

1 Research Article

2 ***Solanum sessiliflorum* (mana-cubiu) antioxidant protective effect**
3 **towards cholesterol oxidation: influence of docosahexaenoic acid**

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18 **Running Title:** *Mana-cubiu* protective effect towards cholesterol oxidation

19 **Keywords:** oxysterols, docosahexaenoic acid, oxidation, natural antioxidants

20 **Abbreviations:** COPs, cholesterol oxidation products; DHA, docosahexaenoic acid; MCE, mana-cubiu
21 extract

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1 **Abstract**

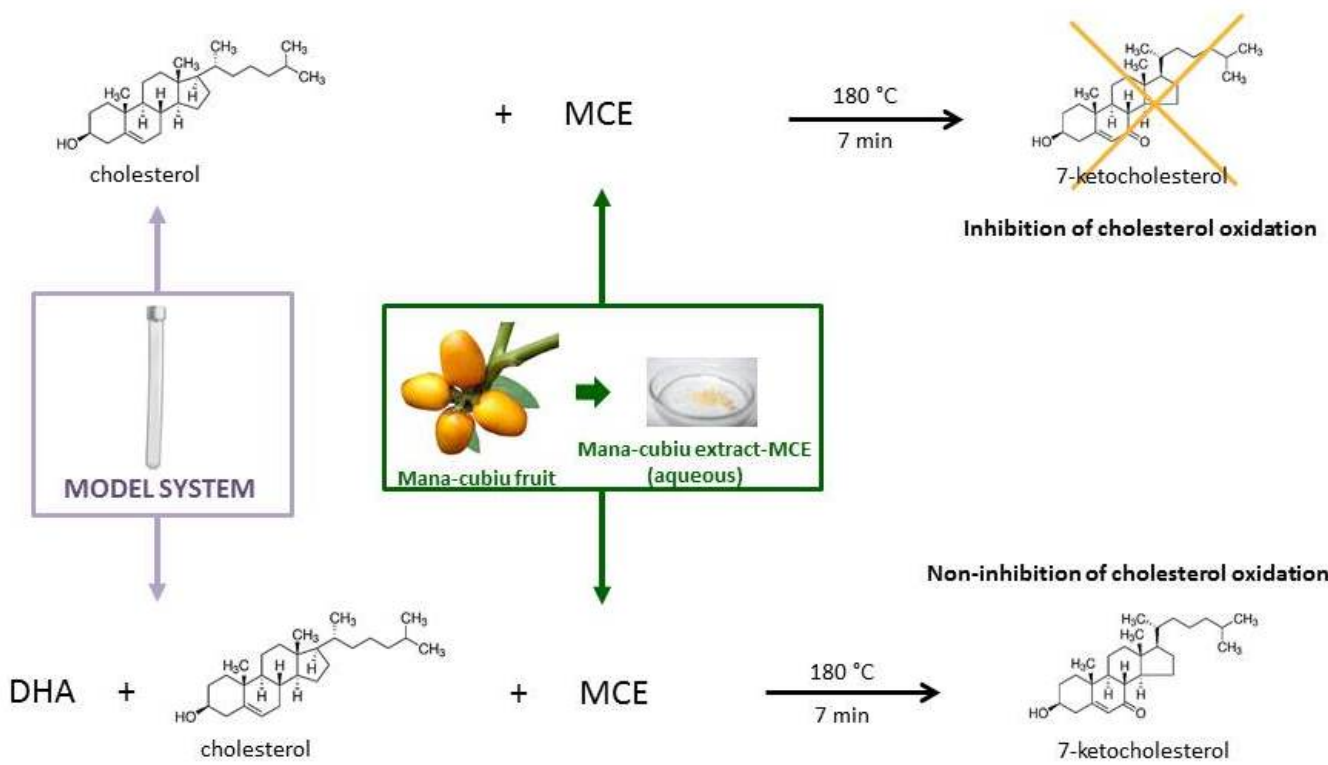
2 Harmful health effects have been attributed to cholesterol oxidation products (COPs). Factors that
3 modulate their formation in foods are light, oxygen, heat, and food matrix (such as antioxidants content or
4 unsaturation degree of lipids), among others. The objective of this work was to assess the effectiveness of
5 an extract obtained from *Solanum sessiliflorum* (mana-cubiu) (MCE) as a potential inhibitor of cholesterol
6 oxidation under heating conditions. The influence of free DHA presence in the system was also evaluated.
7 Results showed that MCE inhibited cholesterol degradation (44 % vs 18 % without and with MCE,
8 respectively) and reduced 9-fold COPs formation in the absence of DHA. However, when DHA was present,
9 the MCE was not effective towards cholesterol oxidation. In this case, MCE showed its antioxidant effect
10 protecting DHA from degradation (89 % vs 64 %).

11 12 **Practical applications**

13 Antioxidant properties of this solvent free natural extract make MCE a potential good ingredient in food
14 products containing highly polyunsaturated lipids to protect them from oxidation and in food products
15 lacking polyunsaturated lipids to protect cholesterol from oxidation.

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17 **Graphical abstract**



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20 Mana-cubiu inhibits cholesterol oxidation in absence of DHA.

21 **1. Introduction**

22 Cholesterol chemical structure makes it an easy-to-oxidize molecule, leading to the formation of oxysterols.
 23 These oxidation products, usually named as Cholesterol Oxidation Products (COPs), have been related to
 24 several diseases (atherosclerosis, neurodegenerative diseases, mutagenic and carcinogenic effects, etc) [1-3].
 25 They have been found in a variety of animal food samples [1], and some studies have pointed out their
 26 potential absorption through the diet [4-5]. Therefore, minimizing their formation during food
 27 manufacturing, processing and / or cooking is of great interest to reduce health risk. Use of additives or
 28 oxygen-restriction methods is commonly applied for that purpose.

29 In this sense, a growing interest in natural antioxidants found in plants is noticed, not only because of
 30 technological reasons, but also due to their potential ability to suppress oxidative stress and related diseases
 31 [6]. Regarding their usefulness in food systems, successful cases in controlling cholesterol oxidation have
 32 been reported in several types of matrices [7-10]. Mana-cubiu (*Solanum sessiliflorum*) is a fruit native to

33 Amazonia, which possess antioxidant properties attributed to the presence of carotenoids and phenolic
34 compounds in its composition [11].

35 On the other hand, the interest in highly unsaturated fatty acids has recently increased due to their health-
36 related properties. Particularly, long-chain omega-3 polyunsaturated fatty acids have demonstrated
37 cardiovascular disease lowering effects [12-13]. Nevertheless, inadequate manufacturing and cooking
38 conditions can lead to some loss in the content of these interesting compounds. A number of studies have
39 dealt with the prevention of fatty acid degradation through antioxidant addition, after different cooking and
40 storage conditions [8, 14-16]. A possible interaction between cholesterol oxidation and the surrounding fatty
41 acids has been proposed by several authors as a factor that modulates cholesterol oxidation susceptibility,
42 although no consensus on the subject has been found [17-20].

43 Foods are usually complex matrices where interferences among several components may hamper a clear
44 view about the mechanisms of cholesterol oxidation. Therefore, model systems are a very useful tool to
45 evaluate separately the factors that exert an influence in this process. A variety of antioxidants [19, 21-24],
46 and lipid matrices [20, 25, 26] have been tested in model systems.

47 Considering the exposed above, the aim of this study was to evaluate the antioxidant protective effect of a
48 *Solanum sessiliflorum* lyophilized food-grade aqueous extract against cholesterol degradation and
49 cholesterol oxidation products formation in a model system, with and without the presence of
50 docosahexaenoic acid (DHA). Furthermore, the effect of refrigeration storage was also evaluated.

51 **2. Material and methods**

52 **2.1 Material and reagents**

53 *Mana-cubiu* fruits (~ 21 Kg) were acquired at CEAGESP (São Paulo General Warehousing and Centers
54 Company, São Paulo, Brazil). Cholesterol, 22R-hydroxycholesterol, 22S-hydroxycholesterol, 20 α -
55 hydroxycholesterol, 25-hydroxycholesterol, 5,6 β -epoxycholesterol, 5,6 α -epoxycholesterol, 7-ketocholesterol
56 and DHA standards were purchased from Sigma-Aldrich (Newport, RI, USA). 7 α -hydroxycholesterol and
57 7 β -hydroxycholesterol were purchased from Steraloids (Newport, RI, USA). The purity of the standards was
58 at least 95% as determined by HPLC or GC analyses. Chloroform and methanol were purchased from Synth

(Diadema, SP, Brazil). Chromatographic grade hexane (minimum 63% n-hexane) and 2-propanol were purchased from Panreac (Alvorada, RS, Brazil).

2.2 Mana-cubiu extract preparation and characterization

Mana-cubiu fruits were lyophilized before extraction [11]. Fifty grams of lyophilized mana-cubiu fruit were homogenized with ultrapure water in a vortex for 5 min and centrifuged at 20000 g at 10 °C. The aqueous layer was lyophilized during 120 h at -92 °C below 40 µHg (Liobras, São Paulo, Brazil). The identification and quantification of the phenolic compounds of the mana-cubiu extract (MCE) was carried out according to Rodrigues et al. [11].

2.3 Sample preparation and heating

Stock solutions of cholesterol (1 mg/mL in chloroform), DHA (1 mg/mL in chloroform) and MCE (2.5 mg/mL in methanol) were prepared. Four types of samples were prepared: cholesterol alone, cholesterol with MCE, cholesterol with DHA, cholesterol with DHA and MCE. Aliquots of cholesterol solution (1 mL) were poured in test tubes. For DHA and MCE containing samples, 1 mL and 0.2 mL of the corresponding stock solution was added, respectively. Solvent was evaporated under a stream of N₂ and uncapped tubes were placed in a dry block (Marconi, Brazil) at 180 °C. After 7 min heating (modelling the medium cooking time for fish), tubes were taken out and introduced into an ice water bath for 4 min and capped. Then, they were kept in the fridge (4 °C) for 72 h or in the freezer (- 30 °C) until analysis. Samples were named as unheated, heated-0 h stored and heated-72 h stored. The experiment was carried out in triplicate.

2.4 Cholesterol and COPs determination

Each sample was dissolved with 1 mL hexane:2-propanol (97:3, v:v), filtered through a 22 µm filter (Millipore, Maryland, MD, USA) and injected into the HPLC system. A Shimadzu liquid chromatograph (Kyoto, Japan) equipped with on-line UV-visible and refractive index detectors was used. The analytical column used was a Nova-Pack CN HP 300 mm x 3.9 mm, 4 µm (Waters, Milford, MA, USA). The mobile-phase was hexane:2-propanol (97:3, v:v) at a flow rate of 1 mL/min and an analysis time of 30 min [27]. 7 α -Hydroxycholesterol, 7 β -hydroxycholesterol, 5,6 β -epoxycholesterol, 5,6 α -epoxycholesterol and 7-ketocholesterol were determined. Quantification was done by external standardization. The identification of

85 COPs was confirmed by HPLC-APCI-MS/MS (HPLC Shimadzu, Kyoto, Japan; MS Bruker Daltonics,
86 model Amazon ETD, Bremen, Germany) using the chromatographic conditions described in detail by
87 Zardetto et al. [28], and the MS conditions previously optimized by Mariutti et al. [27]. Chromatograms of
88 standards separation and MS and MS/MS data and spectra are shown as supplementary material in this
89 manuscript, as well as chromatograms of samples separation (Figures S1-S3 and Table S1).

90 **2.5 DHA determination**

91 Docosahexaenoic acid was converted into its methyl ester according to Joseph & Ackman [29] and analyzed
92 with a gas chromatograph (GC 2010 model, Shimadzu, Kyoto, Japan) equipped with a fused silica CP-SIL
93 88 capillary column 100 m x 0.25 mm i.d., 0.20 μ m film thickness (Chrompack, Middelburg, The
94 Netherlands) and flame ionization detector. Chromatographic conditions were described in detail by Sancho
95 et al. [16].

96 **2.6 Statistics**

97 The data obtained were analyzed by means of the software Stata 12 (SataCorp LP, Texas, U.S.A.). For the
98 evaluation of the significant differences among the amounts of cholesterol and COPs of different samples,
99 one factor ANOVA with Bonferroni post hoc multiple comparisons ($p < 0.05$) was applied. For the
100 evaluation of the significant differences between the amounts of cholesterol, COPs and DHA in samples
101 heated and stored for 0 h and 72 h, Student-t test was applied.

102 **3. Results and discussion**

103 **3.1 MCE properties**

104 The HPLC-DAD chromatogram processed at 280 nm showed the separation of two phenolic compounds
105 from mana-cubiuextract (Figure 1). The major phenolic compound in the extract was 5-caffeoylquinic acid
106 (5-CQA) (2.48 ± 0.08 mg/g extract), while N^1, N^5 or N^5, N^{10} -bis-(dihydrocaffeoyl) spermidine was found in
107 small amounts ($<$ limit of detection, < 2 μ g/mL). Table 1 shows the chromatographic and spectroscopic
108 characteristics of the two identified compounds and the MS and MS/MS spectra are shown in Figure S4.
109 Since a detailed description of the identification of these compounds, in a methanol:water lyophilized mana-
110 cubiu fruit extract was previously reported by Rodrigues et al. [11], these data will not be extensively

111 discussed. On the other hand, as expected, due to the changes in the polarity of the extraction system and the
112 lower solubility of these compounds in water, the water extract prepared in this work showed lower 5-CQA
113 content than the methanol:water one (4.49 mg/g extract) [11].

114 Despite its lower phenolic content, the aqueous extract was interesting, since 5-CQA has demonstrated high
115 antioxidant capacity. This compound is usually found in high amounts in coffee, especially green coffee, or
116 coffee extracts, which have been applied both in model and food systems to protect them from oxidation or
117 to increase their antioxidant capacity [30-32]. It is important to highlight that the identified phenolic
118 compounds represent only about 0.25% of the extract composition; therefore, other water soluble
119 compounds such as carbohydrates, proteins, peptides, amino acids, present in the extract probably
120 contributed for the protection against oxidation. On the other hand, this extract was safe, environmentally
121 friendly and potentially applicable in foodstuffs, since it was free from organic solvents. Hence, the aqueous
122 MCE was selected for the experiments carried out in this work.

123 **3.2 Effect of MCE on cholesterol and DHA degradation**

124 The initial amount of cholesterol (before heating) was 1.05 mg (Table 2) in all samples. All samples showed
125 a significant decrease in the cholesterol content after heating (presenting values below 0.82 mg in all cases).
126 As it can be observed in Figure 2, higher amounts of remaining cholesterol were found when MCE was
127 added to the sample (80 %), compared to the remaining amount present when cholesterol was heated alone
128 (55 %). This reduction in cholesterol degradation was attributed to the high content in 5-CQA of MCE and
129 its antioxidant capacity. Similar reductions in cholesterol degradation have also been noticed in other model
130 systems using phenolic compounds such as green tea catechins and quercetin [19, 21].

131 On the other hand, similar values of remaining cholesterol (around 20%) were found for the two types of
132 samples that included DHA in the mixture, regardless the presence of MCE. As compared to cholesterol
133 alone, the presence of free DHA enhanced cholesterol degradation, and MCE could not counteract this
134 effect. Therefore, MCE seemed to protect cholesterol from oxidation in the absence of DHA, but not in the
135 presence of this fatty acid.

136 The initial amount of DHA (before heating) was 1.00 mg (Table 2). DHA remaining content after the
137 thermal treatment was also analyzed (Fig 3). Both MCE lacking and containing samples showed a
138 significant decrease in the DHA content after heating (0.11 and 0.36 mg remaining, respectively). Heating of
139 DHA alone (without cholesterol nor MCE) resulted in 11.53 ± 3.05 % of remaining compound, so
140 cholesterol had no effect on DHA thermal degradation given that the presence of cholesterol in the mixture
141 yielded the same remaining amount (11 %). Results showed that DHA content was much higher in the
142 presence of MCE (36 %), compared to the previously mentioned remaining amount found in the absence of
143 the extract (11 %). Similar protective effects of natural extracts against DHA degradation have been also
144 reported in studies dealing with fish meatballs and fish oil [14, 16]. Hence, it could be assumed that MCE
145 antioxidant properties were devoted to protect DHA from degradation, lessening the protective effect
146 towards cholesterol degradation.

147 **3.3 Effect of MCE on COPs formation**

148 After heating, COPs content was much higher in cholesterol-alone sample (227 μg COP / mg cholesterol)
149 than in the presence of MCE (25 μg COP / mg cholesterol), as it can be observed in Table 3. On the other
150 hand, similar values (around 87 μg COP / mg cholesterol) were found for both MCE containing and MCE
151 lacking samples when DHA was present in the medium. So again, as it occurred with cholesterol
152 degradation, MCE seemed to prevent from COPs formation in the absence of DHA, but not in the presence
153 of this compound. COPs formation has been previously reported to be depleted in the presence of phenolic
154 compounds [19, 33].

155 Nine COPs were analyzed and only five were found in the samples. From these, 7-KC was the main one in
156 most cases, followed by β -EC and 7 β -HC (Table 3). A number of studies dealing with cholesterol oxidation
157 in model systems have reported this profile of COPs [19, 33-35]. The dominance of β -isomer was supported
158 by the steric hindrance at C3 position. Interestingly, when comparing cholesterol and cholesterol+MCE
159 samples, whereas a 90 % reduction in 7-KC was noticed; only a 40% reduction was reported in 7 α -HC,
160 becoming the main compound. So the antioxidant extract seemed to show differential behavior towards
161 individual COPs formation. In this sense, reaction rate might be slowed down in the presence of MCE,

162 remaining as 7 α -HC for longer time before starting the conversion into 7-KC. Similarly, Kmiecik and co-
163 workers [36] found differences among sterol oxides distribution depending on the antioxidant applied. This
164 selective inhibition towards individual derivatives could be related to the differences in chemical structure,
165 that could hamper certain positions to be attacked and, hence, certain oxidation derivatives to be formed.

166 **3.4 Effect of DHA on cholesterol degradation and COPs formation**

167 Cholesterol degradation was higher when heated within DHA than when heated alone, as it can be observed
168 in figure 2. The presence of a lipid unsaturated surrounding has been reported to protect cholesterol from
169 oxidation [20, 37]. This discordance could be related to the higher ratio cholesterol:lipid matrix used in the
170 current study (1:2) compared to those ones (1:100). Higher amounts of cholesterol could have hampered the
171 physical protection and favoured cholesterol interaction with highly oxidated DHA. This way, Lehtonen and
172 co-workers [26], using cholesteryl esters (stechiometry 1:1) found higher levels of oxidation in cholesteryl
173 linoleate than in free cholesterol (0.17 % and 0.084 %), which was attributed to the linoleate double bonds
174 likelihood to radical formation. Additionally, using free DHA as compared to triglycerides (main
175 constituents of the matrix in [20, 37]) makes also an important difference regarding physical protection,
176 chemical group interaction and viscosity, which are key factors in the process [34].

177 On the other hand, even though cholesterol degradation was enhanced by DHA, COPs formation was lower
178 than in the absence of DHA, denoting that the routes of cholesterol oxidation were different. Consequently,
179 the oxidation products formed were different, probably oligomers [35, 38, 39] or volatile compounds [35]. It
180 was also possible that reaction rates for COP degradation were higher than for COP formation in the
181 presence of DHA, giving rise to the aforementioned compounds. Previous studies have shown no correlation
182 between the sterols degradation and the oxides formed [24, 40].

183 **3.5 Effect of refrigerated storage**

184 Storage under refrigeration conditions (4 °C, 72 h) modified neither cholesterol levels nor COPs
185 concentration in most cases, except for two samples. Chol+DHA sample slightly decreased its content in
186 COPs, possibly due to degradation of the compounds [35]. DHA levels suffered no changes along the time

187 either. This behaviour was attributed to the lack of water or any other solvents in the samples, what retarded
188 the oxidation processes.

189 **4. Conclusion**

190 In conclusion, MCE protected against cholesterol degradation and COPs formation when there was no other
191 lipid compound in the system, but not in the presence of DHA. On the other hand, DHA was effectively
192 protected from oxidation by MCE addition. Considering that it implies a solvent-free extraction process, this
193 food-grade mana-cubiu extract could be a potential good ingredient in food products containing highly
194 polyunsaturated lipids to protect them from oxidation and in food products lacking polyunsaturated lipids to
195 protect cholesterol from oxidation.

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202 **Conflict of interest**

203 Authors have declared no conflict of interest.

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Table 1. Chromatographic and spectroscopic characteristics of phenolic compounds from mana-cubiu extract.

Compound	tr ^a (min)	λ_{max} (nm) ^b	[M+H] ⁺ (<i>m/z</i>)	Fragment ions (<i>m/z</i>) MS ² (+)	[M-H] ⁻ (<i>m/z</i>)	fragment ions (<i>m/z</i>) MS ² (-)
5-caffeoylquinic acid ^d	18.3	300sh ^c , 326	355	163, 145	353	191, 179
N ¹ ,N ⁵ or N ⁵ ,N ¹⁰ -bis(dihydrocaffeoyl) spermidine ^e	19.6	280	474	457, 236, 222, 165	472	350, 308, 186

^a Retention time on the C₁₈ column. ^b Solvent: linear gradient of water and acetonitrile both with 0.5% formic acid. ^c sh: shoulder. ^d identified (standard available).
^e tentatively identified. Supplementary Figure S4 presents the MS and MS² spectra of the identified phenolic compounds.

309 **Table 2.** Cholesterol and DHA content (mg) of unheated cholesterol and DHA, and the four heated samples during
 310 storage at 4 °C for 0 and 72 h.

	unheated	chol		chol+MCE		chol+DHA		chol+DHA+MCE	
		0 h	72 h	0 h	72 h	0 h	72 h	0 h	72 h
cholesterol	1.05 aA	0.56 c	0.54 C ^{ns}	0.82 b	0.81 B ^{ns}	0.22 d	0.22 D ^{ns}	0.25 d	0.29 D *
DHA	1.00 aA	-	-	-	-	0.11 c	0.07 C *	0.36 b	0.41 B ^{ns}

311 Different lower case letters denote statistical differences among the unheated sample and the heated samples stored for 0 h.

312 Different capital letters denote statistical differences among the unheated sample and the heated samples stored for 72 h.

313 ns: non-significantly different content between heated samples stored for 0 and 72 h within each type of sample.

314 *: significantly different content between heated samples stored for 0 and 72 h within each type of sample.

315 **Table 3.** Cholesterol oxidation products (µg/mg cholesterol) content of the unheated sample and the four heated
 316 samples during storage at 4 °C for 0 and 72 h.

	unheated	chol		chol+MCE		chol+DHA		chol+DHA+MCE	
		0 h	72 h	0 h	72 h	0 h	72 h	0 h	72 h
7α-HC	nd	28.90 c	27.78 C ^{ns}	17.07 b	9.55 B *	6.47 a	0.18 A *	10.42 ab	0.18 A *
7β-HC	nd	48.25 c	47.17 C ^{ns}	1.84 a	4.74 A *	13.01 b	11.06 B ^{ns}	13.25 b	15.04 B ^{ns}
β-EC	nd	61.06 c	63.12 C ^{ns}	1.21 a	1.11 A ^{ns}	20.78 b	15.29 B *	20.11 b	18.49 B ^{ns}
α-EC	nd	29.22 c	31.81 C ^{ns}	0.65 a	0.59 A ^{ns}	15.33 b	10.78 B *	12.58 b	10.88 B ^{ns}
7-KC	nq	59.65 c	68.37 C ^{ns}	3.26 a	7.97 A *	31.10 b	25.71 B *	30.78 b	30.64 B ^{ns}
Total COPs	-	227.07 c	238.24 C ^{ns}	24.03 a	23.96 A ^{ns}	86.69 b	63.02 B *	87.14 b	75.22 B ^{ns}

317 Different lower case letters denote statistical differences among heated samples stored for 0 h. Different capital letters denote
 318 statistical differences among heated samples stored for 72h.

319 nd: not detected (detection limit: 7α-HC = 0.98 µg/mg, 7β-HC = 0.46 µg/mg, β-EC = 4.99 µg/mg, α-EC = 3.67 µg/mg)

320 nq: not quantitated (quantification limit: 7-KC = 1.01 µg/mg)

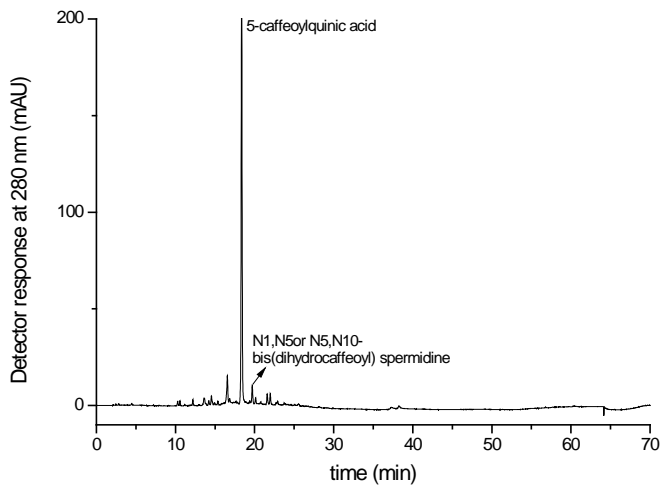
321 ns: non-significantly different content between heated samples stored for 0 and 72 h within each type of sample.

322 *: significantly different content between heated samples stored for 0 and 72 h within each type of sample.

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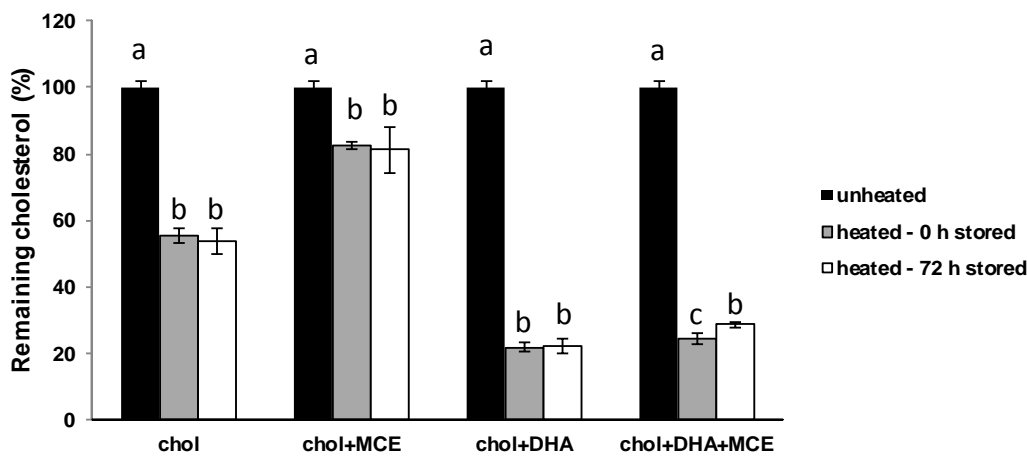


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Figure 1. Chromatogram obtained by HPLC-DAD of the phenolic compounds from the aqueous mana-cubiu extract.



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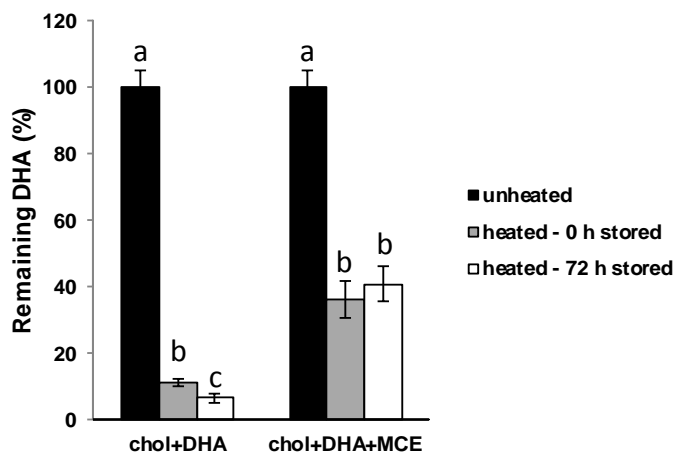
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Figure 2. Remaining percentage of cholesterol of the unheated sample and the four heated samples after 0 and 72 h storage. Different letters for each sample denote statistical differences among the unheated, the 0 h stored and the 72 h stored samples.



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Figure 3. Remaining percentage of DHA of the unheated sample and the two heated samples after 0 and 72 h storage. Different letters for each sample denote statistical differences among the unheated, the 0 h stored and the 72 h stored samples.

Figure captions

Fig. 1 Chromatogram obtained by HPLC-DAD of the phenolic compounds from the aqueous mana-cubiu extract

Fig. 2 Remaining percentage of cholesterol of the unheated sample and the four heated samples after 0 and 72 h storage. Different letters for each sample denote statistical differences among the unheated, the 0 h stored and the 72 h stored samples

Fig. 3 Remaining percentage of DHA of the unheated sample and the two heated samples after 0 and 72 h storage. Different letters for each sample denote statistical differences among the unheated, the 0 h stored and the 72 h stored samples

Fig. S1 Extracted ion chromatograms at m/z 385 and m/z 401, obtained by HPLC-MS/MS, of cholesterol oxide standards. Peak characterization is given in Table S1.

Fig. S2 Extracted ion chromatograms at m/z 385 and m/z 401, obtained by HPLC-MS/MS, of cholesterol oxides in model system. Peak characterization is given in Table S1.

Fig S3 Mass spectra of cholesterol oxide standards.

Fig. S4 Mass spectra of the phenolic compounds in mana-cubiu water extract.

358 **Supplementary Table S1.** Chromatographic and mass spectrometry characteristics of cholesterol oxides
359 obtained by HPLC-MS/MS.

Cholesterol oxide	t _r (min)	[M+H] ⁺ (m/z)	Fragment ions (m/z)
22R-hydroxycholesterol	3.7	nd	385 ⁺ [M+H-18] ⁺ , 367 [M+H-18-18] ⁺
22S-hydroxycholesterol	4.2	nd	385 ⁺ [M+H-18] ⁺ , 367 [M+H-18-18] ⁺
20 α -hydroxycholesterol	4.4	nd	385 ⁺ [M+H-18] ⁺ , 367 [M+H-18-18] ⁺
25-hydroxycholesterol	4.7	403	385 [M+H-18] ⁺ , 367 [M+H-18-18] ⁺
7 α -hydroxycholesterol	5.5	nd	385 ⁺ [M+H-18] ⁺ , 367 [M+H-18-18] ⁺
7-ketocholesterol	5.7	401	383 [M+H-18] ⁺ , 365 [M+H-18-18] ⁺
7 β -hydroxycholesterol	5.8	nd	385 ⁺ [M+H-18] ⁺ , 367 [M+H-18-18] ⁺
5,6 β -epoxycholesterol	7.0	403	385 [M+H-18] ⁺ , 367 [M+H-18-18] ⁺
5,6 α -epoxycholesterol	7.6	403	385 [M+H-18] ⁺ , 367 [M+H-18-18] ⁺

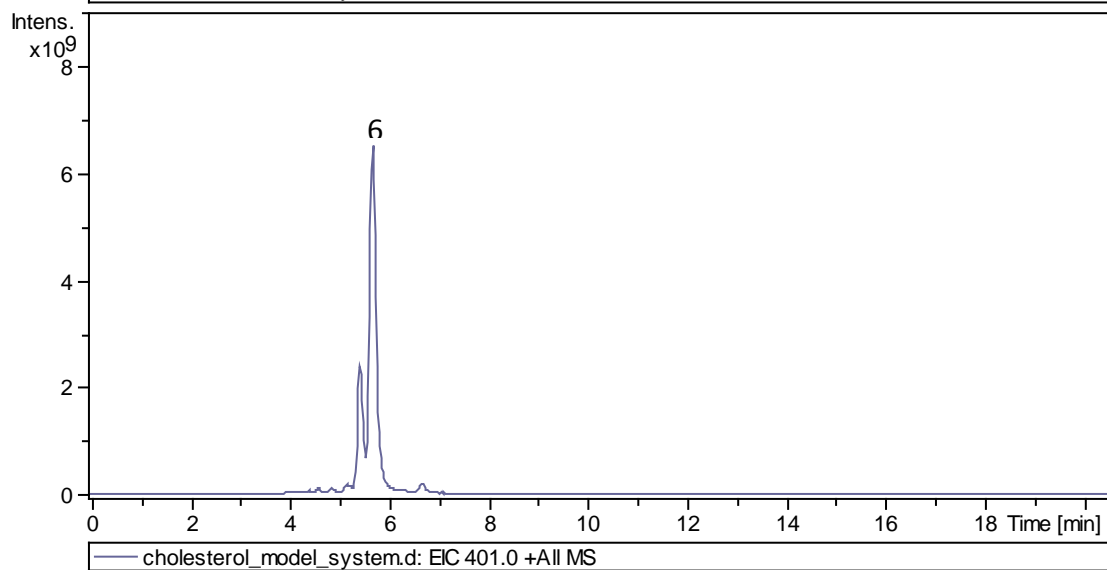
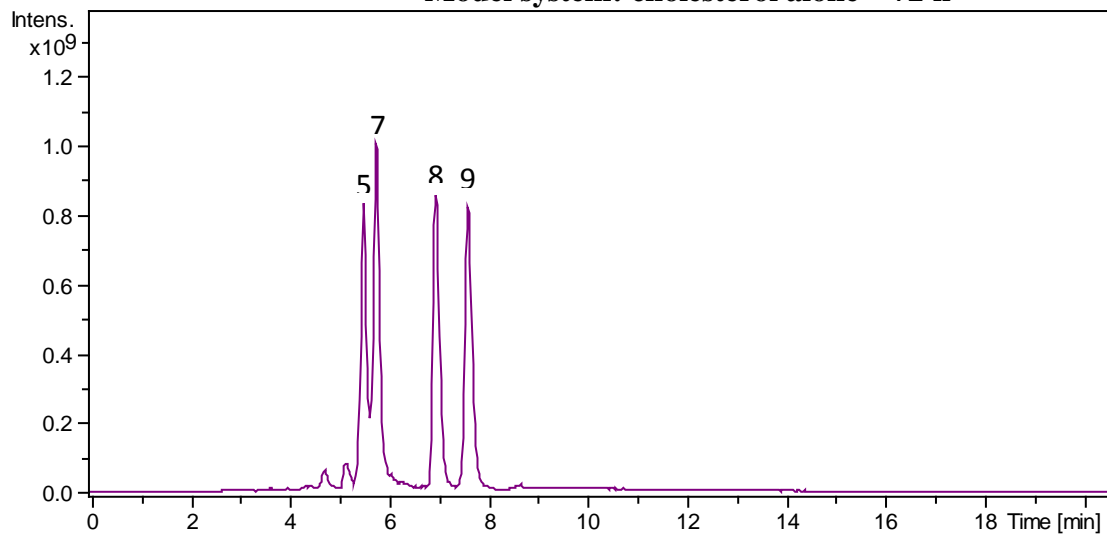
360 nd: Not detected. * In source fragmentation.

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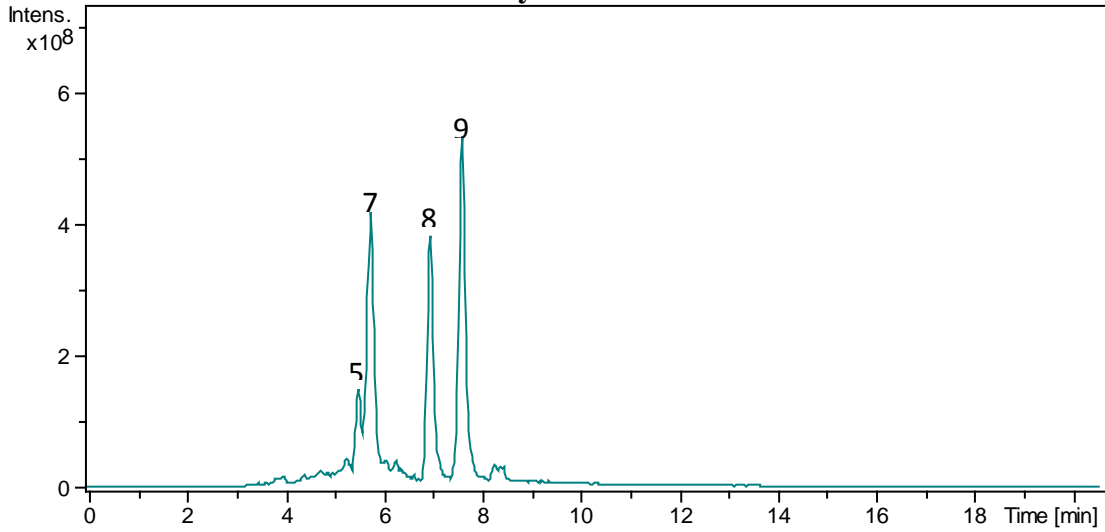
363 **Supplementary Figure S2.** Extracted ion chromatograms at m/z 385 and m/z 401, obtained by HPLC-
364 MS/MS, of cholesterol oxides in model system. Peak characterization is given in Table S1.

365 **Model system: cholesterol alone – 72 h**

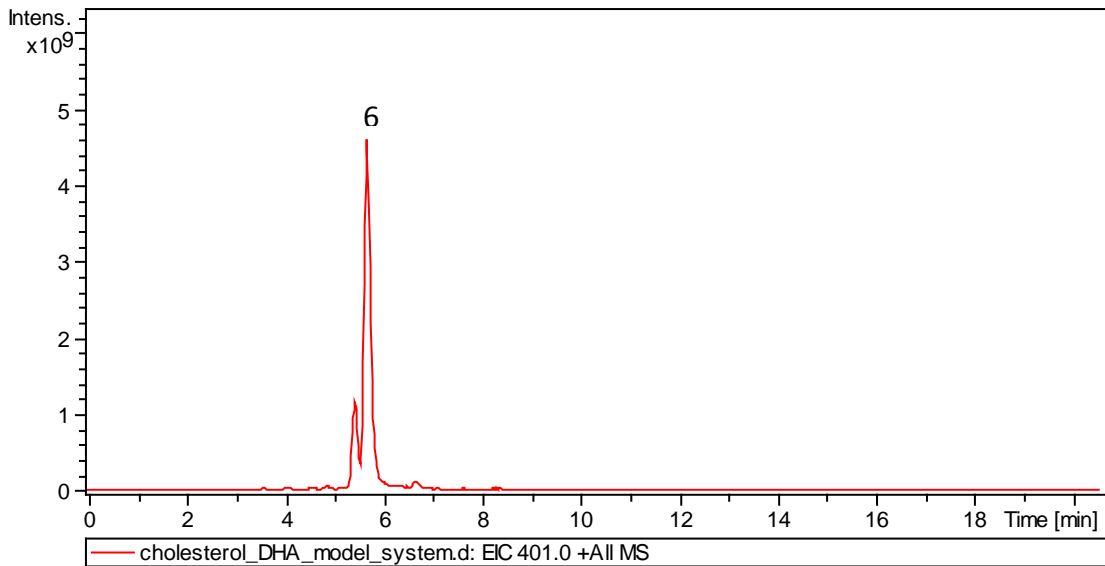


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Model system: cholesterol + DHA – 72 h



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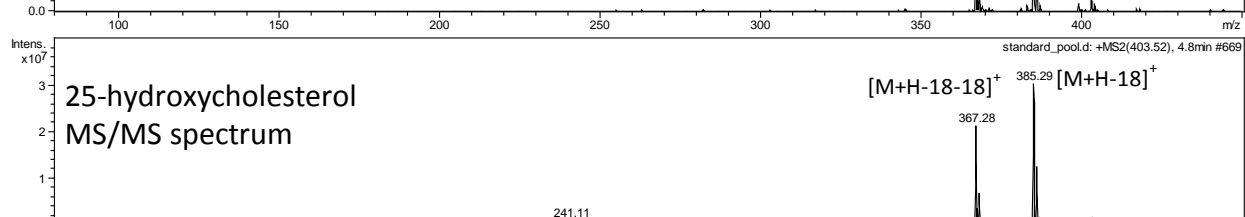
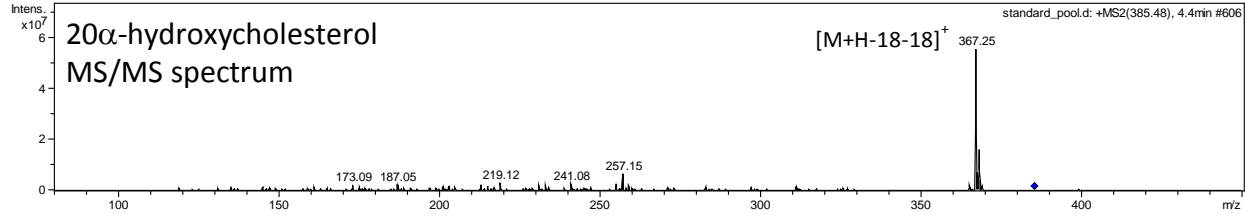
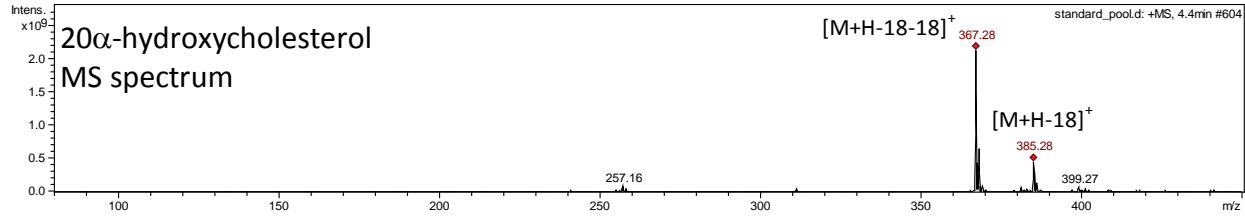
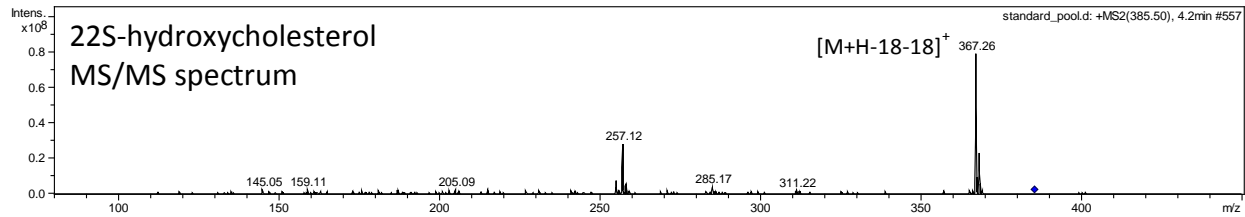
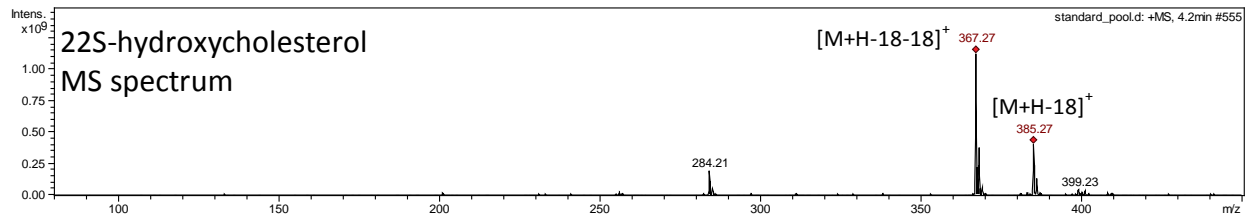
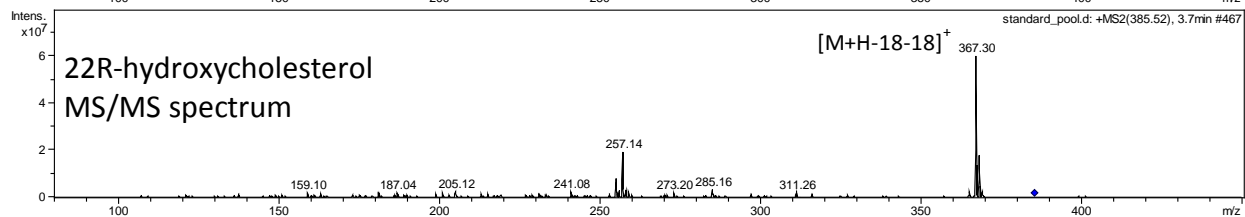
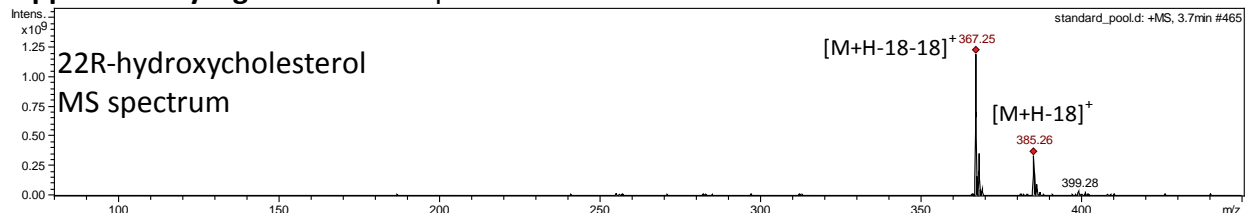
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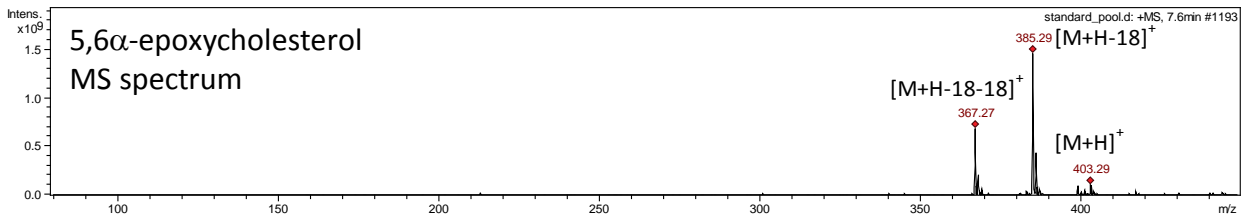
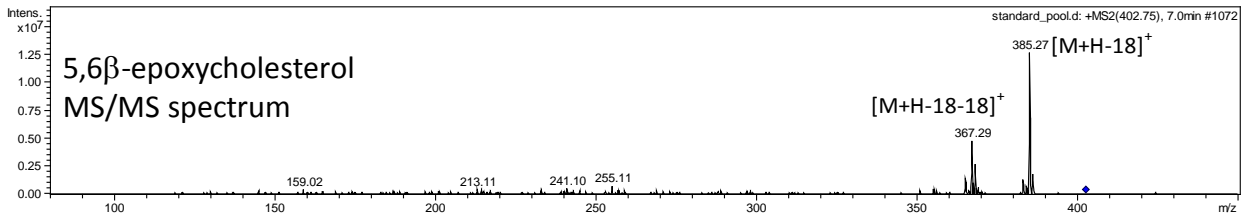
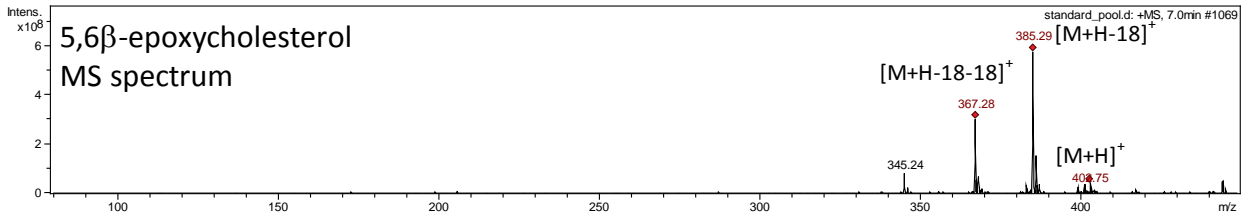
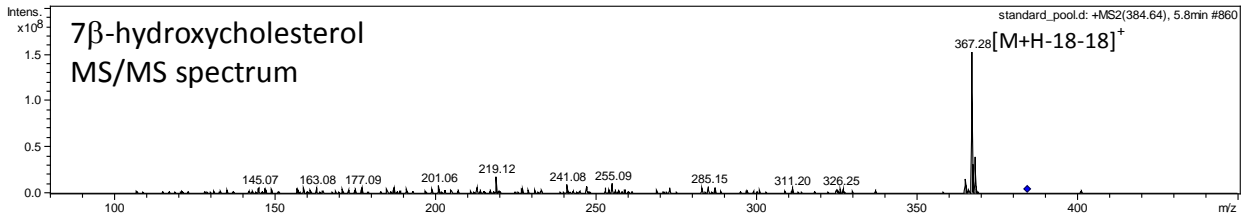
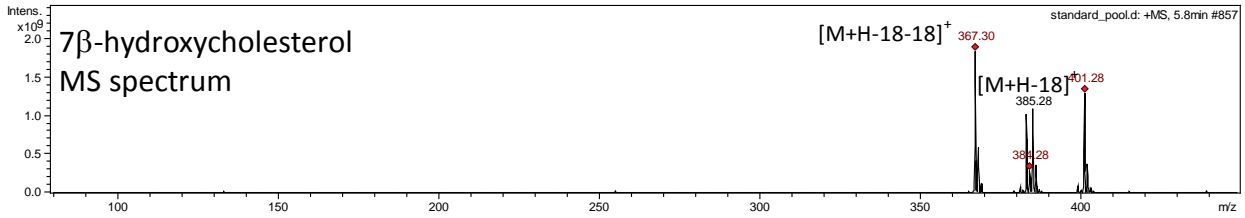
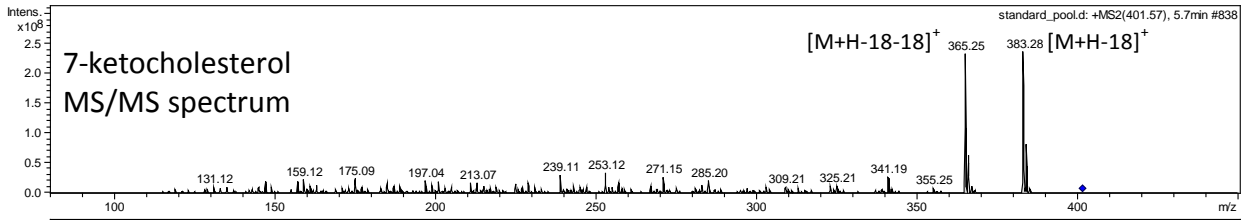
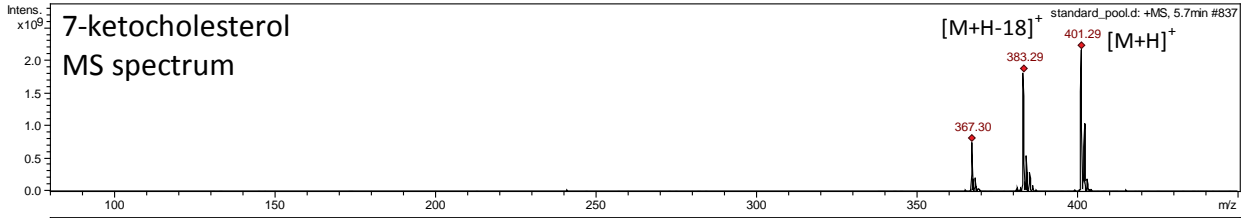
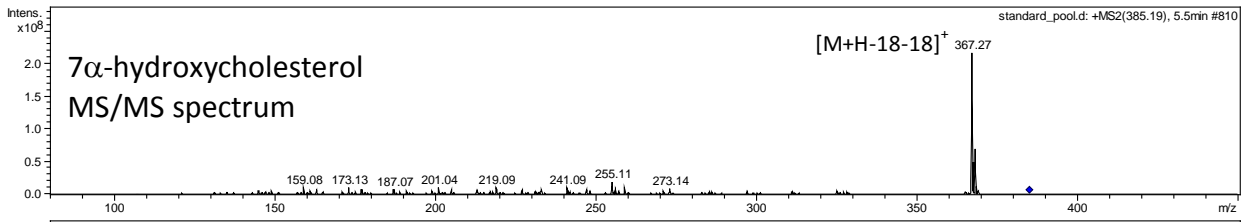
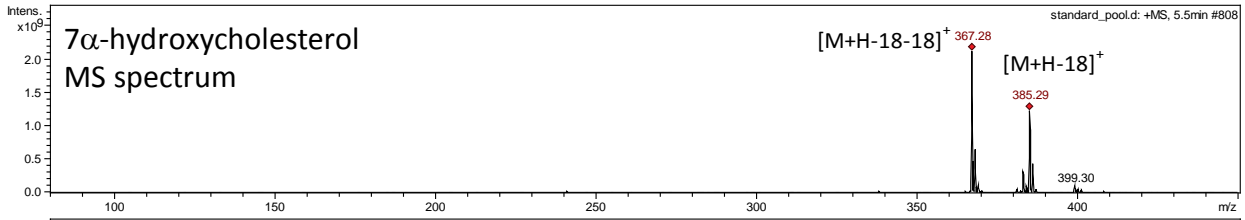
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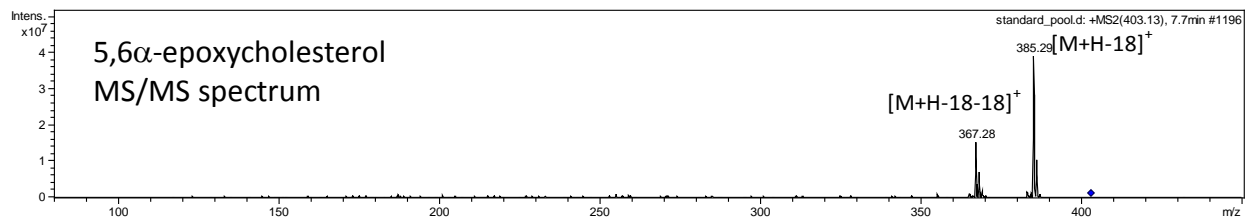
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376 **Supplementary Figure S3. Mass spectra of cholesterol oxide standards.**



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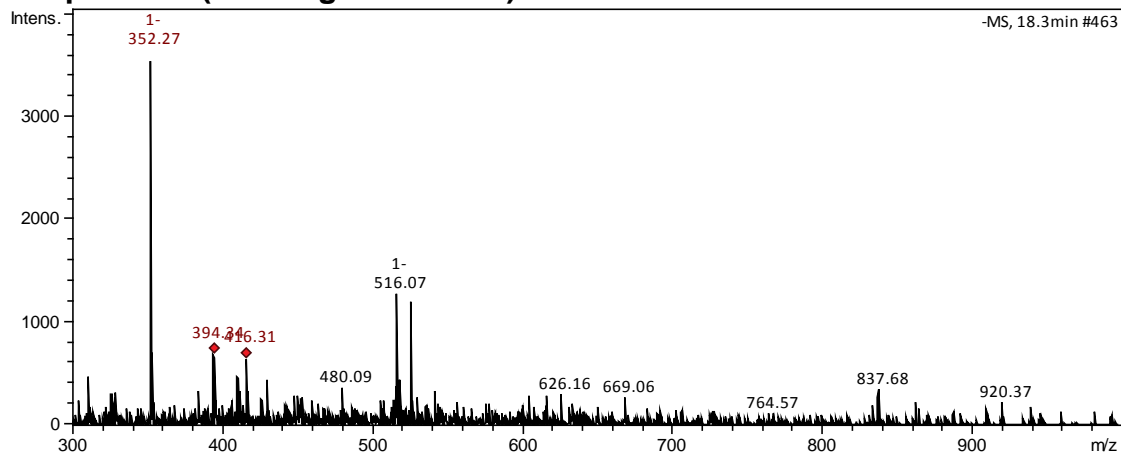




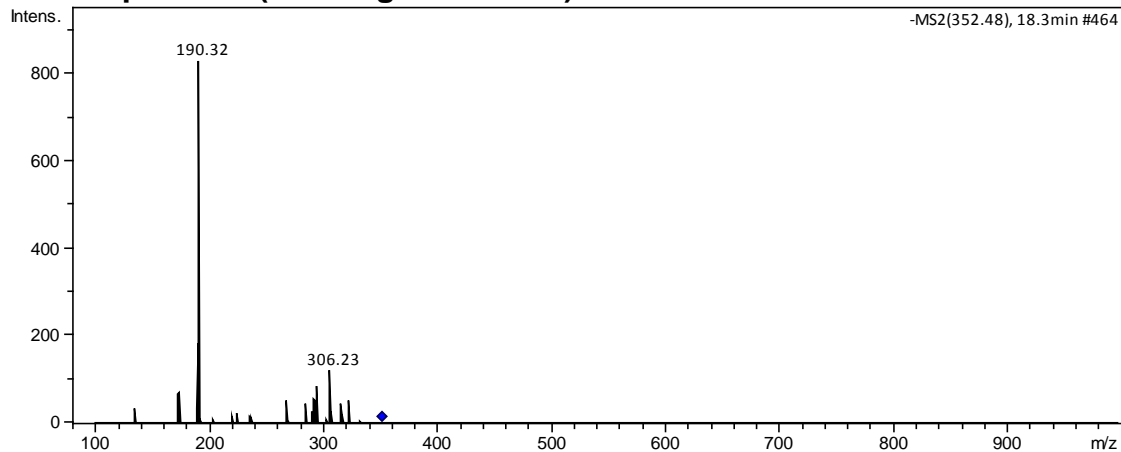
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398 **Supplementary Figure S4.** Mass spectra of the phenolic compounds in mana-cubiu water
399 extract.

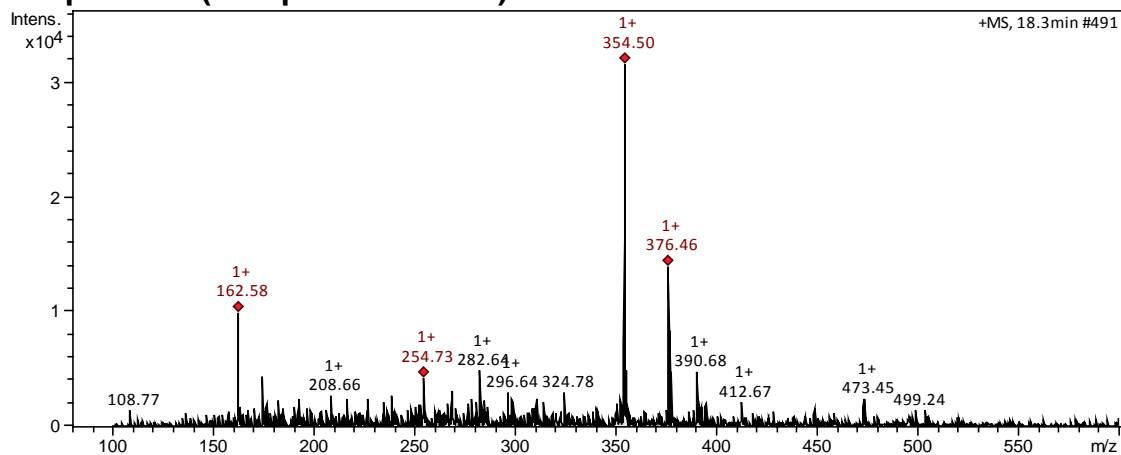
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401 **5-CQA**
402 **MS spectrum (ESI- negative mode)**



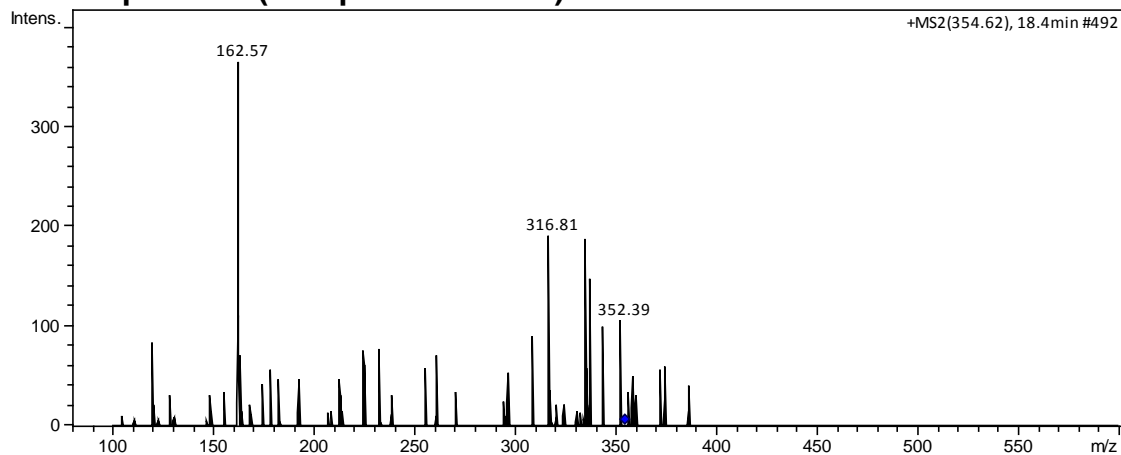
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405 **MS/MS spectrum (ESI- negative mode)**



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412 **5-CQA**
413 **MS spectrum (ESI- positive mode)**



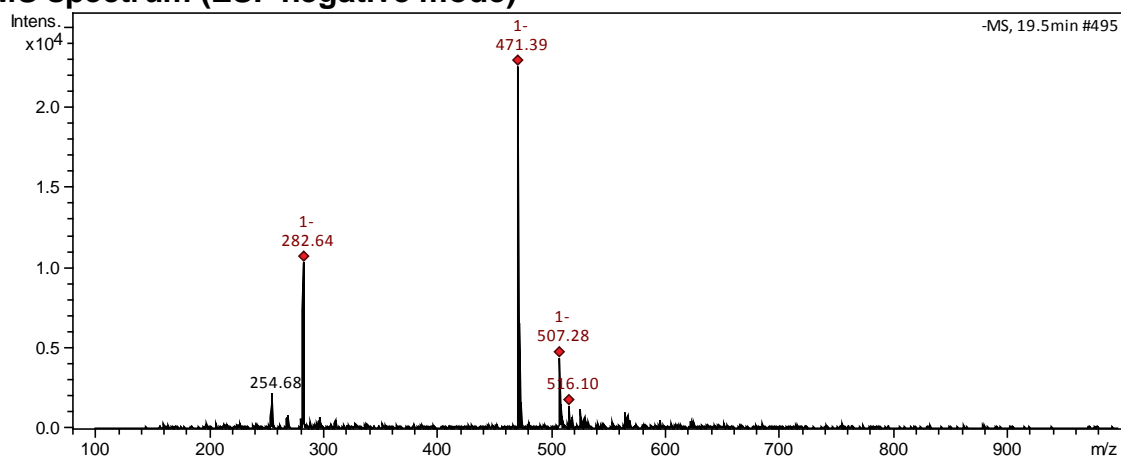
416 **MS/MS spectrum (ESI- positive mode)**



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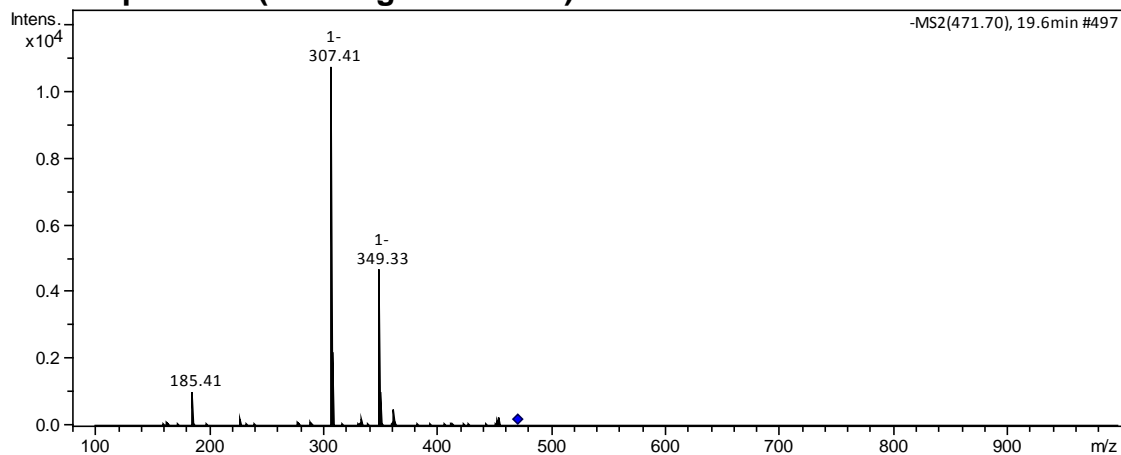
N₁, N₅ or N₅, N₁₀ -bis-(dihydrocaffeoyl)spermidine

427 **MS spectrum (ESI- negative mode)**



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430 **MS/MS spectrum (ESI- negative mode)**

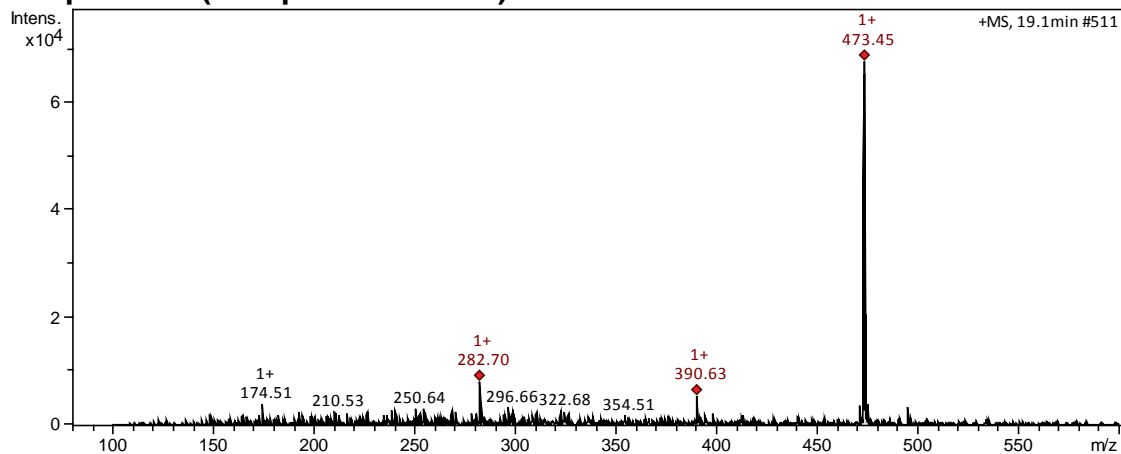


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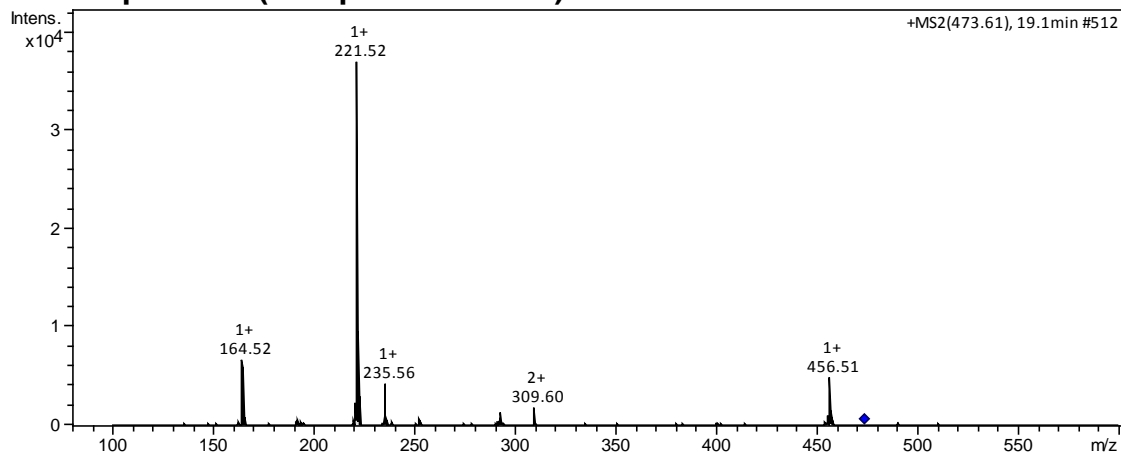
N_1, N_5 or N_5, N_{10} -bis-(dihydrocaffeoyl)spermidine

MS spectrum (ESI- positive mode)



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MS/MS spectrum (ESI- positive mode)



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