*, all others had at least one alkanes/glycosidic member among the fourteen observed as present with no one species possessing all the compounds. With the exception of 9,9-Dichloro-9-silafluorene occuring in *P. macrophylla* and *L. leucocephala*, no other compound was observed in occurring in more than one species, implying thirteen Alkanes and Glycosides compounds specificity among the species. Seven compounds were observed unique to *P. biglobosa*, two to *T. tetraptera*, three to *P. macrophylla* and one to *L. leucocephala*. Possession of alkanes and glycosidic compounds is therefore an important criterion for which these species can be authenticated in field studies as shown in Fig. 12.

Figure 12: Alkanes and Glycosides taxonomic key for the six species:

1a Plants containing Alkanes and Glycosidic compounds

(*P. biglobosa, T. tetraptera, P. macrophylla, L. leucocephala*)

2a Plants containing 9,9-Dichloro-9-silafluorene

(*P. macrophylla, L. leucocephala)*

3a Plant containing Pentane, 1,2-dichloro-

P. macrophylla **1**

3b Plant without Pentane, 1,2-dichloro-, but Glucitol, O-2,3,4,6-tetra-O-methyl-_-D-galactopyranosyl-(1.fwdarw.3)-O-2,4,6-tri-O-methyl-_-D-galactopyranosy

L. leucocephala **2**

2b Plants without 9,9-Dichloro-9-silafluorene

(*P. biglobosa, T. tetraptera*)

4a Plant containing Oxirane, tetradecyl-

P. biglobosa **3**

4b Plant without Oxirane, tetradecyl-, but 1,4,7,10-Cyclododecatetraene

T. tetraptera **4**

1b Plants without alkanes and glycosidic compounds

Albizia adiantifolia* and *Prosopis afrcana

Plants possessing alkanes and glycosidic compounds have been credited with attraction of pollinators or seed dispersers and the repulsion or inhibition of herbivores, microorganisms, and livestock poisoning by toxic glycosides as in cycasin (Rhoades 1979; Charlton et al. 1992). Specifically, Musa *et al.* (2015) reported the use of oxirane, hexadecyl-, hexadecanoic acid, ethyl ester, etc. as having activities against a wide range of human pathogenic microorganisms. cyanogenic glucosides, 2- Trifluoroacetoxydodecane and Hydroperoxide, 1-methylhexyl compounds have also been reported in most species of Fabaceae.

Phenolics and Alcohols: 4-tert-Octylphenol, TMS derivative and Cyclohexaneethanol were the only phenolic and alcohol respectively found in the chemical library of the investigated species. The former was observed in *P biglobosa* while the latter was observed in *T. tetraptera*.

Amines: Six amine compounds observed in *A. adiantifolia, L leucocephala, P. biglobosa, and P. macrophylla* had the first two mentioned species contributing two compounds each. The compounds observed in *P. macrophylla* and *P. biglobosa* were of aliphatic diamines type as against polyamine and monoamine types in *L. leucocephala* and polyamine and diamine type in *A. adiantifolia.* Expression of fish-like and/or offensive odours and activities stimulating growths in plants have long been credited with the presence of monoamines, diamines and polyamines (Harborne, 1973). *A. adianthifolia* with about 25% amine contribution would act as insect attractant, a basic amine function as reported by Harborne, 1973 and Wagner *et al.*, 2008. Fig. 13 is a taxonomic key for the six species based on amine occurence.

Figure 13: Amine taxonomic key for the six species:

1a Plants containing Amine compounds

(*P. biglobosa, Albizia adiantifolia, P. macrophylla, L. leucocephala*)

2a Plant containing Propanenitrile, 3-[4-diethylamino-1-methyl-1-(1-methylethyl)-2-butynyloxy]-

P. biglobosa **1**

2b Plants without Propanenitrile, 3-[4-diethylamino-1-methyl-1-(1-methylethyl)-2-butynyloxy]-

(*Albizia adiantifolia, P. macrophylla, L. leucocephala*)

3a Plant containing benzenamine, 2,6-bis (1,1-dimethylethyl)-4-nitro-

Albizia adiantifolia **2**

3b Plants without benzenamine, 2,6-bis (1,1-dimethylethyl)-4-nitro-

(*P. macrophylla, L. leucocephala*)

4a Plant containing 4-Pyridinamine, 3,5-dibromo-

P. macrophylla **3**

4b Plant without 4-Pyridinamine, 3,5-dibromo-, but 2-Amino-6-benzyl-4-hydroxypteridine

L. leucocephala **4**

1b Plants without amine compounds

T. tetraptera* and *Prosopis afrcana

Vitamin E: α-tocopherols, β - Tocopherols and γ-tocopherols were the three Tocopherol groups observed in the study with the former present in all except *A. adiantifolia* and *T. tetraptera*. The absence of other tocopherol types except α-tocopherols in *P. biglobosa*, *P. macrophylla* and *L. leucocephala* would expose the aforementioned species to membrane fluidity and more oxygen toxicity (Asada, 2006). Presence of tocopherols in plant leafy parts could perhaps explain their usage in part as excellent fodders for herbivory and vegetables by man. The huge concentration of α-tocopherols in *Prosopsis africana* could be harnessed for skin moisturizing through neutralization of free skin radicals. The unique occurrence of β- Tocopherols and γ-tocopherols in *T. tetraptera* and *P. africana* could account for their usage as potent antioxidants, conferring protection to vulnerable lipids in biological tissues and food (Jiang *et al.*, 2001; Woollard and Indyk, 2003; Angerhofer *et al.*, 2009; Ayanwuyi *et al.*, 2010; Mariko *et al.*, 2016; Famobuwa *et al.*, 2016; Erukainure *et al.*, 2017).

Conclusion

The study observed obvious chemical fingerprints for each of the six species. The chemical marker was found effective in species authentication.

References

Aderibigbe S. A., Adetunji O. A., & Odeniyi M. A. (2011). Antimicrobial and pharmaceutical properties of the seed oil of *Leucaena leucocephala* (Lam.) De Wit (Leguminosae). *African Journal of Biomedical Research. 14*(1): 63-68

Al-Daihan, S., Al-Faham, M., Al-shawi, N., Almayman, R., Brnawi, A., Zargar, S., & Bhat, R. S. (2013). Antibacterial activity and phytochemical screening of some medicinal plants commonly used in Saudi Arabia against selected pathogenic microorganisms. *Journal of King Saud University – Science. 25*:115-120. doi:10.1016/j.jksus.2012.11.003

.

Akah, P., Aguwa, C. N., & Agu, R. (1999). Studies on the antidiarrhoeal properties of Pentaclethra macrophylla leaf extracts. *Phytotherapy Research 13*(4):292-295. doi:10.1002/(SICI)1099-1573(199906)13:4<292::AID-PTR415>3.0.CO;2-N

Ahmad, M., Khan, M. A., Zafar, M., Arshad, M., Sultana, S., Abbasi, B. H., &

Din, S. U. (2010b). Use of chemotaxonomic markers for misidentified medicinal plants used in traditional medicines. *Journal of Medicinal Plant Resources, 4*(13):1244–1252

Akintokun, A. K., Akande, G. A., Akintokun, P. O. Popoola, T. O. S., & Babalola, A. O. (2007). Solubilization of insoluble phosphate by organic acid-producing fungi isolated from Nigerian soil. *International Journal of Soil Science*. *2*: 301-307. doi:10.3923/ijss.2007.301.307

Akubor, P. I. (2007). Processing, utilization and storage properties of locust bean. (*Parkia biglobosa*) fruit pulp. A thesis submitted to the Department of Food Science and Technology, Faculty of Agriculture, University of Nigeria.

Akiyama, H., Fujii, K., Yamasaki, O., Oono, T., & Iwatsuki, K. (2001). Antibacterial action of several tannins against *Staphylococcus aureus*. J. Antimicrob. *Chemother*., 48: 487-491

Ajayi, I. A., Nwozo, S. O., Adewuyi, A. (2010). Antimicrobial activity and phytochemical screening of five selected seeds from Nigeria. *International Journal of Biomedical and Pharmaceutical Sciences. 4*(2): 104-106

Amusa, O., Adesoye, A., Ogunkanmi, A., Omoche, O., Olowe, O., Akinyosoye, S., & Omodele, T. (2014) Genetic diversity of *Parkia biglobosa* from different agroecological zones of Nigeria using RAPD markers: Hindawi Publishing Corporation: *International Journal of Biodiversity*. Article ID 457309. Retrieved from http://dx.doi.org/10.1155/2014/457309

Angerhofer, C. K., Maes, D., & Giacomoni, P. U. (2009). The use of natural compounds and botanicals in the development of anti-aging skin care products. In: An integrated approach to biochemistry and product development personal care & cosmetic technology.

Elsevier Inc. Pp 205-263. Retrieved from https://doi.org/10.1016/B978-0-8155-1584-5.50014-4

Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their function. *Plant Physiol*. *141*:391–396

Ayan, A. K., Radusiene, J., Cirak, C., Ivanauskas, L., & Janulis, V. (2009). Secondary metabolites of *Hypericum scabrum* and *Hypericum bupleuroides*. *Pharmaceutical Biology*. *47*(9)nn: 847-853. Retrieved from https://doi.org/10.1080/13880200902942436

Ayanwuyi, L. O., Yaro, A. H., & Abodunde, O. M. (2010). Analgesic and anti-inflammatory effects of the methanol stem bark extract of *Prosopis Africana. Pharmaceutical Biology, 48*(3): 296-299. doi:10.3109/13880200903121006

Baranska, M., Schultz, H., Kruger, H., & Quilitzsch, R. (2005). Chemotaxonomy of aromatic plants of the genus Origanum via vibrational spectroscopy. *Analytical Bioanalytical Chemistry 381*: 1241–1247

Butnariu, M. (2016). Methods of analysis (extraction, separation, identification and **quantification) of carotenoids from natural products. *Journal of Ecosystem & Ecography. 6*(2): 193. doi:10.4172/2157-7625.1000193**

Charlton, T. S., Marini, A. M., Markey, S.P., Norstog, K., & Duncan, M. W. (1992). Quantification of the neurotoxin 2–amino–3–(methylamino)–propanoic acid (BMAA) in Cycadales. *Phytochemistry* 31:3429–3432.

Chen, X., Zhou, L., Tian, K., Kumar, A., Singh, S., & Prior, B. A., *et al*. (2012). Metabolic engineering of *Escherichia coli*: a sustainable industrial platform for bio-based chemical production. *Biotechnol. Adv*. *31*:1200–1223.

Ch'ng, H. Y., Ahmed, O. H., & Majid, N. M. A. (2014). Improving phosphorus availability in an acid soil using organic amendments produced from agroindustrial wastes. *The Scientific World Journal.* Article ID 506356. Retrieved from http://dx.doi.org/10.1155/2014/506356

Chuang, D.Y., Chan, M. H., Zong, Y., Sheng, W., & He, Y. *et al*. (2013). Magnolia polyphenols attenuate oxidative and inflammatory responses in neurons and microglia cells. J. Neuroinflammat., Vol. 10.

Cuthbertson, D. J., Johnson, S. R., Zegarac, J. P., Kappel, J., Schafer, S., Wust, M.,…, Lange, B. M. (2013). Accurate mass-time tag library for LC/MS-based metabolite profiling of medicinal plants. Elsevier Ltd. *91*:187-197. https://doi.org/10.1016/j.phytochem.2013.02.018

Demirci, F., Paper, D. H., Franz, G., Bas¸er, K. H. C. (2004): Investigation of the *Origanum onites* L. essential oil using the chorioallantoic membrane (CAM)-assay. *J Agric Food Chem 52*: 251–254

Dewick, P. M. (2002). *Medicinal natural products, a biosynthetic approach*. John Wiley and Sons Ltd., Chichester, UK, 2nd ed., ISBN 0-471-49641- 3, 507 p.

Ebuehi, O. A., & Okorie, N. A. (2009). Phytochemical screening and quantification of **flavonoids from leaf extract of *Jatropha curcas* Linn.. *Nigerian Quarterly Journal Of Hospital Medicine. 19*(4):200-205**

Erukainure, O. L., Onifade, O. F., Odjobo, B. O., Olasehinde, T. A., Adesioye, T. A., Tugbobo-Amisu, A. O., Adenekan, S. O., Okonrokwo, G. I. (2017). Ethanol extract of *Tetrapleura tetraptera* fruit peels: Chemicalcharacterization, and antioxidant potentials against free radicals andlipid peroxidation in hepatic tissues. *Journal of Taibah University for Science. 11*: 861-867.

Famobuwa, O. E., Lajide, L., Owolabi, B. J., Osho, I. B., & Amuho, U. E. (2016). Antioxidant activity of the fruit and stem bark of *Tetrapleura tetraptera* Taub (Mimosaceae). *British Journal of Pharmaceutical Research. 9*(3): 1-4. doi:10.9734/BJPR/2016/21462

Hanus L. O., Tchilibon S., Ponde D. E., Breuer A., Fride E., & Mechoulam R. (2005). Enantiomeric cannabidiol derivatives: synthesis and binding to cannabinoid receptors. *Org. Biomol. Chem. 3*: 1116–1123. 10.1039/b416943c

Harborne, J. B. (1973). *Phytochemical methods.* Chapman and Hall Ltd, London. Pp 49-188.

Hussain, I., Khan, A. U., Ullah, R., Alsaid, M. S., Salman, S., Iftikhar, S., Marwat, G. A., Sadique, M., Jan, S., Adnan, M., & Tariq, A. (2017). Chemical composition, antioxidant and anti-bacterial potential of essential oil of medicinal plant *Isodon rugosus. Journal of Essential Oil Bearing Plants. 20*(6):1607-1613. doi: 10.1080/0972060X.2017.1407677

Igwenyi, I. O., Isiguzo, O. E., Aja, P. M., Ugwu-Okechukwu, P. C., Ezeani, N. N., & Uraku, A.J. (2015). Proximate composition, mineral content and phytochemical analysis of the african oil bean (*Pentaclethra macrophylla*) Seed. *American-Eurasian Journal Agriculture & Environmental Science*. *15*(9):1873-1875. doi:10.5829/idosi.aejaes.2015.15.9.9679

Iwashina, T. (2003). Flavonoid function and activity to plants and other organisms. *Biological Science Space*. *17*(1): 24-44.

Iwu, I. C., Onu, U. L., Okere, M. C., Ukaoma, A. A., & Azorji, J. N. (2016). GC-MS, **phytochemical and antimicrobial analysis of *Pentaclethra macrophylla* Leaf. *The International Journal of Science and Technoledge*. *4*(7): 151-159**

Kemigisha, E., Nanjebe, D., Boum, Y., Langendorf, C., Aberrane, S., Nyehangane, D., Nackers, F., Mueller, Y., Charrel, R., Murphy, R. A., Page, A. L., & Mwanga-Amumpaire, J. (2018). Antimicrobial treatment practices among Ugandan children with suspicion of central nervous system infection. *PLoS One. 13*(10) e0205316 doi:10.1371/journal.pone.0205316

Kimura, I., Yoshikawa, M., Kobayashi, S., Sugihara, Y., & Suzuki, M. *et al*. (2001). New **triterpenes, myrrhanol A and myrrhanone A, from *Guggulgum resins* and their potent anti-inflammatory effect on adjuvant-induced air-pouch granuloma of mice. *Bioorg. Med. Chem. Lett.*, 11: 985-989.**

Koelmel, J., Prasad, M. N. V., Velvizhi, G., Butti, S. K., & Mohan, S. V. (2016). Metalliferous waste in india and knowledge explosion in metal recovery techniques and processes for the prevention of pollution. In: Environmental materials and waste. *Elsevier B. V.* Pp 339-390. Retrieved from https://doi.org/10.1016/B978-0-12-803837-6.00015-9

Kumar, A. T. G., & Matthew, L. (2014). Preliminary phytochemical and pharmacognostic studies of *Helixanthera wallichiana*- an endemic plant of western ghats. *International journal of ayurvedic and herbal medicine 4*(3): 1494-1498.

Jiang, Q., Christen, S., Shigenaga, M. K., & Ames, B. N. (2001). γ-Tocopherol, the major form of vitamin E in the US diet, deserves more attention. *American Journal of Clinical Nutrition. 74*:714–22

Li, L., Nasr-El-Din, H. A., Chang, F. F., & Lindvig, T. (2008). Reaction of simple organic acids and chelating agents with calcite. International Petroleum Technology Conference, 3-5 December, Kuala Lumpur, Malaysia. Retrieved from https://doi.org/10.2523/IPTC-12886-MS

Liman, A. A., Egwin, P., Vunchi, M. A., & Ayansi, C. (2010). Lipase activity in fermented oil seeds of Africa locust bean, (*Parkia Biglobosa)*, castor seeds *(Ricinu Communis)* and African oil bean (*Pentaclethra Macrophylla). Nigerian Journal of Basic and Applied Science*. *18*(1):136-140

Lytovchenko, A., Beleggia, R., Schauer, N., Isaacson, T., Leuendorf, J. E., Hellmann, H., Rose, J. K. C., & Fernie, A. R. (2009). Application of GC-MS for the detection of lipophilic compounds in diverse plant tissues. *Plant Methods*. *5*(4). doi:10.1186/1746-4811-5-4

Mahdi, S. S., Talat, M. A., Dar, M. H., Hamid, A., & Ahmad, L. (2012). Soil phosphorus fixation chemistry and role of phosphate solubilizing bacteria in enhancing its efficiency for sustainable cropping-a review. *Journal of Pure and Applied Microbiology*. *6*(4).

McNally, D. J., Wurms, K. V., Labbe, C., Belanger, R. R. (2003). Synthesis of C-glycosyl flavonoid phytoalexins as a site-specific response to fungal penetration in cucumber. *Physiol. Mol. Plant Path. 63*(6):293-303.

Mariko, M., Sarr, S. O., Diop, A., Modi, I. A, Dackouo, B., & Diop, Y. M. (2016). Antioxidant activity study and total phenolic determination of leaf extracts of Ximenia americana L. (Olacaceae) an antitumor plant used traditionally in Mali. *Journal of Applied Biosciences. 106*:10258-10265. Retrieved from http://dx.doi.org/10.4314/jab.v106i1.5

Middleton, D. J. (2008). A chemotaxonomic survey of flavonoids and simple phenols in the leaves of *Gaultheria* L. and related genera (Ericaceae). *Botanical Journal of the Linnean Society*. *110*(4): 313-324. https://doi.org/10.1111/j.1095-8339.1992.tb00296.x

Mierziak, J., Kostyn, K., & Kulma, A. (2014). Flavonoids as important molecules of plant interactions with the environment. *Molecules. 19*: 16240-16265. doi:10.3390/molecules191016240

Musa, A. M., Ibrahim, M. A., Aliyu, A. B., Abdullahi, M. S., Tajudeen, N., Ibrahim, H., & Oyewale, A. O. (2015). Chemical composition and antimicrobial activity of hexane leaf extract of *Anisopus mannii* (Asclepiadaceae). *Journal of Intercultural Ethnopharmacology*. *4*(2).

Palma-Tenango, M., Soto-Hernández, M., Aguirre-Hernández, A. (2017). Flavonoids - from biosynthesis to human health. In: Flavonoids in agriculture. *InTech Open*. 189-201. Retrieved from http://dx.doi.org/10.5772/intechopen.68626

Rasool R., Ganai B. A., Akbar S., Kamili A. N., & Masood A. (2010). Phytochemical screening of *Prunella vulgaris* l. – an important medicinal plant of Kashmir. *Pakistan Journal of Pharmaceutical Science*. 23:399-402.

Rhoades, D. F. (1979). *Evolution of plant chemical defense against herbivores* In: Gerald A. Rosenthal and Daniel H. Janzen (eds.), Herbivores, Their interaction with secondary plant metabolites. Academic Press, New York, N.Y. Pp. 3–54.

Samanta, A., Das, G., & Das, S. K. (2011). Roles of flavonoids in plants. *International Journal of Pharmaceutical Science Technology*. *6*(1): 12-35.

Singh, R. (2016). Chemotaxonomy: a tool for plant classification. *Journal of Medicinal Plants Studies*. *4*(2):90-93

Singh, R., Geetanjali, & Singh, V. (2011). Exploring Alkaloids as inhibitors of selected enzymes. *Asian Journal of Chemistry*. *23*(2): 483-490.

Speight, J. G. (2017). Removal of inorganic compounds from the environment. In: environmental inorganic chemistry for engineers. *Elsevier B. V.* Pp 427-478. Retrieved from https://doi.org/10.1016/B978-0-12-849891-0.00009-6

Syahidah, A., Saad, C. R., Hassan, M. D., Rukayadi, Y. Norazian, M. H., & Kamarudin, M. S. (2017). Phytochemical analysis, identification and quantification of antibacterial active compounds in betel leaves, piper betle methanolic extract. *Pakistan Journal of Biological Sciences. 20*(2): 70-81. doi:10.3923/pjbs.2017.70.81

Tevini, M., Teramura, A. H. (1989). UV-B effects on terrestrial plants. *Photochem. Photobiol. 50*:479-487.

Treutter, D. (2008). Significance of flavonoids in plant resistance and enhancement of their biosynthesis. *Plant biology.* Retrieved from https://doi.org/10.1055/s-2005-873009

Umaru, I. J., Samling, B., & Umaru, H. A. (2018). Phytochemical screening of *Leucaena leucocephala* leaf essential oil and its antibacterial potentials. *MOJ Drug Design Development and Therapy. 2*(6):224–228. doi:10.15406/mojddt.2018.02.00066

Wagner, M. R., Cobbinah, J. R., & Bosu, P. P. (2008). *Forest entomology in West Tropical Africa: Forest insects of Ghana,* 2nd edition. Springer Science, Dordrecht, The Netherlands.

Wink, M. (2010a). *Functions and Biotechnology of Plant Secondary Metabolites*. Annual Plant Reviews, vol. 39. Wiley-Blackwell, Oxford.

Wink, M. (2010b). *Biochemistry of Plant Secondary Metabolism.* Annual Plant Reviews, vol. 40. Wiley-Blackwell, Oxford.

Woollard, D. C., & Indyk, H. E. (2003). Tocopherols: properties and determination. ***Encyclopedia of Food Sciences and Nutrition*, 2nd *Edition. Elsevier Science Ltd*Wink, M. (2008). Plant secondary metabolism: diversity, function and its evolution. *Natural Products Communications*. 3:1205–1216**

Yusuf, A. Z., Zakir, A., Shemau, Z., Abdullahi, M., & Halima, S. A. (2014). Phytochemical analysis of the methanol leaves extract of *Paullinia pinnata* linn. *Journal of Pharmacognosy and phytotherapy*. *6*(2): 10-16.

.Zarina, M. A., Wan, H. Y., Isha, A., Armania, N. (2016). Antioxidant, antimicrobial and cytotoxic potential of condensed tannins from *Leucaena leucocephala* hybrid-Rendang. *Food Science and Human Wellness 5*: 65–75

Zayed, M. Z., & Samling, B. (2016). Phytochemical constituents of the leaves of *Leucaena leucocephala* from Malaysia. *International Journal of Pharmacy and Pharmaceutical Sciences*. *8*(12)

.

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