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Chemical Profiles of *Terminalia catappa* LINN Nut and *Terminalia subspathulata* KING Fruit

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ABSTRACT

Terminalia catappa and *Terminalia subspathulata* are two species of the Combretaceae family of medium to large forest trees. The fruits of *T. catappa* are known for the edible nuts commonly known as tropical almonds due to their similarity in taste with almonds of commerce. Therefore, the chemical profiles of the fruits of the two *Terminalia* species were examined to ascertain their potential value for food or health uses. Gas chromatography-mass spectrometry (GCMS) and ultrahigh-pressure liquid chromatography-electrospray ionisation tandem mass spectrometry (UHPLC-ESI-MS/MS) techniques were employed to profile the extracts to ensure good coverage of the classes of metabolites of the fruit extracts. The GCMS results revealed that *T. catappa* nuts were rich in palmitic acid (33.2%), linoleoyl chloride (29.1%), and oxacyclohexadecan-2-one commonly known as pentadecanolide (16.2%). In comparison, the major constituents of *T. subspathulata* fruits were palmitic acid (18.1%) and its methyl ester, methyl palmitate (9.3%). Furthermore, a total of 38 compounds were putatively identified in the 70% aqueous methanolic extracts of both species via UHPLC-ESI-MS/MS analysis, comprising three organic acids, sixteen hydrolysable tannins, ten phenolic acids, eight flavonoids, and a diarylheptanoid. The GCMS- and liquid chromatography-mass spectrometry-(LCMS-) LCMS-based metabolite profiles obtained in the present study have revealed the diversity of chemical constituents in the *T. catappa* nuts and *T. subspathulata* fruits, potentially valorised as functional foods

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nutraceutical ingredients for plant-based health products.

Keywords: Curcuminoids, fatty acids, flavonoids, GCMS, hydrolysable tannins, *Terminalia catappa*, *Terminalia subspathulata*, UHPLC-ESI-MS/MS

INTRODUCTION

Plants have served as a source of food and health remedies for man and animals from time immemorial (Tugume & Nyakoojo, 2019). Today, plants still play an important role in traditional and complementary medicine, featuring in many countries' primary health care systems (Shewamene et al., 2020). It is because plants contain a diverse array of metabolites with useful pharmacological properties. However, only a small percentage of the plant kingdom has been explored for their nutritive and/or therapeutic potentials (Noorhosseini et al., 2020). One possible reason for this phenomenon is the absence or lack of scientific evidence supporting their importance and potential value (Liebelt et al., 2019). Plants are valued based on their chemical composition, the relative concentrations of their chemical constituents, and their biological potentials. Therefore, there is a need to fill this gap in information so that their potential value as food, nutraceutical, and future medicines can be fully realised and valorised.

The genus *Terminalia* belongs to the Combretaceae family of plants, which comprises flowering plants of about 530 species of trees, shrubs, and lianas (Christenhusz & Byng, 2016). Several *Terminalia* species are reported to be medicinal and commonly used in Indian traditional medicine or Ayurveda. Despite its widespread use and reports on the efficacy of several of its members, many remain unexplored for their potential uses (Cock, 2015). Studies on several *Terminalia* species in the past, including *Terminalia arjuna*, *Terminalia bellirica*, *Terminalia chebula*, and *Terminalia catappa*, have shown them to be rich sources of phenolic compounds, flavonoids, triterpenoids, and saponins (Cock, 2015; Zhang et al., 2019). These species were also reported to exhibit important pharmacological properties such an anti-inflammatory, hepatoprotective, antimalarial, antidiabetic, and antimicrobial activities (Cock, 2015; Das et al., 2020; Zhang et al., 2019). However, many of these reports were mainly focused on the properties of the leaf, root, stem, and bark or stem bark parts of the plants. Other parts or organs' chemical and biological properties notably the fruits and nuts plentiful from some of these species, are lesser-known and remain unexplored.

Terminalia catappa Linn. is regarded as a wonder plant among the *Terminalia* species and also seemed to be the most prescribed medicinally (Cock, 2015). It is a large tropical tree that grows predominantly in the tropical regions of Asia, Africa, and Australia (Anand et al., 2015). The leaves, bark, and roots have been used in Ayurvedic medicine to treat several ailments such as hypertension, dysentery, and diarrhoea (Cock, 2015; Oyeleye et al., 2018). The ripe fruits of *T. catappa* are the source of

an edible nut known as 'tropical almonds' or 'Indian almonds', which can be eaten raw or roasted, and reported to be a rich source of fatty acids with high nutritive properties (Siew et al., 2015), and anti-cardiovascular potential (Kalita et al., 2018). A phytochemical study on an antifungal fraction of *T. catappa* leaf revealed the presence of punicalin, punicalagin, gallic acid, and isovitexin derivative (Terças et al., 2017). In addition, chemical characterisation of the phenolic-rich extracts of *T. catappa* revealed eleven (gallic acid, catechin, chlorogenic acid, caffeic acid, ellagic acid, epicatechin, rutin, quercitrin, isoquercitrin, quercetin, and kaempferol), and eight (gallic acid, catechin, caffeic acid, ellagic acid, resveratrol, rutin, quercetin, and kaempferol) phenolic compounds in the leaf and stem bark, respectively (Oyeleye et al., 2018). Furthermore, the flavonoid profiles of the bark, fruit, and wood have also been reported (Venkatalakshmi et al., 2016).

Meanwhile, *T. subspathulata* is native to Malaysia and Singapore (National Board Parks [NParks], 2015). Aside from being highly valued for the use of its hardwood, especially in making canoes, very little is known about its medicinal use by the local population of Malaysia, as well as its chemical profile. However, the recent listing of this wild species in the list of endangered species prompted its inclusion in this research study (NParks, 2015).

Gas chromatograph (GC) and liquid chromatography (LC) are two chromatographic separation techniques extensively used to study phytochemical constituents. Over the past few decades, the development of mass spectroscopy (MS) has contributed substantially to the scope of applications of both GC and LC. Hyphenation of GC or LC with MS technique has made the separation and identification of compounds in complex mixtures more effective and efficient. Gas chromatography-mass spectrometry (GC-MS) is primarily used to analyse compounds that are adequately volatile and stable under the high temperature of GC conditions. Some polar compounds, such as those with a number of hydroxyl groups, can be derivatised and subsequently subjected to GC-MS analysis (Patel et al., 2010). In contrast, compounds with low volatility, whose volatility cannot be increased even on derivatisation, can be analysed using liquid chromatography-mass spectrometry (LC-MS). Generally, LC-MS allows the analysis of compounds with a broader range of polarity and minimal sample preparation (Perez et al., 2016). Given the different scope of application of GC-MS and LC-MS, using both platforms can offer a more comprehensive view of the metabolite profiles of plant extracts, which are usually complex mixtures of compounds with diverse polarity.

While there is a number of studies regarding the phytochemical contents of the different parts of *T. catappa* (Oyeleye et al., 2018; Terças et al., 2017; Venkatalakshmi et al., 2016), there is still a lack of comprehensive phytochemical profile of its fruits. Only flavonoids from fruits of *T. catappa* have been reported

previously by Venkatalakshmi et al. (2016). Besides, the chemical composition of *T. subspathulata* has not yet been reported. Therefore, in the present study, the chemical constituents of *T. catappa* nuts and *T. subspathulata* fruits were profiled using GC-MS and ultrahigh-pressure liquid chromatography-electrospray ionisation tandem mass spectrometry (UPLC-ESI-MS/MS) techniques. Application of both GC-MS and LC-MS will allow the establishment of a more comprehensive phytochemical profile of the fruits of the two *Terminalia* species.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The fresh, unripe fruits of *T. catappa* and *T. subspathulata* were collected from the campus grounds of Universiti Putra Malaysia (UPM) and the Sultan Idris Shah Forest Education Center (SISFEC) in May 2017. Voucher specimens of each species, SK3336/18 and SK3337/18, respectively, were deposited in the mini-herbarium of the Biodiversity Unit, Institute of Bioscience (IBS), UPM.

Solvents and Chemicals

Analytical grade methanol, n-hexane, and LC-MS grade water were purchased from Merck (Germany). LC-MS grade methanol and formic acid were supplied by Fisher Scientific (Belgium). Deionised water was obtained from the Milli-Q purification system (Millipore, USA).

Sample Processing and Extraction

The fresh fruits of *T. catappa* and *T. subspathulata* were cleaned under running water immediately after collection, drained, and pat-dried off any excess water. The edible kernel (nut) of *T. catappa* was removed from the fibrous husk for chemical analysis. For *T. subspathulata*, however, the whole fruit was used for the chemical analysis due to its smaller size. The processed nuts of the former and fruits of the latter were air-dried at ambient temperature. The dried samples were pulverised and extracted with 70% aqueous methanol using a solid to liquid ratio of 1:30 (w/v). The extraction was assisted with 30 min ultrasonication (SK8210HP Shanghai KUDOS Ultrasonic Instrument Co. Ltd., China) with the sonication frequency set to 53 kHz. The sonicator bath temperature was maintained between 25–30 °C. The solvent extract was drained, collected, and set aside, while the plant residue was re-extracted with fresh solvent. The extraction procedure was repeated four times to ensure that the extraction was adequately exhaustive, and the solvent extract was collected for each batch. The collected extracts were pooled, filtered, and concentrated under *vacuo*, using a rotary evaporator (Heidolph GmbH and Co. K.G., Germany), freeze-dried, and kept at -20 °C prior to analysis.

GCMS Analysis

One gram of the crude methanolic extracts of the *T. catappa* nuts and *T. subspathulata* fruits were separately reconstituted in methanol and then solvent-partitioned into n-hexane to separate the fatty acids

and other non-polar metabolites from other metabolites of higher polarity. The hexane fractions were freeze-dried and subjected to GCMS analysis using the QP2010 Ultra GCMS system (Shimadzu, Japan). The system was equipped with Rxi-5ms fused silica capillary column (30 m length, 0.25 mm ID, 0.25 µm film thicknesses, composed of 5% diphenyl and 95% dimethyl polysiloxane). The analysis was carried out by gradient temperature program, starting with an initial temperature of 50 °C for 3 min, increased to 300 °C at 3 °C/min, and then 300 °C constantly for another 10 min. The sample was eluted at 1 µL, with the mass conditions: ionization voltage of 70 eV, helium flowing at 11.8 mL/min, ion source temperature of 250 °C, and scan range m/z 40-700 amu. The compound identification was carried out by comparing the mass data of the hexane fractions to NIST 11 (National Institute of Standard Technologies, Mass Spectra) and FFNSC 1.3 (Flavor and Fragrance Natural and Synthetic Compounds) libraries.

UHPLC-ESI-MS/MS Analysis

The UHPLC-ESI-MS/MS analysis of the 70% aqueous methanolic extracts of *T. catappa* nuts and *T. subspathulata* fruits was carried out using Q Exactive™ Focus Hybrid Quadrupole Orbitrap mass spectrometer (Thermo Scientific, USA). The system was equipped with Dionex Ultimate 3000 UHPLC. Separation was performed by Acquity UHPLC BEH C18 column (100 x 2.1 mm, 1.7 µm) at a flow rate of 0.4 mL/min using a gradient elution of LC-MS grade water (A): acetonitrile (solvent B) with an additional 0.1% formic acid. The solvent was eluted gradient as follows: 5% B (0-2 min), 5-70% B (2–32 min), 70-100% B (32–37 min), 100% B (37-40 min), and 100-5% B (40-48 min). The sample injection volume for the analysis was 2 µL and observed under UV at 210, 254, 270, and 360 nm wavelengths. Mass data acquisition was performed in negative and positive ion modes, using electrospray ionization (ESI) technique at capillary voltage 3.5 kV, sheath gas 80 arb, and non-transfer tube temperature 320 °C, where the total ion chromatogram (TIC) was recorded from 150 to 1,500 amu. Data processing was performed using Thermo Xcalibur™ 2.2 software (Thermo Scientific, USA).

RESULTS AND DISCUSSION

GCMS Metabolite Profile of Hexane Fractions

The GCMS spectral data of the hexane fractions of *T. catappa* nuts and *T. subspathulata* fruits are presented in Figures 1 and 2, respectively, together with the total ion chromatograms of the individual samples. Several of the detected metabolites were common to both *T. catappa* nuts and *T. subspathulata* fruits. These were the saturated fatty acids, palmitic acid and stearic acid, fatty acid esters, methyl palmitate, methyl linoleate, and decanedioic acid bis(2-ethylhexyl) ester, as well as a fatty acid chloride, linoleoyl chloride. Although both *T. catappa* nuts and *T. subspathulata* fruits were rich in palmitic acid, the content in *T. catappa* nuts was almost twice (33.2%) of that found in *T.*

subspathulata fruits (18.1%). *Terminalia catappa* nuts were also rich in fatty acid chloride, linoleoyl chloride (29.1%), and the cyclic lactone, oxacyclohexadecan-2-one, commonly known as pentadecanolide (16.2%). Linoleoyl chloride, however, was a minor constituent (2.1%) in *T. subspathulata* fruits, whereas pentadecanolide was not detected in *T. subspathulata* fruits. The major constituents of *T. subspathulata* were palmitic acid and its methyl ester, methyl palmitate (9.3%). Meanwhile, moderate amounts of stearic acid (4.8-5.7%) were present in both species.

Saturated and polyunsaturated fatty acids are important ingredients for cosmetics and other botanical-based health products. These fatty constituents have been linked to antioxidants (Bouazzi et al., 2020) and antimicrobial activities (Desbois & Smith, 2010; Pinto et al., 2017; Oyeleye et al., 2018). Palmitic acid and stearic acid are very common saturated fatty acids found in both plants and animals and have exhibited good antibacterial and antifungal properties (Karimi et al., 2015). Meanwhile, linoleoyl chloride identified from the essential oils of leaf *Kaempferia galanga* Linn. showed promising anti-nociceptive and anti-inflammatory activities, as well as larvicidal and repellent effects (Bhuiyan et al., 2008). The compound is a useful reagent for synthesising fatty acid esters of hydroxy fatty acids (FAHFAs), which are known to have beneficial biological effects such as antidiabetes and anti-inflammation (Gowda et al., 2020). Meanwhile, pentadecanolide is one of the major components of *Angelica archangelica* L. species. It has been reported to be a potent and selective inhibitor of rat liver cyclic AMP-dependent protein kinase (Wang & Polya, 1996). Pentadecanolide has been synthesised and has important applications as a fragrance ingredient (Belsito et al., 2011). Decanedioic acid bis-(2-ethylhexyl) ester was previously isolated from endophytic bacteria, which exhibited antimicrobial activity (Mohamad et al., 2018; Tambekar et al., 2017).

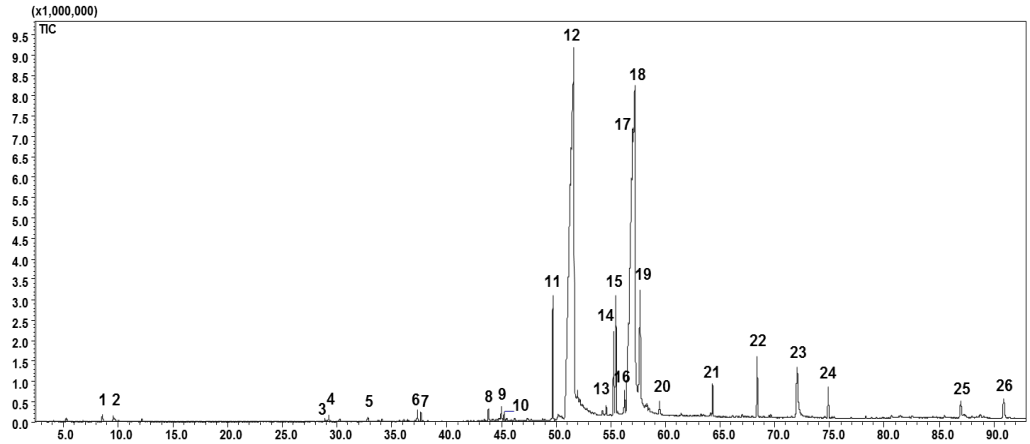
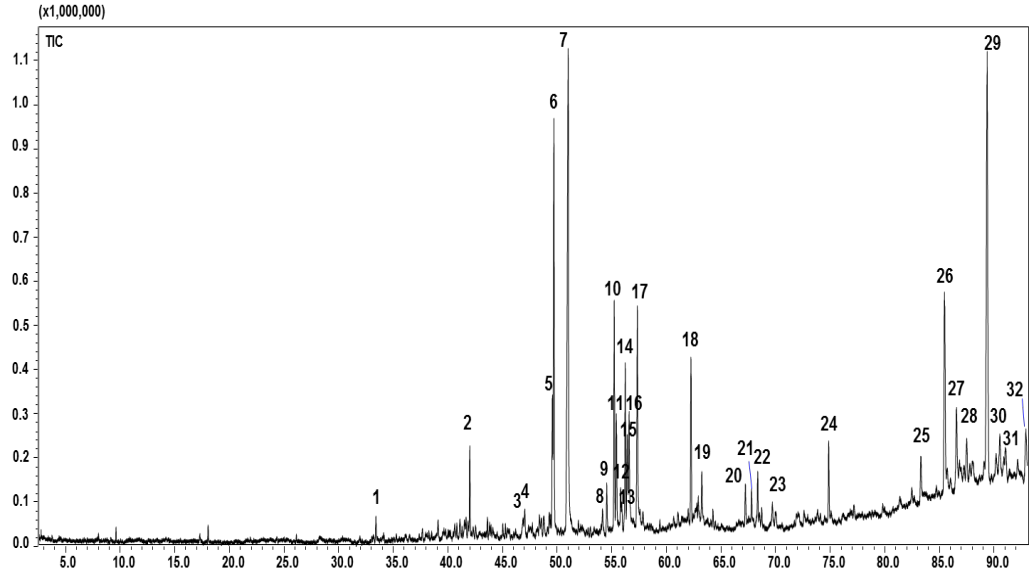


Figure 1. GCMS spectral data of metabolites identified in hexane fraction of *Terminalia catappa* nut

Peak	RT (mins)	Compound ID	Molecular weight	Molecular formula	RI	RI*	SI %	Area %
1	8.58	5,6-Dihydro-2*H*-pyran-2-one	98	$C_5H_6O_2$	939	927	83	0.13
3	28.91	Tetradecene	196	$C_{14}H_{28}$	1391	1403	94	0.06
4	29.27	Tetradecane	198	$C_{14}H_{30}$	1399	1400	95	0.10
5	32.86	Propyl 4-hydroxybenzoate	180	$C_{10}H_{12}O_3$	1482	1480	87	0.12
6	37.38	1-Hexadecene	224	$C_{16}H_{32}$	1592	1602	94	0.19
7	37.68	Hexadecane	226	$C_{16}H_{34}$	1599	1600	96	0.17
8	43.85	Myristic acid	228	$C_{14}H_{28}O_2$	1760	1769	95	0.24
9	45.02	1-Octadecene	252	$C_{18}H_{36}$	1792	1801	95	0.24
10	45.27	Octadecane	254	$C_{18}H_{38}$	1799	1800	95	0.16
11	49.73	Methyl palmitate	270	$C_{17}H_{34}O_2$	1926	1925	95	2.78
12	51.55	Palmitic acid	256	$C_{16}H_{32}O_2$	1980	1977	80	33.15
14	55.28	Methyl linoleate	294	$C_{19}H_{34}O_2$	2095	2093	93	1.92
15	55.48	1,16-Hexadecanediol	258	$C_{16}H_{34}O_2$	2101	2097	78	2.78
16	56.27	Stearic acid methyl ester	298	$C_{19}H_{38}O_2$	2127	2127	90	0.49
17	57.02	Linoleoyl chloride	298	$C_{18}H_{31}ClO$	2151	2139	77	29.10
18	57.21	Oxacyclohexadecan-2-one	240	$C_{15}H_{28}O_2$	2157	2144	83	16.22
19	57.70	Stearic acid	284	$C_{18}H_{36}O_2$	2173	2167	91	4.79
21	64.31	Bis(2-ethylhexyl) adipate	370	$C_{22}H_{42}O_4$	2399	2414	96	0.77
22	68.40	Phthalic acid, 2,4-dimethylpent-3-yl octyl ester	376	$C_{23}H_{36}O_4$	2551	2540	81	1.42
23	72.06	Glyceryl monooleate	356	$C_{21}H_{40}O_4$	2693	2689	86	2.48
24	74.88	Decanedioic acid bis-(2-ethylhexyl) ester	426	$C_{26}H_{50}O_4$	2808	2812	94	0.71

Note. RT: Retention time; RI: Retention index of identified compound; RI*: Retention index of compound identified in NIST library; SI: Similarity index; A%: Percentage composition; nd: Not determined

Figure 1. (Continued)



Peak	RT (min)	Compound ID	Molecular weight	Molecular Formula	RI	RI*	SI %	Area %
1	33.42	Pentadecane	212	$C_{15}H_{32}$	1497	1500	88	0.45
2	41.98	Heptadecane	240	$C_{17}H_{36}$	1712	1700	90	1.55
3	46.87	Phytone	268	$C_{18}H_{36}O$	1846	1841	80	0.18
4	47.03	2,6,10,15-Tetramethylheptadecane	296	$C_{21}H_{44}$	1851	1852	88	0.62
6	49.69	Methyl palmitate	270	$C_{17}H_{34}O_2$	1927	1925	95	9.29
7	51.01	Palmitic acid	256	$C_{16}H_{32}O_2$	1967	1977	94	18.09
8	54.16	2,6,10,14-Tetramethylnonadecane	324	$C_{23}H_{48}$	2063	2051	80	0.34
10	55.24	Methyl linoleate	294	$C_{19}H_{34}O_2$	2096	2093	91	4.92
11	55.44	Methyl linolenate	292	$C_{19}H_{32}O_2$	2103	2098	84	2.83
12	55.83	Phytol	296	$C_{20}H_{40}O$	2115	2106	85	0.82
13	55.92	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	312	$C_{19}H_{36}O_3$	2118	2129	74	0.55
14	56.25	Methyl stearate	298	$C_{19}H_{38}O_2$	2129	2127	88	3.20
15	56.43	Linoleoyl chloride	298	$C_{18}H_{31}ClO$	2135	2139	86	2.14
16	56.60	Eicosyl heptafluorobutyrate	494	$C_{24}H_{41}F_7O_2$	2140	2131	78	3.25
17	57.35	Stearic acid	284	$C_{18}H_{36}O_2$	2165	2167	91	5.74
18	62.26	Methyl eicosanoate	326	$C_{21}H_{42}O_2$	2331	2328	94	3.75
19	63.25	Arachidic acid	312	$C_{20}H_{40}O_2$	2366	2366	86	1.01
20	67.23	2-Monopalmitin	330	$C_{19}H_{38}O_4$	2510	2498	86	0.98
21	67.80	Methyl docosanoate	354	$C_{23}H_{46}O_2$	2532	2530	91	0.51
22	68.36	Phthalic acid, 2,4-dimethylpent-3-yl octyl ester	376	$C_{23}H_{36}O_4$	2553	2540	76	0.92
24	74.86	Decanedioic acid bis(2-ethylhexyl) ester	426	$C_{26}H_{50}O_4$	2812	2812	92	1.73

Note. RT: Retention time; RI: Retention index of identified compound; RI*: Retention index of compound identified in NIST library; SI: Similarity index; A%: Percentage composition; nd: Not determined

Figure 2. GCMS spectral data of metabolites identified in hexane fraction of *Terminalia subspathulata* fruit

UHPLC-ESI-MS/MS Metabolite Profile of 70% Aqueous Methanolic Extracts

UHPLC-ESI-MS/MS gives accurate molecular ion mass and fragmentation patterns of analytes. It gives an exact identity of metabolite present without ambiguity (Xiao et al., 2012). In the present study, the UHPLC-ESI-MS/MS spectral data of the 70% aqueous methanolic extracts of *T. catappa* nuts and *T. subspathulata* fruits were acquired in both negative and positive ionisation modes. However, the metabolites in both extracts were poorly ionized in the positive ionisation mode. Thus, metabolite identification and annotation were carried out only on the negative ionisation mode. Metabolite identification was carried out by comparing the mass spectrometry (MS) data (accurate mass, negative, and positive ion modes) of the compounds analysed using Thermo Xcalibur 2.0 (Thermo Fisher Scientific Inc., USA) with MS data obtained from the literature and open-source databases such as Metabolomics Workbench, Human Metabolome Database (HMDB), PubChem, MassBank, and Metlin. For gallic acid and isovitexin, the identification was based on comparison with the pure standards. The total ion chromatogram (TIC) of the *T. catappa* nuts and *T. subspathulata* fruits extracts are provided as supplementary data SD1(A) and SD1(B), respectively. The metabolites identified in the extracts comprise organic acids, hydrolysable tannins, phenolic acids, flavonoids, and diarylheptanoids. These identified compounds were tabulated in Table 1, consisting of the retention times (RT), molecular formula, molecular ion mass, and tandem mass.

Organic Acids. Organic acids greatly impact organoleptic properties, especially related to flavour, colour, and scent (Famiani et al., 2015; Flores et al., 2012; Sandín-España et al., 2016). In addition, they are major components of ripe fruits (Walker et al., 2018) and have high antioxidant activity, which makes them an excellent remedy against many ailments (Liu et al., 2019).

The citric acid (**1**) was identified in both *T. catappa* nuts and *T. subspathulata* fruits. It showed a pseudomolecular ion at m/z 191 and tandem mass with characteristic sequential losses of carbon dioxide (CO_2) and water moieties, for example, at m/z 129 for $[M\text{-}H\text{-}CO_2\text{-}H_2O]^-$, 111 for $[M\text{-}H\text{-}CO_2\text{-}2H_2O]^-$, 85 for $[M\text{-}H\text{-}2CO_2]^-$, and m/z 67 for $[M\text{-}H\text{-}2CO_2\text{-}2H_2O]^-$. Homocitric acid (**6**), which has an additional methylene (CH_2) unit in its structure, showed a pseudomolecular ion at m/z 205 and a similar fragmentation pattern as citric acid. Comparison with literature supported the identification of the two organic acids (Al Kadhi et al., 2017; Mena et al., 2012). Compound **1** is found in many fruits, especially citrus fruits and vegetables (Abdel-Salam et al., 2014; Penniston et al., 2008). Studies have shown **1** to decrease brain lipid peroxidation and inflammation and liver damage, and DNA fragmentation (Abdel-Salam et al., 2014). Meanwhile, chebulic acid (**33**) was identified based on its pseudo molecular ion at m/z 355 and compared with the literature (Yang et al., 2012). The compound was

Table 1

Identified compounds from Terminalia catappa *nut and* Terminalia subspathulata *fruit extracts using UHPLC-ESI-MS/MS*

No.	RT (min)	Compound ID	Molecular formula	$[M-H]^-$ (m/z)*	Mass error (ppm)	MS/MS fragments (m/z)	TCN	TSF
	Organic acids							
1	0.73	Citric acid	$C_6H_8O_7$	191.0192	0.00	191, 173, 155, 147, 129, 111, 87, 85, 67	+	+
6	1.45	Homocitric acid	$C_7H_{10}O_7$	205.0347	-0.49	191, 173, 155, 143, 125, 111, 87, 67	+	+
33	13.24	Chebulic acid	$C_{14}H_{12}O_{11}$	355.0304	0.84	355, 325, 337 310, 307, 175	+	-
	Hydrolysable tannins							
2	0.87	HHDP glucose	$C_{20}H_{18}O_{14}$	481.0615	-0.62	481, 421, 301, 275,	+	+
4	1.07	Punicalin (α/β isomer)	$C_{34}H_{22}O_{22}$	781.0522	-0.38	781, 601, 600, 575, 448, 392, 301, 298	+	+
5	1.38	Punicalagin	$C_{48}H_{28}O_{30}$	1083.0590	0.28	1083, 781, 601, 600, 301	+	+
7	1.63	bis-HHDP glucose	$C_{34}H_{24}O_{22}$	783.0682	0.13	783, 481, 451, 301, 275, 229	+	+
8	1.72	2-*O*-Galloypunicalin	$C_{41}H_{26}O_{26}$	933.0644	1.07	781, 721, 600, 575, 450, 425, 301	+	+
10	2:42	Punicalagin isomer	$C_{48}H_{28}O_{30}$	1083.0590	0.28	1083, 781, 601, 600, 301	+	+
12	3.36	bis-HHDP glucose isomer	$C_{34}H_{24}O_{22}$	783.0682	0.13	783, 481, 301, 275, 221	+	+
13	4.25	Corilagin	$C_{27}H_{22}O_{18}$	633.0733	0.79	633, 463, 301, 174	-	+
17	5.22	Galloy-bis-HHDP glucose	$C_{41}H_{28}O_{26}$	935.0809	1.92	935, 633, 481, 301, 299,	+	+
18	5.73	DigalloylHHDP glucose	$C_{34}H_{26}O_{22}$	785.0844	0.76	785, 633, 483, 419, 169	+	-

Table 1 *(Continued)*

No.	RT (min)	Compound ID	Molecular formula	$[M-H]^-$ (m/z)*	Mass error (ppm)	MS/MS fragments (m/z)	TCN	TSF
	Hydrolysable tannins							
19	5.87	Flavogallonic acid	$C_{21}H_{11}O_{13}$	469.0046	0.64	469, 425, 301, 300	+	+
20	6.15	Corilagin isomer	$C_{27}H_{22}O_{18}$	633.0733	0.79	633, 463, 301, 174	+	+
24	7.57	Ellagic acid pentoside	$C_{19}H_{14}O_{12}$	433.0412	1.15	433, 301, 229	+	+
29	9.41	Ellagic acid deoxyhexoside	$C_{20}H_{16}O_{12}$	447.0571	1.57	447, 315, 301, 299, 270, 151	+	-
32	11.61	2,3-Di-*O*-methylellagic acid	$C_{16}H_{10}O_8$	329.0303	1.82	329, 314, 299, 298	+	+
38	15.71	Tri-*O*-methylellagic acid	$C_{17}H_{12}O_8$	343.0463	2.62	328, 313, 298, 270, 269	+	-
	Phenolic acids							
3	0.98	Glucogallin	$C_{13}H_{16}O_{10}$	331.0669	1.21	331, 271, 211, 169, 125	+	+
9	1.98	Protocatechuic acid	$C_7H_6O_4$	153.0183	-3.27	153, 109, 108	+	+
11	2.93	Gallic acid	$C_7H_6O_5$	169.0132	-2.96	169, 125, 107	+	+
14	4.51	Digalloyglucose	$C_{20}H_{20}O_{14}$	483.0783	1.65	483, 331, 313, 169	+	+
15	4.94	Caffeic acid	$C_9H_8O_4$	179.0344	0.00	179, 135, 117, 107	+	+
16	4.97	Brevifolin carboxylic acid	$C_{13}H_8O_8$	291.0151	3.44	291, 247, 219, 191, 175	+	-
21	6.28	Trigalloylglucoside	$C_{27}H_{24}O_{18}$	635.0884	0.00	635, 483, 465, 313, 301, 169	+	-
22	6.53	*p*-Coumaric acid	$C_9H_8O_3$	163.0391	-2.45	163, 119, 93	+	-

Table 1 *(Continued)*

No.	RT (min)	Compound ID	Molecular formula	$[M-H]^-$ (m/z)*	Mass error (ppm)	MS/MS fragments (m/z)	TCN	TSF
	Phenolic acids							
28	9.31	Methyl gallate	$C_8H_8O_5$	183.0293	0.00	183, 169, 168, 139	+	+
35	13.32	Fertaric acid	$C_{14}H_{14}O_9$	325.0565	1.54	325, 310, 193, 149	+	+
	Flavonoids							
23	7.25	Luteolin-*C*-hexoside	$C_{21}H_{20}O_{11}$	447.0933	1.34	447, 429, 357, 327, 285	-	+
25	8.28	Isovitexin	$C_{21}H_{20}O_{10}$	431.0985	1.62	431, 341, 311, 283, 281, 269	-	+
26	8.30	Quercetin-3-*O*-deoxy-hexosylhexoside	$C_{27}H_{30}O_{16}$	609.1475	3.12	609, 301, 300, 271, 151	+	+
27	8.52	Quercetin-3-*O*-hexoside	$C_{21}H_{20}O_{12}$	463.0891	3.02	463, 445, 301, 300, 271, 151	+	+
30	11.31	Apigenin	$C_{15}H_{10}O_5$	269.0455	1.86	269, 241, 225, 183, 157, 151, 117	-	+
31	11.49	Isookanin	$C_{15}H_{12}O_6$	287.0562	2.09	287, 269, 177, 151, 135, 125, 109, 107	-	+
36	13.40	Naringenin	$C_{15}H_{12}O_5$	271.0613	2.21	271, 187, 177, 151, 119	-	+
37	14.26	Isorhamnetin	$C_{16}H_{12}O_7$	315.0508	0.95	315, 301, 300, 283, 271, 151	-	+
	Other compounds							
34	13.27	Demethoxycurcumin	$C_{20}H_{18}O_5$	337.1079	0.89	337, 321, 306, 291, 191, 177, 161	+	-

Note. RT: Retention time; CPD ID: Compound identity; TCN: *Terminalia catappa* nuts; TSF: *Terminalia subspathulata* fruits; +: Detected; -: Not detected; HHDP: Galloyl-hexahydroxydiphenoyl

* Values based on total ion chromatogram (TIC) of *Terminalia catappa* for compounds common to both extracts

detected only in the nuts of *T. catappa* but not in the fruits of *T. subspathulata*. It has been previously reported in the fruits of *T. chebula* (Avula et al., 2017).

Hydrolysable Tannins. The two extracts were found to be rich in hydrolysable tannins, comprising of twelve ellagitannins (**2**, **4**, **5**, **7**, **8**, **10**, **12**, **13**, **17**, **18**-**20**) and four ellagic acid derivatives (**24**, **29**, **32**, and **38**), as shown in Table 3. Ellagitannins are polymeric structures consisting of galloyl and hexahydroxydiphenoyl (HHDP) units esterified with a polyol, usually glucose. A characteristic reaction of ellagitannins is releasing the bislactone and forming an HHDP group, which eventually lactonizes to produce ellagic acid (Zhu et al., 2015). It leads to the observation of a characteristic base peak at m/z 301 corresponding to that of a deprotonated ellagic acid in the mass spectra of ellagitannins, in addition to the characteristic neutral losses of galloyl (152 amu), gallic acid (170 amu), HHDP (302 amu), galloylglucose (332 amu), HHDP glucose (482 amu), and galloyl-HHDP-glucose (634 amu) residues (Regueiro et al., 2014). Compound **2** was identified as HHDP glucose based on $[M-H]^-$ at m/z 481 and tandem mass at m/z 301 ($[M-H\text{-glucose}]^-$) corresponding to the HHDP residue, in agreement with the report of Singh et al. (2016). Compounds **7** and **12** were identified as bis-HHDP glucose isomers based on pseudomolecular ions at m/z 783 ($[M-H]^-$) and tandem mass at m/z 481 ($[M-H-HHDP]^-$). Meanwhile, **17**, with $[M-H]^-$ at m/z 935, tandem mass at m/z 633 ([M-H-HHDP] and m/z 481 for a further loss of galloyl moiety, was identified as galloyl-bis-HHDP glucose. This tandem fragmentation pattern was also observed for **18** with $[M-H]^-$ at m/z 785. Compound **18** was thus identified as digalloylHHDP glucose, present only in *T. catappa*. This class of ellagitannins have been reported previously in the bark of *Eucalyptus globulus* Labill. (Santos et al., 2011) and the unripe fruits of *T. arjuna* (Singh et al., 2016). Based on comparison with literature data, **4**, with $[M-H]^-$ at m/z 781, was identified as punicalin. Both compounds **5** and **10** showed the same $[M-H]^-$ (m/z 1083) and tandem mass, including losing an HHDP moiety at m/z 781. Thus **5** and **10** were identified as isomeric forms of punicalagin, differing from **4** by an additional moiety of HHDP (Mena et al., 2012; Mininel et al., 2014). The two compounds have been previously reported in the fraction *T. catappa* leaf, which exhibited antifungal activity against *Candida* species (Venkatalakshmi et al., 2016). Meanwhile, **8**, which exhibited $[M-H]^-$ at m/z 933, 152 amu higher than **4**, was identified as 2-*O*-galloylpunicalin. Isomeric forms of corilagin were also identified in the extracts, as **13** and **20**, with $[M-H]^-$ at m/z 633 and tandem mass at m/z 463 $[M-H-170]^-$ and 301 $[M-H-169-162]^-$, resulting from inductive cleavage of galloyl acid and galloylglucose (Nuengchamnong & Ingkaninan, 2017; Pfundstein et al., 2010). The fragmentation pathways of the ellagitannins are provided in the supplementary data SD2 and SD3. Corilagin has been reported in the leaves of *T. catappa* and is known for its strong

antioxidant property (Kinoshita et al., 2007), in addition to anti-tumour, hepatoprotective, and anti-inflammatory activities (Li et al., 2018).

Flavogallonic acid (**19**) was identified based on its $[M-H]^-$ at m/z 469, and tandem mass at m/z 425 for $[M-H-CO_2]^-$, and 300 for $[M-H-169]^-$ indicating the loss of gallic acid (Pfundstein et al., 2010). Flavogallonic acid has been previously reported in the fruits of *T. chebula* (Sarabhai et al., 2013). Several ellagic acid derivatives (**24**, **29**, **32**, and **38**) were also detected in the extracts. Ellagic acid has been reported previously as a constituent in the leaf and stem bark of *T. catappa* (Oyeleye et al., 2018), thus the detection of these ellagic derivatives in the present work is not surprising. Compounds **24** and **29**, with $[M-H]^-$ at m/z 433 and 447, were identified as ellagic acid pentoxide and ellagic acid deoxyhexoside, respectively. Both compounds exhibited characteristic tandem mass for ellagic acid at m/z 301, produced following the losses of pentosyl (132 amu) and deoxyhexosyl (146 amu) moieties from the respective pseudomolecular ions (Pinheiro et al., 2018). Compounds **32** and **38**, with $[M-H]^-$ at m/z 329 and 343, respectively, were identified as methoxylated derivatives of ellagic acid, based on tandem mass resulting from successive losses of methyl group (CH_3) groups (Kumar et al., 2015). The fragmentation pathways of the ellagic acid derivatives are provided in the supplementary data SD4 and SD5. Ellagic acid has been reported to have strong antioxidant, antiproliferative, chemopreventive, and antiatherogenic properties (Larrosa et al., 2010). The antioxidant activity of ellagic acid has been attributed to the stability of its free radical (Regueiro et al., 2014). Ellagic acid pentoside has been reported to be a constituent of the vitamin C rich berries of camu-camu (*Myrciaria dubia*) (Fracassetti et al., 2013). It is also present in walnuts, together with ellagic acid-2-rhamnoside (Bulló et al., 2011).

Phenolic Acids. Phenolic acids have been reported as one of the major contributors to sensory quality, colour, nutritional, and antioxidant properties of edible foods from the plant kingdom (Cheynier, 2012; Kumar & Goel, 2019). Compounds **3**, **11**, **14**, **21**, and **28** were identified as gallic acid and its derivatives. Gallic acid (**11**), with $[M-H]^-$ at m/z 169 and tandem mass at m/z 125 ($[M-H-CO_2]^-$), was identified by comparison with the standard compound. Oyeleye et al. (2018) has previously reported the presence of gallic acid in the leaf and stembark of *T. catappa*. Compound **3**, with $[M-H]^-$ at m/z 331, was identified as glucogallin, a monogalloylglucose, based on tandem mass at m/z 211 and 169 for sequential loss of the glucose moiety, 162 amu (Avula et al., 2017; Kumar et al., 2015). Compounds **14** and **21**, with $[M-H]^-$ at m/z 483 and m/z 635, were identified as digalloylglucose and trigalloylglucose, respectively, based on the tandem mass arising from losses of the corresponding number of galloyl moieties (Singh et al., 2016). Compound **28**, with $[M-H]^-$ at m/z 183, identified as methyl gallate, showed characteristic tandem mass at m/z 168 and 125 for sequential methyl and CO_2 moieties losses.

Other phenolic acids present in the extracts were the naturally common acids, protocatechuic (**9**) and caffeic acids (**15**), identified based on comparison with literature values (Buiarelli et al., 2010; Maity et al., 2013; Wang et al., 2012). Compound **9** is found in many edible and medicinal plants and protects against cardiovascular diseases and neoplasms (Oniszczuk et al., 2019; Szumiło, 2005). Compound **15** has been reported to be one of the metabolites in hazelnut and most edible fruits and has been reported to have anticancer potential (Ghirardello et al., 2010).

Compound **35**, with $[M-H]^-$ at m/z 325, was identified as fertaric acid, an ester formed from ferulic acid bound to tartaric acid. The compound showed characteristic losses of the corresponding fragments of ferulic acid and tartaric acid moieties (Pati et al., 2014). Fertaric acid is mostly found in grapes (Gris et al., 2013; Mozetič et al., 2006). The nut extract of *T. catappa* showed the presence of *p*-coumaric acid (**22**) and its isocoumarin derivative, brevifolin carboxylic acid (**16**). The former acid, with $[M-H]^-$ at m/z 163, was identified from the tandem mass at m/z 119 for $[M-H-CO_2]^-$ and 93 for $[M-H-CO_2-C_2H_2]^-$ (Kumar et al., 2015), while the latter acid, with $[M-H]^-$ at m/z 291, was identified based on the tandem mass at m/z 247 for $[M-H-CO_2]^-$, 219 for $[M-H-CO_2-CO]^-$, and m/z 191 for $[M-H-CO_2-2CO]^-$ (Zhu et al., 2015). Hydroxycinnamic acids such as **15**, **22**, and **35** have been reported to provide many health benefits, including antioxidant, anti-inflammatory, anti-collagenase, antimicrobial, and anti-tyrosinase (Adisakwattana, 2017; Alam et al., 2016; Taofiq et al., 2017).

Flavonoids. Flavonoids have been reported for numerous positive effects on human health and are present in many plants (Ganeshpurkar & Saluja, 2017; Howes, 2018). In the present study, eight flavonoids were identified in the fruit extract of *T. subspathulata* (**23**, **26**-**27**, **30**, **31**, **36**, and **37**). Compound **23**, with $[M-H]^-$ at m/z 447 and tandem mass of m/z 357 for $[M-H-90]^-$ and m/z 327 for $[M-H-120]^-$, was identified as luteolin-*C*-hexoside (Chen et al., 2016; Otłowska et al., 2018). Compounds **26** and **27**, with $[M-H]^-$ at m/z 609 and 463, respectively, were identified as quercetin derivatives based on the characteristic base peak ions at m/z 301 and tandem mass m/z 271 and 151 (Kumar et al., 2017; Yang et al., 2012). Losses of the deoxyhexosylhexose (m/z 308) and hexose (m/z 162) moieties allowed the identification of **26** as quercetin-3-*O*-deoxyhexosylhexoside and **27** as quercetin-3-*O*-hexoside (Kumar et al., 2015). Glycosylation at the C-3 position of these compounds was determined by the higher relative abundance of their radical aglycone $[Y_0 - H]^-$ ion (m/z 300) than the Y_0^- ion (m/z 301) (Buzgaia et al., 2020). Compound **27** has been reported in the fruit, leaf, and stem bark of *T. catappa*, while quercetin-3-*O*-glucoside was present in the leaf (Oyeleye et al., 2018). Compound **25**, with $[M-H]^-$ at m/z 431, was identified as isovitexin by comparison with a commercial standard. The tandem mass for the apigenin aglycone was observed at m/z 269 ($[M-H-gluc]^-$)

while apigenin itself could be identified as compound **30**, which exhibited [M-H]$^-$ at m/z 269. It showed characteristic fragments at m/z 151 and 117, arising from $^{1,3}A^-$ and $^{1,3}B^-$ retro-Diels Alder (rDA) cleavages of the flavonoid skeleton (Deseo et al., 2020; Otłowska et al., 2018). While the tandem mass m/z 151 is common for flavonoids, m/z 117 is characteristic apigenin, a flavone with a hydroxy group (OH) at the B ring. Compound **31**, with [M-H]$^-$ at m/z 287, and tandem mass m/z 269 for water loss was identified as isookanin based on the proposed fragmentation pathway shown in Figure 3. The respective losses of $C_6H_5O_2$ and $C_9H_7O_4$ moieties from the molecular ion to give the fragment ions at m/z 177 and 109 were also in agreement with Yang et al. (2016). Compound **36** with [M-H]$^-$ at m/z 271, and compound **37** with [M-H]$^-$ at m/z 315, were identified as naringenin and isorhamnetin, respectively, in agreement with literature values (Fathoni et al., 2017; Kumar et al., 2015). Compound **37** has been detected previously in the bark, fruit, and wood of *T. catappa* (Venkatalakshmi et al., 2016).

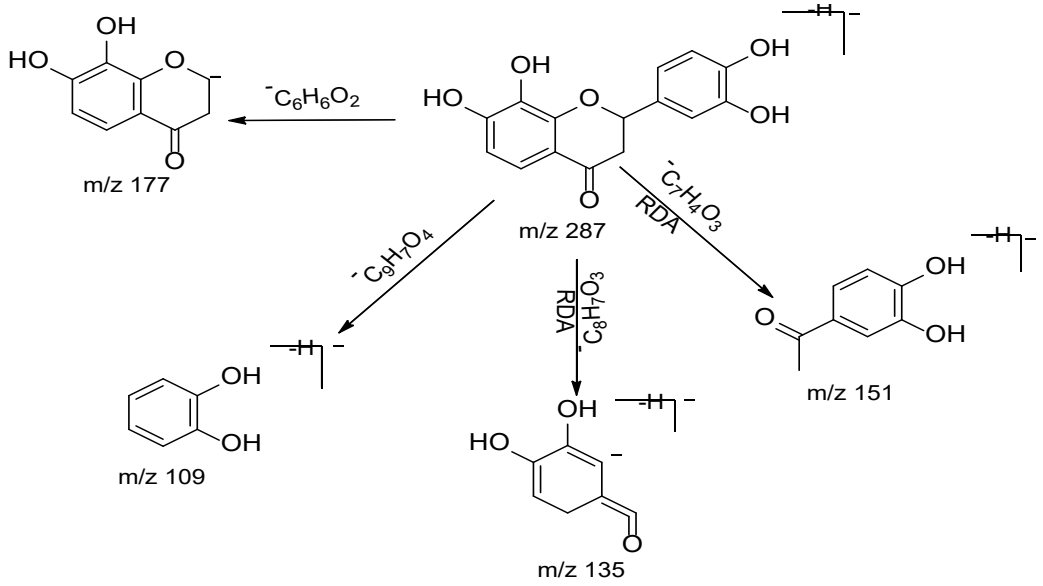


Figure 3. Proposed fragmentation pathways for isookanin **31**

Other Constituents. Compound **34**, with [M-H]$^-$ at m/z 337, was identified as demethoxycurcumin, based on a comparison of tandem mass proposed fragmentation pathway depicted in Figure 4. The compound loses a methoxy group (CH_3O) to give tandem mass m/z 306 [M-H-31]$^-$, while hetero cleavages of the -CO-CH_2-CO- linkage gave rise to tandem masses m/z 161 and m/z 177. Diarylheptanoids are characteristic constituents of the rhizomes of *Curcuma* species (Bresciani et al.,

2020; Jiang et al., 2006), which makes its detection highly surprising due to the difference in the plant family. Further isolation and purification studies will need to be carried out to validate its identification. As a class of compounds, curcuminoids have demonstrated strong antioxidant and anti-inflammatory activities, among others (Opara & Chohan, 2014).

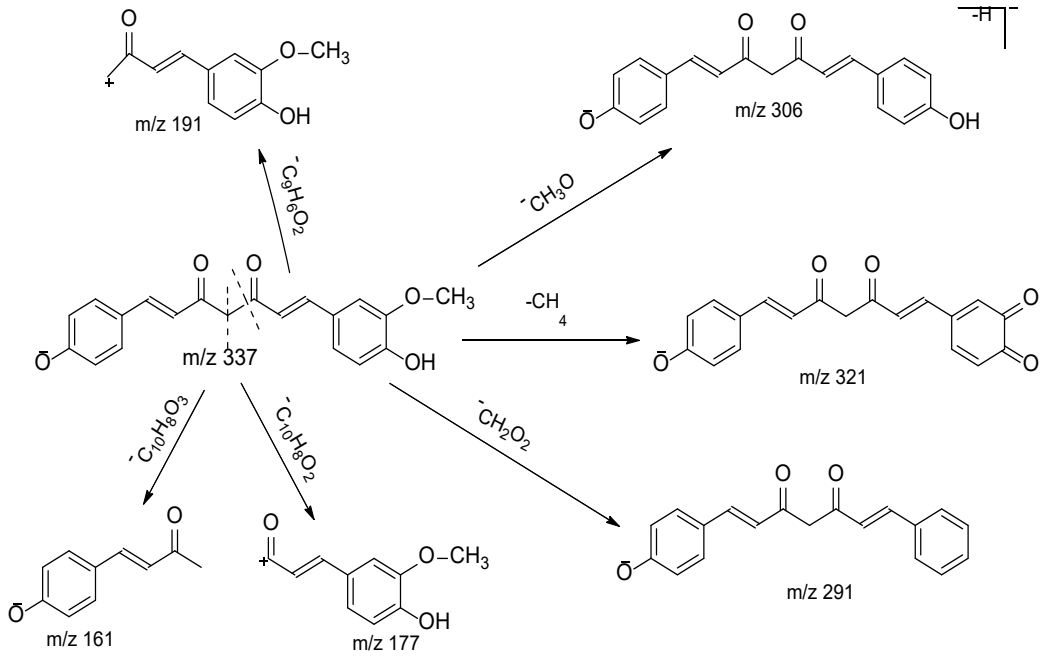


Figure 4. Proposed fragmentation pathways for demethoxycurcumin 34

CONCLUSION

The mass spectrometric analysis revealed the phytochemical diversity of *T. catappa* nuts and *T. subspathulata* fruits. The GCMS profiling of the n-hexane fractions of the crude extracts revealed the presence of metabolites from the classes of saturated and polyunsaturated fatty acid, fatty acid ester, fatty acid chloride, fatty alcohol, macrocyclic lactone, alkane, alkenes, and unsaturated carboxylic acid. On the other hand, via UHPLC-ESI-MS/MS analysis, a total of 38 compounds, comprising organic acids, hydrolysable tannins, phenolic acids, flavonoids, and diarylheptanoids were identified in the methanolic extracts of both species. The outcome of this study provides insight into the chemical profile of *T. catappa* nuts and *T. subspathulata* fruits. The phytochemical diversity of these underutilised fruits and nuts reveals their potential for further exploitation and development as functional foods and nutraceutical ingredients for plant-

based health products. However, a detailed quantitative and toxicity analysis of the extracts or their bioactive components is necessary to reach these goals.

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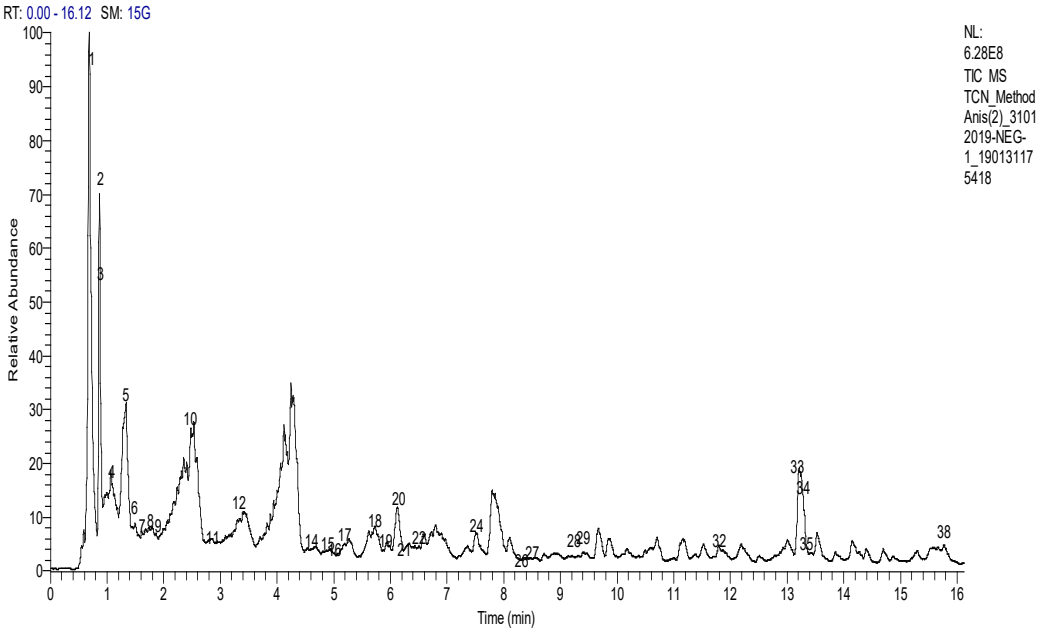
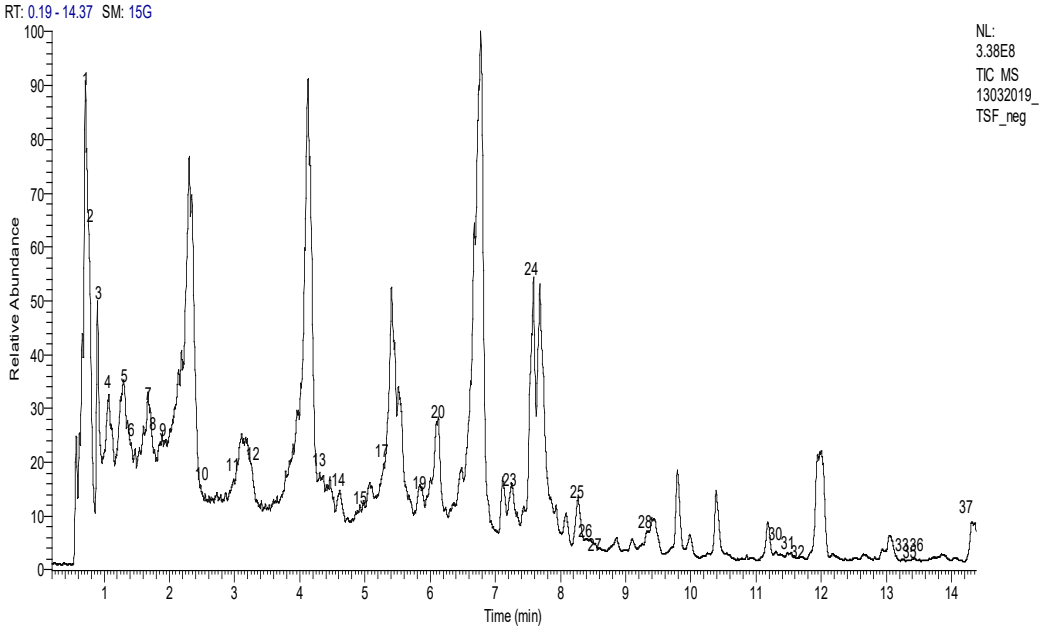
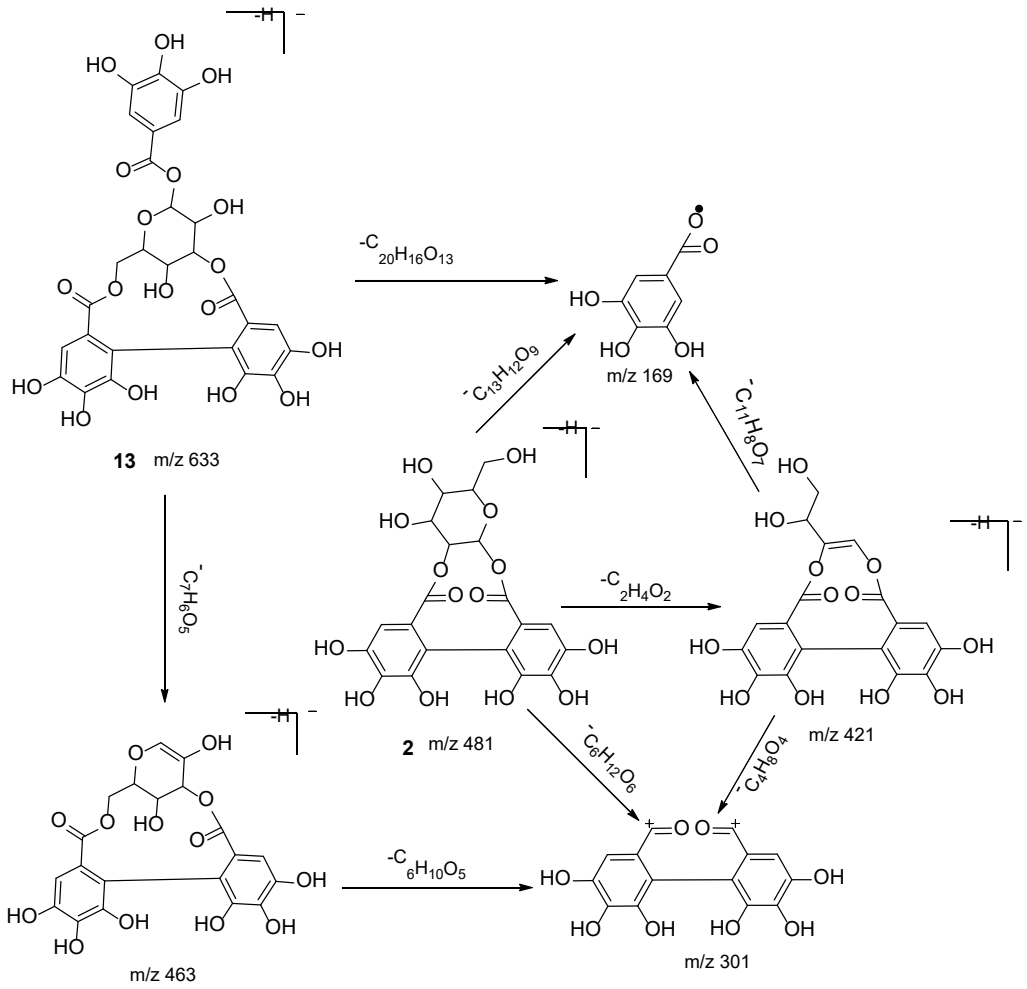
SUPPLEMENTARY DATA



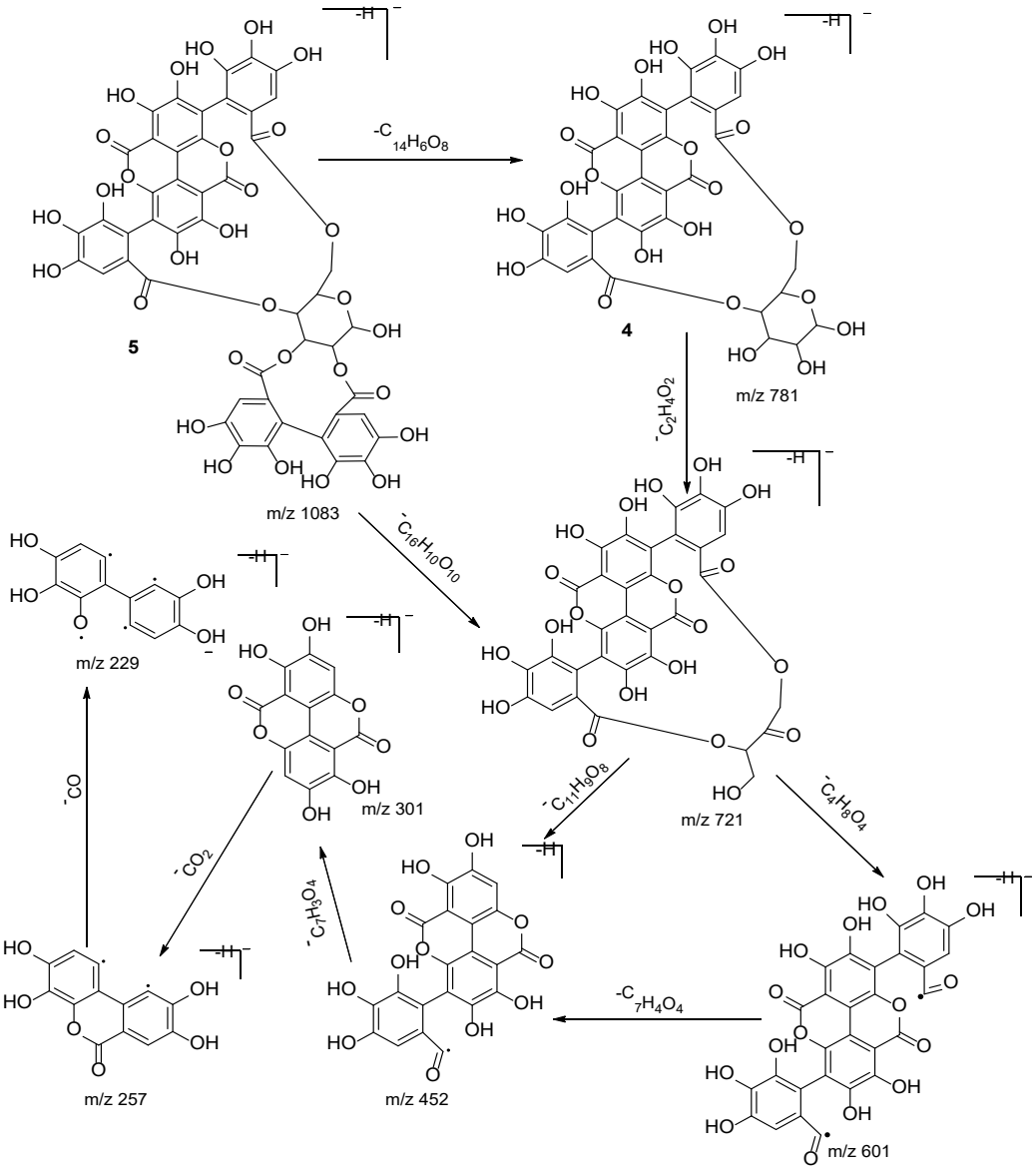
SD1(A). LCMS total ion chromatogram of *Terminalia catappa* nuts in negative mode



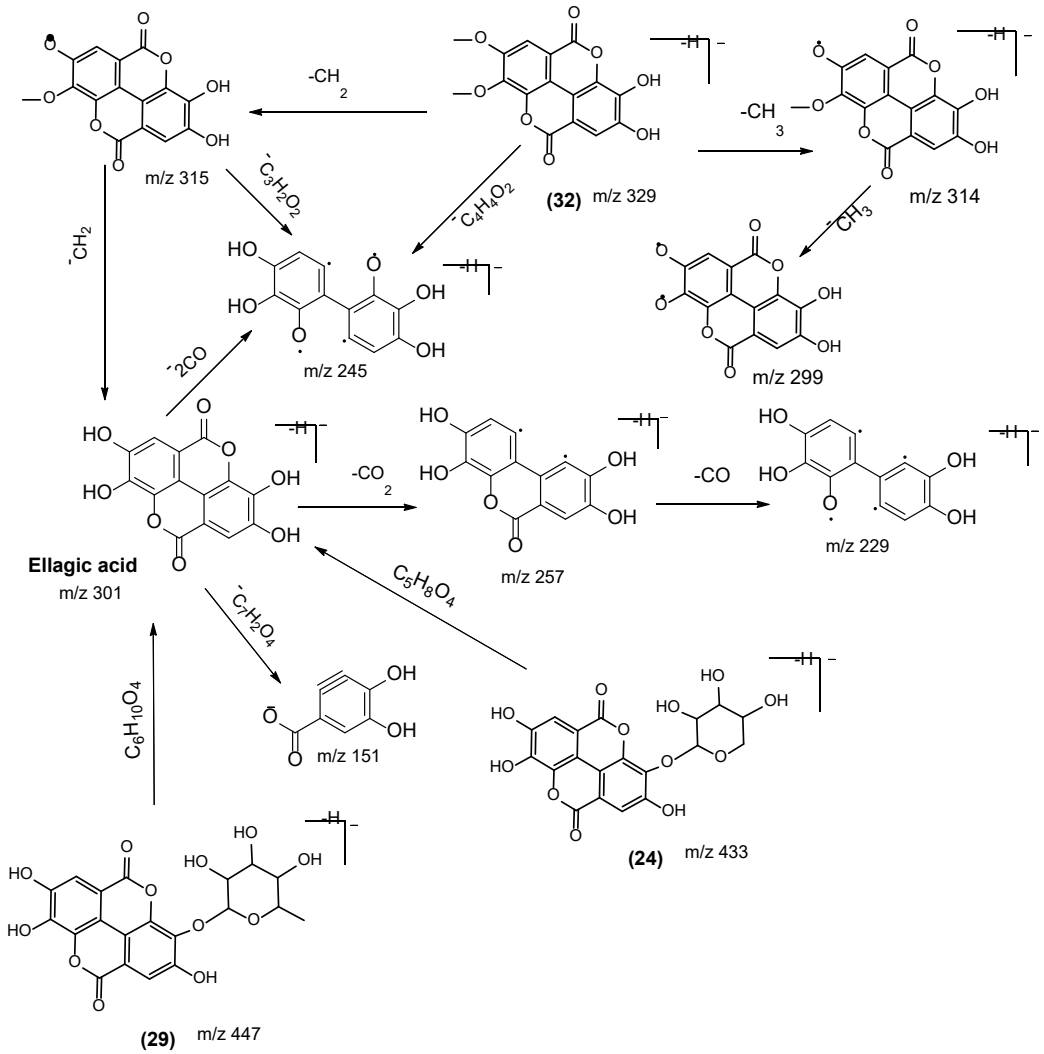
SD1(B). LCMS total ion chromatogram of *Terminalia subspathulata* fruits in negative mode



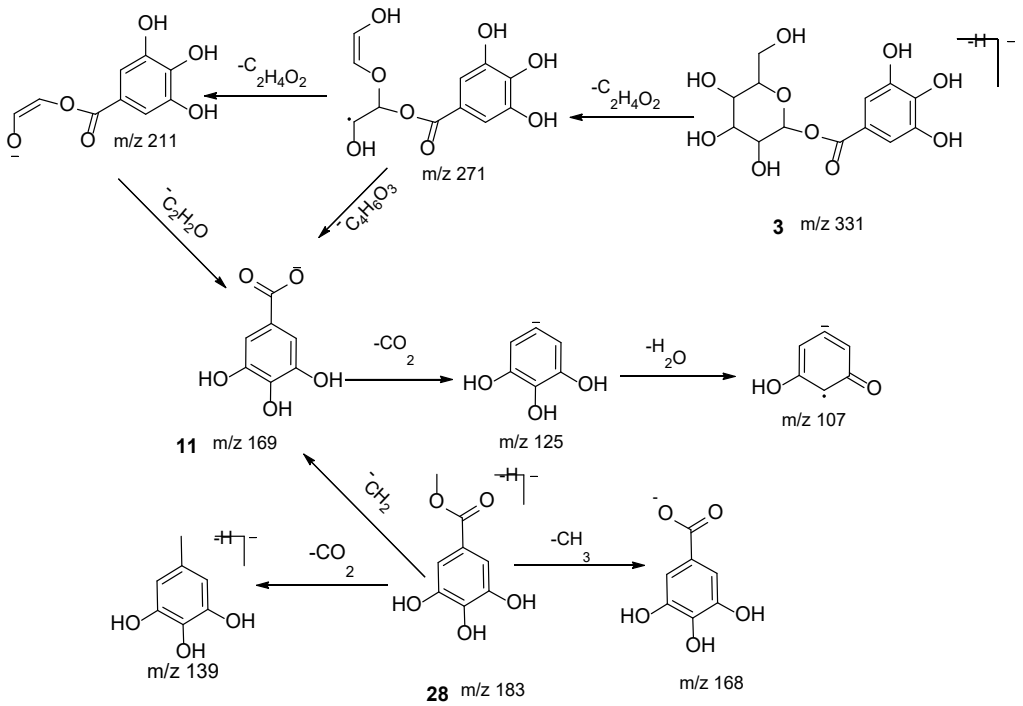
SD2. Proposed fragmentation pattern of HHDP glucose **2** and corilagin **13**



SD3. Proposed fragmentation pattern of punicalagin **5** and punicalin α/β **4**



SD4. Proposed fragmentation pattern of ellagic acid pentoside **24**, ellagic acid deoxyhexoside **29** and 2,3-di-*O*-methylellagic acid **32**



SD5. Proposed fragmentation pattern of gallic acid **11**, glucogallin **3**, and methyl gallate **28**