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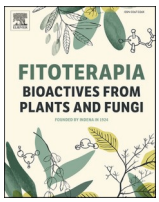

Antidiabetic and antioxidant profiling of 67 African trifoliate yam accessions by planar on-surface assays *versus in vitro* assays

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ABSTRACT

Trifoliate yam (*Dioscorea dumetorum*) is traditionally used to treat diabetics in Nigeria. However, almost no information is available on its antidiabetic constituents and their natural variance. Hence, the activity of methanolic tuber extracts of 67 trifoliate yam accessions from the largest collection in Africa was proven by four colorimetric antidiabetic and antioxidant *in vitro* assays, as diabetes is also linked with oxidative stress. For the first time, selected accessions were also analyzed by planar bioactivity profiling. It has a comparatively higher, more differentiated information content, is more sustainable in terms of material consumption, and enables straightforward compound prioritization and characterization. Up to a dozen individual antioxidant zones were revealed as well as one prominent zone inhibiting α-glucosidase and α-amylase. The latter inhibition zone was tentatively assigned to palmitic, linoleic, oleic, linolenic, oxo-nonanoic fatty acids by direct elution to heated electrospray ionization high-resolution mass spectrometry.

1. Introduction

Diabetes is classified as a civilization disease and a major health burden worldwide [1]. According to the Diabetes Atlas, there has been a continued increase in diabetes prevalence globally with approximately 537 million adults (20–79 years) living with diabetes and 6.7 million deaths caused by diabetes in 2021. An increase to 643 million by 2030 and 783 million by 2045 was also projected [2]. Diabetes is considered to be the leading cause of cardiovascular diseases, blindness, kidney failure and lower limb amputation in most high-income countries, and 3 out of every 4 adults live with diabetes in low- and middle-income countries. Different synthetic medications used to treat diabetes, such as biguanides (*e.g.*, metformin), sulfonylureas (*e.g.*, glibenclamide), thiazolidinediones (*e.g.*, rosiglitazone and pioglitazone), α-glucosidase inhibitors (*e.g.*, acarbose, miglitol and voglibose) are frequently inefficient and have side effects. Such undesirable impacts include weight gain, lactic acidosis, hepatoxicity, kidney toxicity, pancreatitis, cardiovascular diseases, hypoglycemia, and gastrointestinal symptoms such as nausea, vomiting, indigestion, diarrhea, belching, and flatulence [3–5].

Searching for more effective and safer antidiabetic agents, natural plant-based products as ethnomedicinal functional food have always been more sought after as the basis for the treatment of human diseases [6]. Medicinal plants have comparatively few side effects, contain fewer heavy metals, and show a broader distribution of molecular properties, partition coefficient, and structural diversity [7,8]. They also have more interactions with proteins, enzymes and other biological molecules and have greater molecular rigidity when compared with synthetic compounds [9]. One severely understudied plant is the trifoliate yam *Dioscorea dumetorum* (Kunth) Pax belonging to the family Dioscoreaceae. It is one of the most important plant families in the economy of many sub-Saharan African countries. With over 600 species worldwide, the tubers of many *Dioscorea* species (yams) including *D. dumetorum* are highly important for subsistence as proven by their continued cultivation for

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Table 1
Antioxidant and antidiabetic screening of the 67 *Dioscorea dumetorum* accessions by colorimetric *in vitro* assays (in bold: twelve samples selected for reproducibility study as in Table 2 and for comparison with the HPTLC−UV/Vis/FLD − EDA profiling as in Figs. 1–3).

Landraces of *Dioscorea dumetorum*				Colorimetric *in vitro* assays		<LOD: below detection limit.	
No.	Accession TDd-xxxx	Origin	Cultivar name	TPC (mg GAE/g)	DPPH• SC_{50} (mg/mL)	α-Glucosidase IC_{50} (mg/mL)	α-Amylase IC_{50} (mg/mL)
1	2788	Nigeria	Dumetorum	53 ± 0.42	1.2 ± 0.03	155 ± 0.36	4 ± 0.05
2	3093	Togo	Vote 333	165 ± 0.33	0.7 ± 0.01	150 ± 0.25	<LOD
3	3094	Togo	Ic-35	157 ± 0.46	1.6 ± 0.02	125 ± 0.23	<LOD
4	3095	Togo	Koute 281	<LOD	0.7 ± 0.01	148.3 ± 0.31	<LOD
5	3096	Togo	*D. dumentorum*	101 ± 0.34	1.4 ± 0.01	150 ± 0.23	<LOD
6	3097	Togo	Votedre 340	224 ± 0.22	0.9 ± 0.01	201 ± 0.27	<LOD
7	3098	Togo	Fa 26	174 ± 0.51	3.4 ± 0.01	129 ± 0.26	<LOD
8	3100	Togo	Dokoute 33	75 ± 0.37	<LOD	118 ± 0.24	<LOD
9	3102	Togo	Votehe 341	115 ± 0.32	1.0 ± 0.01	148 ± 0.25	<LOD
10	3103	Ghana	Kamfo	101 ± 0.27	1.4 ± 0.01	153 ± 0.24	11 ± 0.04
11	**3104**	**Togo**	**Agbote 93**	**357 ± 0.43**	**0.2 ± 0.00**	**85 ± 0.23**	**13 ± 0.03**
12	3105	Nigeria	*D. domentorum* (44)	99 ± 0.40	<LOD	210 ± 0.35	<LOD
13	3106	Togo	Agbote 19	206 ± 0.39	1.0 ± 0.01	127 ± 0.24	14 ± 0.06
14	3107	Togo	Agbote 104	182 ± 0.47	0.5 ± 0.01	111 ± 0.22	<LOD
15	3108	Ghana	Fa/89/007	163 ± 0.24	0.7 ± 0.01	163 ± 0.25	<LOD
16	3109	Ghana	Nya	160 ± 0.44	1.4 ± 0.01	190 ± 0.21	<LOD
17	3110	Togo	Agbota 457	38 ± 0.16	2.2 ± 0.01	243 ± 0.23	<LOD
18	3111	Togo	Vote 293	98 ± 0.38	2.2 ± 0.01	<LOD	<LOD
19	**3112**	**Nigeria**	***D. domentorum***	**29 ± 0.20**	**2.4 ± 0.01**	**93 ± 0.23**	<LOD
20	3113	Gabon	–	138 ± 0.27	1.2 ± 0.01	126.6 ± 0.22	<LOD
21	3114	Gabon	–	104 ± 0.25	11 ± 0.01	103 ± 0.21	<LOD
22	3686	Nigeria	Ighu(wild)	114 ± 0.36	0.8 ± 0.01	118 ± 0.21	11 ± 0.04
23	3687	Nigeria	Wild(ighu)	83 ± 0.27	1.2 ± 0.01	131 ± 0.23	<LOD
24	**3710**	**Congo**	**Isciuli**	**321 ± 0.46**	**0.1 ± 0.00**	**114 ± 0.20**	<LOD
25	3717	Congo	Assoulu cui	77 ± 0.19	2.3 ± 0.01	154 ± 0.24	<LOD
26	3770	Nigeria	–	121 ± 0.41	1.9 ± 0.01	162 ± 0.24	<LOD
27	**3771**	**Nigeria**	**–**	**279 ± 0.40**	**0.6 ± 0.01**	**73 ± 0.21**	**6 ± 0.04**
28	**3775**	**Nigeria**	**Unu-oji**	**602 ± 0.51**	**0.4 ± 0.00**	**129 ± 0.26**	**9 ± 0.05**
29	**3776**	**Nigeria**	**Obiauturugo**	**229 ± 0.39**	**0.9 ± 0.01**	**83 ± 0.23**	<LOD
30	3777	Nigeria	Oyoyo	132 ± 0.33	0.9 ± 0.01	113 ± 0.22	<LOD
31	3778	Nigeria	Ameh	63 ± 0.39	<LOD	129 ± 0.23	<LOD
32	**3779**	**Nigeria**	**Amola**	**126 ± 0.37**	**0.4 ± 0.01**	**36 ± 0.21**	**5 ± 0.05**
33	3790	Nigeria	Peeba	154 ± 0.33	2.1 ± 0.01	104 ± 0.25	<LOD
34	**3804**	**Benin**	**–**	**310 ± 0.34**	**0.5 ± 0.01**	**90 ± 0.23**	<LOD
35	3810	Benin	Assan-te	91 ± 0.28	1.5 ± 0.01	144 ± 0.27	<LOD
36	3829	Benin	Klipm-36	252 ± 0.30	0.5 ± 0.01	150 ± 0.21	<LOD
37	**3848**	**Benin**	**Leife**	**413 ± 0.46**	**0.4 ± 0.01**	**74 ± 0.21**	<LOD
38	3906	Nigeria	–	87 ± 0.37	1.5 ± 0.01	209 ± 0.37	21 ± 0.05
39	3910	Nigeria	–	456 ± 0.38	0.5 ± 0.01	126 ± 0.22	<LOD
40	3935	Benin	–	48 ± 0.18	1.2 ± 0.01	110 ± 0.22	4 ± 0.04
41	**3946**	**Nigeria**	**Esuru-funfun**	**740 ± 0.43**	**0.2 ± 0.00**	**27 ± 0.21**	<LOD
42	**3947**	**Nigeria**	**–**	**100 ± 0.35**	**2.5 ± 0.01**	**115 ± 0.25**	<LOD
43	4117	Togo	Ikafugaga	481 ± 0.44	0.6 ± 0.01	182 ± 0.24	<LOD
44	4118	Togo	N'kafo	144 ± 0.27	0.8 ± 0.01	114 ± 0.21	<LOD
Cultivated							
45	4119	Nigeria	Esuru (white)	225 ± 0.44	0.8 ± 0.01	152 ± 0.23	<LOD
46	4120	Nigeria	Esuru (white)	165 ± 0.27	1.0 ± 0.01	153 ± 0.22	<LOD
47	4122	Nigeria	Esuru	133 ± 0.31	2.7 ± 0.01	123 ± 0.23	<LOD
48	4123	Nigeria	Esuru	76 ± 0.18	1.5 ± 0.01	130 ± 0.36	<LOD
49	4124	Nigeria	Esuru	121 ± 0.39	1.5 ± 0.01	105 ± 0.22	<LOD
50	4125	Nigeria	Esuru	120 ± 0.24	1.3 ± 0.01	126 ± 0.23	<LOD
51	4126	Nigeria	Esuru	253 ± 0.35	0.8 ± 0.01	105 ± 0.22	<LOD
52	4127	Nigeria	Esuru	220 ± 0.46	0.7 ± 0.01	198 ± 0.22	<LOD
53	**4128**	**Nigeria**	**Esuru**	**156 ± 0.25**	**1.0 ± 0.01**	**35 ± 0.21**	**3 ± 0.03**
54	4129	Nigeria	Esuru	163 ± 0.38	1.4 ± 0.01	151.3 ± 0.27	<LOD
55	4130	Nigeria	Esuru	63 ± 0.24	0.9 ± 0.01	232 ± 0.26	<LOD
56	4131	Nigeria	Esuru	120 ± 0.34	0.9 ± 0.01	208 ± 0.25	<LOD
57	4132	Nigeria	Esuru	157 ± 0.38	3.2 ± 0.01	200 ± 0.26	<LOD
58	4133	Nigeria	Esuru	155 ± 0.49	1.5 ± 0.01	189 ± 0.26	<LOD
59	4134	Nigeria	Gududu (poison)	116 ± 0.32	2.3 ± 0.01	144 ± 0.27	<LOD
60	4135	Nigeria	Esuru	76 ± 0.26	2.0 ± 0.01	208 ± 0.27	<LOD
61	4136	Nigeria	Esuru	82 ± 0.36	1.7 ± 0.01	108 ± 0.22	<LOD
62	4137	Nigeria	Esuru	53 ± 0.27	2.7 ± 0.01	173 ± 0.28	<LOD
63	4138	Nigeria	Esuru	144 ± 0.36	0.8 ± 0.01	131 ± 0.25	<LOD
64	4139	Nigeria	Esuru	257 ± 0.40	0.9 ± 0.01	102 ± 0.22	<LOD
65	4140	Nigeria	Esuru	81 ± 0.43	1.2 ± 0.01	173 ± 0.25	<LOD
66	4141	Nigeria	Esuru	24 ± 0.22	4.7 ± 0.01	237 ± 0.22	<LOD
67	4142	Nigeria	Esuru	42 ± 0.29	2.4 ± 0.01	216 ± 0.25	19 ± 0.04

food and medicinal purposes in Africa [10]. In West African folk medicine practices, *D. dumetorum* is used in the treatment of several free radical-mediated diseases [11] such as hemorrhoid, measles, jaundice, fever, malaria, ulcers, scorpion bites, sexual weakness [12–14]. The alcohol and water extracts of tubers are also used to treat confirmed cases of diabetes [15,16]. Several studies have reported *in vitro* and/or *in vivo* hypoglycemic [13], antioxidant, anti-nociceptive [17], antifungal [18], anticholinesterase [19], and analgesic [20] activities of *D. dumetorum* most likely due to the presence of phenols, flavonoids, alkaloids, tannins, phytates, and oxalates. The ability of a plant extract to inhibit digestive starch-hydrolyzing enzymes (salivary and pancreatic α-amylase, or intestinal α-glucosidase) is indicative of the antidiabetic effect. Researchers have reported the general antidiabetic effects of *D. dumetorum* without narrowing it down to the specific compounds responsible [13,21–25]. So far, only one study has proposed an active compound, *i.e.* dioscoretine, as hypoglycemic agent [26].

Difficulties were faced in discovering drug compounds from plants [6,27]. Apart from tedious workflows, *in vitro* assay protocols may discriminate lipophilic compounds by removal in a defattening step or by insolubility in the polar assay medium or by adsorption to plastic material. Mixed effect mechanisms can mislead the decision taken from results of *in vitro* assays, which only provide a sum value for a complex mixture. Hence, comparing *in vitro* assays with on-surface assays is essential for sound information on bioactivity. The combination of a planar separation technique and a planar biological or enzymatic assay detection has unique benefits [28]. Planar multiplex assays allow differentiation of the various effects caused by agonists, antagonists, false-positives, and synergists [29,30]. As a straightforward hyphenation, high-performance thin-layer chromatography–effect-directed analysis (HPTLC–EDA) including multi-imaging by ultraviolet (UV), visible (Vis, white light illumination), and fluorescence detection (FLD) directly points to bioactive compound zones. This is a great assistance in deciding which specific compounds should be further characterized out of the abundance of compounds present in natural samples [31,32].

In this study, 67 African *D. dumetorum* accessions were analyzed to provide an understanding of their variability regarding total phenolic content (TPC) as well as antioxidative and antidiabetic properties across the botanical diversity. It was hypothesized that ethnomedicinal uses of *D. dumetorum* will be confirmed and support its properties as a functional food, that *in vitro* assays will at best correlate with planar assays, that bioactivity profiles of *D. dumetorum* obtained for the first time are meaningful and can be used for compound prioritization and future authenticity verification, and that a straightforward hyphenation to heated electrospray ionization high-resolution mass spectrometry (HESI-HRMS) will be able to tentatively assign prominent bioactive compounds.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were of analytical grade. Acetic acid (100 %), anisaldehyde (4-methoxy benzaldehyde) sulfuric acid (96 %), gallic acid (≥98 %), citric acid (p. a.), disodium hydrogen phosphate (≥99 %) were obtained from Carl Roth, Karlsruhe, Germany. Potassium sodium tartrate tetrahydrate was purchased from Molychem, Mumbai, India. Potato starch, sodium dihydrogen phosphate dihydrate (≥99 %), disodium hydrogen phosphate dihydrate (≥99 %), sodium carbonate, sodium hydroxide pellets (≥98 %), methanol (MS quality) and formic acid (99 %) were from VWR, Darmstadt, Germany, whereas 3,5-dinitrosalicylic acid was from Kemlight Spechem Labs, Mumbai, India (97 %). Gram's iodine solution, acarbose (for pharm.), α-glucosidase from *Saccharomyces cerevisiae* (1000 U/vial), sodium acetate, sodium chloride, α-amylase from hog pancreas (50 U/mg), α-amylase from porcine pancreas (9 U/mg), *p*-nitrophenyl-α-D-glucopyranoside (≥99 %), L-ascorbic acid, dimethyl sulfoxide, methanol (99.6 %) and ethanol (99 %) were delivered by Sigma-Aldrich, Steinheim, Germany. 2-Naphthyl-α-D-glucopyranoside (Fluorochem, Hadfield Derbyshire, UK), and Fast Blue B salt (95 %) was purchased from MP Biomedicals, Eschwege, Germany. 2,2-Diphenyl-1-picrylhydrazyl (DPPH•, 95 %) was delivered by Alfa Aesar, Schwerte, Germany. Acetonitrile (Honeywell) was from Fluka, Seelze, Germany. Ethyl acetate (≥99.8 %) was purchased from Th. Geyer, Renningen, Germany. HPTLC plates silica gel 60 F_{254} MS-grade and silica gel 60 (both 20 cm × 10 cm) were provided by Merck, Darmstadt, Germany. Folin–Ciocalteu reagent was delivered by LOBA-Chemie, Tarapur, India. Bidistilled water was produced by Purelab Chorus (ElgaVEOLIA, Woodridge, IL, USA).

2.2. Plant materials and preparation

Field-grown tubers of 67 yam accessions were harvested in December 2018, at the International Institute of Tropical Agriculture, Ibadan, Nigeria. These originate from the six African countries Nigeria, Benin, Ghana, Gabon, Togo, and Congo (Table 1), representing the genetic diversity of *Dioscorea dumetorum* in the field bank at the Genetic Resource Center of the Institute. The tubers were washed thoroughly with running tap water and peeled with a sharp knife. The flesh was sliced into thin strips, freeze-dried for 120 h (freeze dryer FreeZone, Labconco, Kansas City, MO, USA), and pulverized (50–300 mesh, speed 1, grinder HYDDNice 2500 W, Hangzhou, China). Each powder (50 g) was macerated in methanol (500 mL) at room temperature with regular stirring for 72 h. The extracts were filtered using a Buchner funnel fitted with a filter paper (185 mm diameter, Whatman, GE Healthcare, Buckinghamshire, UK) and connected to a vacuum pump. The filtrates were concentrated *in vacuo* at 40 °C and allowed to dry in the open air for 48 h. The dry extracts were stored in a refrigerator (4 °C) and dissolved in solvent or buffer as described for the respective *in vitro* assay analysis. For HPTLC, extract solutions (50 mg/mL) were prepared in ethyl acetate – ethanol – water, 1:1:1 (*V/V/V*), ultra-sonicated (10 min), and centrifuged (17,000 x*g*, 5 min).

2.3. TPC cuvette assay

The TPC of the extracts was determined by the colorimetric Folin–Ciocalteu assay [33]. Briefly, 2.5 mL Folin–Ciocalteu reagent – bidistilled water (1,10, *V/V*) was mixed with 0.5 mL extract solution (1 mg/mL in methanol) and 2 mL of sodium carbonate solution (7.5 g/L in bidistilled water). The resultant mixture was incubated at 25 °C for 30 min and the absorbance of the resulting blue-colored complex was measured at 765 nm in a cuvette spectrophotometer (UV-3100PC, VWR International, Radnor, PA, USA). The higher the amount of reducing phenolic compounds, the deeper the intensity of the blue-colored complex formed [34]. A calibration curve (6.25–200 μg/mL, $y = 9.766\times + 0.0538$, $R^2 = 0.9978$) was constructed for gallic acid (6.25, 12.5, 25, 50, 100, and 200 μg/mL in methanol). TPC of the plant extracts was expressed as milligrams gallic acid equivalent (GAE) per g of extract.

2.4. DPPH• scavenging cuvette assay

In the colorimetric assay, the antioxidant activity of the extracts was determined by their ability to scavenge the stable DPPH• [35,36]. Briefly, six 2-mL extract solutions (ranged 15, 30, 60, 125, 250, and 500 μg/mL in methanol) were mixed each with 3 mL DPPH• solution (freshly prepared, 0.1 mM in methanol) and incubated at 25 °C for 30 min in the dark. The deep purple color of the DPPH• changes to yellow in the presence of antioxidant compounds [37]. The absorbance of the purple color was measured at 517 nm in the UV-3100PC cuvette spectrophotometer. The negative control was methanol in equal volume instead of extract.

2.5. α-glucosidase inhibition microtiter plate assay

The colorimetric assay was carried out as described [38]. Briefly, five 20-µL extract solutions (ranged 50, 40, 30, 20 and 10 mg/mL in dimethyl sulfoxide – distilled water, 1:1, *V/V*) were placed in a 96 well plate and 50 µL α-glucosidase (1 U/mL) in 100 mM phosphate buffer (pH 6.9) were added. Each mixture was incubated 37 °C for 10 min, after which 30 µL substrate solution (5 mM *p*-nitrophenyl-α-D-glucopyranoside in 0.1 M sodium phosphate buffer of pH 6.9) were added and incubated at 37 °C for 30 min. The absorbance of the yellow color (formed nitrophenol) was measured at 405 nm at 37 °C in a microplate reader (Infinite 200 PRO, Tecan, Switzerland). Sample blanks were prepared as described [39] to quantify the absorbance contributed due to colored and/or turbidity. The positive control was acarbose (ranged 50, 40, 30, 20 and 10 mg/mL in dimethyl sulfoxide – distilled water, 1:1, *V/V*). The negative control contained dimethyl sulfoxide and distilled water in equal volume instead of extract.

2.6. α-amylase inhibition microtiter plate assay

The colorimetric assay was carried out as described [40,41]. Briefly, five 50-µL extract solutions (ranged 50, 25, 12.5, 6.25, 3.125 mg/mL in a 4:1 mixture, *V/V,* of methanol and 0.02 M sodium phosphate buffer of pH 6.9 containing 0.006 M sodium chloride) were placed in a 96-well plate and 50 µL α-amylase solution (0.5 mg/mL in the mentioned buffer) were added. Each mixture was incubated at 25 °C for 10 min, after which 50 µL starch solution (1 % in the mentioned buffer) were added and again incubated at 25 °C for 10 min. The reaction was terminated by adding 100 µL of dinitrosalicylic acid reagent (96 mM 3,5-dinitrosalicylic acid, 5.31 M sodium potassium tartrate in 2 N sodium hydroxide) to each mixture, which was then heated at 100 °C (boiling water vapor) for 10 min, cooled down to room temperature, and diluted with 100 µL distilled water. The absorbance of the red color (formed 3-amino-5-nitrosalicylic acid) was measured at 540 nm in a microplate reader (LT-4500, Labtech, Uckfield, UK). The positive control was acarbose (1 mg/mL in methanol – mentioned buffer, 4,1, *V/V*). The negative control was 80 % methanol in equal volume instead of extract.

2.7. Calculations and statistical analysis

The *in vitro* assay measurements were carried out in triplicates and the results are expressed as the mean including its standard error. The percentage inhibition was calculated as absorbance difference (negative control minus sample) times 100 divided by the absorbance of the negative control. The 50 % scavenging (SC_{50}) or 50 % inhibition concentrations (IC_{50}) were determined graphically from dose-response curves. The individual sample results were compared by one-way analysis of variance (ANOVA) using the R Statistical Package (Version 4.1.2, R Foundation, Vienna, Austria), whereby significant differences were compared by Duncan's multiple range test in R. Pearson's correlation analysis was performed using SAS (Version 9.4, SAS Institute, Cary, NC, USA) to assess the correlation between assays considered significant for $p < 0.05$.

2.8. HPTLC-UV/Vis/FLD method

The extracts (3 µL/band, but 1.5 µL/band for enzyme inhibition assays) were applied as bands on a prewashed (with methanol-water, 4:1 (*V/V*), and dried at 100 °C for 20 min) HPTLC plate silica gel 60 F_{254} (Automatic TLC Sampler 4, CAMAG, Muttenz, Switzerland). The HPTLC separation was performed in a twin trough chamber (20 × 10 cm, CAMAG) using 6 mL mobile phase up to a migration distance of 70 mm. Acetonitrile – water 4:1 (*V/V*) was used as a polar mobile phase system (plates after derivatization and DPPH• assay), whereas ethyl acetate – *n*-hexane 7:5 (*V/V*) was applied as an apolar mobile phase system (used for both enzyme assays). The ambient air had 20–33 % relative humidity depending on the day. Each chromatogram was dried in a cold air stream (hair dryer) and detected at Vis, UV 254 nm, and FLD 366 nm (TLC Visualizer, CAMAG).

2.8.1. Detection via derivatization reagent

The chromatogram was derivatized with the anisaldehyde sulfuric acid reagent (1.5 mL 4-methoxy benzaldehyde in 210 mL methanol, 25 mL acetic acid, and 13 mL concentrated sulfuric acid added dropwise) *via* piezoelectrical spraying (3 mL, blue nozzle, level 5), followed by heating at 110 °C and 170 °C each for 3 min (TLC Plate Heater, CAMAG), accompanied by detection at Vis and FLD 366 nm.

2.8.2. Detection via planar DPPH• assay

The positive control (0.125 mg/mL gallic acid in ethanol, 0.5, 1.3 and 2.0 µL/band) was applied on the upper right plate edge, followed by drying for 0.5 min. The chromatogram was treated with the DPPH• assay reagent (0.04 % DPPH• in methanol containing 10 % ethanol and 10 mM sodium chloride, 9,1, *V/V*) *via* piezoelectrical spraying (3 mL, green or blue nozzle, level 5) followed by plate heating at 60 °C for 1 min and detection at Vis (repeated after one day as zones can partially be more intense).

2.8.3. Detection via planar α-glucosidase inhibition assay

The positive control (3 mg/mL acarbose in methanol; 1, 3, and 6 µL/band) was applied on the upper right plate edge, followed by drying for 0.5 min. The substrate solution (12 mg 2-naphthyl-α-D-glucopyranoside in 9 mL ethanol and 1 mL 10 mM aqueous sodium chloride solution) was sprayed piezoelectrically on the chromatogram (2 mL, yellow nozzle, level 6), and dried for 2 min. Then, α-glucosidase solution (10 U/mL in sodium acetate buffer pH 7.5) was sprayed onto the chromatogram (2 mL, yellow nozzle, level 6), followed by incubation at 37 °C for 15 min. Finally, the chromogenic reagent solution (4 mg/mL Fast blue B salt in water) was sprayed piezoelectrically on the chromatogram (0.5 mL, red nozzle, level 6) followed by drying at 50 °C for 3 min and detection at Vis.

2.8.4. Detection via planar α-amylase inhibition assay

The positive control (0.1 mg/mL acarbose in methanol; 0.3, 0.6, and 0.9 µL/band) was applied on the upper right plate edge, followed by drying for 0.5 min. The α-amylase solution (62.5 U/mL in sodium acetate buffer pH 7.5) was piezoelectrically sprayed onto the chromatogram (2 mL, red nozzle, level 5), followed by incubation at 37 °C for 30 min. Then, the substrate solution (2 % soluble starch solution in water) was piezoelectrically sprayed (1 mL, red nozzle, level 5) onto the chromatogram, followed by incubation at 37 °C for 20 min. For visualization, Gram's iodine solution was piezoelectrically sprayed (500 µL, yellow nozzle, level 5), followed by plate heating and detection at Vis.

2.9. Zone characterization via HESI-HRMS

Zones of interest were eluted with methanol at a flow rate of 0.1 mL/min. The TLC-MS Interface with an oval elution head (CAMAG) and inline C18 guard cartridge (SecurityGuard cartridge, Phenomenex, Torrance, CA, USA) was connected to the pump (Ultimate HPG-3200SD standard binary pump, Dionex Softron, Germering, Germany) and the heated electrospray ionization source (Ion-Max HESI-II probe Q) of the Exactive Plus Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific, Dreieich, Germany) with the following ionization parameters set: sheath gas 20 AU, aux gas 10 AU, spray voltage ±3.5 kV, capillary temperature 270 °C, probe heater temperature 200 °C and S-lens RF level 50 AU. HRMS spectra were recorded as full scan (*m/z* 50–750) in the positive and negative ionization mode with a resolution of 280,000 full widths at half-maximum (FWHM) at *m/z* 200 with the following settings: automatic gain control (AGC) target 3e6, maximum inject time 100 ms, without lock mass injection. External mass calibration was performed weekly (using Pierce LTQ Velos ESI positive and negative ion

Table 2
Reproducibility of the antioxidant and antidiabetic activities of twelve selected *Dioscorea dumetorum* accessions (Table 1) by colorimetric *in vitro* assays.

Landraces			Colorimetric *in vitro* assays			
			Antioxidant activity		Antidiabetic activity	
No.	Accession TDd-xxxx	Origin	TPC (mg GAE/g)	DPPH• SC_{50} (mg/mL)	α-Glucosidase IC_{50} (mg/mL)	α-Amylase IC_{50} (mg/mL)
11	3104	Togo	357 ± 0.43^{d}	0.2 ± 0.00^{k}	85 ± 0.23^{f}	13 ± 0.03^{a}
19	3112	Nigeria	29 ± 0.20^{l}	2.4 ± 0.01^{b}	93 ± 0.23^{d}	<LOD
24	3710	Congo	321 ± 0.46^{e}	0.1 ± 0.00^{l}	114 ± 0.19^{c}	<LOD
27	3771	Nigeria	279 ± 0.40^{g}	0.6 ± 0.01^{e}	73 ± 0.21^{i}	6 ± 0.04c
28	3775	Nigeria	602 ± 0.51^{b}	0.3 ± 0.00^{i}	129 ± 0.26^{a}	9 ± 0.05^{b}
29	3776	Nigeria	229 ± 0.39^{h}	0.8 ± 0.01^{d}	83 ± 0.23^{g}	<LOD
32	3779	Nigeria	126 ± 0.37^{j}	0.4 ± 0.01^{h}	36 ± 0.21^{j}	5 ± 0.05^{d}
34	3804	Benin	310 ± 0.34^{f}	0.4 ± 0.01^{f}	90 ± 0.23^{e}	<LOD
37	3848	Benin	413 ± 0.46^{c}	0.4 ± 0.01^{g}	74 ± 0.21^{h}	<LOD
41	3946	Nigeria	740 ± 0.43^{a}	0.2 ± 0.00^{j}	27 ± 0.21^{l}	<LOD
42	3947	Nigeria	100 ± 0.35^{k}	2.5 ± 0.01^{a}	115 ± 0.25^{b}	<LOD
53	4128	Nigeria	156 ± 0.25^{i}	1.0 ± 0.01^{c}	35 ± 0.21^{k}	3 ± 0.03^{e}

Values with the same letter are not significantly different. < LOD: below detection limit.

calibration solution, Thermo Scientific). The instruments were operated by Xcalibur 4.2.47 with Foundation 3.1.261.0 and SII for Xcalibur 1.5.0.10747, and spectra processing was performed using Qual Browser Excalibur 3.0.63 (all Thermo Scientific). Mass spectra of the plate background were recorded and subtracted from the analyte spectrum.

3. Results and discussion

The 67 African trifoliate yam accessions (Table 1) of the largest collection of *D. dumetorum* in Africa were grown and harvested in December 2018. Methanolic tuber extracts were prepared. Their TPC as well as the DPPH• scavenging, α-amylase, and α-glucosidase inhibition properties were analyzed using colorimetric *in vitro* assays. Based on these results of the colorimetric *in vitro* assays, twelve accessions (Table 2) were selected for HPTLC–EDA profiling. The method protocol of the latter had to be developed first. These effect-directed techniques were used to clarify the rationale for ethnomedicinal applications, how much the accessions differ in terms of antioxidant and antidiabetic activities, whether the results of the *in vitro* assays are consistent with the HPTLC-EDA profiles, whether the bioactivity profiles can be meaningful, and whether prominent bioactive compounds can be tentatively assigned.

3.1. TPC cuvette assay results

The TPC was analyzed using the Folin−Ciocalteu method (colorimetric assay), which measures the reducing activity of the sample. The Folin−Ciocalteu reagent reacts with all reducing molecules, considered to be mostly phenolic compounds, and thus is ought to resemble the TPC. The TPC varied considerably among the accessions ranging from 24 to 740 mg GAE/g extract with accession 3946 having the highest phenolic content and accession 4141 the lowest (Table 1). There was no geographic bias in the TPC of the accessions, since those samples that showed the highest total phenolic content (TPC ≥ 300 mg GAE/g) originated from different countries. There was a significant difference in the TPC across the accessions (Table 1). Phenolic compounds, such as phenolic acids, flavonoids, tannins, and polyphenols that make up the phenolic content of the accessions, have been reported to be the most abundant antioxidants in medicinal plants. These can also express various bioactivities including antidiabetic, anti-inflammatory, and anti-cancer. Some plant-derived antidiabetic phenolic compounds include kaempferitin, isoscutellarein, vitexin, isovitexin, and isorhamnetin rutinoside [42–47]. The Folin–Ciocalteu assay could also be seen as a measure of total antioxidant capacity and not just phenolic content since the Folin–Ciocalteu reagent also reacts with other antioxidants other than phenolics [34,48]. These other non-phenolic antioxidants include proteins, vitamins, amines, aldehydes and ketones, carbohydrates, thiols, and unsaturated fatty acids among others.

3.2. DPPH• radical scavenging cuvette assay results

The samples showed a dose-dependent DPPH• activity. The SC_{50} (the value is inversely proportional to the extract activity and concentration that will neutralize 50 % of the oxidative potential of DPPH•) ranged from 0.1 mg/mL for accession 3710 to 2.5 mg/mL for accession 3947 (Table 1) when compared with that of the standard ascorbic acid (0.02 mg/mL). Accession 3946, which had the highest TPC, also had a relatively low SC_{50} of 0.2 mg/mL (third lowest), which indicates a high antioxidant activity. A similar SC_{50} (0.1 mg/mL) was recently reported for the DPPH• scavenging activity of the methanol extract of *D. dumetorum* tubers collected in Nigeria [49]. Consequently, this indicated that the reducing activity of phenolic compounds in the accessions studied seems to contribute to their corresponding strong radical scavenging activities. The general DPPH• scavenging activity of *Dioscorea* species has been reported previously [50–56]. Specific phenolic compounds, such as chrysin, quercetin rhamnoside, quercitrin, catechin, epicatechin, hyperin, procyanidin B2, quercetin arabinofuranoside, and hydroxyphloretin were detected in several plants [57,58]. The antioxidative activity of polyphenolic compounds was also reported as a mechanism by which they exhibited antidiabetic effects [59].

3.3. α-glucosidase inhibition microtiter plate assay

The results revealed that all samples exhibited a dose-dependent inhibition of the α-glucosidase with IC_{50} ranging from 27 mg/mL to 129 mg/mL (Table 1). The IC_{50} of the Nigerian accessions 3946 (27 mg/mL) and 4128 (35 mg/mL) elicited the most potent effects, compared to extracts of other accessions and the positive control acarbose (63 mg/mL). The high TPC (740 mg GAE/g) and α-glucosidase inhibition of accession 3946 are consistent with the trends in previous studies [60], in which the α-glucosidase inhibitory activity of medicinal plants was attributed to being a function of their phenolic content. Other studies have also established the presence of α-glucosidase inhibitors in tubers of *D. dumetorum* [21] and attributed this activity in extracts of *Dioscorea* species to their high phenolic content [61]. However, this correlation is not necessarily valid, suggesting that the phenolic compounds are not mainly responsible for their anti-diabetic effect as given in the two following examples. The extract from accession 4128 (IC_{50} 35 mg/mL) showed the second-highest antidiabetic activity but had a low TPC (156

Table 3
Pearson's correlation coefficients (*r;* *significant correlation at $p < 0.05$) of the TPC and of enzyme inhibition activities, all analyzed by *in vitro* assays.

Parameters	TPC	DPPH• scavenging	α-Glucosidase inhibition
TPC	1		
DPPH• scavenging	−0.6516*	1	
α-Glucosidase inhibition	−0.0611	0.30915	1

mg GAE/g). Accession 3775 with a high TPC (602 mg/mL) showed a low α-glucosidase inhibition.

The majority (75 %) of the selected accessions showed an inhibition of the α-glucosidase ($IC_{50} \leq 100$ mg/mL). In literature, the IC_{50} of acarbose (71 mg/mL) at the same enzyme concentration is comparable [38]. However, with acarbose, severe gastrointestinal side effects were observed, such as flatulence, diarrhea, meteorism and abdominal distention [62–66]. Hence, the inhibition of the α-glucosidase *via* natural multi-component mixtures such as *D. dumetorum* is of pharmacological importance to substitute synthetic α-glucosidase inhibitors with side effects, such as acarbose, miglitol, and voglibose.

3.4. α-amylase inhibition microtiter plate assay

The majority (82 %) of the selected accessions showed no inhibition of porcine pancreatic α-amylase. The results revealed that 9 samples exhibited a dose-dependent inhibition of α-amylase with IC_{50} ranging from 3 mg/mL to 23 mg/mL while three accessions exhibited a dose-independent inhibition (Table 1). Eight samples that showed activity originate from Nigeria, two from Togo and one each from Benin and Ghana (Table 1). The positive control acarbose exhibited a dose-dependent inhibition of the pancreatic α-amylase with an IC_{50} of 0.05 mg/mL and a percentage inhibition range between 75 % at 1 mg/mL and 45 % at 0.03 mg/mL. The results supported previous studies that found acarbose at the same concentration of 1 mg/mL to show 57 % [67] and 66 % [66] inhibition of pancreatic α-amylase. Accession 4128 which showed high inhibition of α-glucosidase (IC_{50} 35 mg/mL) elicited the most potent α-amylase inhibition (IC_{50} 3 mg/mL), followed by accessions 3935 and 2788 (both with IC_{50} 4 mg/mL). This suggests that the antidiabetic compound present in accession 4128 inhibits both α-amylase and α-glucosidase and is not a phenolic compound since it had a low TPC (156 mg GAE/g). The accessions 3779, 3771, 3775, 3104, which showed high inhibition of α-glucosidase, also showed antidiabetic activity *via* inhibition of α-amylase. However, accession 3946, which showed the highest inhibition of α-glucosidase (IC_{50} 27 mg/mL), did not inhibit α-amylase. Extracts from accessions 3935 (IC_{50} 4 mg/mL), 2788 (IC_{50} 4 mg/mL), 3686 (IC_{50} 11 mg/mL), 3103 (IC_{50} 11 mg/mL), 3106 (IC_{50} 14 mg/mL), 4142 (IC_{50} 19 mg/mL) and 3906 (IC_{50} 21 mg/mL) showed inhibition of α-amylase despite having low TPC, DPPH• scavenging activity and α-glucosidase inhibition. There has been little research into the inhibitory effects of *D. dumetorum* tuber *in vitro* [21,68], whereas several studies have established its antidiabetic effect *in vivo* [13,21,25,69]. Previous studies have also established a higher inhibitory effect on α-glucosidase than on α-amylase in *D. dumetorum* tuber [21] and other plants [64]. However, the inhibitory effects of other *Dioscorea* species on these two key diabetes-related enzymes have been established [50,61,70].

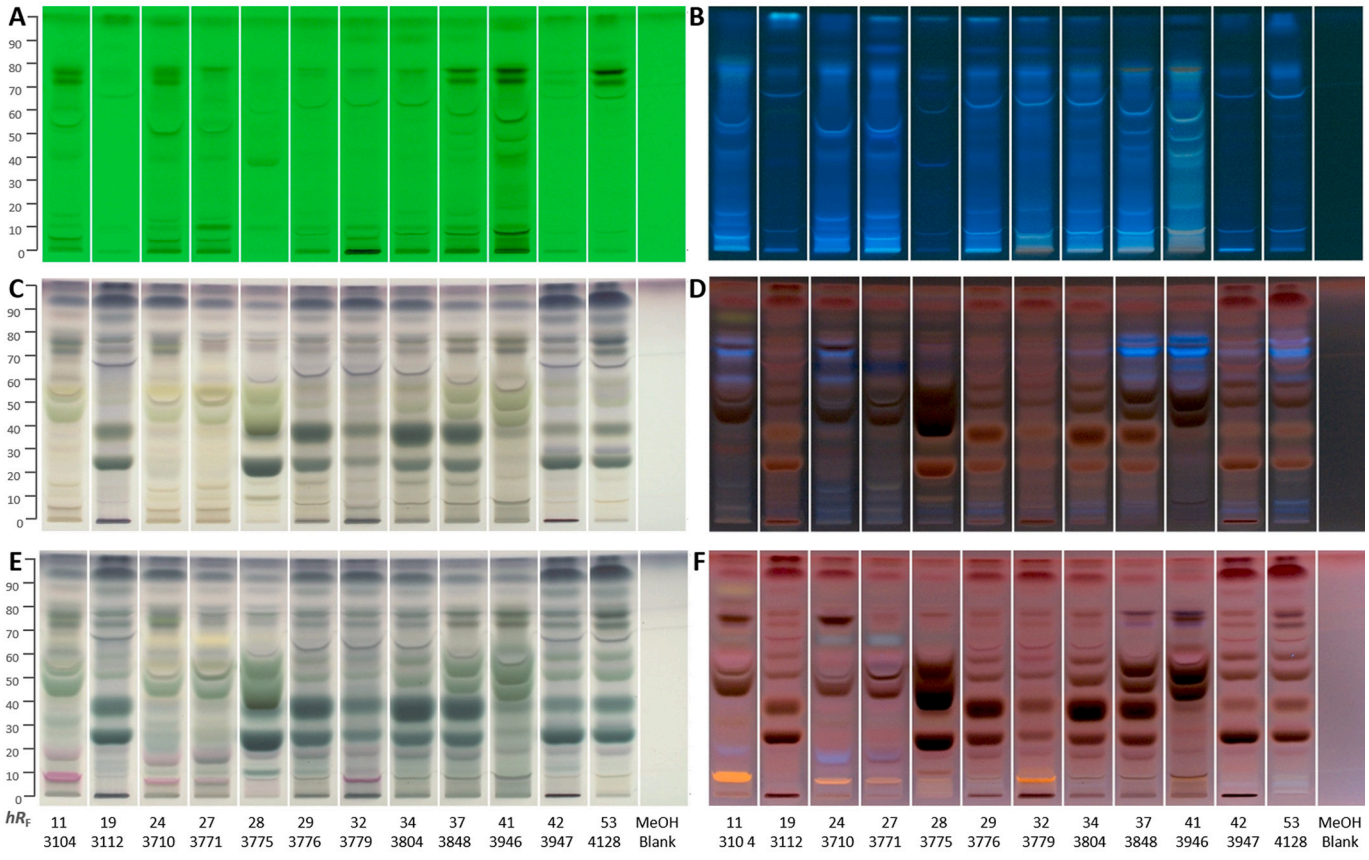

Fig. 1. HPTLC–UV/Vis/FLD profiles of twelve selected *Dioscorea dumetorum* accessions (Table 2, 150 μg/band, 3 μL/band of 50 mg/mL extract solution, solvent blank for comparison) applied on the HPTLC plate silica gel 60 F_{254} with acetonitrile – water 4:1 (*V/V*) up to 70 mm, detected at (**A**) UV 254 nm, (**B**) FLD 366 nm, and (**C/D**) after derivatization with anisaldehyde sulfuric acid reagent, followed by plate heating at 110 °C and then (**E/F**) 170 °C at white light illumination (C/E, reflectance/transmission mode, white balance adjusted) and FLD 366 nm (D/F).

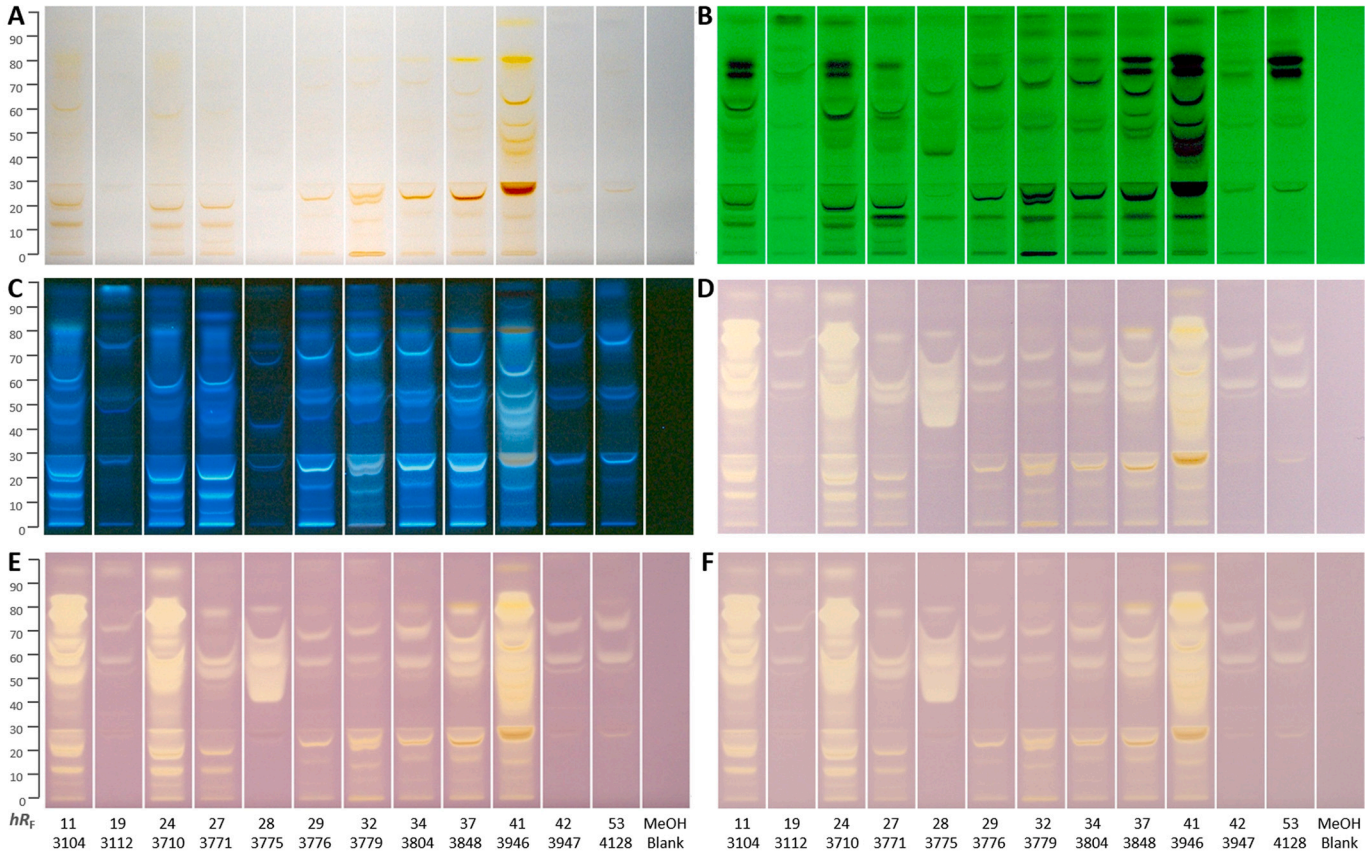

Fig. 2. HPTLC–UV/Vis/FLD – DPPH• profiles of twelve selected *Dioscorea dumetorum* accessions (Table 2, 150 μg/band, 3 μL/band of 50 mg/mL extract solution, solvent blank for comparison) applied on the HPTLC plate silica gel 60 F_{254} with acetonitrile – water 4:1 (*V*/V) up to 70 mm, and then 3:1 up to 30 mm to better separate the polar antioxidants, detected at (**A**) Vis, (**B**) UV 254 nm (enhanced), (**C**) FLD 366 nm (enhanced), and (**D** – **F**) after the DPPH• assay at white light illumination *via* (**D**) reflectance mode, (**E**) transmission mode and (**F**) both latter modes.

3.5. Correlation analysis of the colorimetric in vitro assay results

A significantly negative ($r = -0.6516$, $p < 0.05$) correlation was observed between TPC and DPPH• scavenging activity (Table 3) through Pearson's correlation matrix. This means that an increase in the TPC decreases the IC_{50} values, connoting high antioxidant activity. This corroborates the use of both assays as a measurement of antioxidant activity of plant extracts in previous reports since the magnitude of IC_{50} of DPPH• scavenging activity is inversely proportional to the antioxidant activity of samples, while TPC values have a direct proportion to antioxidant capacity. The observed correlation confirms that all DPPH• scavenging constituents in the phenolic compounds act in synergy to make up the overall antioxidant activity of the extracts. In contrast, the α-glucosidase inhibition assay had no significant associations with the TPC ($r = -0.0611$) and the antioxidant activity expressed as DPPH• scavenging activity ($r = 0.3091$) of the samples.

3.6. Development of the HPTLC method

Based on the *in vitro* results, twelve accessions (Table 2) were selected for HPTLC–EDA profiling, which was performed for the first time in this study. Different mobile phase systems were studied on HPTLC plates silica gel 60 F_{254} for the intended effect-directed profiling. Among the tested ones, acetonitrile – water 4:1 (*V*/*V*) allowed the best polar separation. This mobile phase system was used later for the derivatization experiment (Fig. 1) and DPPH• assay (Fig. 2). As an apolar mobile phase system, the mixture ethyl acetate – *n*-hexane 7:5 (*V*/*V*) was selected and used later for both enzyme assays since a more apolar highly active compound zone was observed (Fig. 3). Both mobile phases took only about 23 min and allowed for fast sustainable profiling in the polar and apolar substance range. Only about 0.5 mL solvent per sample was consumed for each analysis. These developed chromatographic systems were found to be a good start for chemical profiling as well as non-target effect-directed profiling.

3.6.1. Chemical profiling at UV/Vis/FLD including derivatization

The UV/Vis/FLD multi-imaging of the polar HPTLC chromatograms revealed UV-absorbing (Fig. 1A) and mainly blue fluorescent (Fig. 1B) compound bands, which were spread along the migration distance. Accessions 3104, 3710, 3848, 3946, and 4128 with comparatively darker UV-absorbing bands (Fig. 1A) also expressed high TPCs. Previous studies have shown blue fluorescent bands (attributable to plant acids) to be indicative of phenolic compounds [71,72]. In previous studies, phenolic and flavonoids were said to be present in *D. dumetorum* but specific compounds were not identified [49,73–75]. Among the tested derivatization reagents, the anisaldehyde sulfuric acid reagent (Fig. 1C–F) detected most organic compounds. The previously mentioned dark UV-absorbing bands turned blue fluorescent (Fig. 1D) when the plate was heated at 110 °C. Heating the same chromatogram up to 170 °C turned them into a rusty color (Fig. 1F). Additional pink bands were revealed for accessions 3104, 3710, 3771, and 3779 under white light illumination (Fig. 1E), expressed as prominent orange-colored fluorescent bands when viewed at FLD 366 nm (Fig. 1F). All in all, the obtained chemical profiles of *D. dumetorum* are meaningful and can be considered for future authenticity proofs or as reference profiles. Especially the chemical derivatization proved that *D. dumetorum* is rich in phytochemicals, and it was wondered which are the important bioactive ones.

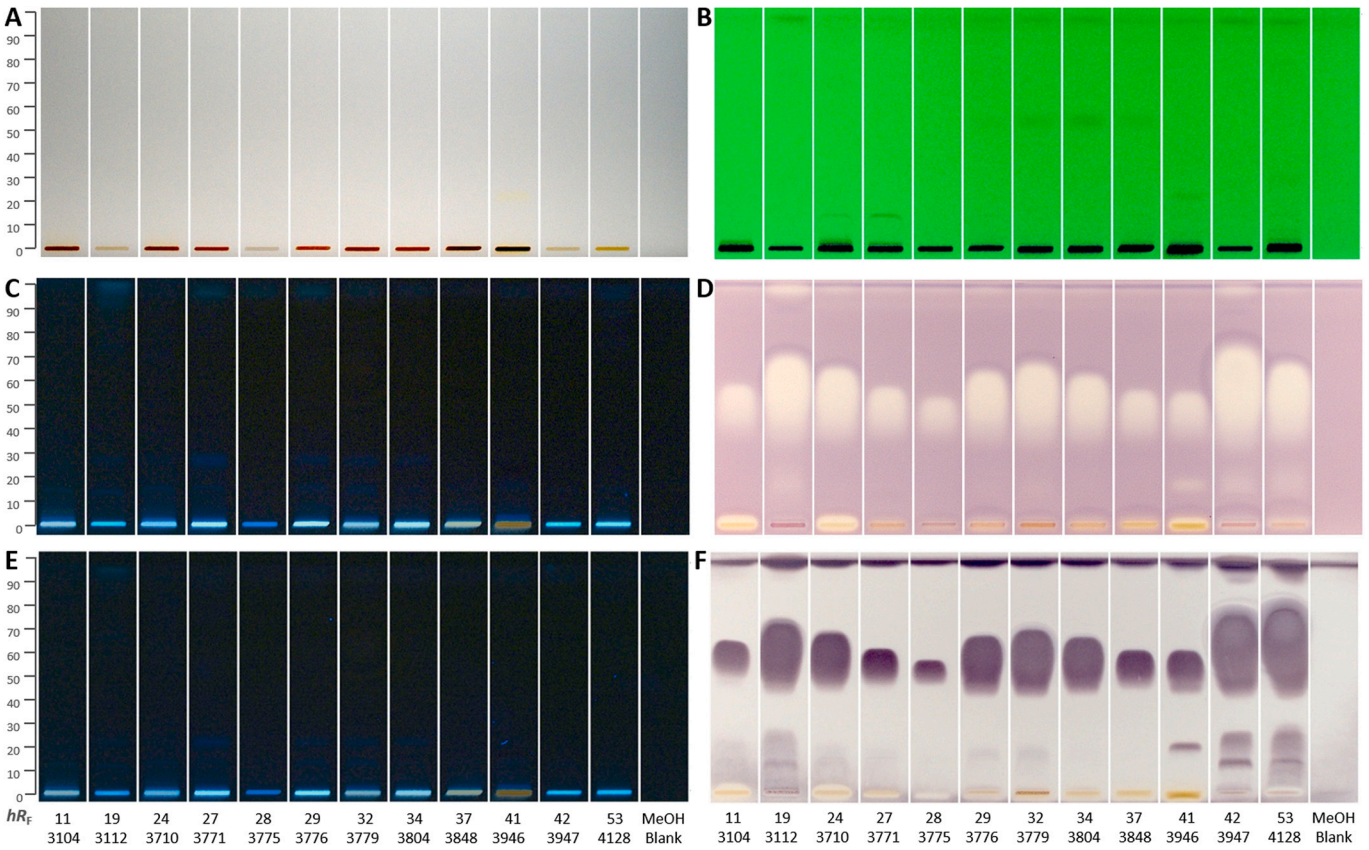

Fig. 3. HPTLC–UV/Vis/FLD – α-glucosidase/α-amylase inhibition profiles of twelve selected *Dioscorea dumetorum* accessions (Table 2, 75 μg/band, 1.5 μL/band of 50 mg/mL extract solution, solvent blank for comparison) applied on the HPTLC plate silica gel 60 F_{254} with ethyl acetate – *n*-hexane 7:5 (V/V) up to 70 mm, detected at (**A**) Vis, (**B**) UV 254 nm, (**C**/**E**) FLD 366 nm, and after the respective (**D**) α-glucosidase and (**F**) α-amylase inhibition assays at white light illumination (reflectance mode; for α-amylase, white balance adjusted).

3.6.2. *HPTLC–DPPH• assay–Vis profiles*

Characteristic antioxidant profiles of *D. dumetorum* were observed. The HPTLC–DPPH• assay–Vis autogram revealed DPPH• scavenging compounds as yellow bands on a purple background under white light illumination (Figs. 2D–F). The previously mentioned pink-colored and orange fluorescent bands in the HPTLC chromatogram derivatized with the anisaldehyde sulfuric acid reagent (Fig. 1E/F) showed no scavenging activity. However, many other natively blue fluorescent compounds (indicating plant acids among others) showed antioxidant activity. The number (up to a dozen compounds per sample) and intensity (from weak to very intense) of the antioxidant compounds substantially differed between the accessions. There was no profile correlation between the accessions and their countries of origin. The results align with what was obtained previously from the cuvette assay as the accessions with the highest SC_{50} (lowest activity) also showed the fewest and least intense bands (Fig. 2D–F; 3947, 4128, and 3112). Our findings are confirmed by several studies reporting the presence of DPPH• scavenging compounds in *D. dumetorum via in vitro* and *in vivo* assays [17,69,74].

3.6.3. *HPTLC–α-glucosidase inhibition assay–Vis profiles*

The polar mobile phase revealed α-glucosidase inhibitors only in the mobile phase front, and thus an apolar mobile phase system was developed and used for this assay. In contrast to the polar separation system, the compounds detected at UV/Vis/FLD (Fig. 3A–C) remained at the start zone due to the weak solvent strength of the apolar mobile phase system. Characteristic α-glucosidase inhibition profiles of *D. dumetorum* are revealed for the first time in this study. The autogram showed α-glucosidase inhibitors as colorless zones on a purple background under white light illumination (Fig. 3D). One prominent intense α-glucosidase inhibitor was detected in all accessions. This suggests the presence of the same bioactive compound that can be a characteristic signature antidiabetic compound of *D. dumetorum* and could be responsible for the inhibitory activities obtained previously from the *in vitro* assay. Depending on the sample, up to two further but substantially weaker inhibitors were observed, apart from some compounds that also showed a weak response and remained in the start zone. There was also no relationship between the variation of enzyme inhibition across the samples and their origin. The accessions 3112, 3710, 3776, 3779, 3804, 3947 and 4128 with the highest response correlated with high activities (ranged from 34.9 mg/mL for 4128 to 115 mg/mL for 3947) which was observed by the colorimetric microtiter plate assay. Our finding is in line with previous studies in which α-glucosidase inhibitors were reported in tuber of *D. dumetorum*, but without assignment to any particular compound [21].

3.6.4. *HPTLC–α-amylase inhibition assay–Vis profiles*

Analogously, the polar mobile phase did reveal inhibitors only in the mobile phase front, and the previous apolar mobile phase system was used also for this assay (Fig. 3E). Characteristic α-amylase inhibition profiles of *D. dumetorum* are revealed for the first time in this study. The autogram showed α-amylase inhibitors as dark purple zones on a bright background under white light illumination (Fig. 3F). Similar to the previous α-glucosidase inhibition profile, the same prominent intense α-glucosidase and now also α-amylase inhibitor was detected in all accessions, since the horizontal intensity pattern of the compounds zones across all accessions was the same. Identical horizontal compound patterns are a clear indication that the same compound is present. Again, accessions 3112, 3710, 3776, 3779, 3804, 3947 and 4128 showed the highest response. This correlated with the inhibition activity observed by the colorimetric microtiter plate assay for accessions 3779 and 4128

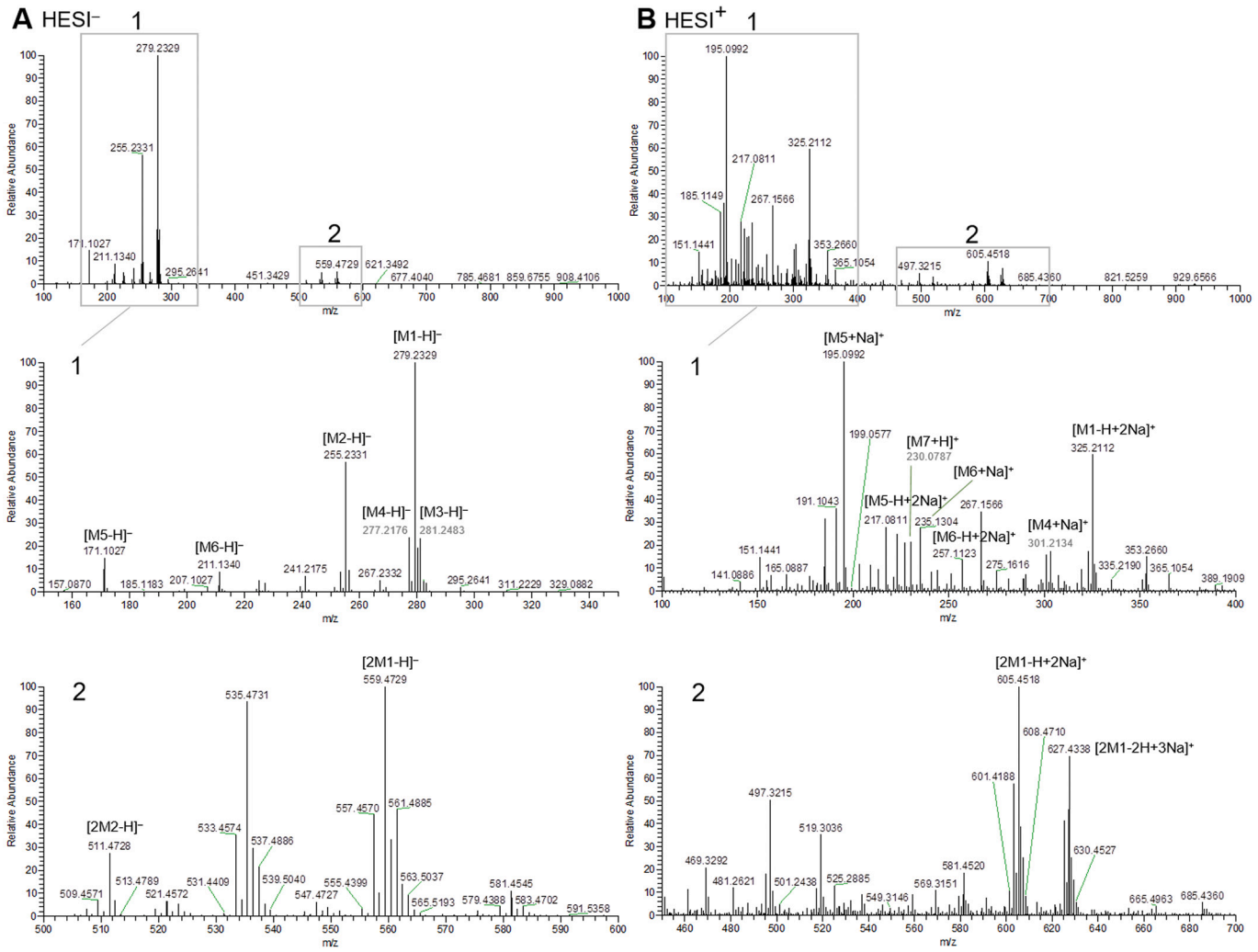

Fig. 4. HPTLC–HESI-HRMS spectra in the (**A**) negative and (**B**) positive ionization mode of the prominent zone inhibiting the α-glucosidase and α-amylase, exemplarily shown for accession 3848 (75 µg/band, 1.5 µL/band of 50 mg/mL extract solution).

but not for 3112, 3710, 3776, 3804, and 3947 which were not observable (below detection limit) by the colorimetric microtiter plate assay. This discrepancy was explained by solubility problems of this obviously very apolar compound in the highly polar bioassay medium or adsorption to plastic material (polystyrene) of the *in vitro* assay since the true concentration of this very apolar extract part was not analytically determined. In contrast to the previous α-glucosidase inhibition assay containing dimethyl sulfoxide as solubilizer, the α-amylase inhibition assay did not contain such a solubilizer and discriminated the more lipophilic compounds present. This demonstrated that the planar assays were more robust regarding solubility issues than the corresponding *in vitro* assays. Although most of the samples did not exhibit α-amylase inhibition in the colorimetric microtiter plate assay, the observed HPTLC data clearly indicate the potential of *D. dumetorum* as a source of antidiabetic α-amylase inhibitors. There was also no relationship between the variation of enzyme inhibition across the samples and their origin. Our results confirm the findings from previous studies [21,68,76], which already described the presence of α-amylase inhibitors (phenolic compounds and phytates) in tubers of *D. dumetorum* but without assignment to any particular compound.

3.6.5. *Characterization of the prominent antidiabetic compound zone*

The prominent antidiabetic compound zone (Fig. 3D/F) was not visible (Fig. 3A), not UV-active (Fig. 3B), and not fluorescent (Fig. 3C), but got colored greyish-blue after derivatization with the anisaldehyde sulfuric acid reagent (Fig. 1C/E, hR_F 93 in the polar mobile phase system). HPTLC–HESI-HRMS spectra (Fig. 4) were recorded from the prominent antidiabetic zone using the apolar separation due to the better zone resolution and less interference from other compounds (left at the start zone). The mass signals at *m/z* 559.4729 [2M1-H]$^-$ (Δ ppm 0.5), *m/z* 279.2329 [M1-H]$^-$ (Δ ppm 0.2), *m/z* 627.4338 [2M1-2H+3Na]$^+$ (Δ ppm −1.3), *m/z* 605.4518 [2M1-H+2Na]$^+$ (Δ ppm −0.3), and *m/z* 325.2112 [M1-H+2Na]$^+$ (Δ ppm 0.5) tentatively indicated linoleic acid ($C_{18}H_{32}O_2$). Further mass signals revealed the coelution of further fatty acids in the same zone, which was expected in a normal phase separation system. Tentatively, palmitic acid ($C_{16}H_{32}O_2$) at *m/z* 511.4728 [2M2-H]$^-$ (Δ ppm 0.7) and *m/z* 255.2331 [M2-H]$^-$ (Δ ppm −0.6), oleic acid ($C_{18}H_{34}O_2$) at *m/z* 281.2483 [M3-H]$^-$ (Δ ppm 1.2), and linolenic acid ($C_{18}H_{30}O_2$) at *m/z* 277.2176 [M4-H]$^-$ (Δ ppm −0.9) and *m/z* 301.2134 [M4+Na]$^+$ (Δ ppm 0.3) were assigned. This tentative assignment to fatty acids is consistent with the earlier hypothesis that the α-amylase and α-glucosidase inhibiting compound was not a phenolic compound because it did not correlate with the TPC. This HPTLC–HESI-HRMS results were in accordance with other studies showing the anti-diabetic effect for individual fatty acids [77–80]. Another oxidized structure evident at *m/z* 171.1027 [M5-H]$^-$ (Δ ppm −0.4), *m/z* 217.0811 [M5-H+2Na]$^+$ (Δ ppm 0.1) and *m/z* 195.0992 [M5+Na]$^+$ (Δ ppm −0.2) was tentatively assigned to oxo-nonanoic acid ($C_9H_{16}O_3$). Further unassigned oxidized structures, such as $C_{12}H_{20}O_3$ at *m/z* 257.1123 [M6-H+2Na]$^+$ (Δ ppm 0.6), *m/z* 235.1304 [M6+Na]$^+$ (Δ

ppm 0.3) and *m/z* 211.1340 $[M6\text{-}H]^-$ (Δ ppm −0.4), and $C_{10}H_{13}O_6$ at *m/z* 230.0787 $[M7+H]^+$ (Δ ppm −1.0), were detected. Oxidized structures with comparatively low HRMS signal intensity can nevertheless have an impact on the bioactivity of samples [81] and are worth to mention.

4. Conclusions

This study provided new insights into the bioactivity of methanolic tuber extracts from 67 different African trifoliate yam accessions, which showed clear differences in the antioxidant and antidiabetic activities. For the first time, characteristic antidiabetic bioactivity profiles were obtained which are helpful as authentic reference profiles for further food processing. Up to a dozen individual antioxidants were revealed as well as one prominent α-glucosidase and α-amylase inhibiting compound zone. The HPTLC−HRMS exemplarily allowed the straightforward characterization and tentative assignment of the latter zone to be coeluting fatty acids. This represents substantial progress in understanding the importance of African trifoliate yam in ethnomedicinal diabetes treatment and could explain the mechanisms by which they lower blood sugar levels. It also suggests it as source of functional food.

The HPTLC−EDA analysis was performed miniaturized on the same planar surface for many samples in parallel, from screening to effect detection and tentative molecular formula assignment. It allowed for the prioritization of compounds, including lipophilic compounds, and provided more differentiated information. The workflow was comparatively faster, simpler, more sustainable and less laborious. The results of *in vitro* assays and planar on-surface assays correlated to a high degree. However, in the colorimetric microtiter plate assay, α-amylase inhibition was comparatively very weak if not absent, which was explained by solubility problems of this obviously very apolar fatty acid structures in the highly polar bioassay medium, containing no dimethyl sulfoxide as solubilizer and thus discriminating the more lipophilic compounds present.

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Compliance with ethical standards

The research did not involve human participants and/or animals.

Declaration of generative AI and AI-assisted technologies in the writing process

Generative AI and AI-assisted technologies were not used during the preparation of this work.

CRediT authorship contribution statement

Priscilla O. Aiyedun: Writing – original draft, Investigation, Formal analysis. **Mubo A. Sonibare:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Badara Gueye:** Writing – review & editing, Supervision, Resources, Methodology. **Dirk C. Albach:** Writing – review & editing, Supervision, Resources, Methodology. **Julia Heil:** Investigation. **Gertrud E. Morlock:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] V.G. Clatici, C. Voicu, C. Voaides, A. Roseanu, M. Icriverzi, S. Jurcoane, Diseases of civilization - cancer, diabetes, obesity and acne - the implication of Milk, IGF-1 and mTORC1, Maedica 13 (4) (2018) 273–281, https://doi.org/10.26574/maedica.2018.13.4.273.
[2] IDF, International Diabetes Federation, in: Diabetes Atlas, 10th ed., 2021, https://doi.org/10.1016/j.diabres.2013.10.013.
[3] J.-H. He, L.-X. Chen, H. Li, Progress in the discovery of naturally occurring antidiabetic drugs and in the identification of their molecular targets, Fitoterapia 134 (2019) 270–289, https://doi.org/10.1016/j.fitote.2019.02.033.
[4] J. Park, H.-J. Jang, Anti-diabetic effects of natural products an overview of therapeutic strategies, Mol. Cell. Toxicol. 13 (1) (2017) 1–20, https://doi.org/10.1007/s13273-017-0001-1.
[5] P.M. Thulé, Mechanisms of current therapies for diabetes mellitus type 2, Adv. Physiol. Educ. 36 (4) (2012) 275–283, https://doi.org/10.1152/advan.00094.2012.
[6] M. Lahlou, Screening of natural products for drug discovery, Expert Opin. Drug Discov. 2 (5) (2007) 697–705, https://doi.org/10.1517/17460441.2.5.697.
[7] G.E. Morlock, J. Heil, V. Bardot, L. Lenoir, C. Cotte, M. Dubourdeaux, Effect-directed profiling of 17 different fortified plant extracts by high-performance thin-layer chromatography combined with six planar assays and high-resolution mass spectrometry, Molecules 26 (5) (2021), https://doi.org/10.3390/molecules26051468.
[8] T. Schreiner, G.E. Morlock, Non-target bioanalytical eight-dimensional hyphenation including bioassay, heart-cut trapping, online desalting, orthogonal separations and mass spectrometry, J. Chromatogr. A 1647 (2021) 462154, https://doi.org/10.1016/j.chroma.2021.462154.
[9] S. Mathur, C. Hoskins, Drug development: lessons from nature (review), Biomed. Rep. 6 (6) (2017) 612–614, https://doi.org/10.3892/br.2017.909.
[10] M. Tamiru, H.C. Becker, B.L. Maass, Diversity, distribution and management of yam landraces (Dioscorea spp.) in Southern Ethiopia, Genet. Resour. Crop. Evol. 55 (1) (2008) 115–131, https://doi.org/10.1007/s10722-007-9219-4.
[11] V. Lobo, A. Patil, A. Phatak, N. Chandra, Free radicals, antioxidants and functional foods: impact on human health, Pharmacogn. Rev. 4 (8) (2010) 118–126, https://doi.org/10.4103/0973-7847.70902.
[12] I.P. Dike, O.O. Obembe, F.E. Adebiyi, Ethnobotanical survey for potential antimalarial plants in South-Western Nigeria, J. Ethnopharmacol. 144 (3) (2012) 618–626, https://doi.org/10.1016/j.jep.2012.10.002.
[13] M.M. Iwu, C.O. Okunji, G.O. Ohiaeri, P. Akah, D. Corley, M.S. Tempesta, Hypoglycaemic activity of dioscoretine from tubers of Dioscorea dumetorum in normal and alloxan diabetic rabbits, Planta Med. 56 (3) (1990) 264–267, https://doi.org/10.1055/s-2006-960952.
[14] J. Laly, D.S.J.C. Gbemavo, A.A. Gbaguidi, I. Dossou-Aminon, A. Dansi, Dioscorea dumetorum (Kunth) Pax, a neglected and underutilized yam species in Benin: folk classification and post-harvest conservation, Genet. Resour. Crop. Evol. 66 (4) (2019) 965–979, https://doi.org/10.1007/s10722-019-00762-0.
[15] P.I. Akubue, G.C. Mittal, Clinical evaluation of a traditional herbal practice in Nigeria: a preliminary report, J. Ethnopharmacol. 6 (3) (1982) 355–359, https://doi.org/10.1016/0378-8741(82)90056-3.
[16] M.M. Iwu. Traditional igbo medicine, Institute of African Studies, University of Nigeria, Nsukka, 1983, p. 75.
[17] C. Ukwueze, S. Onoja, M. Ezeja, Experimental evaluation of analgesic and antioxidant effects of hydromethanolic extract of Dioscorea dumetorum Tuber, J. Adv. Med. Pharm. Sci. 3 (3) (2015) 131–137, https://doi.org/10.9734/jamps/2015/18117.
[18] C. Ifediba, U. Okezie, A. Onyegbule, T. Gugu, E. Chukwudiegwu, M. Ugwu, Antifungal Activity of the Methanol Tuber Extract of Dioscorea Dumetorum (Pax), 2017, pp. 376–380.
[19] S.O. Salawu, P.B. Ajiboye, A.A. Akindahunsi, A.A. Boligon, Antioxidant and anticholinesterase potential of two Nigerian bitter yams using a simulated gastrointestinal digestion model and conventional extraction, Prevent. Nutr. Food Sci. 22 (2) (2017) 107–117, https://doi.org/10.3746/pnf.2017.22.2.107.
[20] A.O. Christy, In vivo evaluation of analgesic activities of Dioscorea dumetorum (Kunth) Pax and Tragia benthamii Bak, Scholars Int. J. Tradit. Complement. Med. 6 (2) (2023) 18–22, https://doi.org/10.36348/sijtcm.2023.v06i02.001.
[21] B.C. Adedayo, B.M. Okeke, S.I. Oyeleye, G. Oboh, Biokemistri, 2018. June.
[22] R. Nimenibo-Uadia, Control of hyperlipidaemia, hypercholesterolaemia and hyperketonaemia by aqueous extract of Dioscorea dumetorum tuber, Trop. J. Pharm. Res. 2 (1) (2003) 183–189, https://doi.org/10.4314/tjpr.v2i1.14584.
[23] H.A. Ogbunugafor, E.E. Ilodigwe, D.L. Ajaghaku, C.N. Ezekwesili, C.S. Okafor, C. F. Ajuzieogu, S.U. Madunatum, Dioscorea dumetorum-fed rats exhibited decreased body weight, blood glucose, and insulin in STZ-induced diabetes, Funct. Foods Health Dis. 4 (2) (2014) 87–97, https://doi.org/10.31989/ffhd.v4i2.23.

[24] A.T. Okolie, C.E. Umeyor, C.D. Nwakile, I. Okoye, T.C. Okeke, E.M. Uronnachi, Formulation design and evaluation of the physicotechnical and hypoglycaemic properties of tablets containing Dioscorea dumetorum fraction in alloxanized diabetic rats, J. Med. Herbs 12 (3) (2021) 49–59.

[25] A.S. Undie, P.I. Akubue, Pharmacological evaluation of Dioscorea dumetorum tuber used in traditional antidiabetic therapy, J. Ethnopharmacol. 15 (2) (1986) 133–144, https://doi.org/10.1016/0378-8741(86)90150-9.

[26] M.M. Iwu, C. Okunji, M.S. Tempesta, D. Corley, Letters Dioscoretine : The Hypoglycemic Principle of *Dioscorea dumetorum* 1, 1988, pp. 0–1.

[27] M. Lahlou, The success of natural products in drug discovery, Pharmacol. Pharm. 04 (03) (2013) 17–31, https://doi.org/10.4236/pp.2013.43a003.

[28] G.E. Morlock, Chromatography combined with bioassays and other hyphenations - the direct link to the compound indicating the effect, in: ACS Symposium Series vol. 1185, American Chemical Society, 2014, pp. 101–121, https://doi.org/10.1021/bk-2014-1185.ch005.

[29] A. Ronzheimer, T. Schreiner, G.E. Morlock, Multiplex planar bioassay detecting estrogens, antiestrogens, false-positives and synergists as sharp zones on normal phase, Phytomedicine 103 (2022) 154230, https://doi.org/10.1016/j.phymed.2022.154230.

[30] T. Schreiner, A. Ronzheimer, M. Friz, G.E. Morlock, Multiplex planar bioassay with reduced diffusion on normal phase, identifying androgens, verified antiandrogens and synergists in botanicals via 12D hyphenation, Food Chem. 395 (2022) 133610, https://doi.org/10.1016/j.foodchem.2022.133610.

[31] M.K. Islam, T. Sostaric, L.Y. Lim, K. Hammer, C. Locher, A validated method for the quantitative determination of sugars in honey using high-performance thin-layer chromatography, JPC – J. Planar Chromatogr. – Mod. TLC 33 (5) (2020) 489–499, https://doi.org/10.1007/s00764-020-00054-9.

[32] G.E. Morlock, High-performance thin-layer chromatography combined with effect-directed assays and high-resolution mass spectrometry as an emerging hyphenated technology: a tutorial review, Anal. Chim. Acta 1180 (2021) 338644, https://doi.org/10.1016/j.aca.2021.338644.

[33] I.O. Oresanya, M.A. Sonibare, B. Gueye, F.O. Balogun, S. Adebayo, A.O.T. Ashafa, G. Morlock, Isolation of flavonoids from Musa acuminata Colla (Simili radjah, ABB) and the in vitro inhibitory effects of its leaf and fruit fractions on free radicals, acetylcholinesterase, 15-lipoxygenase, and carbohydrate hydrolyzing enzymes, J. Food Biochem. 44 (3) (2020) e13137, https://doi.org/10.1111/jfbc.13137.

[34] J.D. Everette, Q.M. Bryant, A.M. Green, Y.A. Abbey, G.W. Wangila, R.B. Walker, Thorough study of reactivity of various compound classes toward the Folin-Ciocalteu reagent, J. Agric. Food Chem. 58 (14) (2010) 8139–8144, https://doi.org/10.1021/jf1005935.

[35] I.O. Ayoola-Oresanya, M.A. Sonibare, B. Gueye, R. Paliwal, M.T. Abberton, G. E. Morlock, Effect-directed profiling and identification of bioactive metabolites from field, in vitro-grown and acclimatized Musa spp. accessions using high-performance thin-layer chromatography-mass spectrometry, J. Chromatogr. A 1616 (2020) 460774, https://doi.org/10.1016/j.chroma.2019.460774.

[36] D. Susanti, H.M. Sirat, F. Ahmad, R.M. Ali, N. Aimi, M. Kitajima, Antioxidant and cytotoxic flavonoids from the flowers of Melastoma malabathricum L, Food Chem. 103 (3) (2007) 710–716, https://doi.org/10.1016/j.foodchem.2006.09.011.

[37] Z. Akar, M. Küçük, H. Doğan, A new colorimetric DPPH(•) scavenging activity method with no need for a spectrophotometer applied on synthetic and natural antioxidants and medicinal herbs, J. Enzyme Inhib. Med. Chem. 32 (1) (2017) 640–647, https://doi.org/10.1080/14756366.2017.1284068.

[38] R. Agada, W.A. Usman, S. Shehu, D. Thagariki, In vitro and in vivo inhibitory effects of Carica papaya seed on α-amylase and α-glucosidase enzymes, Heliyon 6 (3) (2020) e03618, https://doi.org/10.1016/j.heliyon.2020.e03618.

[39] C. Lankatillake, S. Luo, M. Flavel, G.B. Lenon, H. Gill, T. Huynh, D.A. Dias, Screening natural product extracts for potential enzyme inhibitors: protocols, and the standardisation of the usage of blanks in α-amylase, α-glucosidase and lipase assays, Plant Methods 17 (1) (2021) 3, https://doi.org/10.1186/s13007-020-00702-5.

[40] H. Gok, S. Pekacar, D. Deliorman Orhan, Investigation of enzyme inhibitory activities, antioxidant activities, and chemical properties of Pistacia vera leaves using LC-QTOF-MS and RP-HPLC, Iran. J. Pharm. Res. 21 (2022), https://doi.org/10.5812/ijpr-127033.

[41] N.Z. Msomi, Antidiabetic activity of *Warburgia salutaris* (Bertol. f.) Chiov. (Canellaceae). https://core.ac.uk/download/pdf/200989379.pdf, 2017.

[42] R. Ayad, S. Akkal, Chapter 12 - Phytochemistry and biological activities of algerian Centaurea and related genera, in: N.P.C. Atta-Ur-Rahman (Ed.), Bioactive Natural Products vol. 63, Elsevier, 2019, pp. 357–414, https://doi.org/10.1016/B978-0-12-817901-7.00012-5.

[43] L. Bai, X. Li, L. He, Y. Zheng, H. Lu, J. Li, L. Zhong, R. Tong, Z. Jiang, J. Shi, J. Li, Antidiabetic potential of flavonoids from traditional Chinese medicine: a review, Am. J. Chin. Med. 47 (05) (2019) 933–957, https://doi.org/10.1142/S0192415X19500496.

[44] L.A. de la Rosa, J.O. Moreno-Escamilla, J. Rodrigo-García, E. Alvarez-Parrilla, in: E. M. B. T.-P. P. and B. of F, V. Yahia (Eds.), Chapter 12 - Phenolic Compounds, Woodhead Publishing, 2019, pp. 253–271, https://doi.org/10.1016/B978-0-12-813278-4.00012-9.

[45] V.B. Tatipamula, B. Kukavica, Phenolic compounds as antidiabetic, anti-inflammatory, and anticancer agents and improvement of their bioavailability by liposomes, Cell Biochem. Funct. 39 (8) (2021) 926–944, https://doi.org/10.1002/cbf.3667.

[46] D. Tungmunnithum, A. Thongboonyou, A. Pholboon, A. Yangsabai, Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview, Medicines (Basel, Switzerland) 5 (3) (2018) 93, https://doi.org/10.3390/medicines5030093.

[47] A. Umeno, M. Horie, K. Murotomi, Y. Nakajima, Y. Yoshida, Antioxidative and antidiabetic effects of natural polyphenols and isoflavones, Molecules (Basel, Switzerland) 21 (6) (2016), https://doi.org/10.3390/molecules21060708.

[48] R.L. Prior, X. Wu, K. Schaich, Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements, J. Agric. Food Chem. 53 (10) (2005) 4290–4302, https://doi.org/10.1021/jf0502698.

[49] A.A. Adeniran, M.A. Sonibare, In vitro antioxidant activity, brine shrimp lethality and assessment of bioactive constituents of three wild Dioscorea species, J. Food Meas. Charact. 11 (2) (2017) 685–695, https://doi.org/10.1007/s11694-016-9438-5.

[50] J.B. Adeloye, P.A. Aluko, T.D. Oluwajuyitan, In vitro α-amylase and α-glucosidase inhibitory activities, antioxidant activity, in vivo glycemic response and nutritional quality of dough meals from Dioscorea alata and Vernonia amygdalina, J. Food Meas. Charact. 15 (5) (2021) 4083–4097, https://doi.org/10.1007/s11694-021-00965-z.

[51] S. Ghosh, A. Derle, M. Ahire, P. More, S. Jagtap, S.D. Phadatare, A.B. Patil, A. M. Jabgunde, G.K. Sharma, V.S. Shinde, K. Pardesi, D.D. Dhavale, B.A. Chopade, Phytochemical analysis and free radical scavenging activity of medicinal plants Gnidia glauca and Dioscorea bulbifera, PLoS One 8 (12) (2013) e82529, https://doi.org/10.1371/journal.pone.0082529.

[52] F.B. Mukeba, Phytochemical study and anti-nutritional factors in stems of Dioscorea praehensilis benth (Dioscoreaceae), Discov. Phytomed. 8 (3) (2022) 2021. http://phytomedicine.ejournals.ca/index.php/phytomedicine/article/view/178.

[53] O. Odeghe, E. Adikwu, C. Ojiego, Phytochemical and antioxidant assessments of Dioscorea bulbifera stem tuber, Biomed. Biotechnol. Res. J. (BBRJ) 4 (4) (2020) 305–311, https://doi.org/10.4103/bbrj.bbrj_96_20.

[54] B. Padhan, J.K. Nayak, D. Panda, Natural antioxidant potential of selected underutilized wild yams (Dioscorea spp.) for health benefit, J. Food Sci. Technol. 57 (6) (2020) 2370–2376, https://doi.org/10.1007/s13197-020-04470-x.

[55] G. Sakthidevi, V.R. Mohan, Total phenolic, flavonoid contents and in vitro antioxidant activity of Dioscorea alata l. Tuber, J. Pharm. Sci. Res. 5 (5) (2013) 115–119.

[56] A.N. Ukom, P.C. Ojimelukwe, C.F. Ezeama, D.O. Ortiz, I. Aragon, Phenolic content and antioxidant activity of some under-utilized Nigerian yam (Dioscorea spp.) and cocoyam (Xanthosomamaffa (scoth)) tubers, IOSR J. Environ. Sci. Toxicol. Food Technol. 8 (7) (2014) 104–111, https://doi.org/10.9790/2402-0871104111.

[57] Q. Hu, Y.-Y. Chen, Q.-Y. Jiao, A. Khan, J. Shan, G.-D. Cao, F. Li, C. Zhang, H.-X. Lou, Polyphenolic compounds from Malus hupehensis and their free radical scavenging effects, Nat. Prod. Res. 32 (18) (2018) 2152–2158, https://doi.org/10.1080/14786419.2017.1367784.

[58] Z. Xiao, Y. Zhang, X. Chen, Y. Wang, W. Chen, Q. Xu, P. Li, F. Ma, Extraction, identification, and antioxidant and anticancer tests of seven dihydrochalcones from Malus 'red splendor' fruit, Food Chem. 231 (2017) 324–331, https://doi.org/10.1016/j.foodchem.2017.03.111.

[59] T. Nie, G.J.S. Cooper, Mechanisms underlying the antidiabetic activities of polyphenolic compounds: a review, Front. Pharmacol. 12 (2021), https://doi.org/10.3389/fphar.2021.798329.

[60] A. Mohammed, N. Tajuddeen, Antidiabetic compounds from medicinal plants traditionally used for the treatment of diabetes in Africa: a review update (2015–2020), S. Afr. J. Bot. 146 (2022) 585–602, https://doi.org/10.1016/j.sajb.2021.11.018.

[61] X. Guo, X. Sha, J. Liu, S. Cai, Y. Wang, B. Ji, Chinese purple yam (Dioscorea alata L.) extracts inhibit diabetes-related enzymes and protect HepG2 cells against oxidative stress and insulin resistance induced by FFA, Food Sci. Technol. Res. 21 (5) (2015) 677–683, https://doi.org/10.3136/fstr.21.677.

[62] H.B.B. Bischoff, Pharmacology of α-glucosidase inhibition, Eur. J. Clin. Investig. 24 (S3) (1994) 3–10, https://doi.org/10.1111/j.1365-2362.1994.tb02249.x.

[63] M. Dehghan-Kooshkghazi, J.C. Mathers, Starch digestion, large-bowel fermentation and intestinal mucosal cell proliferation in rats treated with the α-glucosidase inhibitor acarbose, Br. J. Nutr. 91 (3) (2004) 357–365, https://doi.org/10.1079/BJN20031063.

[64] G.-N. Kim, Y.-I. Kwon, H.-D. Jang, Mulberry leaf extract reduces postprandial hyperglycemia with few side effects by inhibiting α-glucosidase in normal rats, J. Med. Food 14 (7–8) (2011) 712–717, https://doi.org/10.1089/jmf.2010.1368.

[65] K.-H. Lee, K.-S. Ha, S.-H. Jo, C.M. Lee, Y.-C. Kim, K.-H. Chung, Y.-I. Kwon, Effect of long-term dietary arginyl-fructose (AF) on hyperglycemia and HbA1c in diabetic db/db mice, Int. J. Mol. Sci. 15 (5) (2014), https://doi.org/10.3390/ijms15058352.

[66] G. Oboh, O.B. Ogunsuyi, M.D. Ogunbadejo, S.A. Adefegha, Influence of gallic acid on α-amylase and α-glucosidase inhibitory properties of acarbose, J. Food Drug Anal. 24 (3) (2016) 627–634, https://doi.org/10.1016/j.jfda.2016.03.003.

[67] Gaurav, S. Zahiruddin, B. Parveen, M. Ibrahim, I. Sharma, S. Sharma, A.K. Sharma, R. Parveen, S. Ahmad, TLC-MS bioautography-based identification of free-radical scavenging, α-amylase, and α-glucosidase inhibitor compounds of antidiabetic tablet BGR-34, ACS Omega 5 (46) (2020) 29688–29697, https://doi.org/10.1021/acsomega.0c02995.

[68] G.N. Medoua, I. Lape Mbome, T. Agbor-Egbe, C.M.F. Mbofung, Antinutritional factors changes occurring in trifoliate yam (Dioscorea dumetorum) tubers after harvest, Food Chem. 102 (3) (2007) 716–720, https://doi.org/10.1016/j.foodchem.2006.06.005.

[69] F. Bukatuka, K. Ngombe, K. Mutwale, B. Moni, K. Makengo, L. Pambu, N. Bongo, M. Mbombo, M. Musuyu, U. Maloueki, K.-T.-N. Ngbolua, F. Mbemba, Bioactivity and nutritional values of some Dioscorea species traditionally used as medicinal foods in Bandundu, DR Congo, Eur. J. Med. Plants 14 (1) (2016) 1–11, https://doi.org/10.9734/ejmp/2016/25124.

[70] B.C. Adedayo, A.O. Ademiluyi, G. Oboh, A.A. Akindahunsi, Interaction of aqueous extracts of two varieties of yam tubers (Dioscorea spp) on some key enzymes linked to type 2 diabetes in vitro, Int. J. Food Sci. Technol. 47 (4) (2012) 703–709, https://doi.org/10.1111/j.1365-2621.2011.02896.x.

[71] G.E. Morlock, P. Ristivojevic, E.S. Chernetsova, Combined multivariate data analysis of high-performance thin-layer chromatography fingerprints and direct analysis in real time mass spectra for profiling of natural products like propolis, J. Chromatogr. A 1328 (2014) 104–112, https://doi.org/10.1016/j.chroma.2013.12.053.

[72] P. Ristivojević, J. Trifković, I. Vovk, D. Milojković-Opsenica, Comparative study of different approaches for multivariate image analysis in HPTLC fingerprinting of natural products such as plant resin, Talanta 162 (2017) 72–79, https://doi.org/10.1016/j.talanta.2016.10.023.

[73] R. Nimenibo-Uadia, A. Oriakhi, Proximate, mineral and phytochemical composition of Dioscorea dumetorum Pax, J. Appl. Sci. Environ. Manag. 21 (4) (2017) 771, https://doi.org/10.4314/jasem.v21i4.18.

[74] M.A. Sonibare, R.B. Abegunde, In vitro antimicrobial and antioxidant analysis of Dioscorea dumetorum (Kunth) Pax and Dioscorea hirtiflora (Linn.) and their bioactive metabolites from Nigeria, J. Appl. Biosci. 51 (2012) 3583–3590. http://www.m.elewa.org/JABS/2012/51/4.pdf.

[75] E.N. Uhuo, C.P. Nwuke, O.J. Mba, Proximate, mineral and phytochemical composition of Dioscorea dumetorum (bitter yam) commonly cultivated in south eastern Nigeria, Eur. J. Nutr. Food Saf. 12 (4) (2020) 91–97, https://doi.org/10.9734/ejnfs/2020/v12i430222.

[76] G.N. Medoua, I.L. Mbome, T. Agbor-Egbe, C.M.F. Mbofung, Influence of fermentation on some quality characteristics of trifoliate yam (Dioscorea dumetorum) hardened tubers, Food Chem. 107 (3) (2008) 1180–1186, https://doi.org/10.1016/j.foodchem.2007.09.047.

[77] N.G.A.S.S. Chandana, G.E. Morlock, Comprehensive bioanalytical multi-imaging by planar chromatography in situ combined with biological and biochemical assays highlights bioactive fatty acids in abelmosk, Talanta 223 (2021) 121701, https://doi.org/10.1016/j.talanta.2020.121701.

[78] H. Nikolaichuk, I.M. Choma, G.E. Morlock, Bioactivity profiles on 15 different effect mechanisms for 15 golden root products via high-performance thin-layer chromatography, planar assays, and high-resolution mass spectrometry, Molecules 28 (4) (2023), https://doi.org/10.3390/molecules28041535.

[79] T. Schreiner, N.M. Eggerstorfer, G.E. Morlock, Ten-dimensional hyphenation including simulated static gastro-intestinal digestion on the adsorbent surface, planar assays, and bioactivity evaluation for meal replacement products, Food Funct. 14 (1) (2023) 344–353, https://doi.org/10.1039/D2FO02610D.

[80] N.G.A.S. Sumudu Chandana, G.E. Morlock, Eight different bioactivity profiles of 40 cinnamons by multi-imaging planar chromatography hyphenated with effect–directed assays and high-resolution mass spectrometry, Food Chem. 357 (2021) 129135, https://doi.org/10.1016/j.foodchem.2021.129135.

[81] G.E. Morlock, D. Meyer, Designed genotoxicity profiling detects genotoxic compounds in staple food such as healthy oils, Food Chem. 408 (2023) 135253, https://doi.org/10.1016/j.foodchem.2022.135253.