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# Leaves metabolomic profiling of *Musa acuminata* accessions using UPLC–QTOF–MS/MS and their antioxidant activity

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## Abstract

*Musa acuminata* (Musaceae) is a wild species native to South East Asia. In addition to its potential as a food crop, different non-food morphological parts of the plant have been investigated for various pharmacological activities, including anticholinesterase and antioxidant activity. This study aimed to characterize *Musa* leaf extracts based on their phenolic composition and their agro-morphological traits. A metabolomic approach was applied to discover biomarkers that can be used to separate eight accessions of the species originating from five different countries. Statistical analysis was employed for data analysis. The antioxidant activity and total phenolic content was measured by Ferric Reducing Antioxidant Power (FRAP) assay and Folin–Ciocalteu colorimetric method, respectively. Over 500 metabolites were observed. Thirty-one of them were important for defining variations among the accessions. The identities of some of these markers were confirmed based on their $MS^2$ fragmentation. These include Quercetin *O*-rhamnoside-*O* hexoside (*m*/*z* 609), Kaempferol-3-*O*-rutinoside (*m*/*z* 593), Quercetin *O* hexoside (*m*/*z* 463), Hexadecanoic acid (*m*/*z* 255), Rhamnoside-*O*-rutinoside (*m*/*z* 623). Country of origin and methods of extraction did not play any significant role in the separation, although extraction of accessions by Soxhlet gave better yield (20.0–60.0%) than by sonication (18.4–23.0%). Accession TMp 24 from Nigeria gave the highest yield in both methods of extraction. The sonicated accession TMb 8 exhibited highest antioxidant activity having FRAP values of 49.14 mg GAE/g and 125.10 mg TROLOX/g. The next accession in FRAP activity was the sonicated leaf extract of TMb 116 with 31.69 mg GAE/g and 121.57 mg TROLOX/g. The PCA analysis allowed the separation of the accessions into two groups. The metabolomics approach was found to be informative as a screening tool of the *Musa* accessions. The extracts showed good antioxidant activity and can be a potential source of bioactive metabolites for industrial use.

**Keywords** Polyphenolics · UPLC–QTOF–MS/MS · Morphological traits · Metabolomics · Antioxidant · *Musa acuminata*

## Introduction

*Musa* spp. (bananas and plantains) are good sources of carbohydrates, proteins, other vitamins and minerals. They contain different amino acids like threonine, tryptamine, tryptophan, as well as flavonoids, dopamine, beta-carotene and sterols [1]. Different parts of banana have been studied for various biological activities such as; stem as antidiabetic [2], fruits for wound healing [3], fruits as antiulcer [4, 5], peel as immunomodulatory agent [6]. The antidiabetic activity of the flower extract has also been reported [7]. Also, Shanmuga and Subramanian [8] reported that oral administration of *Musa paradisiaca* tepals extract significantly improved the altered levels of blood glucose, plasma insulin, glycosylated haemoglobin and modulated the activities of carbohydrate metabolizing enzymes. High consumption of plantain is capable of lowering deoxycorticosterone acetate

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(DOCA)-induced elevated mean arterial blood pressure, prevents the onset of DOCA-induced hypertension in rats [9], and enhances antimicrobial activity [10]. In a study by Rai et al. [11], the high potassium (K) and sodium (Na) content of plantains was correlated with the glycaemic effect. Agarwal et al. [12] reported the wound healing activity of both methanolic and aqueous extracts of plantain banana (*M. sapientum* var. *paradisiaca*) in rats. Kumar et al. [13] reported that orally administered banana pulp powder had significant antiulcerogenic activity in rats subjected to aspirin, indomethacin, phenyl butazone, prednisolone and cysteamine, and in guinea pigs subjected to histamine. Banana pulp powder not only increased mucosal thickness, but also significantly increased thymidine incorporation into mucosal DNA. Owing to the numerous medicinal applications of bananas and plantains, it is important to have a precise identification of the different accessions based on their individual phytochemical profile using a metabolomic approach.

The ultra-performance liquid chromatography-quadrupole time of flight mass spectrometric (UPLC–QToF–MS) fingerprinting technology employed in this study allows better separating effects in terms of improved detection limits and chromatographic resolution with greater sensitivity [14]. The precision of the fragmentation pattern obtained in UPLC–QTOF–MS system makes it a powerful analytical technique [15, 16]. It is widely used for quality control of medicinal plants, characterization of metabolites and as a means of cultivar identification [17]. However, there has been no report of the use of the UPLC–QTOF–MS technique to identify the metabolites in the leaf samples of *Musa acuminata*, which is a wild species of *Musa*. Our earlier study on these accessions showed that they possess moderate antioxidant activity. The antioxidant activity of the *Musa* spp. leaf samples was evaluated through their ability to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. The radical scavenging activity of all the plants extracts was dose-dependent [18]. Hence, the aim of this study was to investigate the Ferric Reducing Antioxidant Power (FRAP) and polyphenolic profiles of eight *M. acuminata* accessions from five countries, using the UPLC–QTOF–MS. The profile of the polyphenolic compounds for each accession was studied in the leaves, using two extraction methods in order to possibly generate marker compounds for cultivar identification. Furthermore, the metabolomics profiling was compared to the agro-morphological classification of the same *Musa* spp.

## Materials and methods

### Chemicals and reagents

All chemicals (reagents and analytical grades) were purchased from Sigma-Aldrich (Bremen, Germany). Ultrapure water was obtained from a Milli-Q purification system (Millipore, Molsheim, SA, France). The HPLC grade acetonitrile and formic acid were purchased from Merck (Merck, Darmstadt, Germany).

### Collection of plant material

Leaf samples of the eight *M. acuminata* accessions known as Tropical Musa banana (TMb) and Tropical Musa plantain (TMp): TMb 8, TMb 55, TMb 82, TMb 106, TMb 116, TMb 145, TMp 24 and TMp 36 were obtained from the *Musa* spp. field collection held by the genetic resources centre of the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria. The accessions originated mostly from European and Asian countries (France, Brazil, Sri Lanka, Nigeria, Phillippines) including one accession of unknown origin (Table 1). The harvested leaves of the different accessions were dried in the oven (Gallenkamp Economy incubator) at below 40 °C for about 24 h and pulverized into powder using a Mallex blender.

**Table 1** Passport data of *M. acuminata* accessions from five different countries used for UPLC/QTOF–MS and antioxidant analyses

| IITA accession identifier[a] | Species name | Cultivar name | Type | Genome | Origin |
|---|---|---|---|---|---|
| TMb 8 | *Musa acuminata* | Borneo | Banana | AA | France |
| TMb 55 | *Musa acuminata* | ND | Banana | – | Unknown |
| TMb 82 | *Musa acuminata* | Muga | Banana | AAA | Brazil |
| TMb 106 | *Musa acuminata* | Ouro Mel | Banana | AAA | France |
| TMp 116 | *Musa acuminata* | P. Raja | Plantain | AAB | France |
| TMp 24 | *Musa acuminata* | Egjoga | Plantain | AAB | Nigeria |
| TMp 36 | *Musa acuminata* | Muracho | Plantain | AAB | Phillippines |
| TMb 145 | *Musa acuminata* | P. Awak | Banana | ABB | Sri-Lanka |

[a]International Institute of Tropical Agriculture Sample identification used in this study include *TMb* tropical Musa banana; *TMp* tropical Musa plantain

## Extraction and sample preparation

*Musa acuminata* (TMb 8, TMb 55, TMb 82, TMb 106, TMb 116, TMb 145, TMp 24 and TMp 36) leaf powders (5 g) were extracted in a Soxhlet apparatus with 100 mL of aqueous MeOH (70%) for 9 h and extracted by sonication for 30 min, respectively. The extracts were filtered using Whatman No. 1 filter paper, and filtrates were concentrated in vacuo and freeze dried on a Freeze dryer for 12 h. Extracts were stored at − 20 °C until needed for analysis. Extracts were thawed at room temperature, dissolved in 70% MeOH (5 mg/mL), filtered through a membrane filter (0.45 µm) and used directly for the LC–MS analysis.

## UPLC–QTOF–MS analysis

The spectrometry was performed on an IMPACT HD Q-ToF mass spectrometer (Brucker Daltonics, Bremen, Germany) connected to Agilent 1260 Infinity UPLC system via an electrospray ionization interface (Milford, MA, USA).

## QToF–MS conditions

The Q-ToF consists of a binary solvent delivery system, a Hystar autosampler and photodiode-array detection (PDA) system. The chromatography was performed using an Agilent Poroshell 120 EC-C18 column (3.0 × 50.0 mm, 2.7 µm). The mobile phase consisted of water containing 0.005% formic acid (A) and acetonitrile containing 0.005% formic acid (B). The UPLC eluting conditions were optimized as follows: linear gradient elution from 10% B to 50% B in 10 min, 50–90% B in 6 min followed by isocratic 90% B for 3 min and a return to 10% B at 20 min. The flow rate was 0.6 mL/min, the temperature of the column and autosampler were maintained at 30 and 10 °C, respectively. Equilibration was done for 5 min. The injection volume was 2 µL. The scan range for the PDA was 180–380 nm. Detection was performed in both positive and negative ion modes in the *m*/*z* range of 100–1500 Da, with an acquisition time of 0.5 s in centroid mode.

## Liquid chromatography–tandem-mass spectrometry conditions

The LC equipment (Agilent 1100 series, Karlshuhe, Germany) comprised a binary pump, an auto sampler with a 100 µL loop and a DAD detector with a light-pipe flow cell (recording at 254, 280 and 320 nm and scaning from 200 to 600 nm). This was interfaced with ion-trap mass spectrometer fitted with ESI source (Bruker Daltonics HCT Ultra, Bremen, Germany) operating in full scan, auto $MS^n$ mode to obtain fragment ion *m*/*z*. Tandem mass spectra were acquired in auto $MS^n$ mode (smart fragmentation) using a ramping of the collision energy. MS operating condition was set at 4 precursor ions for $MS^2$, 3 for $MS^3$ both in the negative and positive ion modes, a dry gas flow rate of 10 L/min and a nebulizer pressure of 10 psi, as described in literature [19].

## Data acquisition and analysis

All MS data acquired were processed using Data Analysis 4.2 (Brucker, Bremen Germany) and MZmine 2.14.2 [20]. The threshold for molecular formulas determination was set at 5 ppm. The data were analyzed for total ion chromatogram, mass detection, and chromatogram building. An aligned peak list was generated on the MZmine by using retention time ($t_R$), peak area and mass data *m*/*z*. The aligned peak list was subjected to PCA (axes 1 and 2) using peak area of the most abundant peaks. Extraction method (Soxhlet, sonication) and origin (France, Brazil, Nigeria, Sri Lanka, Phillippines, unknown) were set as variable parameters in the analysis. Unique markers that could explain the variability in the accessions were sought based on their intensities.

## Biomarker identification

The *m*/*z* data obtained through the UPLC–QTOF–MS based on retention time and peak areas were employed to calculate the proposed molecular formula of each marker using MZmine software. The software proposed the formula by matching experimental and theoretical isotopic pattern of the markers. Suggested formulas are arranged according to the percentage of fitness. The formulas proposed were further identified by subjecting them to an online database search (KEGG).

## Agro-morphological data acquisition and analysis

Agro-morphological characters from IITA *Musa* germplasms morphological descriptors, including 32 vegetative

**Table 2** Percentage yield of *M. acuminata* accessions obtained by Soxhlet and sonication extraction methods

| Accession | % Yield (g) | |
|---|---|---|
| | Soxhlet | Sonication |
| TMb 8 | 0.997 (19.94) | 1.126 (22.52) |
| TMb 55 | 1.237 (25.74) | 0.921 (18.42) |
| TMb 82 | 1.165 (23.30) | 0.977 (19.54) |
| TMb 106 | 1.109 (22.18) | 0.958 (19.16) |
| TMb 116 | 1.297 (25.94) | 0.920 (18.40) |
| Tmp 24 | 3.045 (60.90) | 1.150 (23.00) |
| Tmp 36 | 1.618 (32.36) | 0.980 (19.60) |
| TMb 145 | 1.703 (34.06) | 1.030 (20.60) |

and fruit characters of the *Musa* field accessions were utilized to compute a distance matrix before being used for the clustering. The characters utilized are: Pseudostem height (m), main underlying colour of the pseudostem, sap colour, blotches at the petiole base, petiole canal of the third leaf, petiole margin, petiole margin colour, edge of petiole margin, colour of cigar leaf outer surface, bunch position, bunch shape, rachis position, rachis appearance, male bud shape, male bud size (cm), bract apex shape, bract imbrication, colour of the bract external face, bract internal face colour, bract behavior before falling, compound tepal basic colour, lobe colour of compound tepal, anther colour, dominant colour of male flower, number of hands, number of fruits on third hand, fruit length (cm), fruit shape, fruit apex, remains of flower relicts at fruit apex, fruit pedicel length (mm), fusion of pedicels. The DISTANCE procedure in SAS was used to compute the simple matching coefficient between each pair of banana cultivars based on dummy codes. The

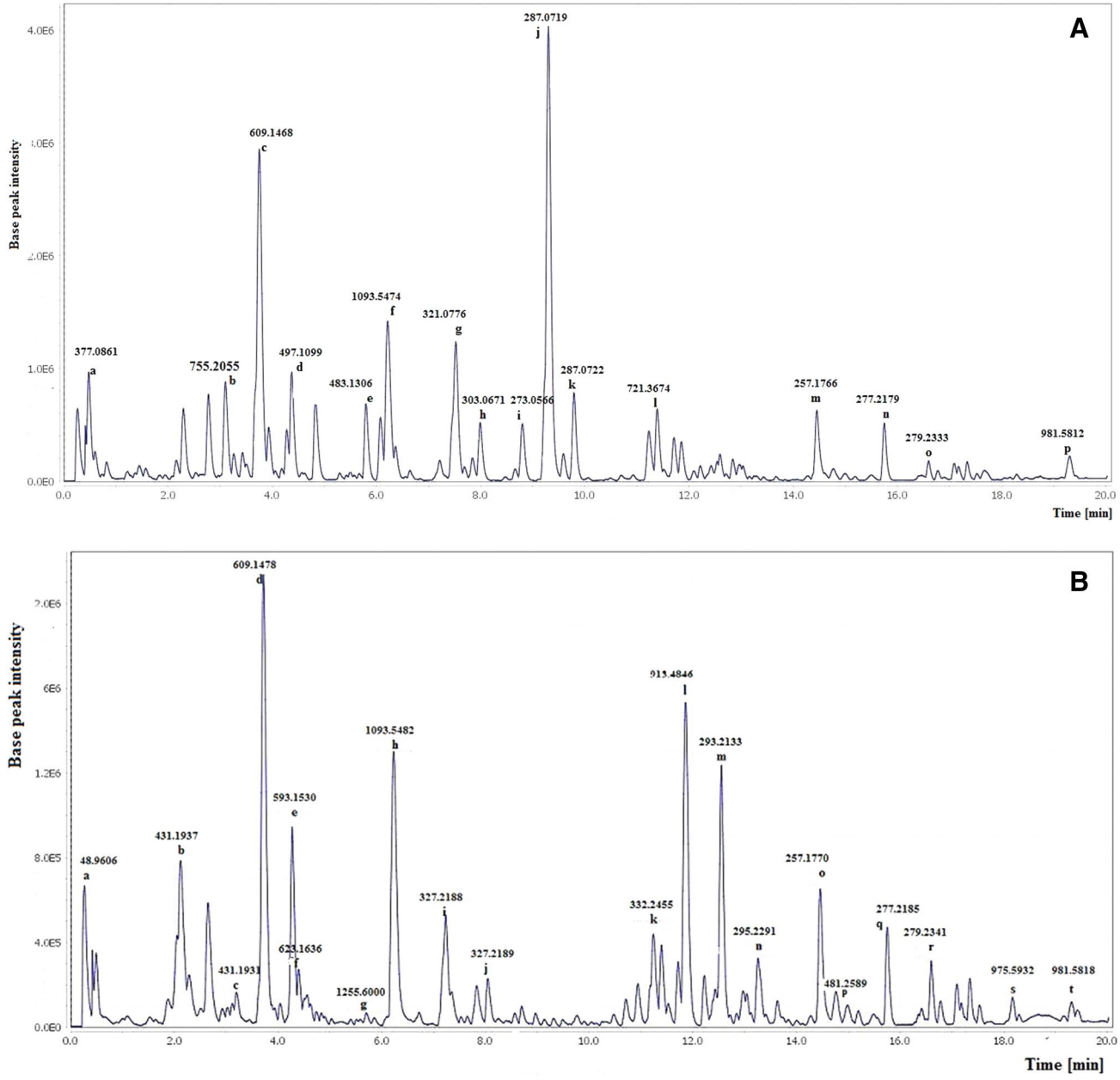


**Fig. 1** Representative base peak chromatograms of *M. acuminata* accessions: **a** TMb 82 from Brazil showing c as Quercetin *O*-rhamnoside-*O*-hexoside, i as Phloretin/Luteolinflavan and o as linoleic acid. **b** TMb 55 from an unknown origin showing e as Kaempferol-7-*O*-neohesperidoside and f as Isorhamnetin 3-*O*-(6-*O*-rhamnosyl-hexoside)

CENTROID method was used to perform the hierarchical cluster analysis using the distance matrix produced by the DISTANCE procedure as input data.

## Antioxidant activity

The accessions were tested for antioxidant activities using the FRAP [21] and the Folin–Ciocalteu total phenolic test [22]. For the FRAP assay, to a 10 µL sample of each accession (500 µg/mL) was added 200 µL of freshly prepared

**Table 3** Selected maker compounds that mostly contributed to variation in *M. acuminata* accessions analyzed by UPLC/Q-TOF–MS

| Peak | RT (min) | Acc. | Proposed compound identity | Molecular formula | $[M–H]^-$ *m/z* | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Measured mass (Da) | Theoretical mass (Da) | Mass accuracy (ppm) | mSigma |
| 1 | 0.5 | 12 | Bergapten | $C_{12}H_8O_4$ | 215.0331 | 215.0350 | 8.9 | 55.8 |
| 2 | 0.5 | 7 | Quinate | $C_7H_{12}O_6$ | 191.0563 | 191.0551 | – | – |
| 3 | 0.5 | 1 | Rosmarinate | $C_{18}H_{16}O_8$ | 359.0746 | 359.0761 | – | – |
| 4 | 0.5 | 14 | Citric acid | $C_6H_8O_7$ | 191.0201 | 191.0197 | −2.0 | 6.1 |
| 5 | 0.6 | 1 | Vanillic acid hexoside | $C_{14}H_{18}O_9$ | 329.0889 | 329.0878 | −3.5 | 4.3 |
| 6 | 1.0 | 1 | *O*-Feruloylgalactarate | $C_{16}H_{18}O_{11}$ | 385.0784 | 385.0878 | −2.0 | 1.9 |
| 7 | 1.1 | 2 | 4-*O*-Caffeoylquinic acid | $C_{16}H_{18}O_9$ | 353.0883 | 353.0878 | −9.0 | 19.3 |
| 8 | 1.7 | 1 | Epicatechin/catechin | $C_{30}H_{26}O_{12}$ | 577.1352 | 577.1351 | −0.2 | 5.6 |
| 9 | 1.9 | 4 | Ferulic acid-*O*-glucoside | $C_{16}H_{20}O_9$ | 355.1037 | 355.1035 | −0.8 | 5.1 |
| 10 | 2.3 | 1 | Catechin/Epicatechin | $C_{15}H_{14}O_6$ | 289.0717 | 289.0718 | 0.2 | 3.4 |
| 11 | 2.5 | 1 | 4-*O*-Feruloylquinic acid | $C_{17}H_{20}O_9$ | 367.1033 | 367.1035 | 0.4 | 3.9 |
| 12 | 2.6 | 1 | Sinapic acid-*O*-glucoside | $C_{17}H_{22}O_{10}$ | 385.1144 | 385.1129 | – | – |
| 13 | 3.0 | 5 | Quercetin 3-*O*-beta-D-glucosyl (1 > 2)-beta-D-glucoside | $C_{27}H_{30}O_{17}$ | 625.1420 | 625.1410 | −1.6 | 6.1 |
| 14 | 3.2 | 3 | Phloretin/Luteoliflavan | $C_{15}H_{14}O_5$ | 273.0771 | 273.0768 | −0.9 | 1.8 |
| 15 | 3.5 | 4 | Kaempferol 3-Sophortrioside | $C_{33}H_{40}O_{21}$ | 771.2006 | 771.1978 | – | – |
| 16 | 3.8 | 1 | Quercetin 3-*O*-hexoside | $C_{21}H_{20}O_{12}$ | 463.0889 | 463.0882 | −1.4 | 6.1 |
| 17 | 3.9 | 1 | Geranyl arabinopyranosyl-glucoside | $C_{21}H_{36}O_{10}$ | 447.2244 | | | 2.3 |
| 18 | 4.2 | 2 | Quercetin 3-*O*-(6-*O*-malonyl-beta-D-glucoside) | $C_{24}H_{22}O_{15}$ | 549.0888 | 549.0886 | −0.4 | 2.3 |
| 19 | 4.5 | 8 | Kaempferol-7-*O*-glucoside | $C_{21}H_{20}O_{11}$ | 447.0942 | 447.0933 | −2.1 | 3.4 |
| 20 | 4.7 | 1 | Furcatin | $C_{20}H_{28}O_{10}$ | 427.1618 | 427.1599 | – | – |
| 21 | 6.1 | 3 | Quercetin | $C_{15}H_{10}O_7$ | 301.0359 | 301.0354 | −1.9 | 16.1 |
| 22 | 7.2 | 1 | Luteolin | $C_{15}H_{10}O_6$ | 285.0413 | 285.0405 | −2.8 | 7.8 |
| 23 | 7.2 | 15 | 9,10-Dihydroxy-8-oxo-12-octadecenoic acid | $C_{18}H_{32}O_5$ | 327.2185 | 327.2177 | −2.6 | 1.0 |
| 24 | 7.4 | 2 | Rhamnetin | $C_{16}H_{12}O_7$ | 315.0519 | 315.0510 | −2.9 | 1.8 |
| 25 | 8.6 | 1 | Flavanol 3-*O*-(alpha-L-rhamnosyl-(1 > 6)-beta-D-glucoside) | $C_{27}H_{30}O_{12}$ | 545.1673 | 545.1664 | −1.5 | 11.4 |
| 26 | 9.0 | 1 | 5-*O*-Methylembelin | $C_{18}H_{28}O_4$ | 307.1924 | 307.1915 | −2.9 | 5.1 |
| 27 | 13.3 | 11 | 12,13-Epoxy 9Z-octadecaenoic acid | $C_{18}H_{32}O_3$ | 295.2286 | 295.2279 | −2.6 | 3.4 |
| 28 | 15.8 | 1 | Corticosterone | $C_{21}H_{30}O_4$ | 345.2059 | 345.2060 | – | – |
| 29 | 17.2 | 15 | 13-Hydroxydocosanoic acid | $C_{22}H_{44}O_3$ | 355.3226 | 355.3218 | −2.5 | 6.1 |
| 30 | 18.7 | 12 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 283.2646 | 283.2643 | −0.7 | 6.4 |
| 31 | 18.8 | 1 | Betulinic acid | $C_{30}H_{48}O_3$ | 455.3536 | 455.3531 | −1.2 | 5.3 |

*m/z* data were obtained through the UPLC–QTOF–MS based on retention time and peak areas

*RT* retention time

*Acc.* number of accessions

**Table 4** Mass spectrum ($MS^n$) fragmentation of compounds in *M. acuminata* in the negative ion mode, showing percentage relative abundance of fragments

| Compounds | Parent ion (M–H) | Characteristic *m/z* of ion in negative mode (relative abundance in %) |
|---|---|---|
| Bergapten | 215.0331 | $MS^2$—179 (100), 213 (47), 177 (11), 143 (6), 113 (6), 89 (5) |
| Quinate | 191.0563 | $MS^2$ |
| Rosmarinate | 359.0746 | $MS^2$— 323 (100), 163 (95), 247 (91), 287 (35), 359 (29) |
| Citric acid | 191.0201 | $MS^2$— 111 (100), 87 (40), 173 (35) |
| Vanillic acid hexoside | 329.0889 | $MS^2$— 167 (100), 209 (97), 267 (30), 239 (21); $MS^3$—108 (100), 140 (40) |
| *O*-Feruloylgalactarate | 385.0784 | $MS^2$—191 (100), 353 (39) |
| 4-*O*-Caffeoylquinic acid | 353.0883 | $MS^2$—173 (100), 135 (9), $MS^3$—93 (100), 111 (61), 72 (36), 155 (13) |
| Procyanidin B dimer | 577.1352 | $MS^2$—407 (100), 451 (24), 289 (12), 559 (7), $MS^3$—285 (100), 389 (18), 339 (5), 255 (3) |
| Ferulic acid-*O*-glucoside | 355.1037 | $MS^2$—193 (100), 134 (7) |
| Catechin/Epicatechin | 289.0717 | $MS^2$—245 (100), 205 (40), 125 (4), $MS^3$—203 (100), 227 (74), 187 (47), 97 (37), 161 (20), 181 (30), 243 (28), 81 (9) |
| 4-*O*-Feruloylquinic acid | 367.1033 | $MS^2$—173 (100), 193 (12); $MS^3$—93 (100), 72 (32) |
| Sinapic acid-*O*-glucoside | 385.1144 | $MS^2$—223 (100), 205 (95), 154 (94), 316 (43), 263 (16) |
| Quercetin 3-*O*-beta-D-glucosyl (1 > 2)-beta-D-glucoside | 625.1420 | $MS^2$—463 (100), 301 (33); $MS^3$—301 (100), 363 (4), 255 (3) |
| Phloretin | 273.0771 | $MS^2$ |
| Kaempferol 3-Sophortrioside | 771.2006 | $MS^2$—301 (100), 343 (13), 609 (10), 657 (3); $MS^3$—179 (100), 151 (94), 300 (56), 398 (5), 233 (11), 107 (8) |
| Quercetin *O*-rhamnoside-*O*-hexoside | 609.1476 | $MS^2$—301 (100), 271 (6), 343 (5), 179 (4); $MS^3$—179 (100), 151 (92), 300 (74), 271 (49), 107 (11) |
| Quercetin *O*-rhamnoside-*O* hexoside dimer | 1219.304 | – |
| Quercetin 3-*O*-hexoside | 463.0889 | $MS^2$—301 (100), $MS^3$—300 (100), 179 (81), 151 (57), 271 (35) |
| Quercetin-*O*-hexoside | 463.0893 | $MS^2$—301 (100), 271 (6), 343 (5), 179 (4); $MS^3$—179 (100), 300 (60), 151 (71), 273 (22), 257 (20), 107 (8) |
| Geranyl arabinopyranosyl-glucoside | 447.2244 | $MS^2$—284 (93), 255 (26), 327 (14), 357 (4), 151 (3) |
| Quercetin 3-*O*-(6-*O*-malonyl-beta-D-glucoside) | 549.0888 | $MS^2$—265 (100), 163 (97), 503 (87), 387 (72), 339 (39), 235 (35) |
| Kaempferol-7-*O*-neohesperidoside | 593.1528 | $MS^2$—285 (100), 327 (6), 429 (3); $MS^3$—257 (100), 267 (41), 229 (34), 163 (17) |
| Isorhamnetin 3-*O*-(6-*O*-rhamnosyl-hexoside) | 623.1633 | $MS^2$—315 (100), 300 (20), 271 (14), 357 (4); $MS^3$—300 (100), 191 (45), 81 (80) |
| Kaempferol-7-*O*-glucoside | 447.0942 | $MS^2$ 285 (100), 255 (31), 327 (10); $MS^3$—255 (100), 83 (31) |
| Furcatin | 427.1618 | $MS^2$—369 (100), 325 (28), 223 (49), 161 (77), $MS^3$—160 (100) |
| Quercetin | 301.0359 | $MS^2$—151 (100), 299 (73), 233 (12), 107 (6) |
| Luteolin | 285.0413 | $MS^2$—285 (100), 151 (10), 257 (8), 213 (3) |
| 9, 10-Dihydroxy-8-oxo-12-octadecenoic acid | 327.2185 | $MS^2$—291 (63), 229 (100), 171 (59), $MS^3$—211 (100), 183 (8), 125 (41) |
| Rhamnetin | 315.0519 | $MS^2$—300 (100) |
| Flavanol 3-*O*-(alpha-L-rhamnosyl-(1 > 6)-beta-D-glucoside) | 545.1673 | 445 (100), $MS^2$—513 (44), 193 (9), 469 (5), $MS^3$—175 (100), 193 (92), 134 (26), 430 (12), 403 (7), 458 (6) |
| 5-*O*-Methylembelin | 307.1924 | $MS^2$—289 (100), 235 (44), 185 (23), 121 (64), $MS^3$—270 (100), 247 (22), 185 (7), 125 (36) |
| Mukaadial | 265.1486 | $MS^2$—97 (100) |
| Unknown | 699.3829 | $MS^2$—653 (100), 397 (20), $MS^3$—595 (4), 397 (100), 305 (16), 235 (13) |
| 12, 13-Epoxy 9Z-octadecaenoic acid | 295.2286 | $MS^2$—277 (100), 171 (70); $MS^3$—233 (100), 275 (68), 143 (10), 97 (6) |
| Unknown | 555.2858 | $MS^2$—225 (100), 299 (33), 255 (2), 485 (18), 207 (14), 243 (10); $MS^3$—207 (100), 125 (95), 165 (79), 81 (71) |

**Table 4** (continued)

| Compounds | Parent ion (M–H) | Characteristic *m/z* of ion in negative mode (relative abundance in %) |
|---|---|---|
| Corticosterone | 345.2059 | $MS^2$ |
| Linoleic acid | 279.2338 | $MS^2$—261 (100), 279 (21), 207 (7), 243 (4); $MS^3$—259 (100), 243 (12), 148 (8), 125 (13) |
| 13-Hydroxydocosanoic acid | 355.3226 | $MS^2$—353 (33), 309 (42); $MS^3$—307 (100) |
| Hexadecanoic acid | 255.2348 | $MS^2$—253 (100); $MS^3$—235 (100), 253 (10) |
| (9Z)-Octadecenoic acid | 281.2491 | 281 (100), 279 (49), 263 (21); $MS^3$—261(100), 279 (98), 97 (11) |
| Octadecanoic acid | 283.2646 | $MS^2$—281 (100), 165 (6), $MS^3$—281 (100), 237 (6), 109 (7) |
| Betulinic acid | 455.3536 | $MS^2$—409 (100), 356 (6) |

FRAP reagent in a 96 well plate. The FRAP solution was prepared by mixing 10 mM acetate buffer, 1 mL of 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) solution and 1 mL of $FeCl_3 \cdot 6H_2O$. The plate was shaken gently, incubated in the dark for 10 min and absorbance was measured at 593 nm with a Biochrom EZ Read 2000 (v. 1.1a) microplate reader. Total phenolic content was measured by pipetting 30 µL of sample (and standard) into eppendorf tubes. This was made up to 100 µL with deionized water before adding 100 µL of Folin–Ciocalteu reagent. The tubes were vortexed for 2–5 s and after 2 min 800 µL of $Na_2CO_3$ solution was added to each tube. The final mixture was vortexed and incubated in the oven at 40 °C for 20 min. The tubes were cooled at room temperature and 200 µL of the mixture was transferred in the 96-well plate for absorbance measurement at 725 nm. For the two assays, Gallic acid and Trolox 0–0.5 mg/mL were used as standard antioxidants.

## Results and discussion

### Extraction methods

A total of eight different *Musa* accessions were chosen for phytochemical analysis. Firstly the extraction protocol was optimized. The methods of extraction (Soxhlet and sonication) affected the yield of extract. The yield of samples extracted with 100 mL of aqueous MeOH (70%) for 9 h relative to the dry weight (5 g) of starting plant material ranged from 20.0 to 60.9% in Soxhlet extracted samples and from 18.4 to 23.0% in sonicated samples (Table 2). Expectedly, the accessions extracted by Soxhlet gave more yield than the ones extracted by sonication. This is in agreement with the report of Sanghi and Kannamkumarath [23] in comparing extraction methods by Soxhlet, sonicator and microwave for screening pesticide residues from solid matrices. They found that sonicator extraction was less efficient than other methods. Accession TMp 24 from Nigeria gave the highest yield in both methods of extraction.

### Identification of phenolic compounds

The UPLC–QTOF–MS/MS analysis allowed the detection of a total of 592 constituents in the negative ion mode in the eight *Musa* accessions using a compound search algorithm of the LC–MS data set. Forty-one compounds were found in all accessions, irrespective to country of origin and method of extraction, while 551 were variable. Figure 1 shows a representative base peak chromatogram of *M. acuminata* accessions TMb 82 and TMb 55 from Brazil and an unknown origin, respectively. 41 out of the 41 common markers were eluted between 3 and 20 min. The relative abundance of the compounds was obtained with reference to their peak area based on 5 g of dried leaf sample per accession (see Supplementary Table A1). The extracted ion chromatograms of the common compounds in negative ion mode at *m/z* 609 for Quercetin *O*-rhamnoside-*O* hexoside (a–c) in TMb 145b and at *m/z* 463 for Quercetin *O* hexoside (d–f) in TMb 106a are presented as Supplementary Figs. A1–A3. The list of selected markers that contributed to the variation in the accessions is also presented in Table 3. Thirty-one of the characteristic markers were found to be informative, 49% of which were found only in one accession each. The identities of some of these markers were confirmed based on their $MS^2$ and $MS^3$ fragmentation (Table 4). These include Quercetin *O*-rhamnoside-*O* hexoside (*m/z* 609), Kaempferol-7-*O*-neohesperidoside (*m/z* 593), Quercetin *O*-hexoside (compound 8; *m/z* 463), Hexadecanoic acid (*m/z* 255) and Rhamnoside-*O*-rutinoside (*m/z* 623). The fragmentation patterns of these compounds correspond to those that had been reported in literature [24–27]. Using these markers, the most distinct accession is TMb 145b from Sri Lanka, extracted by sonication. It contained 16 diagnostic markers such as two proanthocyanidins Epicatechin (4 beta->8) ent-epicatechin (Compound 1; $[M–H]^-$ *m/z* 577.1352) and Catechin/Epicatechin

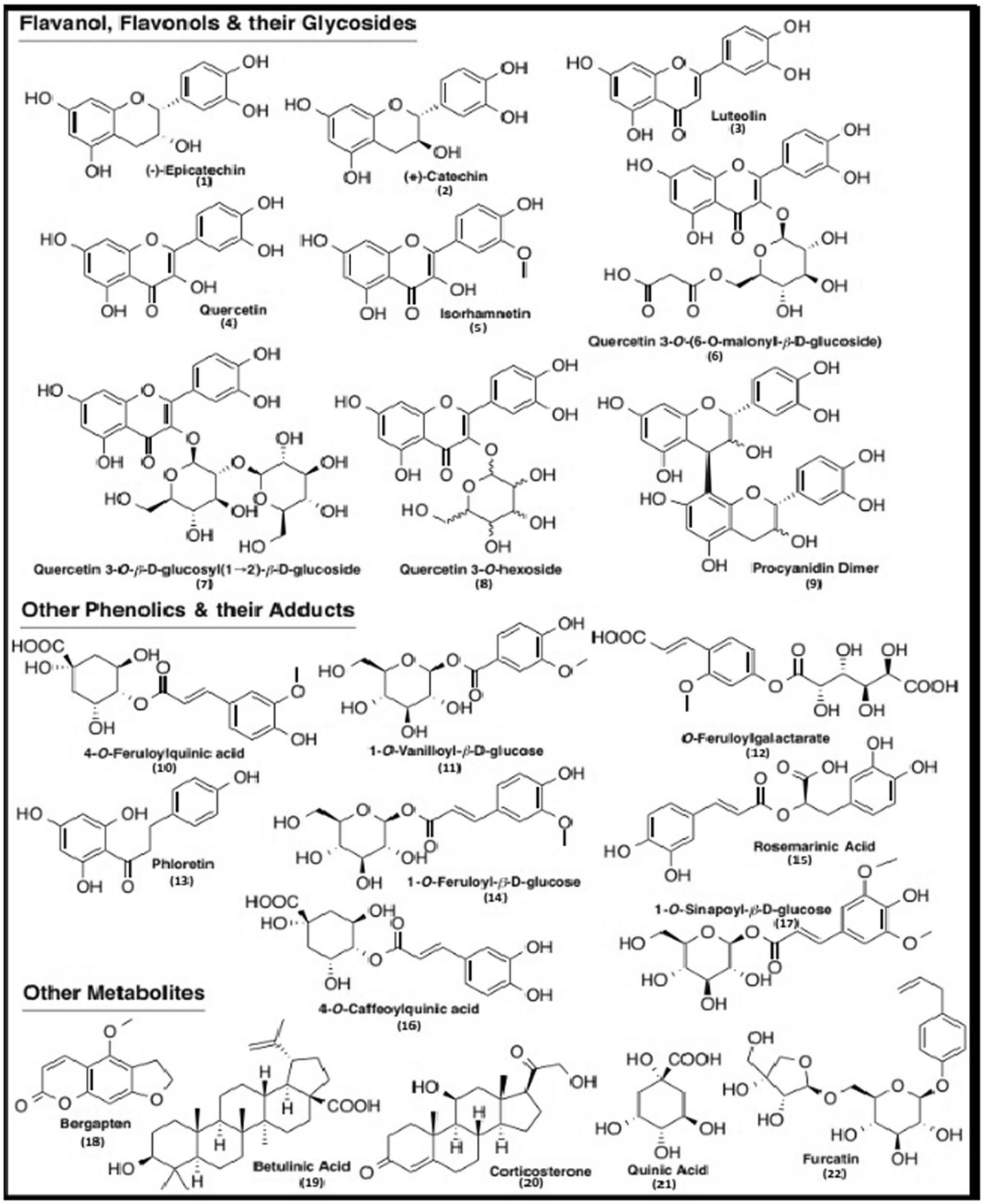


**Fig. 2** Structures of some identified compounds from *Musa* accessions obtained through the UPLC–QTOF–MS analysis based on the proposed molecular formula of each marker using MZmine software

(Compound 2; [M–H]$^-$ *m/z* 289.0717), 4-*O*-Feruloylquinic acid (Compound 10; [M–H]$^-$ *m/z* 367.1033), 5-*O*-Methylembelin ([M–H]$^-$ *m/z* 307.1924) and Corticosterone (Compound 20; [M–H]$^-$ *m/z* 345.2059); that were not found in any other accession. The precursor *m/z* 289 is known to favor fragmentation pattern reported in literature for (epi) catechin [28]. Other markers such as: Rosmarinate (Compound 15; [M–H]$^-$ *m/z* 359.0746), 1-*O*-Vanilloyl-beta-D-glucose(Compound 11; [M–H]$^-$ *m/z* 329.0889), *O*-Feruloylgalactarate (Compound 12; [M–H]$^-$ *m/z* 385.0784), Sinapic acid-*O*-glucoside (Compound 17; [M–H]$^-$ *m/z* 385.1144), Quercetin 3-*O*-glucoside (Compound 6; [M–H]$^-$ *m/z* 625.1420), Luteolin (Compound 3; [M–H]$^-$; *m/z* 285.0413), Flavanol 3-*O*-(alpha-L-rhamnosyl-(1 > 6)-beta-D-glucoside) ([M–H]$^-$ *m/z* 545.1673) and Betulic acid (Compound 19; [M–H]$^-$ *m/z* 455.3536) were found only in TMb 8b, TMb 106b, TMb 106a, TMb 145a, TMb 106a, TMb 8b, TMb 82a and TMb 8a, respectively. Among the detected compounds, Flavonols and related glucosides, other phenolics and their adducts, and other metabolites were identified, as presented in Fig. 2. Some of the metabolites identified in the extracts are known to be bioactive. For instance, quercetin is considered a strong antioxidant due to its ability to scavenge free radicals and bind transition metal ions [29]. Also, quercetin has been reported to protect against the more obvious environmental causes of free radicals such as smoking. Begum and Terao [30] found that quercetin aglycone and its conjugate metabolites (quercetin 3-*O*-ß-glucorionide and quercetin 3-*O*-ß-glycoside) could protect erythrocytes from membrane damage caused by smoking. The antiradical

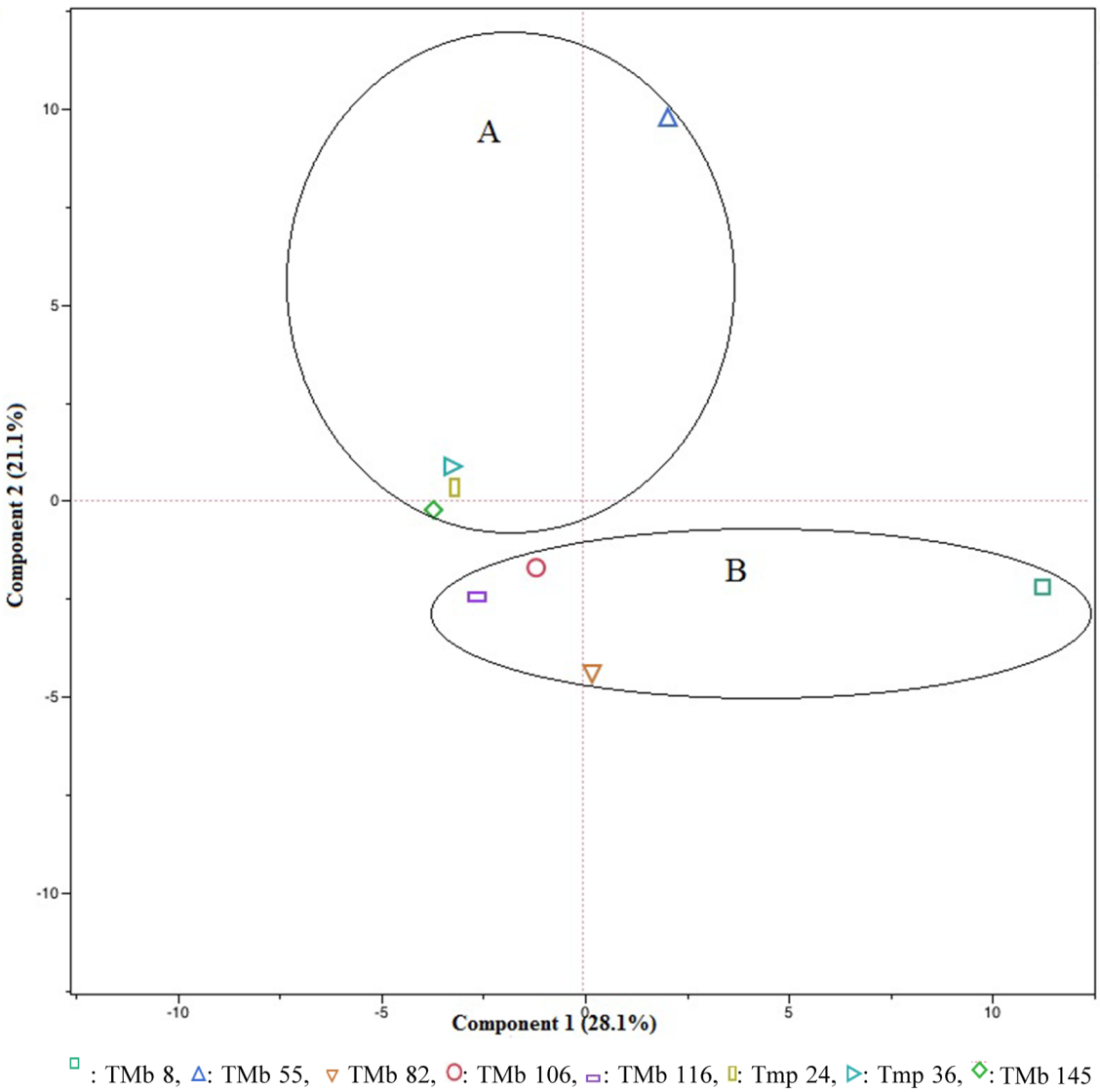


**Fig. 3** Principal component analysis of *M. acuminata* accessions based on most abundant peaks from UPLC–QTOF–MS analysis

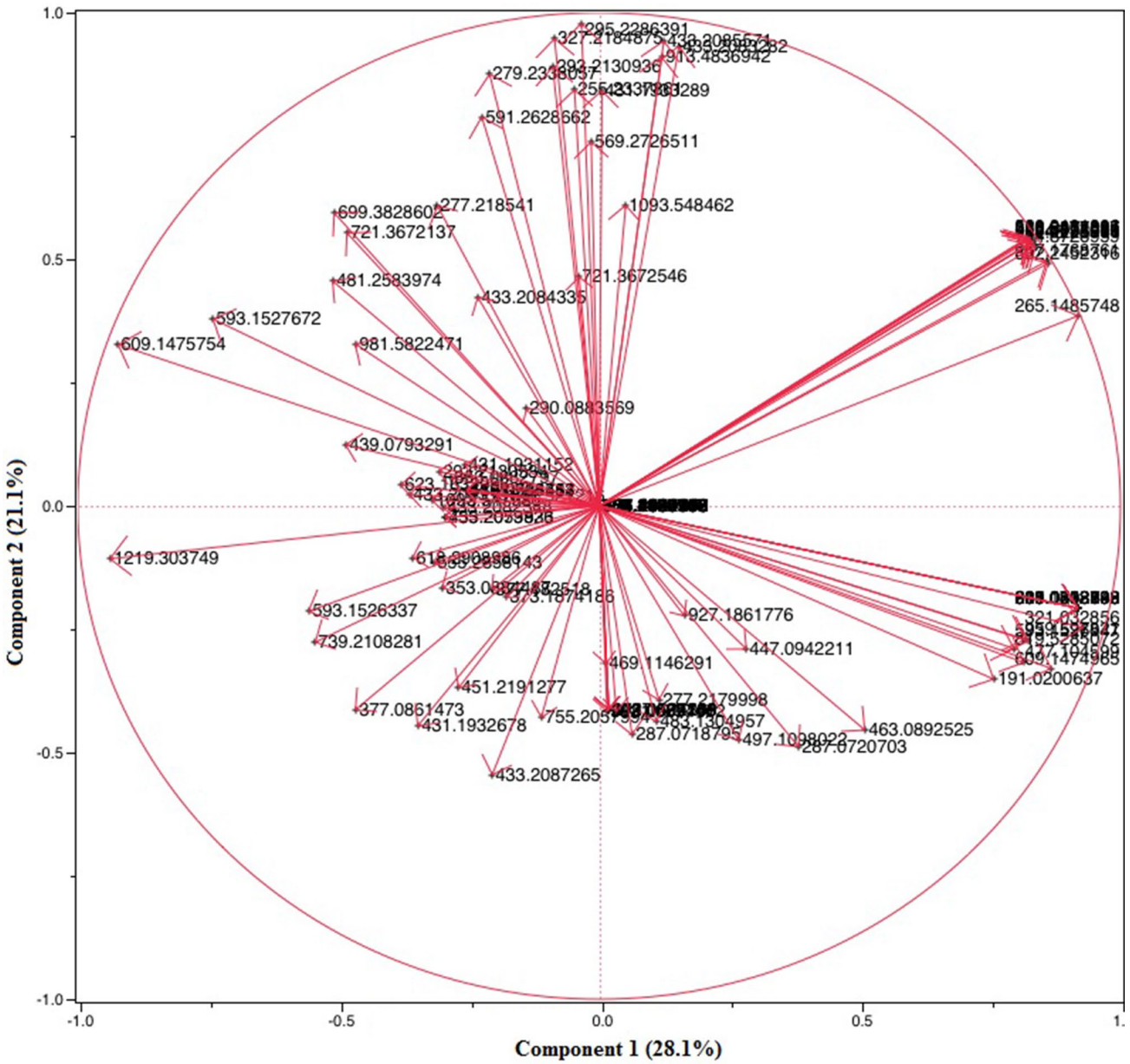


**Fig. 4** Factor loading of peak contribution to separation of *Musa* accessions

capacity of catechin, epicatechin, quercetin and rutin was reported by Iacopini et al. [31]. Caffeoylquinic acid and feruloylquinic acid possess strong antioxidant activity in vitro [32] and in vivo by increasing the resistance of LDL to lipid peroxidation [33].

The PCA analysis using the first two principal components allowed the separation of the accessions into two groups (Fig. 3). Group A is made up of TMp 24, TMp 36 and TMb 145 from Nigeria, Phillipines and Sri Lanka, respectively with TMb 55 being enclosed in the group but very distinct to the other accessions of the group. The distinction of the TMb 55 can be explained by the fact that this accession contains the least number of markers and its unknown origin (information not provided in the available passport data). Group B comprised TMb 106, TMb 116, TMb 82 and TMb 8. In this group, the closeness of the two France-originated accessions TMb 106 and TMb 116 is confirmed. However, TMb 8, which is also from France appeared to be distantly related to the other accessions in this group. The group B seems to be tied by the Caribbean and South American origin, assuming that the France origin refers to the French departments in Caribbean. Though, in Fig. 4, showing the factor loading data points contributing to difference in accessions, the markers related to the origin

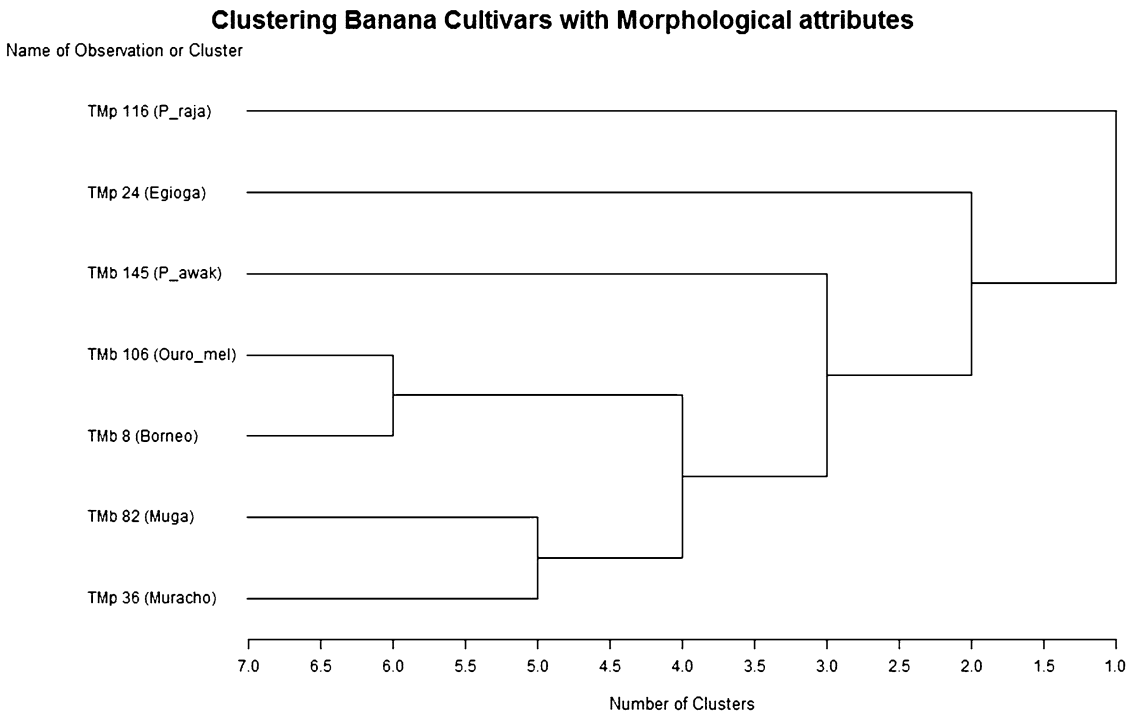


**Fig. 5** Dendogram showing the relationship of *M. acuminata* accessions based on 32 agro-morphological descriptors

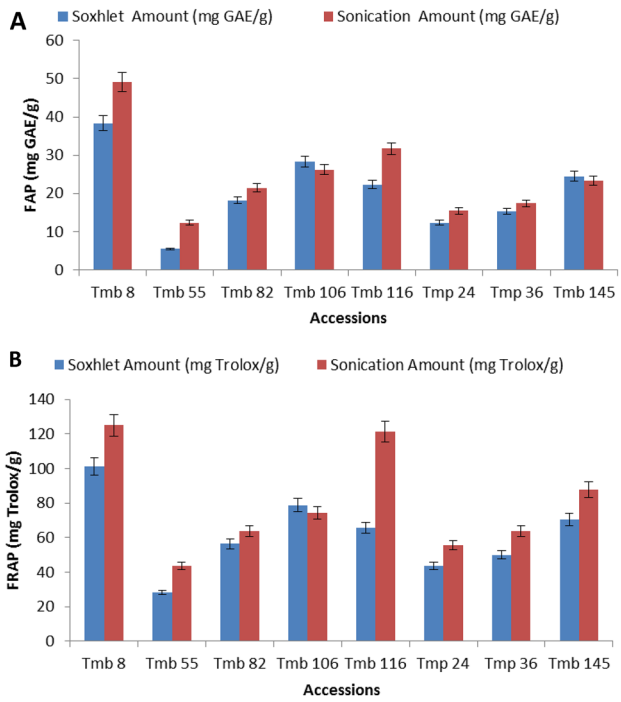


**Fig. 6** Ferric chloride reducing antioxidant power of Soxhlet and sonicated leaf extracts of *M. acuminata* accessions: **a** accessed by Gallic acid equivalent; **b** accessed by Trolox equivalents

contributed significantly less in the accessions' variation, compared to other markers, which were more differentiating. These markers include peaks with *m*/*z* data: 327, 591, 433, 255, 569, 265, 191, 609, 329, 287, 463, 447, 451, 431 and 377.

## Agro-morphological traits

Morphological trait measurements are among the various methods that have been employed to estimate the genetic diversity of species [34]. The morphological traits varied significantly among the accessions and were used to separate the accessions according to their morphological closeness (Fig. 5). The morphological clustering confirmed the closeness of TMb 106 and TMb 8, which originated from France, having similar morphological characters (same pseudostem height ($\leq$2 m), blotches at petiole base, petiole canal of the third leaf, petiole margin, and petiole margin colour, edge of petiole margin and colour of cigar leaf), as suggested by the metabolomics method. But, the two methods diverted in the grouping of TMp 36 and TMb 82, which are grouped as very closed by the morphological characters but very different when metabolomics markers are considered. The divergence between the two methods occurred also for the three accessions (TMb 145, TMp 24 and TMp 116) as they are separated respectively away from the 2 by 2 groups defined precedently by the morphological character analysis. The

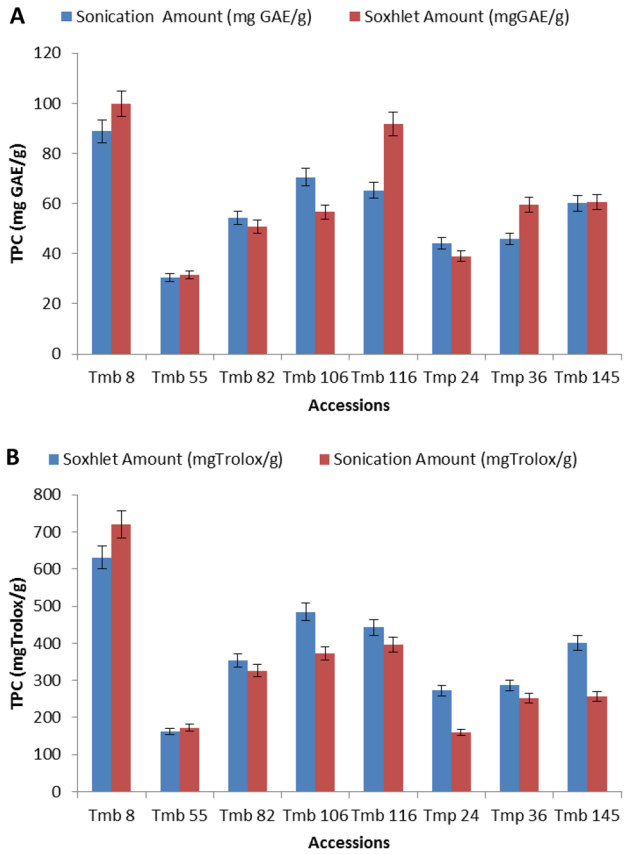


**Fig. 7** Total phenolic content of Soxhlet and sonicated leaf extracts of *M. acuminata* accessions: **a** accessed by Gallic acid equivalent; **b** accessed Trolox equivalent

difference in the clustering observed from the morphological analysis is likely due to phenotypic plasticity of the plants in response to changes in the habitat environment [35], regardless to the metabolomics. Oritz [36] already reported the high influence of the environment on morphological variation in *Musa* germplasm, which is similar to our report. In summary, the two clustering methods converged only in the grouping two accessions.

### Antioxidant activity and total phenolic content

Reactive oxygen species have been implicated in the incidence of many chronic diseases. In recent times, studies aimed at sourcing for new antioxidants from plants have been on the increase [37–39]. This is because the biological activities shown by many plants have been linked to their antioxidant potentials. Therefore, many plant phenolics have been evaluated for their antioxidant activities [40–42]. It follows that the antioxidant potential of many of these compounds may explain their biological activity. In the present study, the antioxidant potential of the accessions of *Musa* was investigated by the FRAP and Folin–Ciocalteu methods. All the accessions demonstrated antioxidant capacity at various levels. The sonicated accessions exhibited higher FRAP ranging from 12.33 to 49.14 mg GAE/g and 43.52 to 125.10 mg TROLOX/g (Fig. 6). The TPC ranged from 30.48 to 88.84 mg GAE/g and 162.97 to 484.51 mg TROLOX/g in Soxhlet extracted accessions (Fig. 7). The two closely related accessions in both metabolomics and agro-morphological analyses; TMb 8 and TMb 106 gave the highest FRAP and TPC values when extracted by Soxhlet using GA and TROLOX as standards. It therefore follows that the presence of important polyphenolics in the *Musa* accessions, especially, TMb 8 and TMb 106 could suggest them as important repositories of bioactive compounds with enormous health benefits.

## Conclusions

In this study, metabolomics markers were identified and used to cluster the *M. acuminata* accessions. The grouping patterns in the *M. acuminata* accessions provided by two methods of diversity analysis based on metabolomics and agro-morphological characters were rather different. This suggests that no correlation link is relevant between the two analytical methods. This study identifies the presence of polyphenolic compounds and significant antioxidant potential of *Musa* leaves extract which indicates their potential as source of bioactive phytochemicals.

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### Compliance with ethical standards

**Conflict of interest** The authors report no declarations of interest.

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