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Metabolites proûling of Sapota fruit pulp *via* a multiplex approach of GC/MS and UPLC/MS in relation to its lipase and glucosidase inhibition eûects

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Background. Sapota fruits, *Manilkara zapota* L., are juicy, and nutrient-rich fruits aside from their several health beneûts. **Methods.**The current study presented an integrated metabolomic proûling of sapota fruits pulp *via* GC/MS and UPLC/MS, quantiûcation of total phenolics and ûavonoids, antioxidant capacity, and inhibitory eûect of pancreatic lipase (PL) and ³-glucosidase enzymes. **Results.** GC/MS analysis of silylated primary polar metabolites led to the identiûcation of 68 compounds belonging to sugars (74%), sugar acids (18%), and sugar alcohols (7%) mediating the fruit sweetness. Headspace SPMEGC/MS analysis led to the detection of 17 volatile compounds belonging to nitrogenous compounds (72%), ethers (7.8%), terpenes (7.6%), and aldehydes (5.8%). Non polar metabolites proûling *via* HR-UPLC/MS/MS-based GNPS molecular networking led to the assignment of 31 peaks, with several novel sphingolipids and fatty acyl amides reported for the ûrst time. The total phenolic content was estimated at 6.79±0.12 mg GAE/g, concurrent with antioxidant capacities of the fruit at 1.62 ±0.2, 1.49±0.11, and 3.58±0.14 mg TE/g *via* DPPH, ABTS, and FRAP assays, respectively. *In vitro* enzyme inhibition assays revealed considerable PL inhibition activity (IC_{50} = 2.2 ± 0.25 mg/mL), whereas no inhibitory eûect towards ³-glucosidase enzyme was detected. This study provides deep insight into sapota fruit9s ûavor, nutritional attributes, secondary metabolites, and its biological eûects.

1 **Metabolites profiling of Sapota fruit pulp *via* a multiplex approach**

2 **of GC/MS and UPLC/MS in relation to its lipase and glucosidase**

3 **inhibition effects**

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inhibition effects

Abstract:

Background. Sapota fruits, *Manilkara zapota* L., are juicy, and nutrient-rich fruits aside from their several health benefits.

Methods. The current study presented an integrated metabolomic profiling of sapota fruits pulp *via* GC/MS and UPLC/MS, quantification of total phenolics and flavonoids, antioxidant capacity, and inhibitory effect of pancreatic lipase (PL) and ³-glucosidase enzymes.

Results. GC/MS analysis of silylated primary polar metabolites led to the identification of 68 compounds belonging to sugars (74%), sugar acids (18%), and sugar alcohols (7%) mediating the fruit sweetness. Headspace SPME-GC/MS analysis led to the detection of 17 volatile compounds belonging to nitrogenous compounds (72%), ethers (7.8%), terpenes (7.6%), and aldehydes (5.8%). Non polar metabolites profiling *via* HR-UPLC/MS/MS-based GNPS molecular networking led to the assignment of 31 peaks, with several novel sphingolipids and fatty acyl amides reported for the first time. The total phenolic content was estimated at 6.79–0.12 mg GAE/g, concurrent with antioxidant capacities of the fruit at 1.62 –0.2, 1.49–0.11, and 3.58–0.14 mg TE/g *via* DPPH, ABTS, and FRAP assays, respectively. *In vitro* enzyme inhibition assays revealed considerable PL inhibition activity (IC_{50} = 2.2 – 0.25 mg/mL), whereas no inhibitory effect towards ³-glucosidase enzyme was detected. This study provides deep insight into sapota fruit s flavor, nutritional attributes, secondary metabolites, and its biological effects.

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Keywords: *Manilkara zapota* L.; SPME-GC/MS; UPLC/MS; GNPS Molecular Networking; lipase inhibitor; ³-glucosidase inhibitor; antioxidant.

1. Introduction

The enormous diversity of fruits worldwide makes them a potential source for a wide range of nutraceuticals that are useful as edible food and health agents. According to studies, regular consumption of vegetables and fruits can aid in lowering the susceptibility to risky diseases (Bazzano et al., 2003).

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Sapotaceae is a family of tropical, evergreen trees and shrubs that comprises more than 50 genera and 1100 species. Their trees are famous for producing latex, gums, timbers from the trunks, oil from the seeds, and most of which produce edible flowers and sweet fruits (Vaghani, 2003). Fruits have been valued for their rich nutritional quality due to their carbohydrates, vitamins, minerals, and fiber

content, aside from their multitude health benefits owing to their remarkable bioactive substances. The family is rich in pentacyclic, triterpenoid saponins and their glycosides, which are thought to be related to folk uses of the family as antimicrobial, antitumor, and anti-inflammatory (Baky et al., 2022).

One of the most famous genera in the Sapotaceae family is *Manilkara*, with *ca*. 80 species. The trees of *Manilkara zapota* (L.) Van Royen is the most extensively grown species, native to Central America, especially Mexico and the Caribbean. The long-lived trees are now broadly cultivated in many tropical Asian countries. *M. zapota* trees are recognized for their wood and latex, in addition as a source of sweet, edible fruits. The name *Manilkara zapota* has numerous synonyms mentioned by the plant list including *M. zapotilla* (Jacq.), *M. achras* (Mill.) Fosberg, *Achras zapota* or (*sapota*) L., *A. zapotilla* (Jacq.) Nutt., or *Sapota zapotilla* (Jacq.) (The Plant List (2010) , Madani et al., 2018). *M. zapota* (L.) fruits, also known as Sapota, Sapodilla, and chicozapote, comprise a wide array of nutrients, minerals, and polyphenols, and hence diverse biological activities. Fruits are uniquely delicious, with a delicate, grainy feel and pleasant smell, covered by thin, yellowish-brown peel (Siddiqui et al., 2014). The nutritional value of fruits is attributed to their high content of carbohydrates 20%, dietary fiber 5 %, with small amounts of proteins and fats, in addition to considerable amounts of vitamins A and C. Fruits are rich in minerals especially calcium and potassium, alongside magnesium, sodium, phosphorus, and a lesser amount of iron (Singh et al., 2021, Rivas-Gastelum et al., 2023). Unripe fruits are astringent in taste due to high levels of catechins, gallic acid, chlorogenic acid, gallotannins, and proanthocyanidins (Ma et al., 2003). Upon maturation, free sugars increase concurrently with a drop in phenolics. Aside from phenolics, fruits are also rich in triterpenoids represented by ³1amyrin13-(3'1dimethyl) butyrate and lupeol131acetate (Fayek et al., 2013). In a comparative study among the different *M. zapota* (L.) parts, the highest level of phenolic acids and flavonoids was found in leaf, followed by seed, peel, and flesh. Consequently, leaves showed the most potent antioxidant effect using ³-carotene bleaching and DPPH *in vitro* assays (Tamsir et al., 2020). Such promising antioxidant actions of the fruit, along with the lipoidal content of oleic, linoleic acids, and glycerol, make the fruits appropriate for dermatological formulations for anti-wrinkles, anti-aging medications (Shafii et al., 2017). Other effects reported in fruit include its aqueous extract reported to exhibit potent anti-hypercholesterolemic, antihyperglycemic, and antioxidant activities (Fayek et al., 2013). Both leaf and fruit extracts showed a strong influence on lowering sugar and cholesterol blood levels. Moreover,

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fruits revealed a decrease in body weight, posing it in obesity and diabetes management 96 (Barbalho et al., 2015).

97 Suppressing pancreatic lipase enzyme helps in the treatment of several metabolic disorders, 98 including diabetes, hyperlipidemia and obesity which exerts severe health issues for all body 99 organs, especially in developing countries (Lunagariya et al., 2014).

100 The current study presents a multiplex approach employing Gas Chromatography coupled with 101 Mass Spectrometry (GC/MS), and High-Resolution Ultra-high performance liquid 102 chromatography coupled with tandem Mass Spectrometry (HR-UPLC/MS/MS) for profiling *M.* 103 *zapota* (L.) fruit pulp targeting its aroma, non volatile polar and non polar metabolites to account 104 for fruit sensory, nutritional, and health attributes. The aroma profile was assessed using solid 105 phase micro-extraction (SPME), whereas primary polar metabolites viz. sugars were analyzed 106 using GC/MS post-silylation. For large molecular weight non polar metabolites analysis, HR107 UPLC/MS/MS was employed aided by Global Natural Products Social (GNPS) molecular 108 networking to aid in metabolites identification. In addition, total phenolics and flavonoids were 109 determined for standardization, alongside lipase and ^{3}-glucosidase inhibition activities of fruit 110 extract.

111 2. Material and Methods

112 2.1. Plant Material and Extraction Process

113 The fresh fruit pulp of sapota (*Manilkara zapota* L.) was collected from Haryana Agriculture
114 University, Hisar, India in December 2022 and was identified by Dr. Rupesh Deshmuk, Central 115 University of Haryana, India. Fruits were immediately lyophilized, peeled and the pulp was taken 116 and ground in liquid nitrogen using mortar and pestle, and stored in closed, tight bags till further 117 analysis at -20 C. The extraction process was carried out following the procedure previously 118 mentioned in (El-Akad et al., 2023). Using a homogenizer (Ultra-Turrax, IKA, Staufen, Germany) 119 at 11,000 rpm, 5 X 60 s with 1 min break intervals, about 150 g of the crushed sample was mixed 120 with 6 mL methanol containing 10 ug/mL umbelliferone (Sigma-Aldrich, St. Louis, MO, USA, 121 purity g 98%) that used as an internal standard and for MS calibration. Further processing, extract 122 was centrifuged at 3000x *g* for 30 min after being vortexed for 1 min., then filtered through a 22 m 123 pore size filter and directly used for HR-UPLC-MS/MS analysis. For GC/MS, 100 ul was aliquoted 124 in a glass vial and left to evaporate till dryness under a nitrogen stream. For bioassay, fruit pulp was 125 extracted using 100% MeOH

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Commented [MA7]: Proof of plant identification missing

Commented [MA8]: Details of lyophilization technique or model missing

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Commented [MA10]: Treated with liquid nitrogen, powdered with pestle and mortar

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till exhaustion and evaporated using rotavap under vacuum to yield 126 dried yellowish residue stored at -20 C till further assays.

127 **2.2. Chemicals and Fibers**

128 The stableflex fiber used for solid phase micro-extraction (SPME) was covered by 129 divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 m) and was obtained 130 from Supelco (Oakville, ON, Canada). Chemicals were acquired from Sigma Aldrich (St. Louis, 131 MO, USA). Milli-Q water and solvents that were used for HR-UPLC/MS/MS analysis; formic acid 132 and acetonitrile were of LC-MS grade and obtained from J. T. Baker (The Netherlands).

133 ABTS [2,202azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] g 98% 134 purity, DPPH (2,2-diphenyl-1-picrylhydrazyl), ferric chloride for FRAP (ferric reducing 135 antioxidant power), Trolox (6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid; g97% 136 purity), Porcine pancreatic lipase (PL) enzyme type II, intestinal ^{3}-glucosidase, Orlistat, Acarbose 137 from Sigma Aldrich Chemie GmbH (St. Louis, MO).

138 **2.3. GC/MS Analysis of Silylated Primary Polar Metabolites of *M. zapota* Fruit Pulp:**

139 Analysis of primary metabolites in fruit pulp followed the exact procedure by (El-Akad et al., 140 2023), in triplicates under the same conditions. The derivatization process was compiled as follows; 141 the dried methanol extract of fruits prepared as in section 2.1. was derivatized using a silylating 142 agent; N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) (150 L equally diluted with 143 anhydrous pyridine), incubated in an oven for 45 min at 60 C (Yamato Scientific DGS400 Oven, 144 QTE TECHNOLOGIES, Hanoi, Vietnam), just prior to GC/MS analysis. Silylated compounds 145 were separated on a column 30 m. x 0.25-mm id x 0.25-m film (Rtx-5MS Restek, Bellefonte, PA, 146 USA), and were analyzed under conditions described in (Farag et al., 2022). Analysis was done 147 in triplicate under the same conditions along with a blank sample to assess for biological variance.

148 **2.4. SPME Analysis of Volatiles in *M. zapota* Fruit Pulp:**

149 Preparation and investigation of aroma profile in fruit pulp were performed following the same
150 conditions reported in (Farag et al., 2022). A quadrupole mass spectrometer connected to an Agilent 151 5977B GC/MSD (Santa Clara, CA, USA) was used fitted with a DB-5 column (Supelco, Bellefonte, 152 PA, USA) 30 m x 0.25 mm i.d. x 0.25m film thickness. The scan range of the MS spectrometer was 153 adjusted at *m/z* 40 500 and EI mode at 70 eV. Analysis was done in triplicate under the same 154 conditions along with a blank sample to assess for biological variance.

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2.5. Identification of Volatile and Non-Volatile Silylated Components Using GC/MS

Deconvolution of the GC/MS spectrum was first applied using AMDIS software (www.amdis.net). Detection of compounds was achieved by matching the retention indices (RI) of the detected peaks with those of the n-alkanes series (C8-C30), along with matching their mass spectra with respected databases; NIST011 and WILEY libraries, and standards whenever available.

2.6. High-resolution Ultrahigh-Performance Liquid Chromatography Coupled with Tandem Mass Spectrometry (HR-UPLC/MS/MS)Analysis of Non Polar Metabolites

HR-UPLC/MS/MS analysis was performed using an ACQUITY UPLC system (Waters, Milford, MA, USA) coupled with an HSS T3 column (100 x 1.0 mm, particle size 1.8 m; Waters). The analysis was accomplished following the precise guidelines as reported by (Hegazi et al., 2022). The tentative identification of compounds was based on the generated molecular formula at an error of 10 ppm or less, and by comparing MS^2 fragments with those reported in literature.

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2.7. Molecular Networking and Metabolites Annotation of HR-UPLC/MS/MS Data

The HR-UPLC/MS/MS data (acquired in positive ion mode) from the fruit extract was used to create molecular network (MN) using GNPS website (http://gnps.ucsd.edu). The raw data underwent conversion to an open-source format (.mzML) using the MS Convert package (Proteowizard Software Foundation, Version 3.0.19330, USA). The transformed (.mzML) files were then uploaded to the GNPS platform using WinSCP (SFTP, FTP, WebDAV, and SCP client). GNPS parameters included fragment ion tolerance (0.5 u), minimum-matched fragments (4 ions), minimum pairs cosine score (0.65), and parent mass tolerance (1.0 u), which were used to generate consensus spectra. To access the generated molecular network, follow this link (https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=4b2bc3c320234c6e804955a6119d1240)

The spectral network was visualized with the aid of Cytoscape 3.9.1. Each spectrum was represented as a node in the visualization, with spectrum-to-spectrum connections forming edges based on structural correspondence identified through MS analysis (Xu et al., 2021, Zia-ur-Rehman et al., 2022)

For natural products dereplication, various databases were searched, including PubChem (https://pubchem.ncbi.nlm.nih.gov/), Metabolome Database (https://hmdb.ca/), Online lipid calculator database (http://www.mslipidomics.info/lipid-calc/), and LIPID MAPS (https://www.lipidmaps.org/). For visualization of metabolite classes with sapota fruit, acquired tandem mass spectrometry data, and molecular networks were (MNS) constructed. Mass spectrometric data were classified according

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to the spectra resemblances in the fragmented ions (Ragheb et al., 2023), and to aid in the identification of unknown peaks.

2.8. Total Phenolic (TP) and Total Flavonoid (TF) Contents Estimation

The evaluation of TP content was based on the Folin-Ciocalteu method, previously described by (Babot et al., 2018). The result was represented as milligrams of gallic acid equivalent per gram sample (mg GAE/g), after triple measurements. For TF content, aluminum chloride assay was used, with results expressed as milligrams of rutin equivalent per gram sample (mg RE/g) (Babot et al., 2018). Fruit pulp methanol extract for both tests were analyzed, after being re-dissolved and diluted, in 96-well plates using a SPECTROstarfi Nano Multi-Detection Microplate Reader (BMG Labtech, Ortenberg, Germany).

Commented [MA16]: Fruit pulp extract was analyzed for TPC and TFC

2.9. *In vitro* Antioxidant Assays

Two assays depending on the free radical scavenging actions; DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS [2,22-azino-bis(3-ethylbenzothiazoline) 6-sulfonic acid], along with the FRAP (Ferric reducing antioxidant power) technique for ferric reducing capacity, were applied following the protocols of (Babot et al., 2018). The resulting data were reported as mg of Trolox equivalents per gram sample (mg TE/g) in each case.

2.10. *In vitro* Enzyme Inhibition Assays

Pancreatic Lipase (PL) inhibition activity was measured colorimetrically based on *p*-nitrophenol release (at 410 nm compared to a blank of denatured enzyme), following a modified method mentioned by (Bustanji et al., 2011). The experiment was run in triplicate and percentage inhibition represented the average of three observations using two concentrations of the extract, expressed in terms of IC_{50} (Half-maximal inhibitory concentration). Orlistat was used as a positive control as a standard PL inhibitor.

Whereas, ^{3}-Glucosidase inhibitory action was measured following the same protocol (Tanase et al., 2019). The color formed due to *p*-nitrophenol was measured at 405 nm, and acarbose was used as a positive control. The result was calculated as the concentration that inhibited 50% of the enzyme (IC_{50}), after three measurements using two concentrations of the extract.

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3. Results & Discussion

3.1. Metabolites profiling of silylated primary polar metabolites in *M. zapota* fruit pulp as analyzed *via* GC/MS

GC/MS analysis of the non-volatile primary metabolites in fruit was carried out post-silylation to present a comprehensive overview of metabolites (Figure 1), and further account for nutritional and sensory attributes in fruits. As listed in Table (1), 68 compounds were detected belonging to 11 chemical classes. The most abundant metabolite classes as typical in fruits included sugars, sugar acids, and sugar alcohols detected at 74.0, 18.3, and 7 %, respectively. Other detected primary metabolites though at much lower levels included fatty acids/esters (0.22%), organic acids (0.22%), and inorganic acids (0.124%), along with traces of alcohols, terpenes, and nitrogenous compounds.

The high content of sugars in fruit imparts a sweet taste and calories as typical in most fruits. As represented in the TIC (Figure 2), sugars represented major primary metabolites detected in 22 peaks, especially mono-sugars to account for 75% of identified sugars. The most prominent forms included fructose (22.1%), D-glucose (16.6%), and mannose (16.5%). Sucrose (17.9%, peak 63) was the predominant disaccharide. Previous reports on sapota fruit for total sugars fruits revealed that they amounted for 46 to 52.2% of its weight (Swami, 2018).

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The high level of sugar acids represented by keto-D-gluconic acid (9.8 %) and L-gluconic acid lactone (7.9%) imparts a slightly tangy and acidic taste, which might provide a balanced sensation alongside the intense sweet taste (Karaffa & Kubicek, 2021).

Sugar alcohols with lower calorie intake than free sugars were represented by 5-deoxy-myo-inositol (6.7%). In addition to its low-calorie level, it reduces the body s resistance to insulin and aids in diabetes management (Corrado & Santamaria, 2015).

Although organic acids were present at minor levels (0.22%), they were represented by 13 compounds, with oxalic and pyruvic acids as major forms.

Fatty acids/esters composition plays a role in nutrition and flavor in fruits, detected at 0.23% including glycerol monostearate, the monoglyceride ester with a sweet taste. Likewise, saturated fatty acid palmitic acid (0.045%) and its monoester, monopalmitin (0.054%), were detected suggesting that fruit is richer in saturated fatty acids. To the best of our knowledge, this is the first detailed report on primary metabolites composition in sapota fruit to account for its nutritive value.

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3.2. Aroma Profiling of *M. zapota* Fruits Pulp *via* SPME Coupled to GC/MS:

SPME-GC/MS analysis of aroma composition in *M. zapota* fruit revealed the detection of 17 compounds belonging to 8 chemical classes mostly dominated by nitrogenous compounds amounting for 71.7%. Other classes included ethers (7.8%), terpenes (7.6%), and aldehydes (5.8%) as represented in Figure (2) and Table (2).

The identified nitrogenous compounds (peaks 3, 5, 10 and 11) were detected for the first time in the fruit belonging to isothiocyanates, a hydrolysis product of glucosinolates. The major compound was 3-butenyl isothiocyanate (64.3%), alongside allyl isothiocyanate (2.8 %). The presence of ethers in fruit provides specific fragrances, represented here by benzyl isoeugenol ether (4.2%), peak 17, in addition to pentyl allyl ether (3.1%), peak 4, and cineole, peak 8 (Kirsch & Buettner, 2013).

As typical in fruit aroma, a considerable amount of mono- and sesquiterpenes were detected amounting (7.6%) of total aroma composition, with ò- caryophyllene (5.42%), peak 16, and limonene (1.54%) peak 7 as major components. ò-Caryophyllene was previously detected in sapota fruit volatiles using steam distillation (Pino et al., 2003). Aldehydes, which accounted for 5.8% of the fruit aroma, likely contribute to the fruit scent and likewise, protect against their deterioration due to potential antibacterial action (Aljaafari et al., 2022). The major form was hexanal at 4% to impart an apple-like odor (Plotto et al., 2017), alongside benzyl alcohol (2.6%) and with light fragrant smell (Kulkarni & Mehendale, 2005) and all to contribute to sapota fruit specific scent.

3.3. Non polar Metabolites Profiling of *M. zapota* Fruit as Analyzed *via* HR-UPLC/MS/MS

Considering that GC/MS can only detect low molecular weight polar phytochemicals in food, and to provide comprehensive composition of sapota fruit metabolome, HR-UPLC/MS/MS was employed to complement GC/MS and target large molecular weight lipids (Islam et al., 2021). Herein, a list of tentatively identified metabolites of *M. zapota* fruit is presented in Table (3), along with their chromatographic and spectroscopic data (Figure 3). Major identified metabolites belonged to lipoidal components e.g. fatty acyl amides, phospholipids, and sphingolipids, and contrary to low levels of lipids detected using GC/MS more suited for polar chemicals profiling. Other classes detected at minor levels included fatty acyl esters, nitrogenous compounds, glycol, amino acids and diethanolamines. To aid in metabolites assignment, molecular networking was used for HR-

UPLC/MS/MS dataset visualization. The MN afforded a total of 346 nodes, of which 276 141 clustered nodes and 205 self-looped nodes were detected (Figure 4). The visual aid of MNS 277 showed the diverse metabolite classes, which assisted in analogs identification. The substantial 278 clusters of positive MN belonged to oxylipids including cluster A (sphingosine and sphinganine), 279 cluster B (fatty acyl amides), cluster C (phytosphingosine), and cluster D (fatty acyl esters), (Fig. 280 4).

281 3.3.1. Identification of fatty acyl amides

282 Fatty acyl amides, a subclass of lipids, exist as bioregulators for lipids in plants and are formed 283 through amidation of fatty acids (Tanvir et al., 2018). Seven fatty acyl amides were identified in 284 sapota fruit extract based on neutral losses of 14 amu, indicative of an acyl group (Suppl. Fig. S1). 285 Further, the annotation of saturated fatty acyl amides $[M + H]^+$ 256.263, 284.293 and 312.325 was 286 based on their abundant fragments at *m/z* 102 ($C_5H_{10}NO$) and *m/z* 116 ($C_6H_{12}NO$). The presence 287 of a single unsaturation in the alkyl chain of acyl amides alters product ion dramatically, as 288 fragmentation differed with daughter ions corresponding to the combined neutral losses (-35 Da) 289 of H_2O and NH_3 in the amide group. Distinct fragments at *m/z* 247 for the successive losses of 290 water and ammonia moieties along with multiple losses of CH_2 were recognized in MS^2 spectra of 291 assigned unsaturated acyl amides. Conclusively, the whole loss of the acyl chain and formation of 292 9-carbon and 10-carbon macrocyclic dieneyl cation yielded daughter ions at *m/z* 135 and 121, and 293 aided in structural elucidation of that subclass (Murphy, 2014).

294 Peaks 17, 18, 21, 22, 26, 27 and 28 exhibited molecular ions $[M+H]^+$ at *m/z* 256.26, 282.27,
295 284.29, 310.30, 312.325, 338.34 and 675.67 in MS/MS spectra with distinctive fragment ions of 296 fatty acyl amides; palmitamide, octadecenamide (oleamide), octadecanamide (steramide), 297 eicosenamide icosanamide, erucamide and erucamide dimer, respectively, cluster B in MN (Fig. 298 4). These metabolites are reported here for the first time in sapota fruit, and likely to account for a 299 wide array of therapeutic indications such as treatment of bacterial infections, cancer, 300 inflammations, and metabolic disorders (Tanvir et al., 2018). Steramide was detected previously in 301 Sapota leaves (Tamsir et al., 2020).

302 3.3.2. Identification of sphingolipids

303 The identified sphingolipids were detected in clusters A and C in GNPS network (Fig. 4).

Sphingosine is the major form present in this class and is assigned in peaks (3, 6, 8, 10, and 11), followed by the sphinganine class which was observed in BPC in peaks (20, 24, 25, and 30). The lipophilicity, formula composition, and fragmentation pattern suggest that these peaks are sphingolipid conjugates.

Most of the sphingolipids and their dihydro equivalents fragment to backbone ions with *m/z* 264 in positive ion mode as a key for the identification of sphingolipids (Otify et al., 2019). Most notably, product ion (*m/z* 284) is for sphinganine, whereas product ion at *m/z* 282 corresponds to sphingosine (Suppl. Fig. S2).

For example, peak 8 exhibited a molecular formula [$C_{18}H_{37}NO_3$ (m/z 316.2836)], such formula matches the class of sphingoid bases (that is non-phosphorylated plant sphingolipids) belonging to basic sphingoid compounds, either dehydrophytosphingosine, or 6-hydroxysphingosine (LØnÆrt et al., 2021). The fragmentation pattern showed product ions at *m/z* 280 and 262 corresponding to losses of 2 and 3 H_2O molecules, respectively, and assigning it as dehydrophytosphingosine.

Peak 3 showed a fragmentation pattern of tetradecaphytosphingosine, based on the neutral loss of two water molecules and alkyl chain ($C_{10}H_{20}$, 140 Da) at *m/z* 226 and 122 (Table 3). Sphingolipid long-chain base (LCB) was detected in peak 5 showing fragment ions at *m*\z 272, 254, 242 (Qu et al., 2018). The cerebroside (peak 29) with $(M+H)^+$ at *m*\z 732.56 ($C_{40}H_{77}NO_{10}$) and abundant ion at *m*\z 570 due to neutral loss of hexosyl and further loss of two water molecules to yield product ion at *m/z* 534 (Kang et al., 1999) and assigned as araliacerebroside (Suppl. Fig. S3).

C16 sphinganine, a sphingolipid conjugate, was identified previously in *M. zapota* leaves (Tamsir et al., 2020), albeit this study represents the first comprehensive profiling of sphingolipids in sapota fruits.

3.3.3. Identification of Lysophosphatidylethanolamines

Lysophosphatidylethanolamines (Lyso-PE) were characterized in peaks 14 and 15 (Suppl. Fig. S4) by the molecular formula of $C_xH_xNO_7P$ (Ragheb et al., 2023). LysoPE (0:0/18:2) and LysoPE (0:0/16:0) exhibited $(M+H)^+$ at *m/z* 478.29 and 454.29, respectively. The most abundant ions at *m/z*

337 and 313, in their positive-ion mass spectra, corresponded to the neutral loss of 141 Da of 332 phosphoethanolamine (Fang et al., 2003), and aiding in their assignment for the first time in sapota 333 fruit.

334 **3.3.4. Identification of ethanolamines**

335 Peak 9 with $[M)+)H]^+$ at *m/z* 302 was assigned as tetradecyl diethanolamine ($C_{18}H_{39}NO_2$). The 336 dehydration of parent ion yielded *m/z* 284, with further cleavage of the carbon chain to yield 337 fragment ion at *m/z* 102. The direct loss of carbon chain from quasi-molecular ion gave product 338 ion at *m/z* 106 (Suppl. Fig. S5), key fragment of this class (Zhang et al., 2022). Peak 13 showed 339 similar fragmentation pattern assigned as N-hexadecyl diethanolamine $[M)+)H]^+$ at *m/z* 330.33 and 340 fragment ions at *m/z* 312, 106 and 102. This is the first report for the presence of ethanolamines 341 in sapota fruit. Ethanolamines are at the hub of various cellular processes, they stimulate the 342 synthesis of phosphoethanolamine, a vital component to maintain human health. Moreover, 343 ethanolamine prevents cardiovascular disease and ischemia (Patel & Witt, 2017).

344 **3.3.5. Identification of fatty acyl esters**

345 Fatty acyl esters were grouped in cluster D (Fig. 4) (peaks 23 & 19). This is the first report for 346 the presence of fatty acyl esters in sapota fruit. Peak 23 showed the dehydration of precursor ion 347 $[M)+)H]^+$ (*m/z* 359) that yielded fragment ion at *m/z* 341 (Suppl. Fig. S6) attributed to an allylic 348 cleavage and loss of glyceryl moiety yielding product ion at *m/z* 267 assigned as glyceryl 349 monostearate. Similarly, peak 19 displayed similar fragmentation scheme, suggesting the 350 presence of hexadecanoyl glycerol with product ions at *m/z* 313 and 239.

351 **3.3.6. Identification of Tocopherols**

352 MS^2 fragments of dehydrotocopherol (*m/z* 429.37) were detected in peak 31 and characterized 353 by successive losses of alkyl groups to show fragmentation pattern; (*m/z* 401, 345 and 303), 354 eventually the complete loss of side-chain together with the cleavage of chromene ring developed 355 the product ion *m/z* 165 (Suppl. Fig. S7).

356 **3.4. Total Phenolics (TP) and Total Flavonoida (TF) Contents**

357 The quantitative estimation of total phenolics and flavonoids in sapota fruit flesh extract 358 revealed that it encompasses a moderate amount of phenolics (6.79 – 0.12 mg GAE/g) and traces 359 of flavonoids below our LOQ (limit of quantitation). The ripeness of the fruit results in a major 360 change in its composition from an astringent taste owing to tannins and catechins, to a sweet taste 361 due to the

elevated sugar content. Fruit ripening had an impact on phenolic content due to the oxidation of phenolic compounds by the action of polyphenol oxidase (PPO) enzyme (Torres Rodr guez et al., 2011). The higher phenolic and flavonoid contents were reported for leaves, then peels and the least was for the flesh, where values detected were at 14.15 – 0.48, 1.23 – 0.06, and 0.73 – 0.1 g GAE/100 g, respectively, for 70% ethanol extract of each organ (Tamsir et al., 2020).

3.5. *In vitro* Antioxidant Assays

Assessment of the antioxidant activity of sapota fruit pulp extract was carried out using DPPH and ABTS scavenging assays, in addition to FRAP assay to estimate its reducing property. Results revealed moderate effects at 1.62 – 0.2, 1.49 – 0.11, and 3.58 – 0.14 mg TE/g as per DPPH, ABTS, and FRAP assays, respectively. According to previous reports, the highest antioxidant activity was exhibited by leaf (92.96 – 0.06 %), then peel (91.98 – 0.71%), much higher than that of fruit pulp (78.21 – 0.04 % of DPPH scavenging activity) as was reported in this study (Tamsir et al., 2020).

The metabolic profiling of fruit pulp showed that sphingoid bases and fatty acyl amides were the most abundant components, they could be one of the major contributors to the antioxidant activities. Prior studies proved that sphinganine inhibits the transport of cholesterol and low density lipoprotein (Tamsir et al., 2020), (Roff et al., 1991). Furthermore, previous findings confirm that monounsaturated fatty acids regulate several biochemical events within the cells (Murphy, 2015). Additionally, other constituents may work together synergistically to boost antioxidant effectiveness. Herein, several metabolites detected in the present study were reported for their antioxidant effect, including sugar alcohols, *viz*, mannitol (Kang et al., 2007), allyl isothiocyanates (Caglayan et al., 2019), palmitic acid, linoleic acid, (Henry et al., 2002), organic acids, *viz*, malic acid (Gsecka et al., 2018), along with anethole (Aprotosoaie et al., 2019), curlone (Jayaprakasha et al., 2002), limonene (El Omari et al., 2023), cineole (Hoch et al., 2023).

3.6. *In Vitro* Enzymes Inhibition Assays

Fruit pulp extract was assessed for its hypolipidemic and antidiabetic activities *via in vitro* assays targeting the inhibition of pancreatic lipase (PL) and ³-glucosidase enzymes, respectively.

PL inhibitory assay tested the extract s influence on enzymatic activity and its potential for obesity management and lipid metabolic disorders. Results revealed that sapota fruit extract inhibited lipase

enzyme by IC_{50}= 4.42– 0.5 and 2.21 – 0.25 at sample concentrations of 10 and 5 391 mg/mL, respectively, compared to the standard drug, Orlistat which showed IC_{50} values of 0.16 392 and 0.08 mg/mL, Table (4).

393 Lipase enzyme plays a major role in fat metabolism. its downregulation leads to a decrease in 394 LDL and an increase in HDL (Liu et al., 2020), and provides health benefits for obesity prevention 395 and its related disorders (Marzouk et al., 2024).

396 In the current study, the major metabolites detected in sapota fruit were sphingolipids, fatty
397 acyl amides and phospholipids which could relate to its potential lipase inhibitory effect. Previous
398 studies reported the potency of dietary sphingolipids in improving metabolic syndrome and
399 associated disorders including atherosclerosis and obesity (Wang et al., 2021). Additionally, the 400 supplementation of sphingolipids has been found to decrease plasma triglycerides, and low-density 401 lipo-protein-cholesterol levels and enhance glucose clearance (Snel et al., 2010). Fatty acyl amide 402 are involved in metabolic heamostasis of human system (Tanvir et al., 2018). Likewise, 403 phospholipids, amphiphilic lipids rich in sapota pulp, have been implicated in exhibiting a favored
404 impact on blood lipids by reducing TG, total cholesterol, and LDL levels (K llenberg et al., 2012).
405 Moreover, terpenoids detected by GC/MS were well-reported as pancreatic lipase inhibitor (Singh
406 et al., 2015). Compared to the potential lipase inhibition effect in fruit pulp, no effect was observed 407 regards ³-glucosidase inhibitory action. Fruits were found inactive against the enzyme compared 408 to the positive drug control acarbose.

409

410 Conclusion

411 Sapota fruit, *Manilkara zapota* L., is well recognized for its delightful taste and satisfying 412 flavor, though with less evidence on its comprehensive chemical makeup. In this study, a 413 metabolites profiling approach for sapota fruit pulp was investigated targeting its non volatile and 414 volatile chemicals using UPLC/MS and GC/MS techniques. SPME-GC/MS analysis resulted in 415 the detection of 17 aroma compounds belonging to nitrogenous isothiocyanates, ethers, terpenes, 416 aldehydes, acids, alcohols, furan and ketone, which emphasized the fruit s delightful, fragrant 417 aroma. With regards to nutrient metabolites to mediate for fruit value and sensory attributes, 418 GC/MS analysis revealed 68

peaks belonging to sugars (mainly fructose, glucose, mannose, and 419 sucrose), sugar acids, and sugar alcohols, as major components and to account for fruit sweetness, 420 and high-calorie content, in addition to some fatty acids/ esters, organic, inorganic acids, alcohols, 421 terpenes, and nitrogenous compounds. HR-UPLC/MS/MS visualized, using GNPS molecular 422 networking, 31 metabolites, including sphingolipids, fatty acyl amides,
423 lysophosphatidylethanolamines, diethanolamines and fatty acyl esters, which were annotated for 424 the first time in sapota fruit. For standardization of fruit pulp in terms of its total phenolics and 425 flavonoids, moderate level of phenolics was detected at 6.79 – 0.12 mg GAE/g.

426 The antioxidant assays revealed a moderate free radical scavenging effect via DPPH (1.62 – 427 0.2 mg TE/g) and ABTS (1.49 – 0.11 mg TE/g) assays, and moderate reducing capacity by FRAP 428 assay (3.58 – 0.14 mg TE/g). Fruit pulp methanol extract exerted a considerable pancreatic lipase 429 inhibitory (PL) action, compared to the standard drug, Orlistat, which has yet to be clarified for 430 which exact chemical, alongside identifying best solvent to be used to insure best recovery of 431 bioactives targeting such an effect. On the other hand, fruits showed no *a*-glucosidase inhibition 432 effect likely attributed for moderate levels of phenolics and the absence of flavonoids in their pulp.
433 The current article presented a comprehensive profiling of phytochemicals to provide better 434 insight into sapota fruits nutritive and health benefits. Future research is recommended to get the 435 best routes for fruit consumption and processing. A comparative approach for exploring 436 metabolites from peels, seeds or fruits from different origins will enhance our insight into the 437 nutritional richness of the fruit and reveal comprehensive chemical profiling. Also, the promising 438 antioxidant and lipase inhibitory action of fruit pulp motivates investigating various extracts to 439 determine the most effective one for discovering promising natural antiobesity medications.

Commented [MA20]: Conclusion is facts based but too lengthy

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616

617

Figure 1

Total ion chromatogram (TIC) of *M. zapota* fruit silylated polar metabolites analyzed using GC/MS.

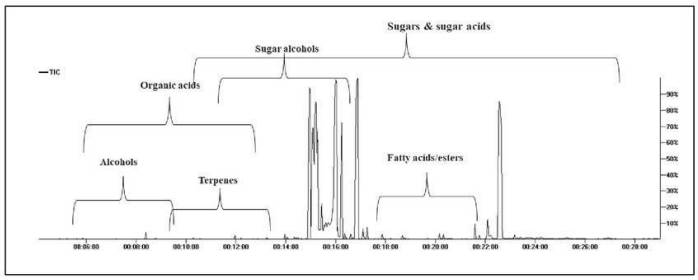
Sugars & sugar acids
Sugar alcohols
TIC
Organic acids
Alcohols
Terpenes
Fatty acids/esters

Figure 2

Total ion chromatogram (TIC) of *M. zapota* fruit volatile constituents analyzed using SPME-GC/MS.

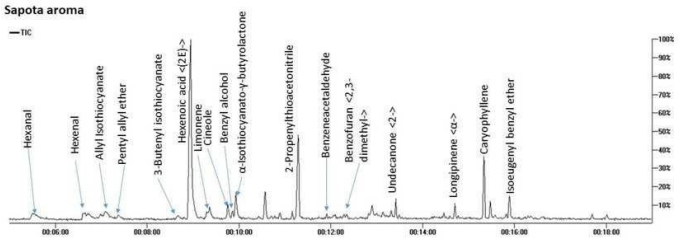
Sapota aroma
TIC
Hexanal
Hexenal
Allyl isothiocyanate
Pentyl allyl ether
3-Butenyl isothiocyanate
Hexanoic acid <(2E)->
Limonene
Citronellal
Benzyl alcohol
α-Isothiocyanato-γ-butyrolactone
2-Propenylthioacetonitrile
Benzeneacetaldehyde
Benzofuran <2,3-dimethyl->
Undecanone <2->
Longipinene <α->
Caryophyllene
Isoeugenyl benzyl ether
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
00:06:00
00:08:00
00:10:00
00:12:00
00:14:00
00:16:00
00:18:00

Figure 3

Base peak chromatogram (BPC) of *M. zapota* fruit non polar metabolites analyzed using HR UPLC/MS/MS, in positive ion mode .

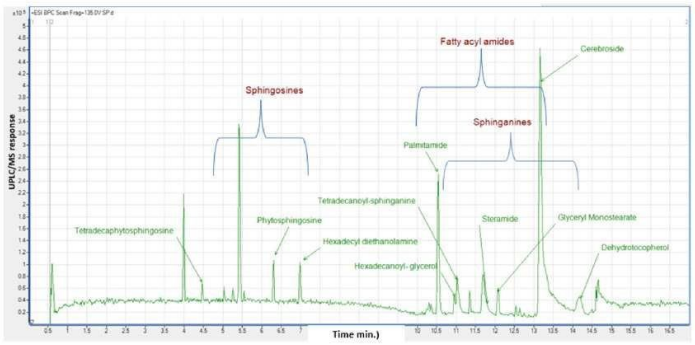
Fatty acyl amides
Cerebroside
Sphingosines
Sphinganines
Palmitamide
Tetradecanoyl-sphinganine
Tetradecaphytosphingosine
Phytosphingosine
Hexadecyl diethanolamine
Hexadecanoyl- glycerol
Steramide
Glyceryl Monostearate
Dehydrotocopherol
UPLC/MS response
Time min.)

Figure 4

Molecular networks created using MS/MS data from M. zapota fruit

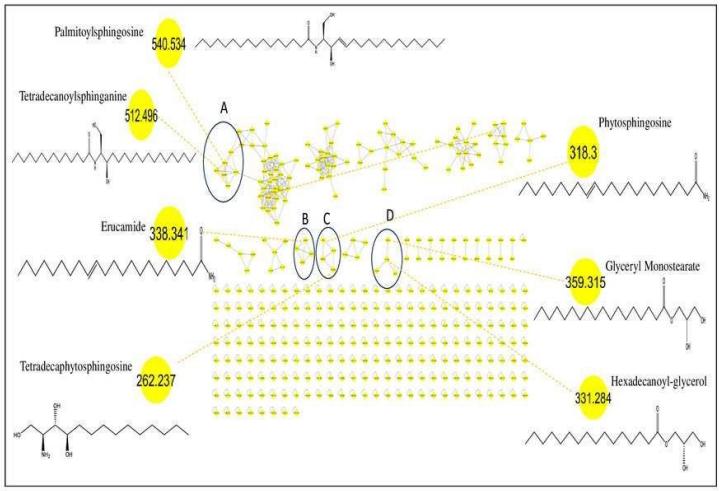
Palmitoylsphingosine
540.534
Tetradecanoylsphinganine
512.496
A
Phytosphingosine
318.3
Erucamide
338.341
B
C
D
Glyceryl Monostearate
359.315
Tetradecaphytosphingosine
262.237
Hexadecanoyl-glycerol
331.284

Table 1(on next page)

Identiûed silylated polar metabolites in *M. zapota* fruits using GC/MS, results expressed as a relative percentile % of the total peak area (n = 3). Tr. Traces.

Results are represented as a relative percentile of the whole peak area (n = 3).

1

Peak	Rt. (min.)	KI	Metabolite	Average – SD
			Alcohols	
1	4.81	1042	2,3-Butanediol, 2TMS	tr.
2	4.93	1049	2,3-Butanediol, 2TMS isomer	0.036–0.002
3	5.127	1061	1,3-Propanediol, 2TMS	tr.
10	7.201	1190	1,2-Glycerol, 2TMS	0.014–0.001
17	8.654	1293	Butanetriol, 3TMS	0.009–0.001
			Total	0.06–0.005
			Fatty acids/esters	
52	17.14	2030	Palmitic acid, TMS	0.045–0.003
55	18.637	2192	Linoleic acid, TMS	tr.
62	21.751	2573	1-Monopalmitin, 2TMS	0.054–0.020
64	23.164	2765	Glycerol monostearate, 2TMS	0.121–0.057
			Total	0.225–0.081
			Organic acids	
4	5.238	1068	Lactic Acid, 2TMS	0.027–0.004
5	5.45	1081	Glycolic acid, 2TMS	tr.
6	5.63	1092	Pyruvic acid, 2TMS	0.048–0.003
7	5.86	1107	Oxalic acid, 2TMS	0.071–0.020
8	6.26	1131	Methylmalonic acid, TMS	tr.
9	6.693	1159	Hydroxybutyric acid, 2TMS	0.006–0.001
13	7.921	1240.5	Benzoic acid, 3TMS	0.006–0.001
18	8.785	1302.5	Malonic acid, 3TMS	0.020–0.005
19	8.859	1308	Succinic acid, 2TMS	0.004–0.002
22	10.185	1403	Ketosuccinic acid, TMS	0.002–0.000
24	11.24	1488	Malic acid, 3TMS	tr.
26	12.207	1566	Erythronic acid, 4TMS	0.025–0.004
27	12.697	1606	Tartaric acid, 4TMS	tr.

			Total	0.220–0.040
			Inorganic acid	
15	8.39	1274	Phosphoric acid, tri-TMS	0.124–0.014
			Nitrogenous compounds/ Amino acid	
11	7.589	1216.7	Uracil, TMS	0.002–0.0
12	7.78	1230	Urea, 2TMS	0.002–0.0
14	8.102	1253	L-Serine, 2TMS	0.001–0.0
			Total	0.005–0.001
			Terpenes	
16	8.517	1283	Anethole	0.001–0.001
21	9.3	1339.4	³-Terpinyl acetate	0.002–0.0
32	13.712	1681	Curlone	0.002–0.003
			Total	0.005–0.005
			Sugar acids	
20	9.18	1331	Glyceric acid, 3TMS	0.005–0.0
39	14.604	1778	2-Keto-l-gluconic acid, 5TMS	0.008–0.0
43	15.167	1831.3	L-gluconic acid, 4TMS, lactone	7.934–0.204
41	15.069	1821.6	Mannonic acid, 5TMS, lactone	0.008–0.007
45	15.182	1833	Keto-gluconic acid, 5TMS	9.790–1.663
51	17.087	2024	D-Gluconic acid, 6TMS	0.101–0.007
53	17.25	2041.6	D-Glucuronic acid, 4TMS	0.292–0.019
56	18.73	2202	D-Galacturonic acid, 5TMS	0.041–0.005
60	20.315	2390	D-Glucuronic acid, 5-TMS	0.095–0.021
			Total	18.274–1.925
			Sugar alcohols	
25	11.96	1546.5	Deoxyribitol, 4TMS	0.026–0.003
34	13.963	1720	Arabitol, 5TMS	0.128–0.018
35	14.061	1728.9	D-Glucitol, 6-deoxy, 5TMS	0.048–0.004
36	14.343	1754.3	D-Mannitol, 6TMS	0.010–0.001

40	14.95	1809.6	Myo-inositol, 5-deoxy, 5TMS	6.691–0.292
54	17.859	2108	Myo-Inositol, 6TMS	0.096–0.011
			Total	7.000–0.329
			Sugars	
23	10.227	1407	L-Threose, 3TMS	0.043–0.003
28	12.87	1622	Arabinose, 4TMS	0.020–0.002
29	13.234	1654.6	Arabinopyranose, 4TMS	0.049–0.007
30	13.277	1658	Galactopyranose, 5TMS	0.002–0.0
31	13.449	1674	Arabinofuranose, 4TMS	0.003–0.0
33	13.774	1695	L-Rhamnose, 4TMS	0.002–0.0
37	14.473	1766	1-Deoxyglucose, 4TMS	0.012–0.002
38	14.52	1770	Mannopyranose, 6-deoxy, 4TMS	0.007–0.0
42	15.077	1822.4	Fructofuranose, 5TMS isomer	0.003–0.003
44	15.172	1831.6	Fructofuranose, 5TMS	11.392–0.212
46	15.245	1839	Fructofuranose, 5TMS isomer	5.919–0.248
47	15.429	1857	D- Galactofuranose, 5TMS	0.207–0.023
48	16.064	1920	Mannose, 5TMS	16.530–0.091
49	16.225	1936	D-Fructose, 5TMS	4.791–0.214
50	16.87	2000.5	D-Glucose, 5TMS	16.652–0.457
57	18.8	2211	Cellobiose, 8TMS	0.006–0.003
61	21.58	2551	Turanose, 8TMS	0.254–0.057
63	22.559	2682	Sucrose, 8TMS	17.963–1.072
65	23.393	2797	3-^{3}-Mannobiose, 8TMS	0.033–0.001
66	24.207	2908.7	Melibiose, 8TMS	0.057–0.004
67	28.528	3503	Maltose, 8TMS	0.006–0.003
68	28.7	3527	^{3}-Gentiobiose, 8TMS	0.052–0.046
			Total	74.002–2.449
			Glycerolipids	
58	19.685	2315	Glycerol-^{3} galactopyranoside, 6TMS	0.041–0.004

59	20.155	2371	Glycerol-galactopyranoside isomer, 6-TMS	0.044–0.004
			Total	0.084–0.008

2

Table 2(on next page)

Volatile compounds in *M. zapota* fruits as analyzed by SPME coupled to GC/MS Results are represented as a relative percentile of the whole peak area (n = 3).

1

Peak	Rt. (min.)	KI	Metabolite	Percent
			Aldehydes	
1	5.492	913	Hexanal	4.133–0.96
2	6.603	1096	3-Hexenal, (Z)-	1.208–0.52
12	12.29	1573	Benzene acetaldehyde	0.438–0.15
			Total	5.78–1.62
			Nitrogenous compounds	
3	7.083	1176	Allyl Isothiocyanate	2.80–0.22
5	8.94	1313	3-Butenyl isothiocyanate	64.33–1.96
10	10.56	1433	³-Isothiocyanato-³-butyrolactone	4.54–0.35
11	11.29	1492	2-Propenylthioacetonitrile	0.02–0.01
			Total	71.69–2.55
4	7.136	1186	Pentyl allyl ether	3.14–0.29
8	9.86	1379	Cineole <1,8->	0.46–0.12
17	15.88	1902	Isoeugenyl benzyl ether	4.23–0.79
			Total	7.825–1.20
			Acids	
6	9.35	1343	Hexenoic acid <(2E)->	1.73–0.51
			Terpenes	
7	9.75	1371	Limonene	1.54–0.27
15	14.699	1786	Longipinene <³->	0.62–0.09
16	15.33	1847	Caryophyllene	5.42–0.63
			Total	7.59–0.99

			Alcohol	
9	9.93	1384	Benzyl alcohol	2.59–0.41
13	12.89	1623	Benzofuran <2,3-dimethyl->	2.01–0.56
			Ketone	
14	13.48	1677	Undecanone <2->	0.79–0.34

2

Table 3(on next page)

Major non polar metabolites annotated in M. zapota fruit methanol extract *via* HRUPLC/MS/MS in positive ion mode

LCBs ; long-chain bases sphingolipid

1

Peak No.	Rt (min.)	Mol. Ion		Molecular		MS/MS fragments	Identification	
1	0.987	166.0862		C_9H_{11}		121, 120, 103	Phenylalanine	Amino acid
2	2.476	139.0755	-1.04	C_8H_{10}	2	124, 121, 120	glycol	Alcohol
3	4.622	262.2371		$C_{14}H_{31}$	3	226, 122	etradecaphytosphingosine	pid
4	5.459	230.2474		$C_{14}H_{31}$		213, 212, 109	Un	us
5	5.631	290.2688		$C_{16}H_{35}$	3	272, 254,242	*LCBs (16;0)*	pid
6	5.645	272.2576		$C_{16}H_{33}$	2	254, 236, 224	Hexadecasphingosine	pid
7	5.73	288.2527		$C_{16}H_{33}$	3	227, 116, 102	Un amide	us lipid
8	5.964	316.2836		$C_{18}H_{37}$	3	298,286, 281, 280, 262, 256, 141	gosine	pid
9	6.388	302.3047		$C_{18}H_{39}$	2	284, 106, 102	amine	us lipid
10	6.41	318.2997		$C_{18}H_{39}$	3	300, 282,	Phytosphingosine	

					264			pid
11	6.624	300.2891		$C_{18}H_{37}$	2	282, 264		pid
12	7.063	415.2108		$C_{20}H_{33}P$	6	354	Un PE	Phospholipid
13	7.145	330.3361		$C_{20}H_{43}$	2	312,106, 102	Hexadecyl diethanolamine	us lipid
14	7.289	478.2936	-1.64	$C_{23}H_{45}P$	7	337	LysoPE(0	Phospholipid
15	7.833	454.2918		$C_{21}H_{44}P$	7	313	LysoPE(0	Phospholipid
16	8.014	358.368	-0.12	$C_{22}H_{47}$	2	340, 322, 270	Un	Un
17	10.59	256.2632		$C_{16}H_{33}$		239, 238,209, 116, 102	Palmitamide	acyl amide
18	10.90	282.2785		$C_{18}H_{35}$		247, 135, 121, 111, 102	(	acyl amide
19	10.94	331.2843	-0.04	$C_{19}H_{38}$	4	313, 109	Hexadecanoyl-glycerol	acyl ester
20	11.10	512.503		$C_{32}H_{65}$	3	284	sphinganine	pid

21	11.90	284.2937		$C_{18}H_{37}$	200, 174,130, 116, 102	(	acyl amide
22	12.02	310.3099		$C_{20}H_{39}$	293,292, 275, 268, 247, 135,121, 111,109	Eicosenamide	acyl amide
23	12.23595	359.3153		$C_{21}H_{42}$	[4] 341, 267, 239,112, 109		acyl ester
24	12.35406	540.5345		$C_{34}H_{69}$	[3] 307,286, 285,284	Palmitoylsphingani ne	pid
25	12.45680	568.566		$C_{36}H_{73}$	[3] 285, 284, 264	Heneicosanoylpentadecasphingani ne	pid
26	13.03128	312.3258		$C_{20}H_{41}$	182, 116,112, 102	Icosanamide	acyl amide
27	13.13385	338.3414		C22H43	339, 321,320, 303. 265,247, 135, 121	Erucamide	acyl amide
28	13.16756	675.6761		$C_{44}H_{86}$	[2] [2] 338, 321, 303,121, 111,109	Erucamide dimer	acyl amide

29	13.2732.5612 7	$C_{40}H_{77}$ $_{0}$	[1]	570, 552, 314, 262	Cerebroside (araliacerebroside)	pid
30	14.1302.305 4	$C_{18}H_{39}$	[2]	285,284, 217	ne	pid
31	14.3429.3715 4	$C_{29}H_{48}$	[2]	401, 371, 345, 205, 203, 187 ,165		l

2

Table 4(on next page)

Enzymes inhibitory actions of sapota fruit extract, at 2 concentrations, compared to the positive controls:

IC_{50} (mg/mL)	Pancreatic Lipase (PL) Inhibition Assay		^{3}-Glucosidase inhibit ory Assay	
Sample Conc.	SF ext.	Orlistat	SF ext.	Acarbose
10 mg/mL	4.42– 0.5	0.16	NA	0.5
5 mg/mL	2.21– 0.25	0.08	NA	0.16

2