SUPPLEMENTARY MATERIALS

Table S1. ^{1}H NMR characteristic signals of identified putative metabolites in *P. cubeba* L. extracts and fractions.

Metabolites	Reported ^{1}H NMR signals	ME	EE	HE	HF	EAF	AMF
1. Cubebin	6.80–6.40 (m)	+	+	+	+	+	+
	5.95 (s)	+	+	+	+	+	+
	4.05 (dd)	+	+	+	+	+	+
	3.72 (dd)	3.74 (m)	3.74 (m)	3.74 (m)	3.73 (m)	3.74 (m)	3.74 (m)
	2.90-2.50 (m)	+	+	+	+	+	+
	2.30-1.90 (m)	+	+	+	+	+	+
2. Yatein	6.69 (d, *J*= 8.3)	+ (*)	nd	+ (*)	+ (*)	+ (*)	+ (*)
	6.36 (s)	+	+	+	+	+	+
	5.94 (d, *J*= 1.5)	5.94 (m)	5.94 (m)	5.94 (m)	5.94 (m)	5.94 (m)	5.94 (m)
	5.93 (d, *J*= 1.5)	5.93 (m)	5.93 (m)	5.93 (m)	5.93 (m)	5.93 (m)	5.93 (m)
	3.82 (s)	+	+	+	+	+	+
	2.92-2.89 (m)	nd	nd	nd	nd	+	nd
	2.63-2.48 (m)	+	+	+	+	+	+
3. Hinokinin	6.80–6.40 (m)	+	+	+	+	+	+
	5.90 (s)	+	+	+	+	+	+
	2.60 (d, *J*= 7.1)	+	+	+	2.60 (m)	+	+
	2.55 (m)	+	+	+	+	+	+
	2.45 (d, *J*= 8.6)	2.45 (m)	2.45 (m)	2.45 (m)	2.45 (m)	2.45 (m)	2.45 (m)
4. Dihydro-cubebin	6.60 (m)	+	+	+	+	+	+
	3.70 (dd, *J*= 1.3, 11.3)	3.74 (m)	3.74 (m)	3.74 (m)	3.73 (m)	3.74 (m)	3.73 (m)
	3.20 (s)	nd	nd	nd	+	+	+
	2.70 (d, *J*= 8.7)	2.70 (m)	2.70 (m)	nd	2.72 (m)	2.72 (m)	2.70 (m)
	2.65 (d, *J*= 8.7)	+	+ (*)	nd	+ (*)	nd	+
	2.56 (d, *J*= 5.7)	2.56 (m)	nd	nd	+ (*)	2.56 (m)	nd
	2.51 (d, *J*= 5.7)	2.50 (m)	+ (*)	nd	+ (*)	2.50 (m)	2.50 (m)
5. Dihydro-clusin	6.72 (d, *J*= 8.0)	+ (*)	+ (*)	+ (*)	+ (*)	+ (*)	+ (*)
	6.59 (d, *J*= 8.0)	+	+	+	+	+ (*)	+
	6.36 (s)	+	+	+	+	+	+
	5.92 (s)	+	+	+	+	+	+
	3.84 (s)	+	+	+	+	+	+
	3.80-3.42 (m)	+	+	+	+	+	+
	1.88 (m)	+	+	+	+	nd	nd
6. Cubebinin	6.47 (s)	+	+	nd	nd	+	+
	6.40 (s)	+	+	+	+	+	+
	6.36 (s)	+	+	+	+	+	+
	5.27 (s)	+	+	+	+	nd	+
	4.03 (t, *J*= 8.0)	+	+	+	+	+	+
	3.84 (s)	+	+	+	+	+	+
	3.82 (s)	+	+	+	+	+	+
	3.80 (s)	+	+	+	+	+	+
	3.62 (t, *J*= 8.0)	+	+	+	+	+	+
	2.04-2.85 (m)	+	+	+	+	+	+
7. Magnosalin	6.46 (s)	+	+	nd	nd	+	+
	3.86 (s)	+	+	nd	nd	+	+
	3.84 (s)	+	+	+	+	+	+
	3.68 (s)	nd	nd	nd	nd	+	+
	1.75 (s)	+	nd	nd	nd	nd	+
	1.19 (d, *J*= 5.5)	+	+	+	+	nd	+
8. *p*-cymene	2.87 (m)	+	+	+	+	nd	+
	2.23 (s)	+	+	+	+	+	+

	1.23 (d, *J*= 8.5)	+	+	+	+	+	+
9. Piperidine	2.79 (t)	2.76 (m)	2.75 (m)	2.75 (m)	2.75 (m)	2.75 (m)	2.75 (m)
	2.18 (s)	nd	nd	nd	nd	2.19 (s)	nd
	1.58 (t)	1.58 (m)	1.58 (m)	1.58 (m)	1.58 (m)	nd	1.58 (m)
	1.55 (d)	1.56 (m)	1.55 (m)	1.56 (m)	1.56 (m)	nd	1.56 (m)
	1.51 (m)	+	+	+	+	nd	+
10. Cubebol	1.84-1.37 (m)	+	+	+	+	nd	+
	1.28 (s)	+	+	+	+	+	+
	0.97 (d, *J*= 6.8)	+ (*)	+ (*)	+ (*)	+ (*)	0.97 (m)	+
	0.94 (d, *J*= 6.5)	+ (*)	+ (*)	+ (*)	+ (*)	0.94 (m)	+
	0.91 (d, *J*= 4.3)	+ (*)	+ (*)	+ (*)	+ (*)	0.91 (m)	+
	0.87 (d, *J*= 3.4)	0.88 (m)	0.88 (m)	0.88 (m)	0.88 (m)	0.87 (m)	0.88 (m)
	0.83 (dd, *J*= 3.4, 3.4)	0.88 (m)	0.88 (m)	0.83 (m)	0.88 (m)	0.87 (m)	0.88 (m)
11. D-Germacrene	4.93 (brs)	+	+	+	+	+	+
	2.30- 190 (m)	+	+	+	+	+	+
	1.45 (brs)	+	+	+	+	+	+
	1.30 (m)	+	+	+	+	+	+
	0.88 (d, *J*= 6.7)	0.88 (m)	0.88 (m)	0.88 (m)	0.88 (m)	0.87 (m)	0.88 (m)
	0.84 (d, *J*= 6.8)	+	+	+	+	0.85 (m)	+ (*)
12. Ledol	1.65-156 (m)	+	+	+	+	+	+
	1.39 (m)	+	+	+	+	nd	+
	1.15 (s)	+	+	+	+	+	+
	1.02 (s)	+	+	+	+	+	+
	0.98 (s)	+	+	+	+	+	+
	0.92 (d, *J*=7.00)	+ (*)	+ (*)	+ (*)	+ (*)	0.91 (m)	+ (*)

(*) Indicates the identification has been confirm with 2D ^{1}H *J*-resolved (JRES) NMR spectroscopy. (nd): not detected. ME: methanol extract; EE: ethanol extract; HE: hexane extract; HF: hexane fraction; EAF: ethyl acetate fraction; AMF: aqueous methanol fraction. Metabolites reported from; **1:** Souza et al. (2004), **2:** Miyata et al. (1998), **3:** Souza et al. (2005), **4:** Laurentiz et al. (2015), **5** and **6:** Prabhu and Mulchandani, (1985), **7:** Ryu et al. (2002), **8:** Human Metabolome Database (HMDB) 05805, **9:** HMDB 0034301, **10:** Chen et al. (2001), **11:** Mori et al. (1990), **12:** Miyazawa et al. (1994).

Table S2. Nitric oxide (NO) production by extracts and fractions of *P. cubeba* L. stimulated with LPS and IFN-γ in RAW 264.7 cells.

	NO production (μg/mL)					
Conc.	**ME**	**EE**	**HE**	**HF**	**EAF**	**AMF**
LPS, IFN-γ	45.53 ± 3.98	45.53 ± 3.98	45.53 ± 3.98	45.53 ± 3.98	45.53 ± 3.98	45.53 ± 3.98
1.95	41.14 ± 3.46	40.49 ± 1.02	n.d	44.32 ± 1.57	44.48 ± 2.70	n.d
3.90	39.33 ± 4.14	35.82 ± 4.52	35.20 ± 9.52	43.13 ± 0.85	42.37 ± 1.69	43.49 ± 2.26
7.81	38.29 ± 3.47	33.13 ± 7.61	34.20 ± 8.94	40.90 ± 0.93	39.28 ± 2.39	40.43 ± 5.85
15.63	31.73 ± 2.95	31.88 ± 5.19	32.99 ± 9.81	37.96 ± 0.48	39.03 ± 1.44	37.79 ± 3.24
31.25	31.16 ± 2.96	24.29 ± 7.36	37.00 ± 5.75	33.72 ± 1.29	36.42 ± 2.09	33.27 ± 2.22
62.50	22.98 ± 5.18	23.13 ± 1.81	28.96 ± 0.54	34.44 ± 0.64	37.68 ± 2.98	27.31 ± 1.83
125.00	n.d	n.d	22.12 ± 6.59	n.d	n.d	18.88 ± 2.15
Control	4.09 ± 0.15	4.09 ± 0.15	4.09 ± 0.15	4.09 ± 0.15	4.09 ± 0.15	4.09 ± 0.15
Curcumin	4.79 ± 0.12	4.79 ± 0.12	4.79 ± 0.12	4.79 ± 0.12	4.79 ± 0.12	4.79 ± 0.12

Values are the mean ± S.D. of replications (n =3). n.d: no data. ME: methanol extract; EE: ethanol extract; HE: hexane extract; HF: hexane fraction; EAF: ethyl acetate fraction; AMF: aqueous methanol fraction.

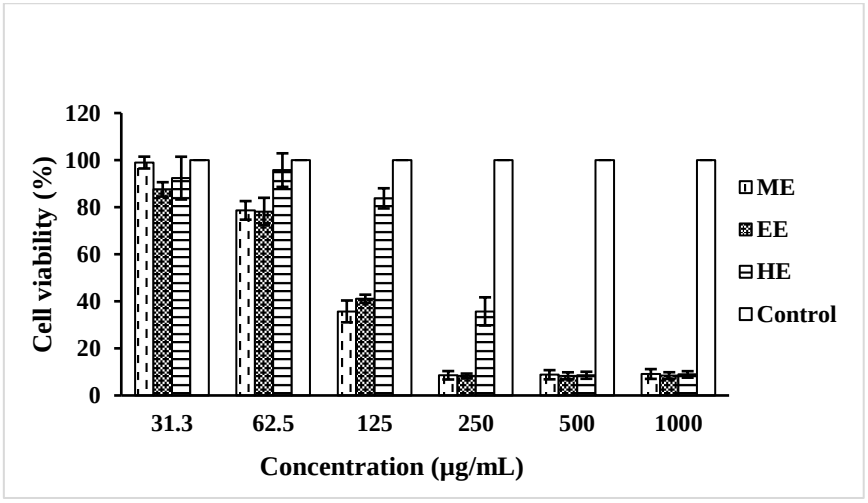


Figure S1. Percentage of cell viability (%) treated with different *P. cubeba* L. extracts. Values are the mean ± S.D. of replications (n=3). ME: methanol extract; EE: ethanol extract; HE: hexane extract.

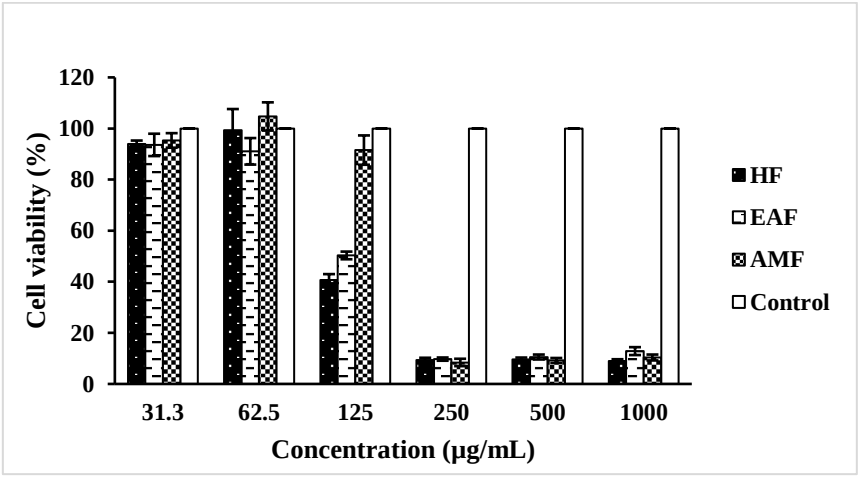


Figure S2. Percentage of cell viability (%) treated with different *P. cubeba* L. fractions. Values are the mean ± S.D. of replications (n=3). HF: hexane fraction; EAF: ethyl acetate fraction; AMF: aqueous methanol fraction.

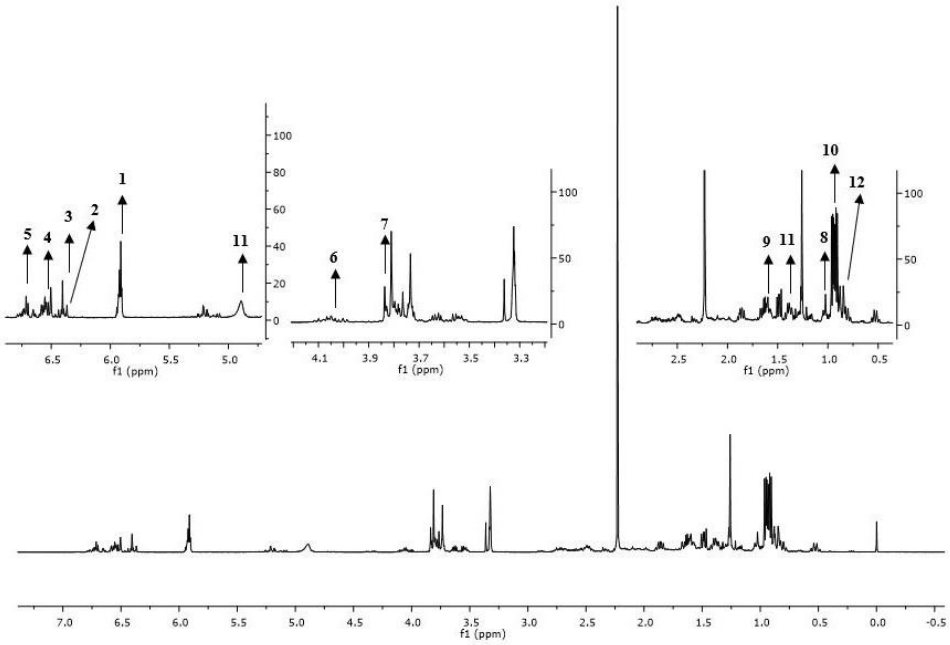


Figure S3. The ^{1}H NMR spectra of ethanol extract of *P. cubeba* L.. The numbers indicate the identified putative metabolites. **1**: cubebin; **2**: yatein; **3**: hinokinin; **4**: dihydrocubebin; **5**: dihydroclusin; **6**: cubebinin; **7**: magnosalin; **8**: *p*-cymene; **9**: piperidine; **10**: cubebol; **11**: D-germacrene; **12**: ledol.

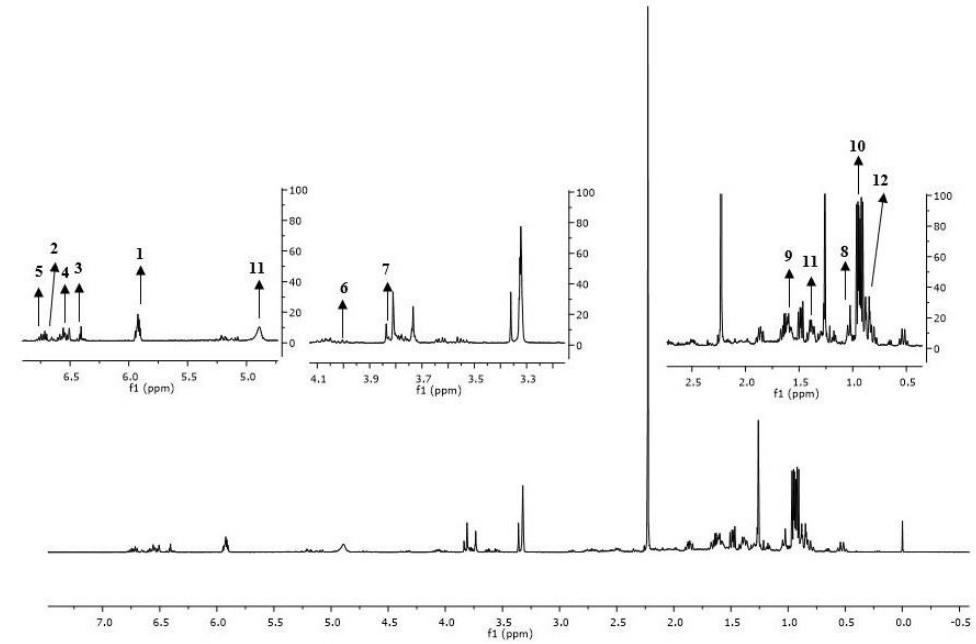


Figure S4. The ^{1}H NMR spectra of hexane extract of *P. cubeba* L.. The numbers indicate the identified putative metabolites. **1**: cubebin; **2**: yatein; **3**: hinokinin; **4**: dihydrocubebin; **5**: dihydroclusin; **6**: cubebinin; **7**: magnosalin; **8**: *p*-cymene; **9**: piperidine; **10**: cubebol; **11**: D-germacrene; **12**: ledol.

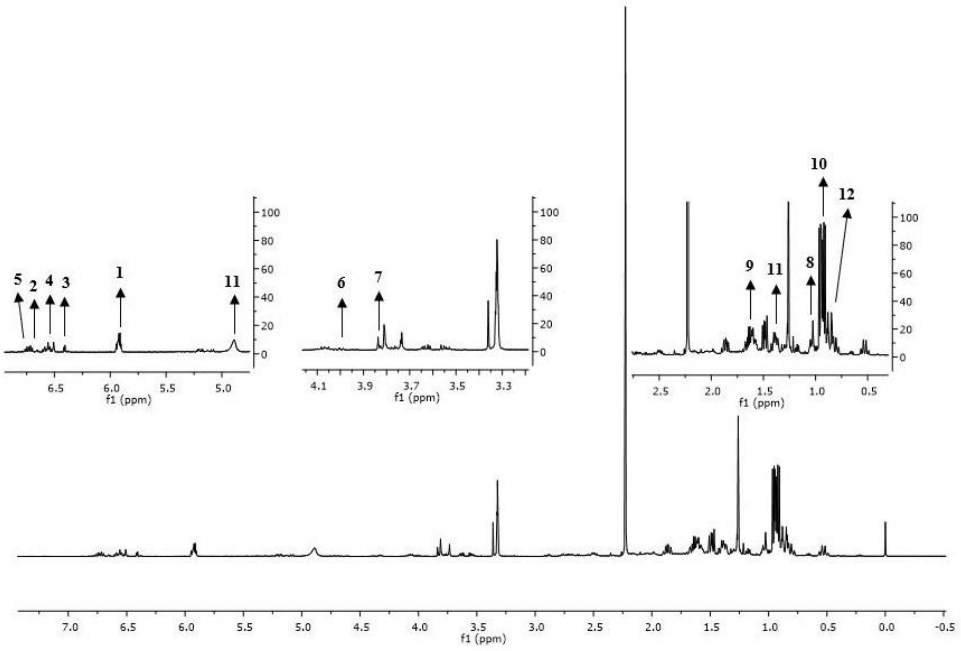


Figure S5. The ^{1}H NMR spectra of hexane fraction of *P. cubeba* L.. The numbers indicate the identified putative metabolites. **1**: cubebin; **2**: yatein; **3**: hinokinin; **4**: dihydrocubebin; **5**: dihydroclusin; **6**: cubebinin; **7**: magnosalin; **8**: *p*-cymene; **9**: piperidine; **10**: cubebol; **11**: D-germacrene; **12**: ledol.

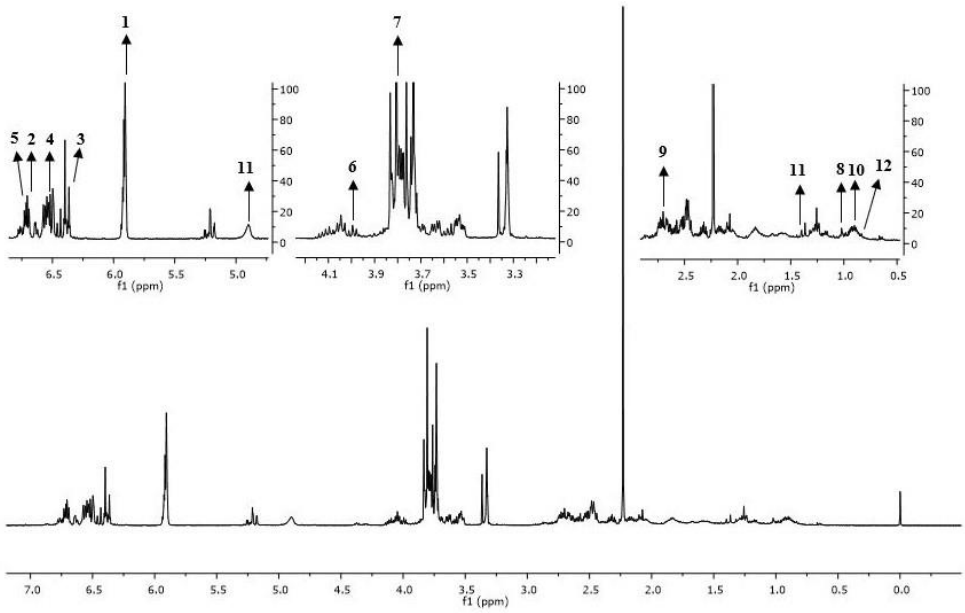


Figure S6. The ^{1}H NMR spectra of ethyl acetate fraction of *P. cubeba* L.. The numbers indicate the identified putative metabolites. **1**: cubebin; **2**: yatein; **3**: hinokinin; **4**: dihydrocubebin; **5**: dihydroclusin; **6**: cubebinin; **7**: magnosalin; **8**: *p*-cymene; **9**: piperidine; **10**: cubebol; **11**: D-germacrene; **12**: ledol.

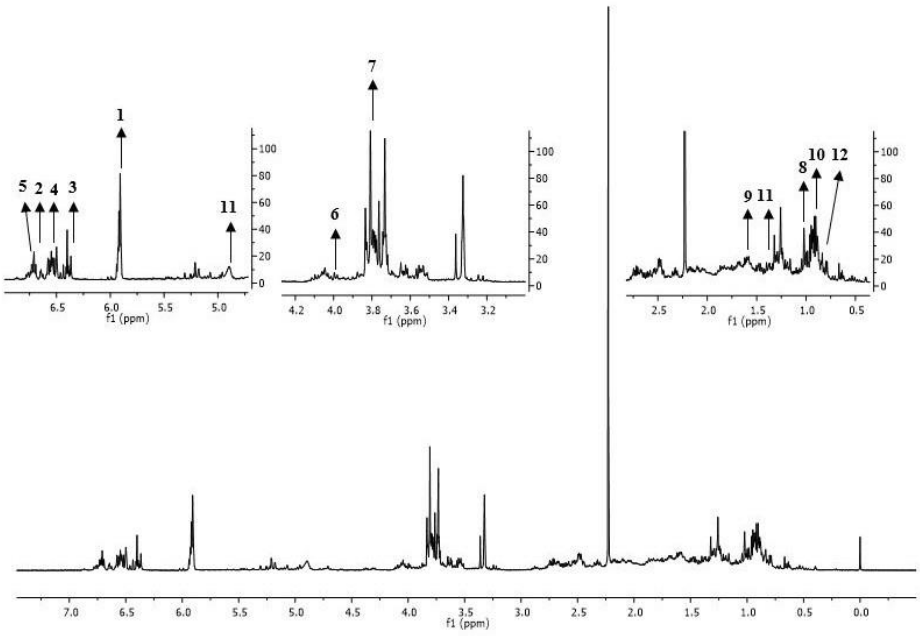


Figure S7. The ^{1}H NMR spectra of aqueous methanol fraction of *P. cubeba* L.. The numbers indicate the identified putative metabolites. **1**: cubebin; **2**: yatein; **3**: hinokinin; **4**: dihydrocubebin; **5**: dihydroclusin; **6**: cubebinin; **7**: magnosalin; **8**: *p*-cymene; **9**: piperidine; **10**: cubebol; **11**: D-germacrene; **12**: ledol.

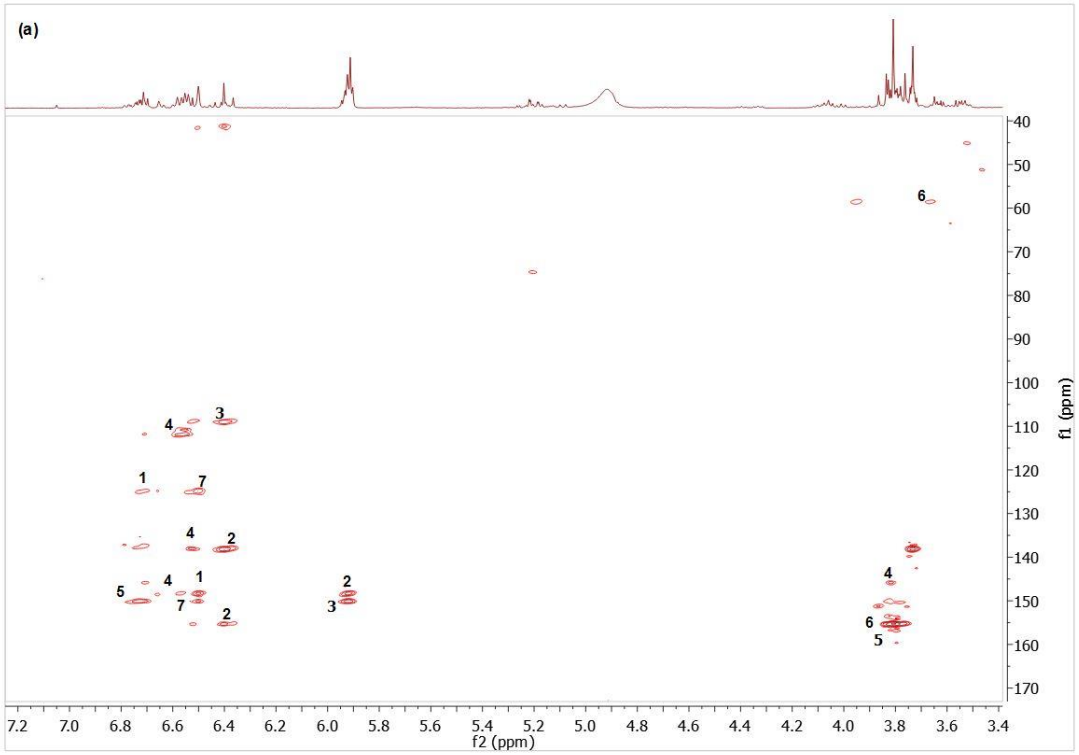


Figure S8 (a). Representative heteronuclear bond correlation (HMBC) spectrum of methanol extract of *P. cubeba* L. in the range of δ 3.40-δ 7.00 of ^{1}H and δ 50-δ 170 of ^{13}C. The numbers indicate the identified putative metabolites. **1**: correlation of H-7′/C-6′, H-2′/C-3′ of cubebin; **2**: correlation of H-2/C-4, H-6/C-5/, H-10′/C-3′ of yatein; **3**: correlation of H-10/C-4, H-6/C-2 of hinokinin; **4**: correlation of H-2′/C-3′, H-2′/C-1′, H-2′/C-5, H-4/C-3 of dihydrocubebin; **5**: correlation of H-6′/C-4′, Ar-OCH_3/C-3′′ of dihydroclusin; **6**: correlation of H-5/C-3, Ar-OCH_3/C-3′ of cubebinin; **7**: correlation of H-3′/C-1′, H-3′/C-4′ of magnosalin.

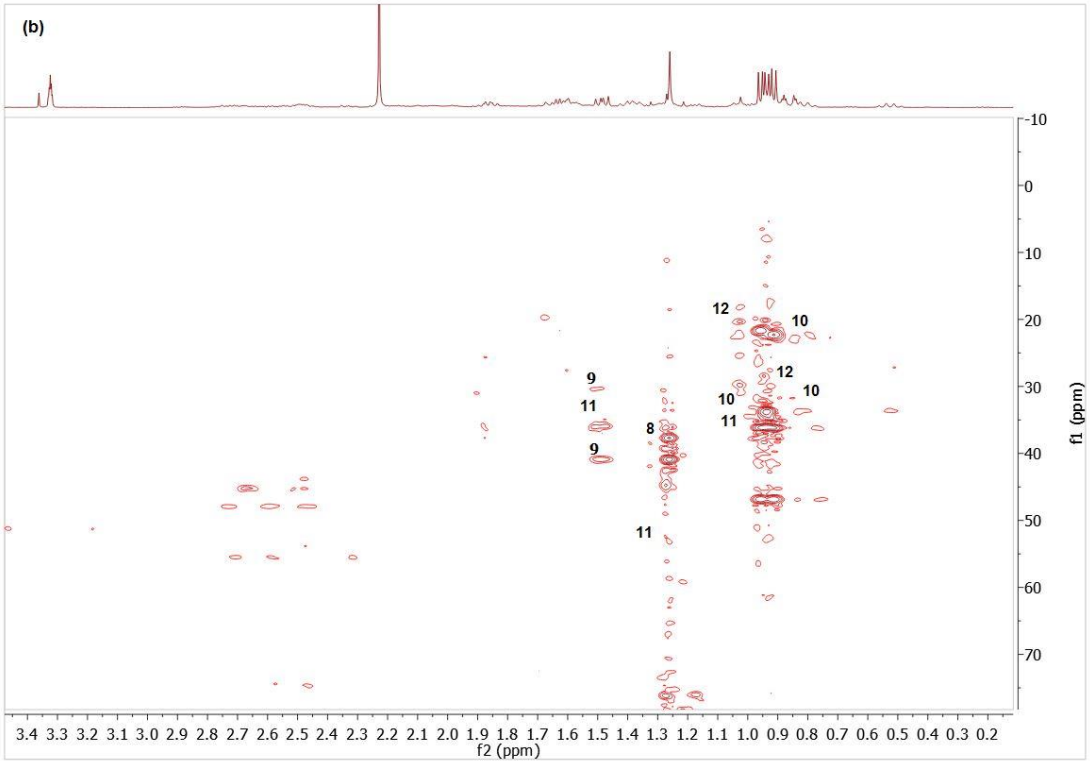


Figure S8 (b). Representative heteronuclear bond correlation (HMBC) spectrum of methanol extract of *P. cubeba* L. in the range of δ 0.20-δ 2.00 of ^{1}H and δ 10-δ 70 of ^{13}C. The numbers indicate the identified putative metabolites. **8**: correlation of H-9/C-7, H-9/C-4 of *p*-cymene; **9**: correlation of H-4/C-5, H-4/C-2 of piperidine; **10**: correlation of H-14/C-10, H-14/C-1, H-5/C-1, H-5/C-6 of cubebol; **11**: correlation of H-13/C-11, H-8/C-7, H14-C9 of D-germacrene; **12**: correlation of H-12/C-11, H-13/C-7 of ledol.

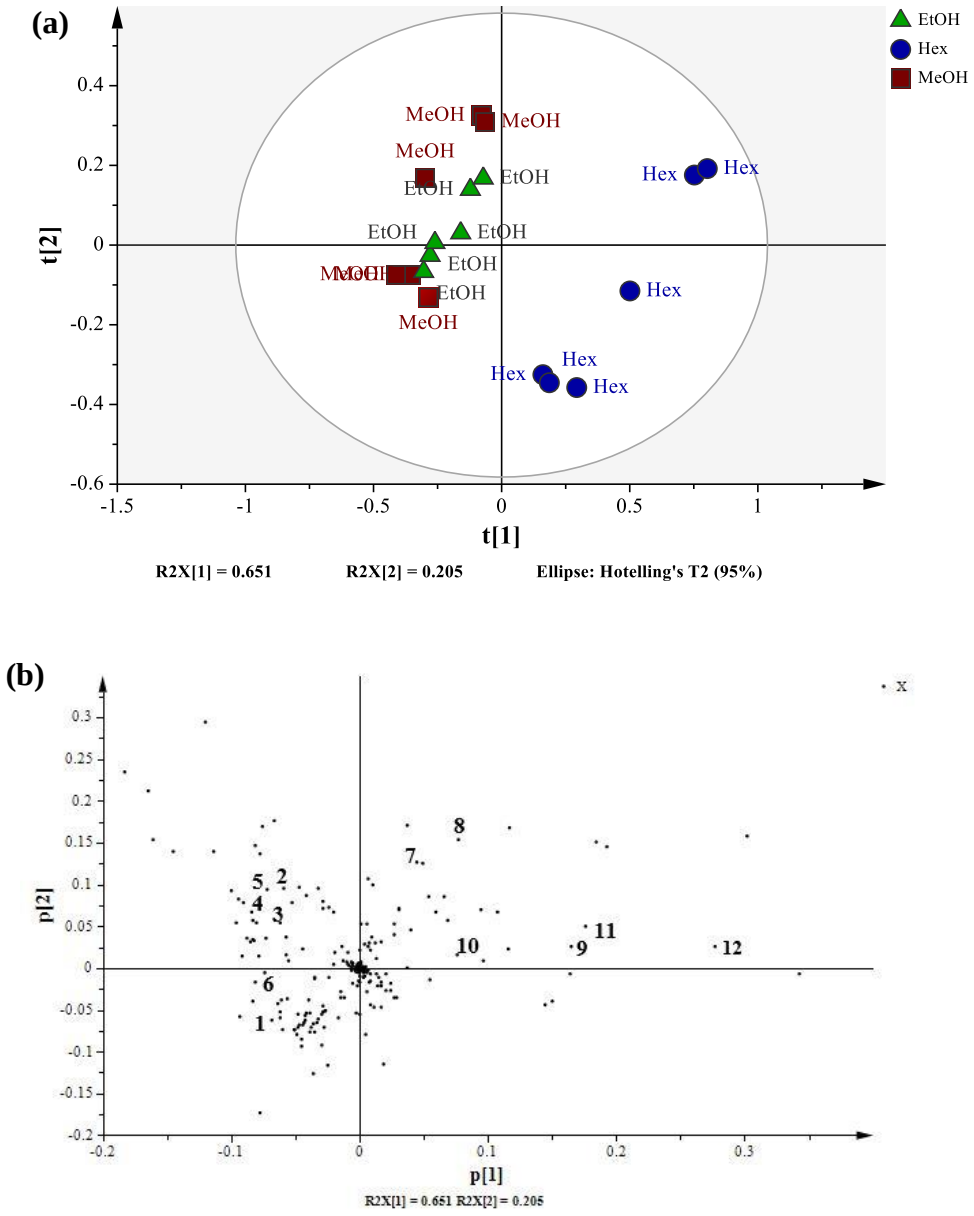


Figure S9. Principal component analysis (PCA) (**a**) score plot (PC1 vs. PC2) and (**b**) loading scatter plot of ^{1}H NMR data of different extracts of *P. cubeba* L. The numbers indicate the identified putative metabolites. **1**: cubebin; **2**: yatein; **3**: hinokinin; **4**: dihydrocubebin; **5**: dihydroclusin; **6**: cubebinin; **7**: magnosalin; **8**: *p*-cymene; **9**: piperidine; **10**: cubebol; **11**: D-germacrene; **12**: ledol, X: metabolites in *P. cubeba* L.

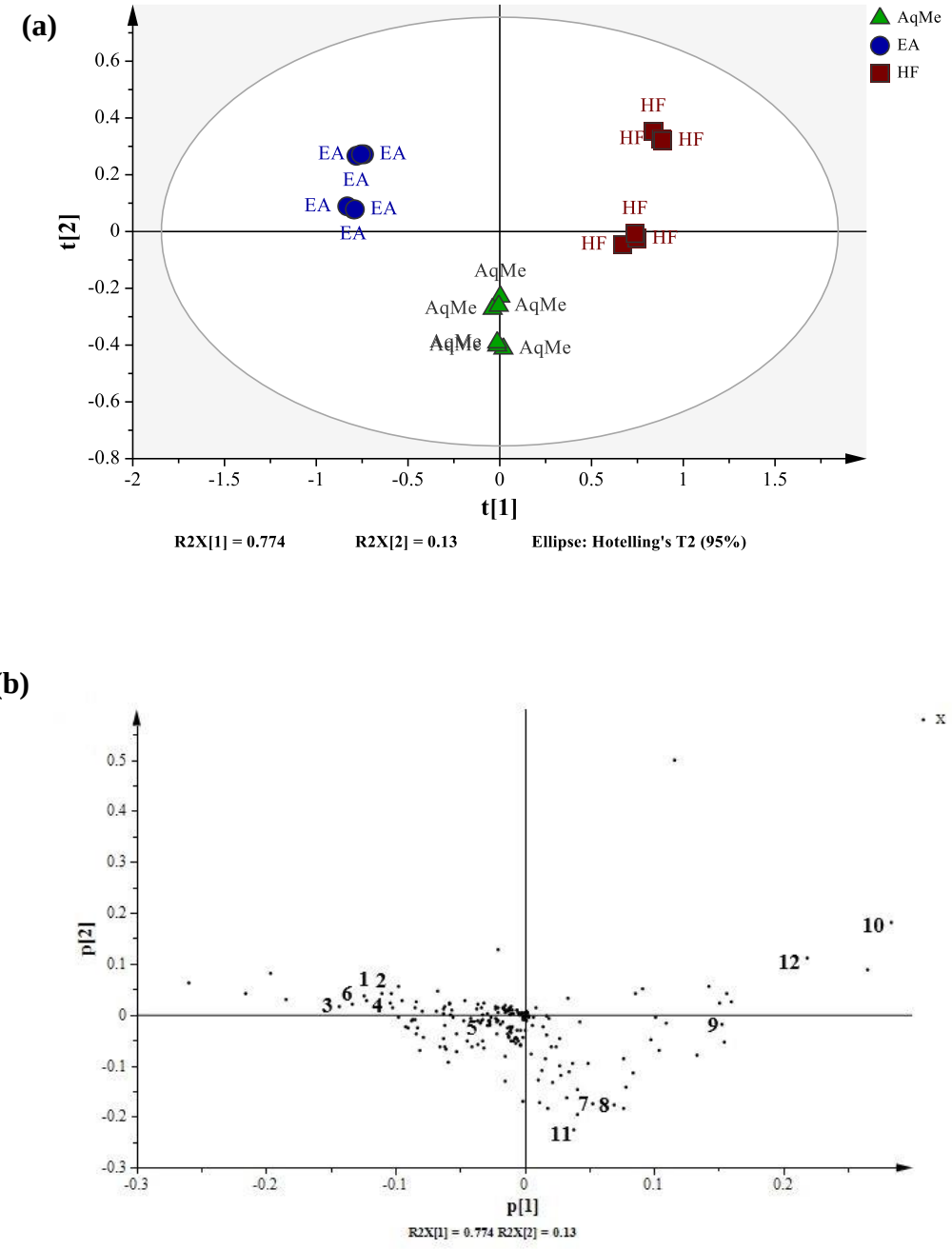


Figure S10. PCA (**a**) score plot (PC1 vs. PC2) and (**b**) loading scatter plot of ^{1}H NMR data of different fractions of *P. cubeba* L. The numbers indicate the identified putative metabolites. **1:** cubebin; **2:** yatein; **3:** hinokinin; **4:** dihydrocubebin; **5:** dihydroclusin; **6:** cubebinin; **7:** magnosalin; **8**: *p*-cymene; **9:** piperidine; **10:** cubebol; **11:** D-germacrene; **12:** ledol, X: metabolites in *P. cubeba* L.

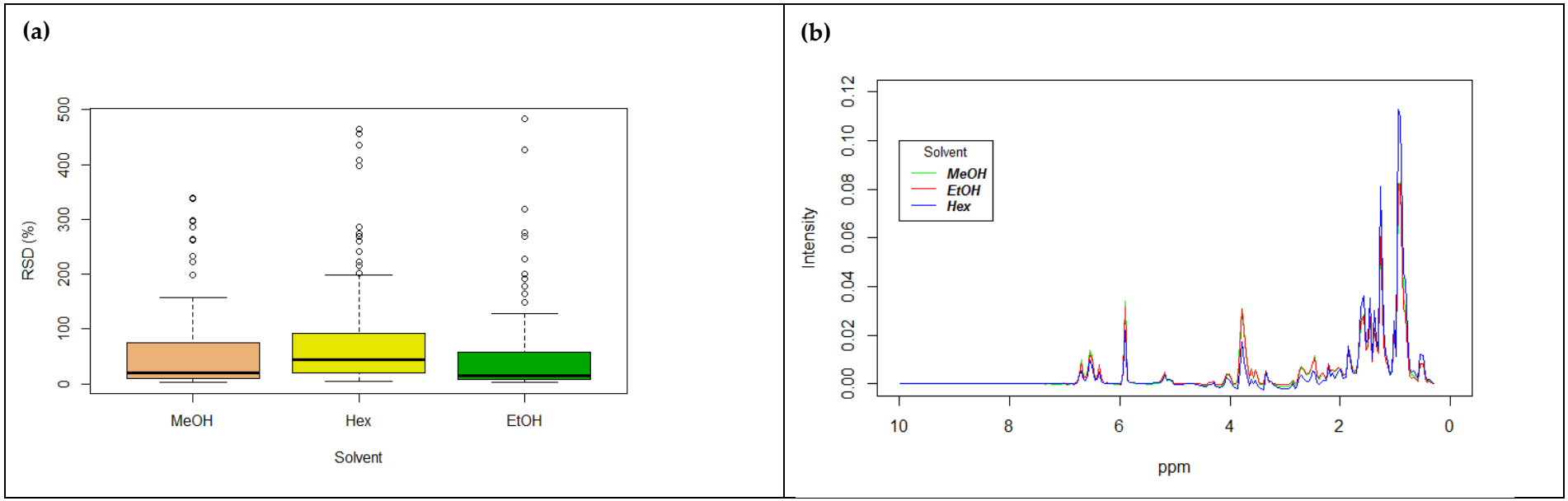
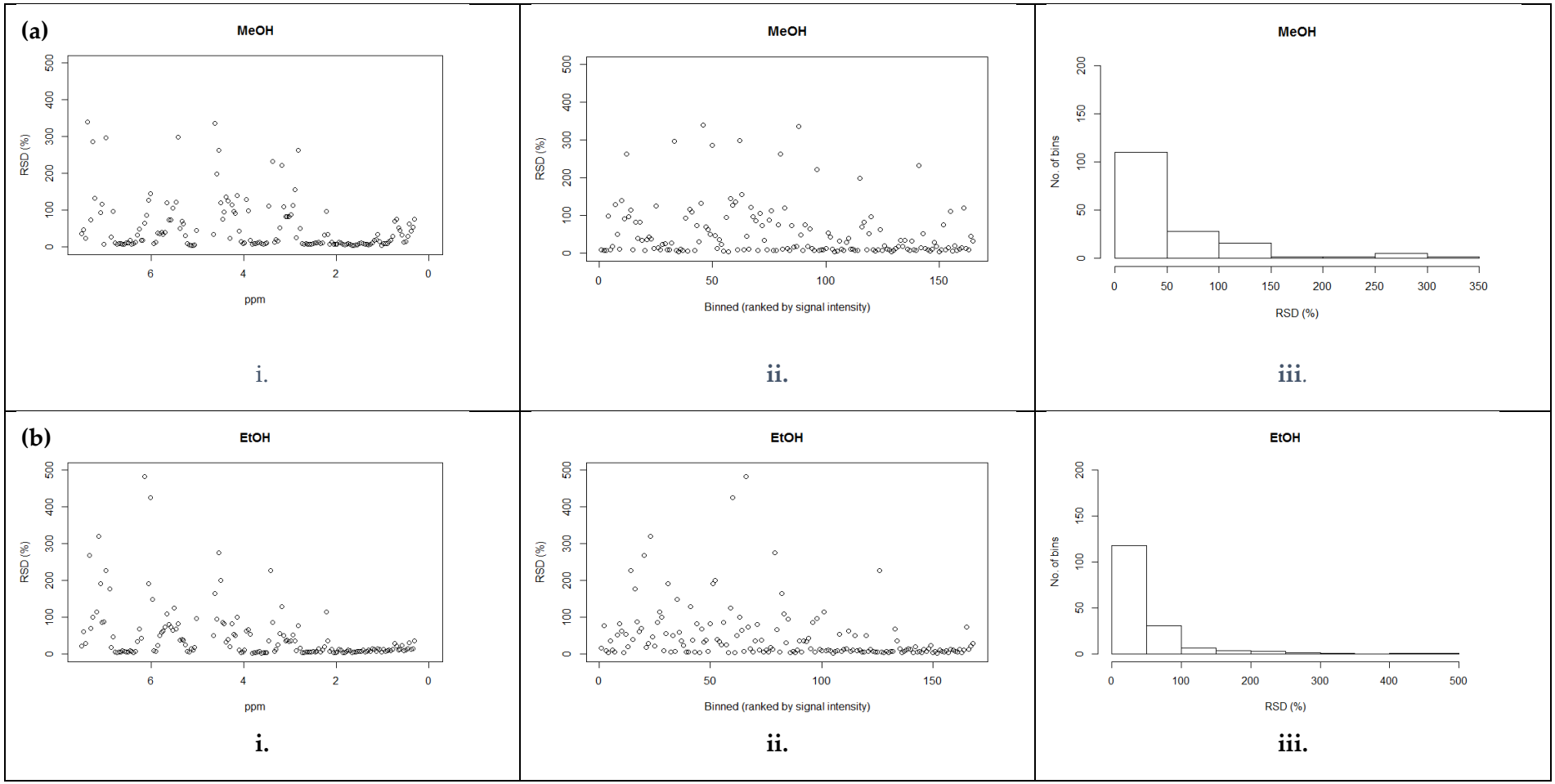


Figure S11. Calculation of spectral relative standard deviation (RSD) values of different solvents of *P. cubeba* L. extracts. **(a)** summary of RSD values **(b)** Three overlaid ^{1}H NMR spectra of *P. cubeba* L. using different extraction solvents (bin width of 0.04 ppm).


(a)
MeOH
RSD (%)
ppm
i.
MeOH
RSD (%)
Binned (ranked by signal intensity)
ii.
MeOH
No. of bins
RSD (%)
iii.
(b)
EtOH
RSD (%)
ppm
i.
EtOH
RSD (%)
Binned (ranked by signal intensity)
ii.
EtOH
No. of bins
RSD (%)
iii.

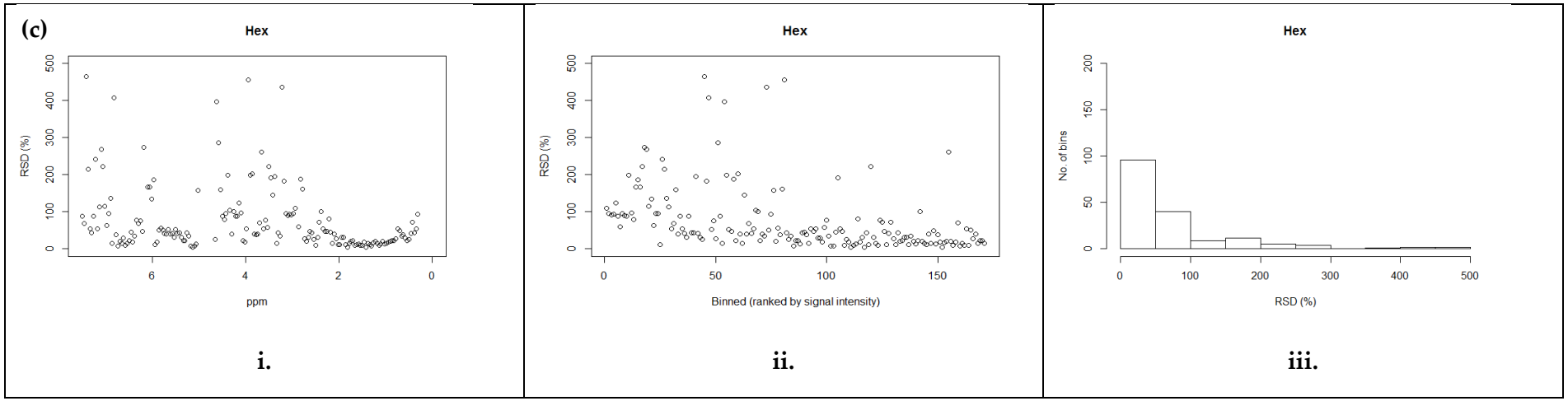


Figure S12. RSD calculation for each extract. The RSD results showed the values range from 46.82-75.88% for all extracts. **(a)** Methanol extract, **(b)** Ethanol extract, **(c)** Hexane extract; i. RSD values, ii. RSD values (ranked according to bin signal intensity), iii. Histogram of RSD values showing a right-skewed distribution, Mean RSD (%) and standard deviation for different extraction solvents, MeOH: 53.20 ± 69.16, EtOH: 46.82 ± 72.51, Hex: 75.88 ± 88.31.

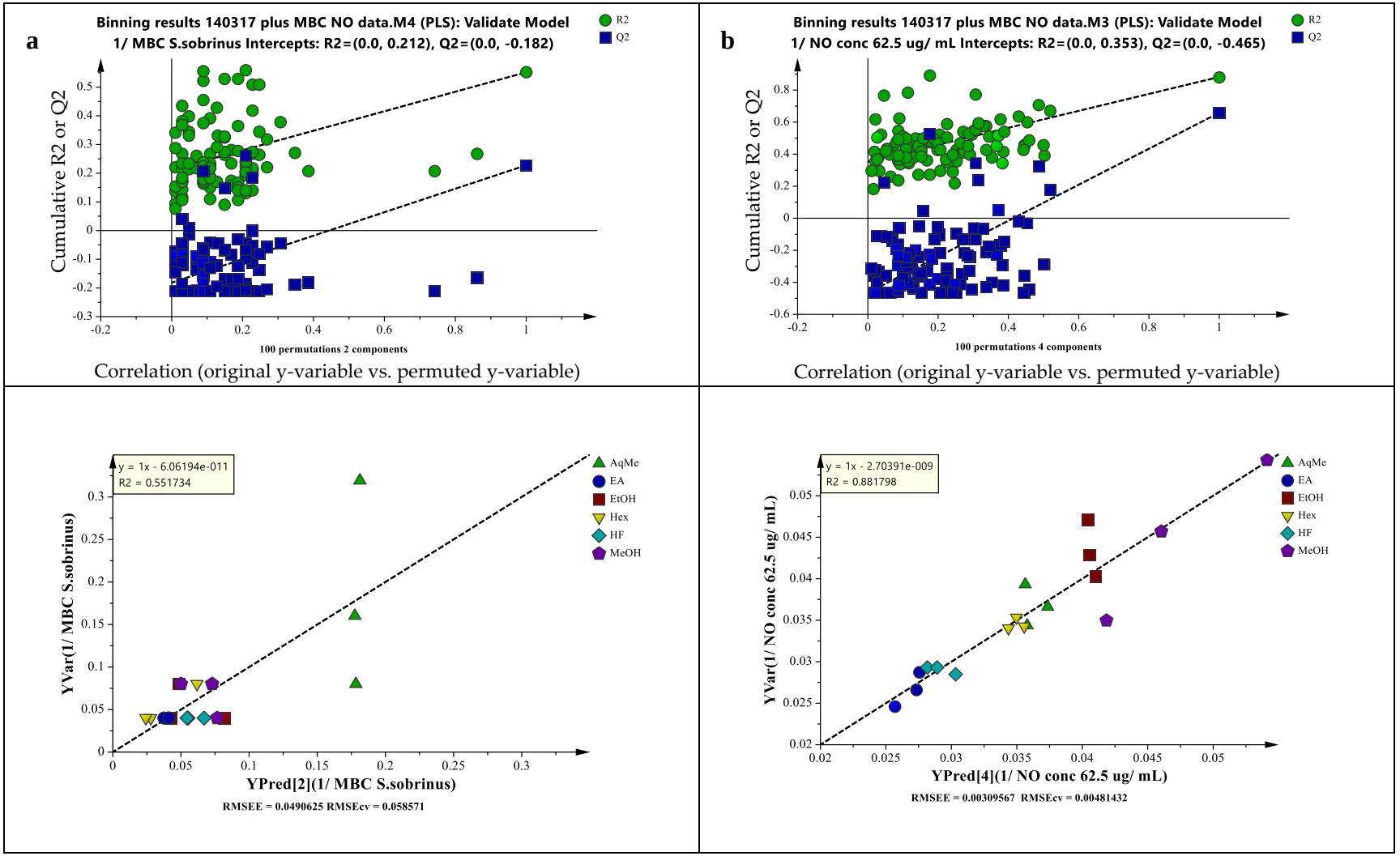


Figure S13. Validation of partial least squares (PLS) model using permutation test and observed and predicted plots for (a) minimum bactericidal concentration (1/MBC), (b) nitrite oxide (1/NO) production.