

**Assessment of metabolome diversity in black and white pepper seeds in response to autoclaving using MS- and NMR-based metabolomics and in relation to its remote and direct antimicrobial effects against food borne pathogens**

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**Table S1: Vapor-phase antimicrobial activity of the tested ground pepper n=3, Average ± SD**

Vapor-phase minimum inhibitory concentration (VP-MIC) mg/mL									
	<i>MRSA USA300</i>	<i>Acinetobacter baumannii AB5075</i>	<i>Salmonella typhi ATCC35664</i>	<i>Enterococcus faecalis ATCC19433</i>	<i>Enterobacter cloacae</i>	<i>Escherichia coli ATCC87</i>	<i>Pseudomonas aeruginosa PAO1</i>	<i>Klebsiella pneumoniae ATCC13883</i>	<i>Candida albicans</i>
<b>White pepper</b>	33.3 ± 11.5	40 ± 0	33.3 ± 11.5	26.6 ± 11.5	20 ± 0	-	16.4 ± 6.1	-	-
<b>White pepper (autoclaved)</b>	LA	LA	40 ± 0	LA	LA	-	40 ± 0	-	-
<b>Black pepper</b>	26.6 ± 11.5	40 ± 0	23.1 ± 15.5	16.4 ± 6.1	12.9 ± 6.1	-	12.9 ± 6.1	-	-
<b>Black pepper (autoclaved)</b>	LA	LA	LA	LA	LA	-	40 ± 0	-	-

(-) indicates that there was no antimicrobial activity detected under the tested conditions

(LA) Indicates the loss of activity after autoclaving.

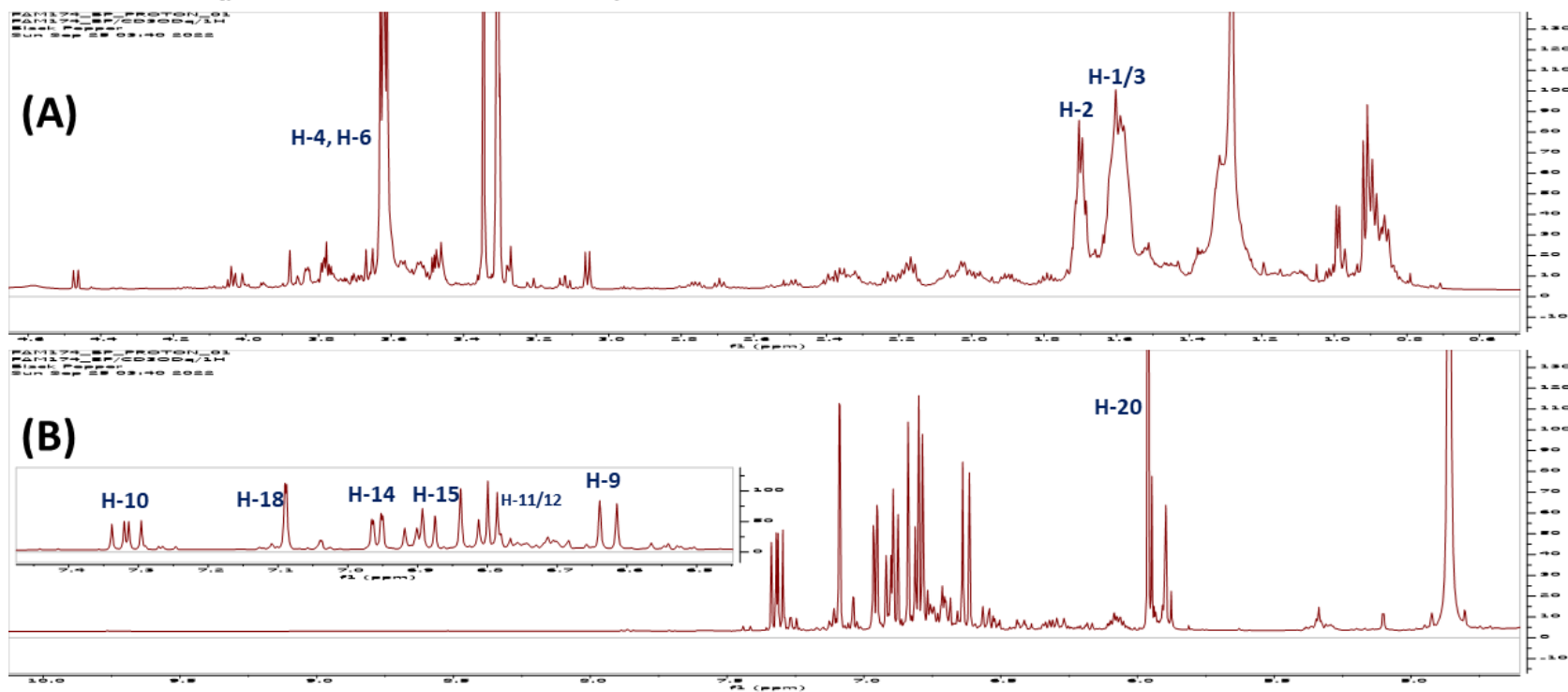
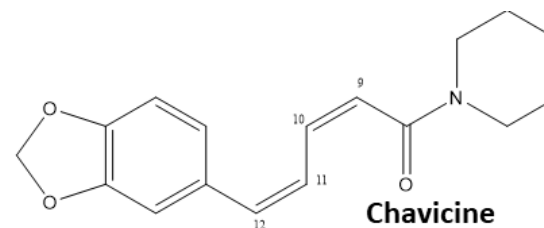
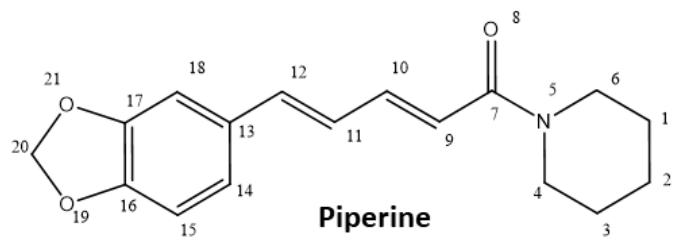
**Table S2: Minimum inhibitory concentration of pepper methanol extracts using microdilution method, n=3, Av±SD.**

Minimum inhibitory concentration (MIC) mg/mL						
	<i>MRSA USA300</i>	<i>Acinetobacter baumannii AB5075</i>	<i>Salmonella typhi ATCC35664</i>	<i>Enterococcus faecalis ATCC19433</i>	<i>Enterobacter cloacae</i>	<i>Pseudomonas aeruginosa PAO1</i>

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<b>White pepper extract</b>	6.6 ± 2.8	26.6 ± 11.5	33.3 ± 0	16.6 ± 5.7	11.6 ± 7.6	20 ± 0
<b>Black pepper extract</b>	2.9 ± 1.9	20.0	33.3 ± 0	13.3 ± 5.7	11.6 ± 7.6	16.6 ± 5.7

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**Figure S1.** Signal assignment of the  $^1\text{H}$ -NMR markers for piperine alkaloid

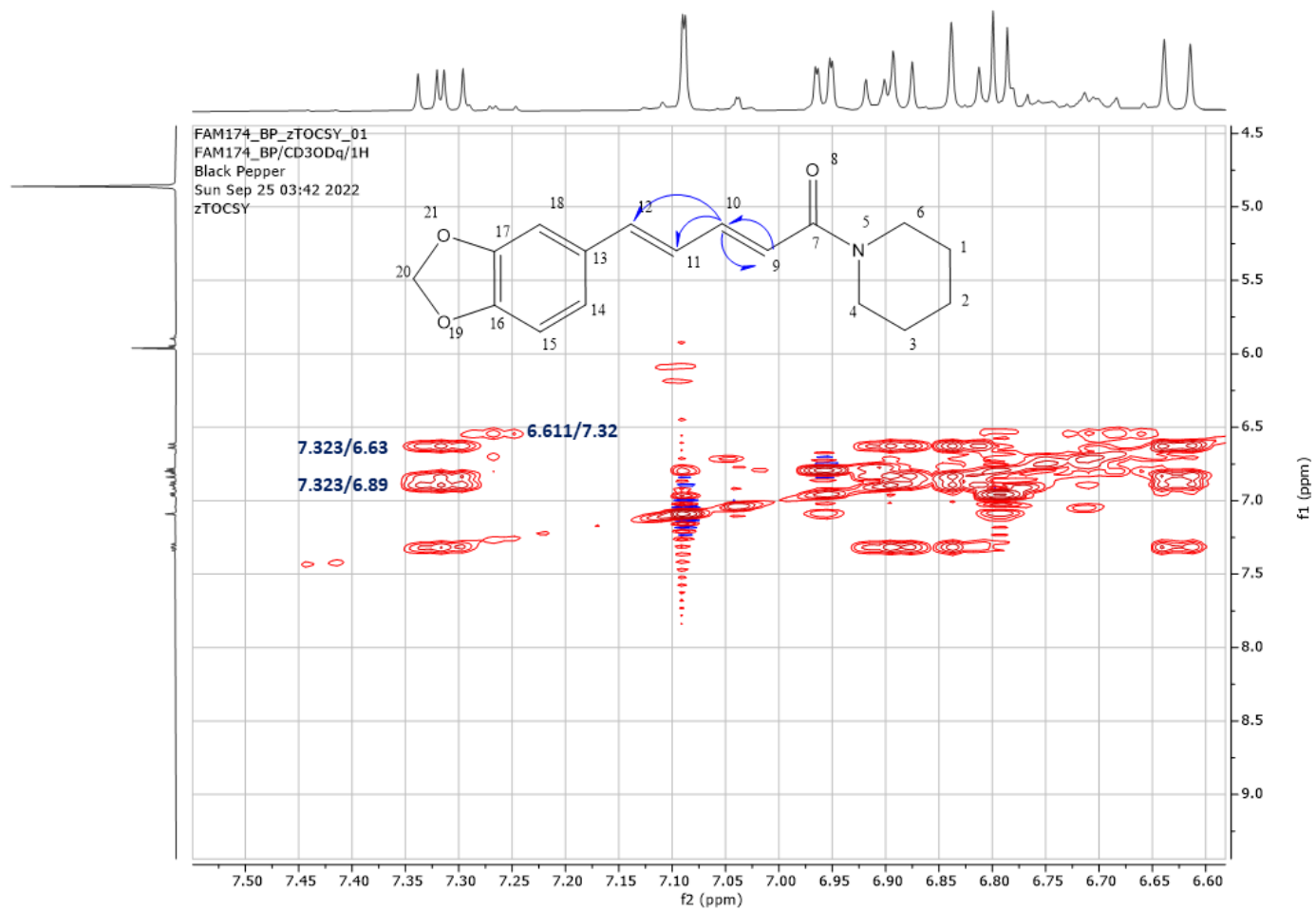
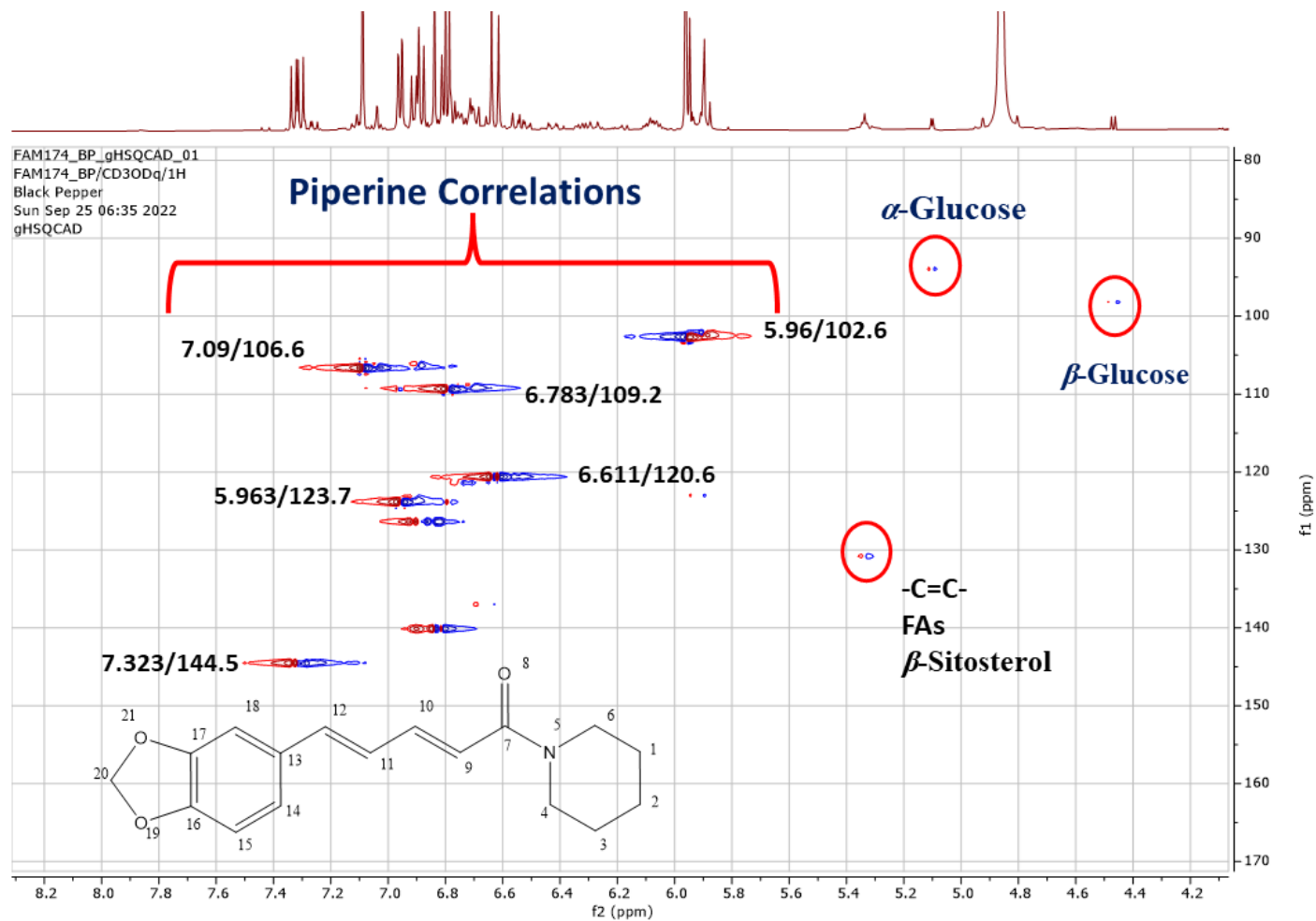


Figure S2.  $^1\text{H}$ - $^1\text{H}$  correlations observed in the TOCSY spectrum for piperine



**Figure S3.**  $^1\text{H}$ - $^{13}\text{C}$  correlations observed in the HSQC spectrum for piperine

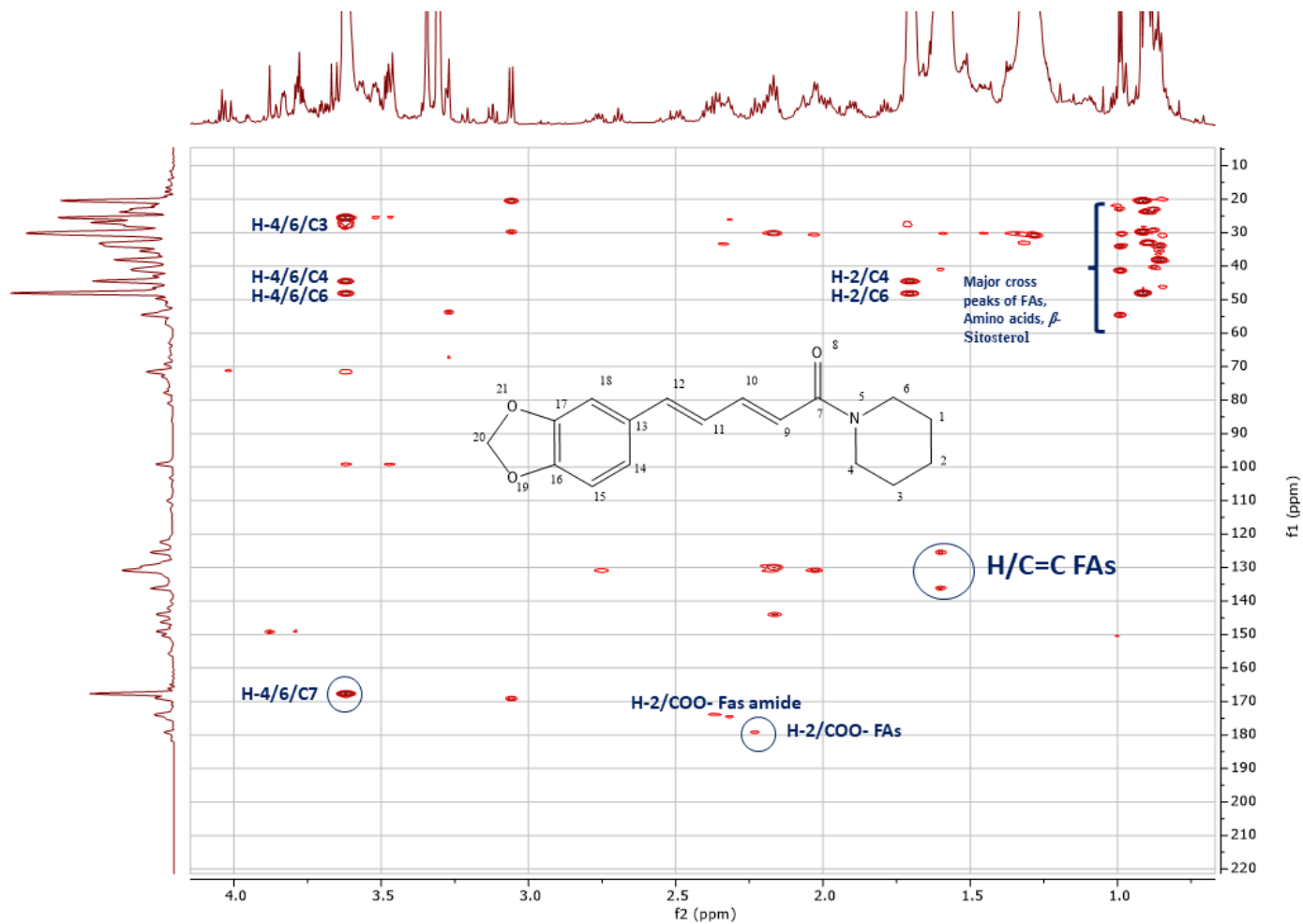


Figure S4.  $^1\text{H}$ - $^{13}\text{C}$  correlations observed in the HMBC spectrum identified metabolites

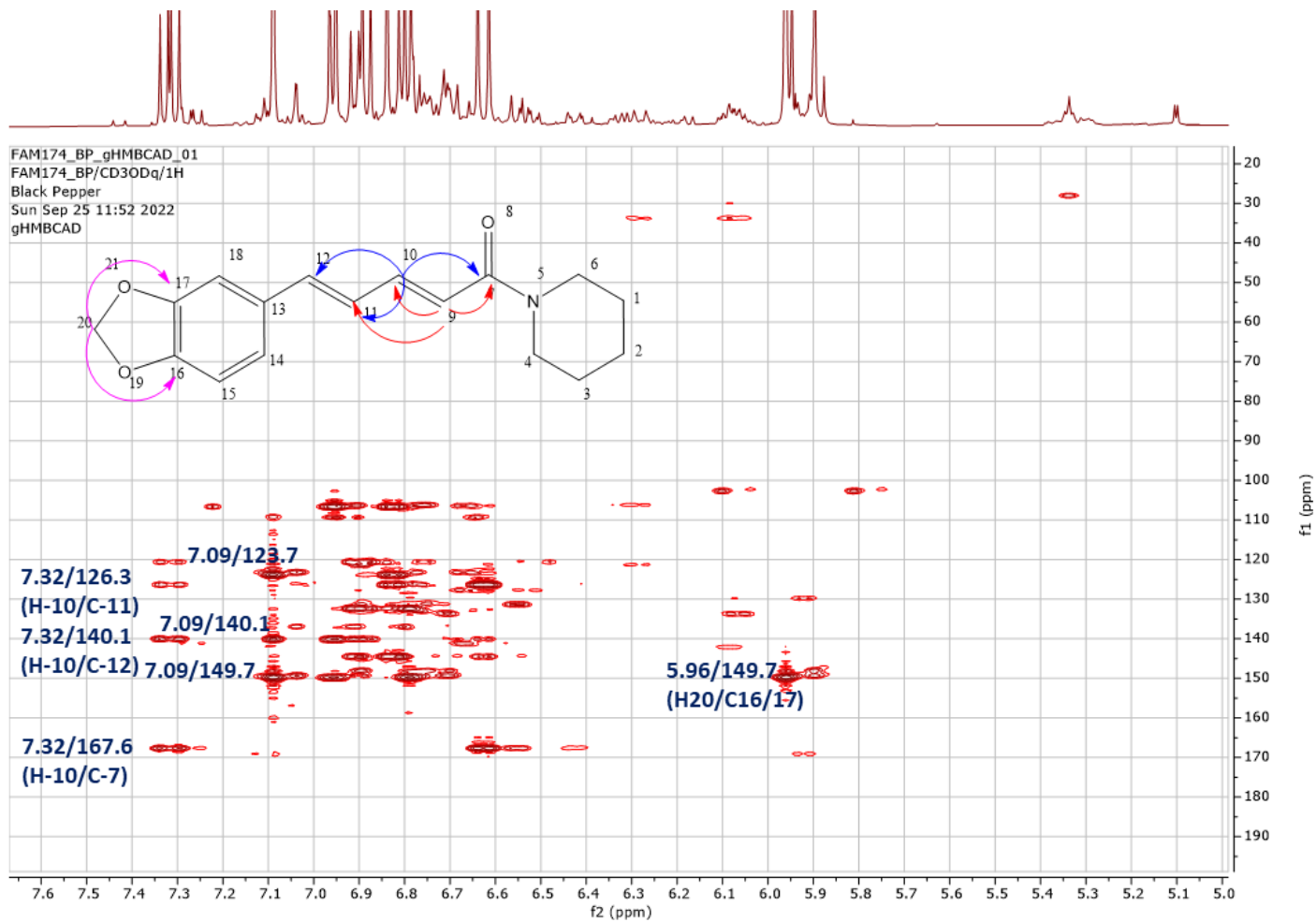
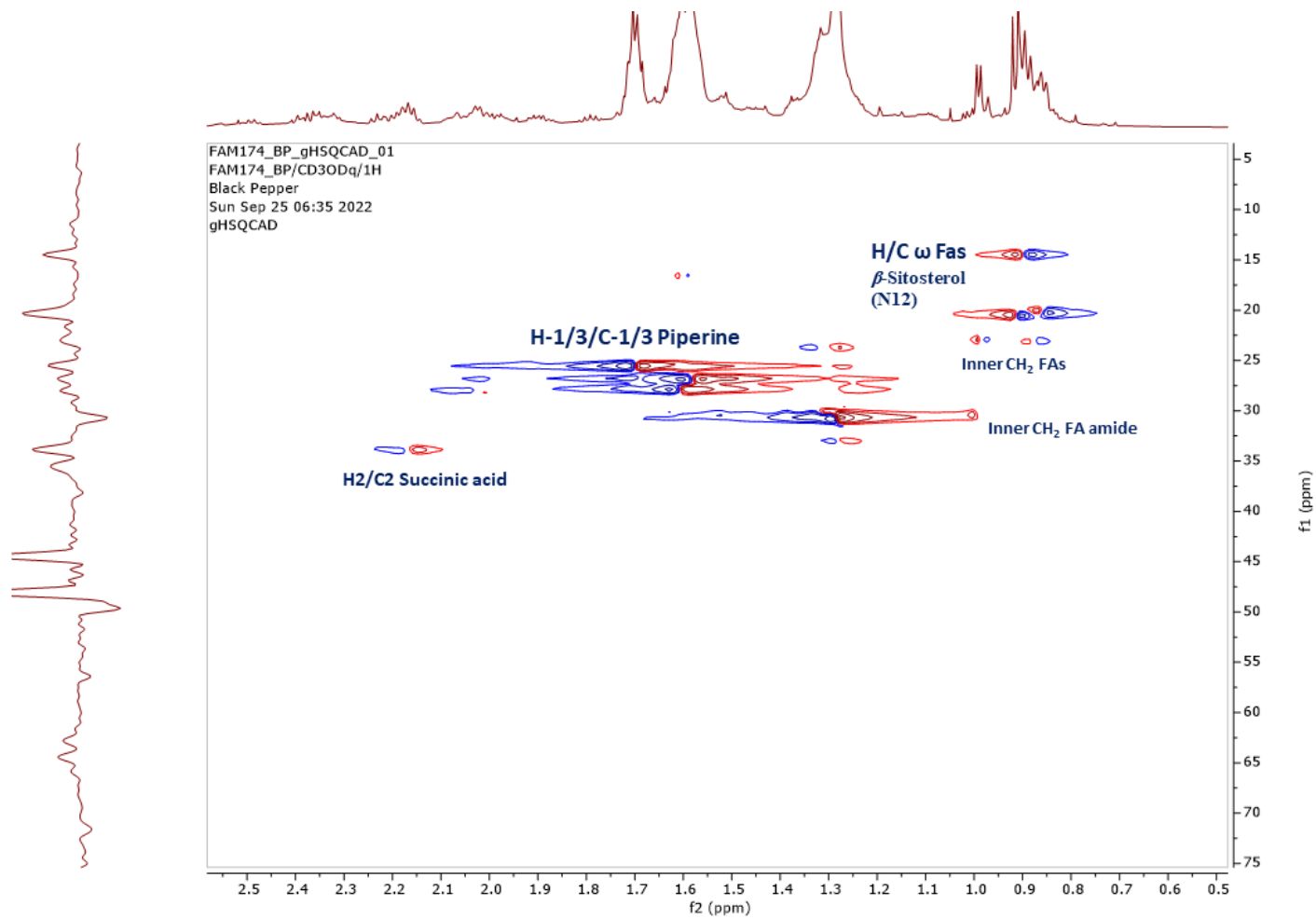
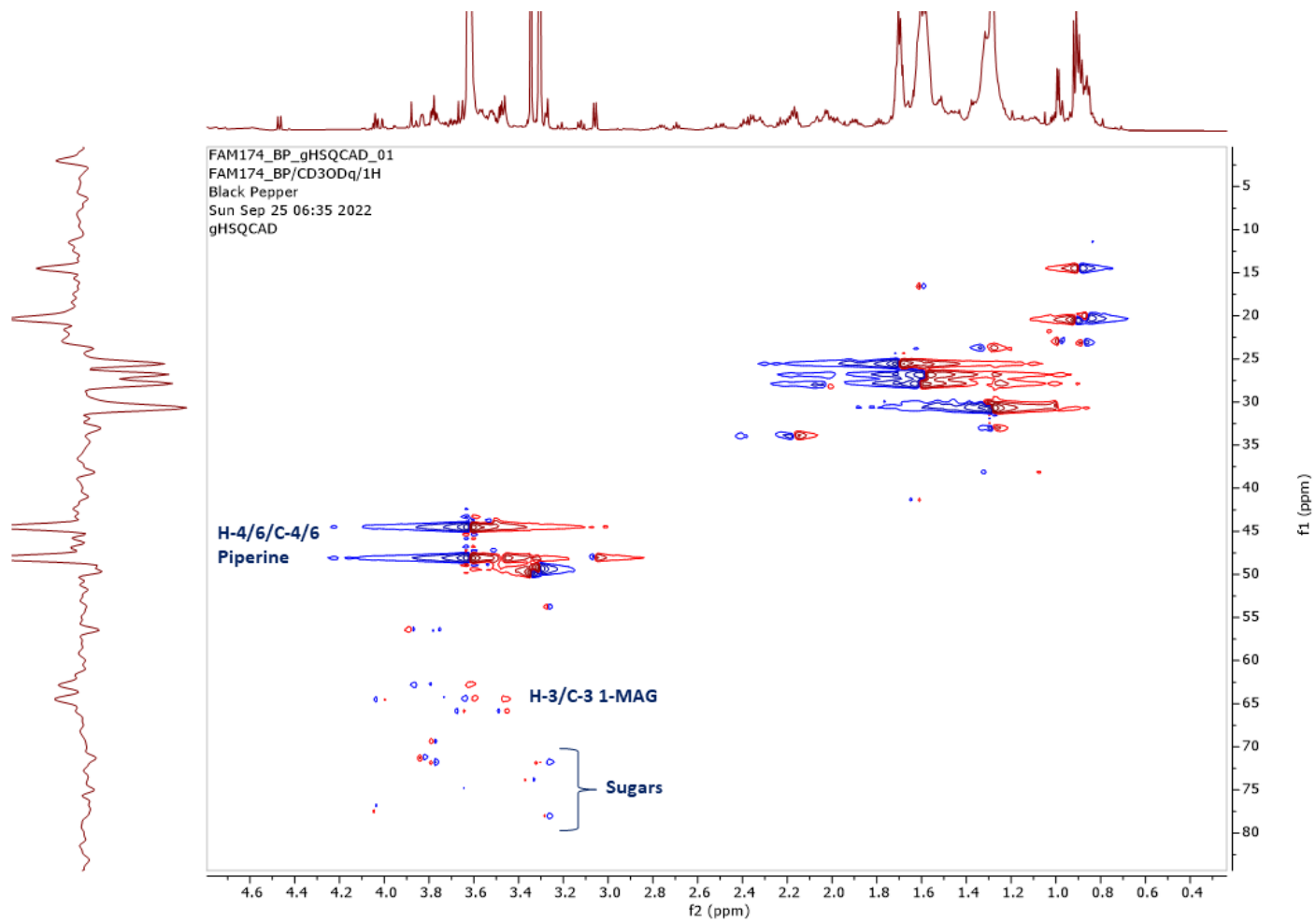


Figure S5.  $^1\text{H}$ - $^{13}\text{C}$  correlations observed in the HMBC spectrum for piperine

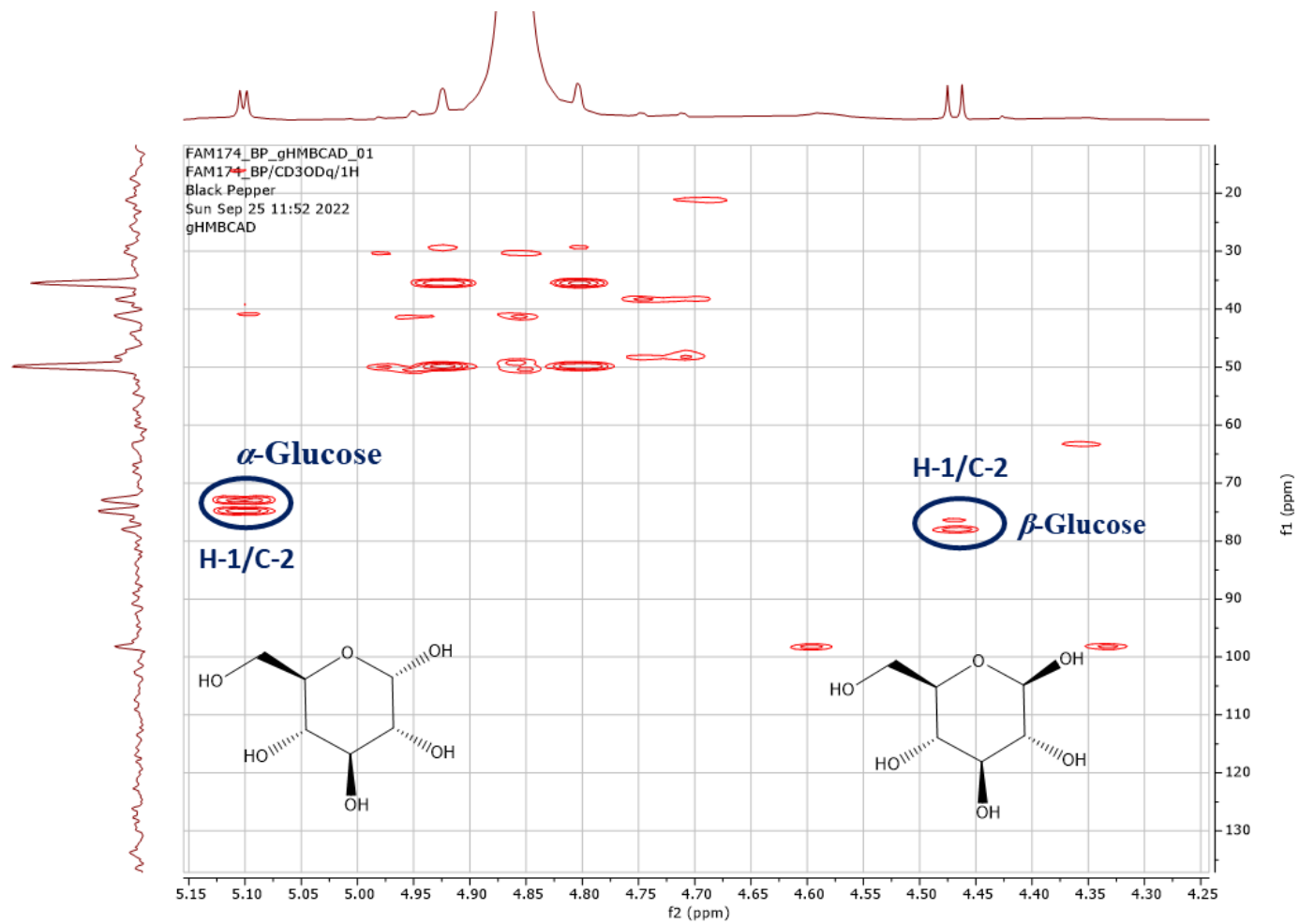




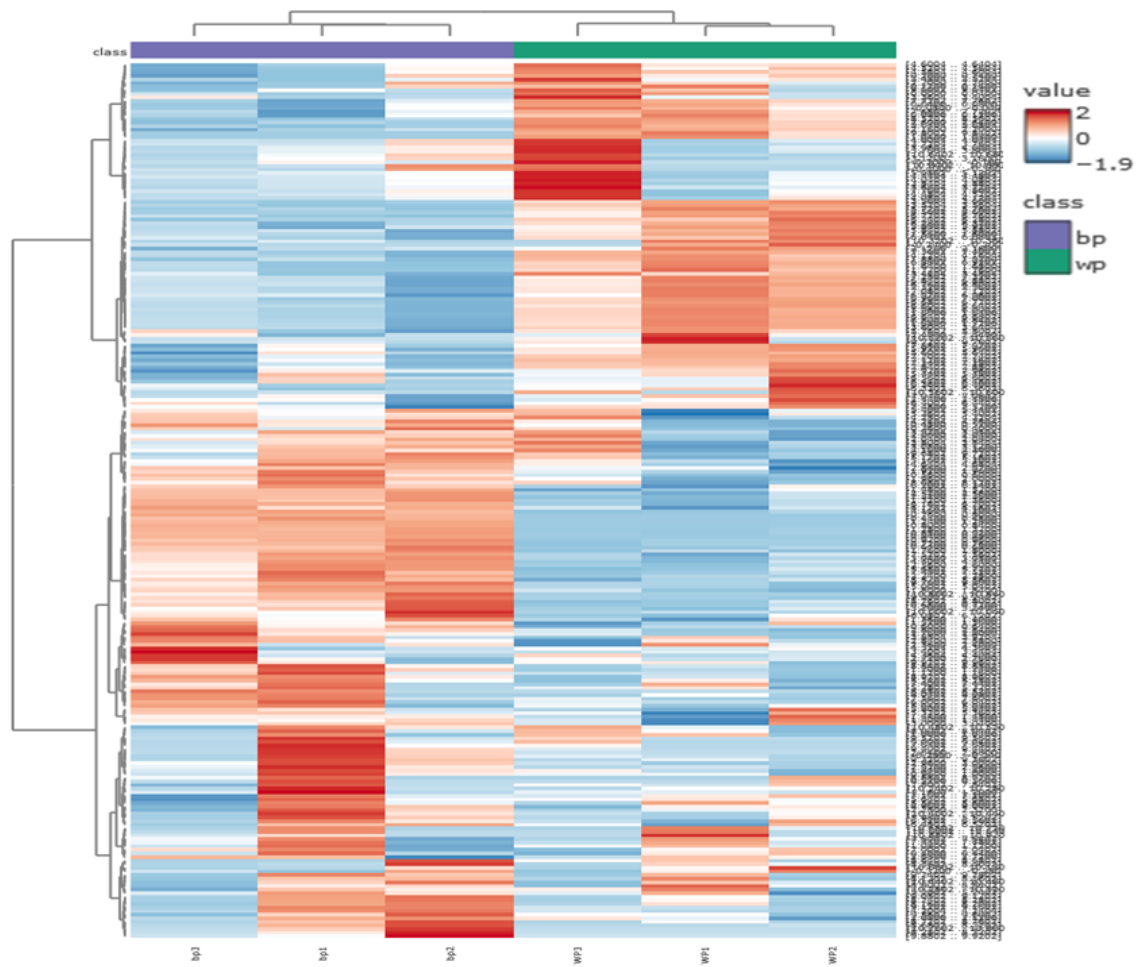
**Figure S6.** <sup>1</sup>H-<sup>13</sup>C correlations observed in the HSQC spectrum for major primary metabolites



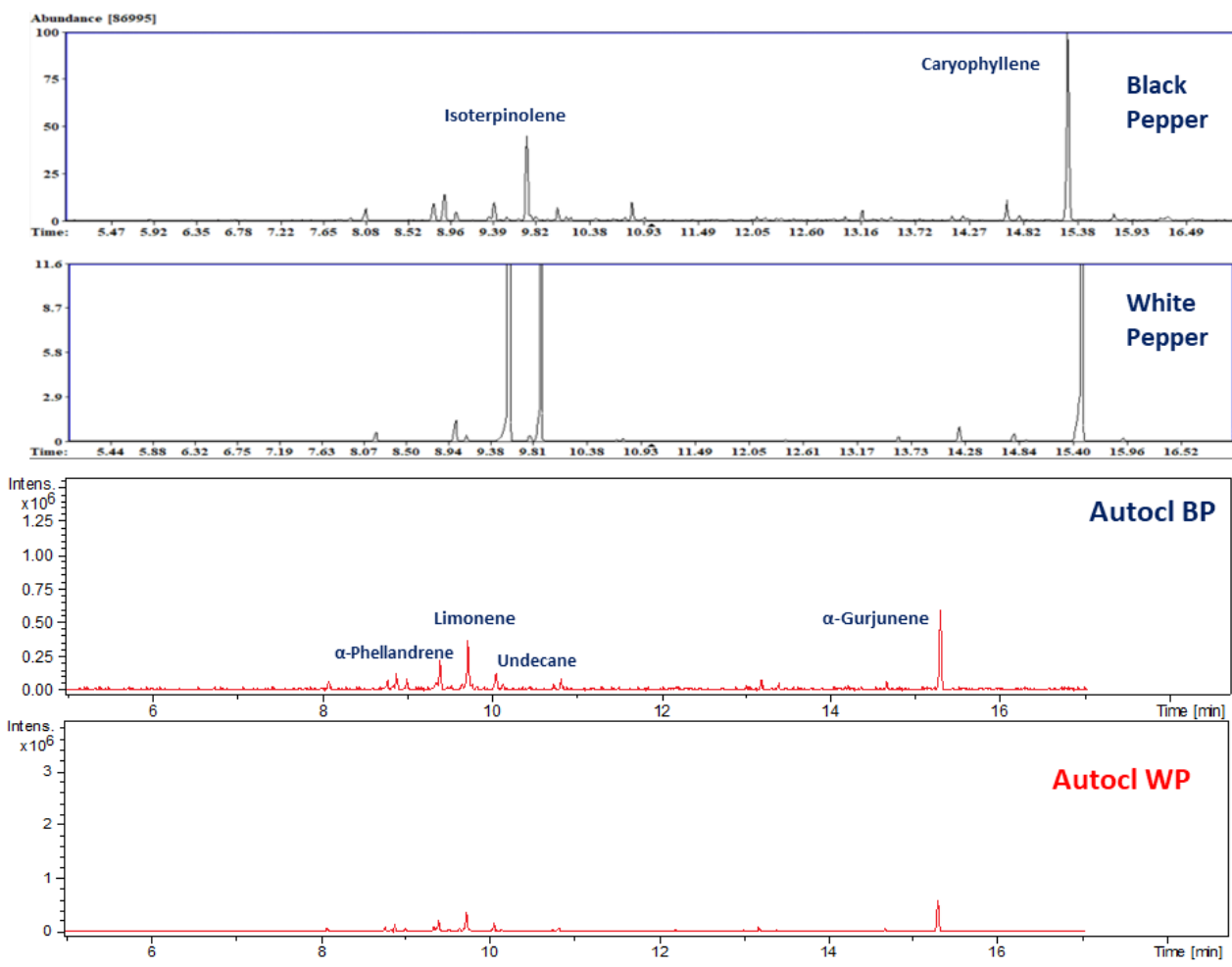
**Figure S7.** <sup>1</sup>H-<sup>13</sup>C correlations observed in the HSQC spectrum for major primary metabolites



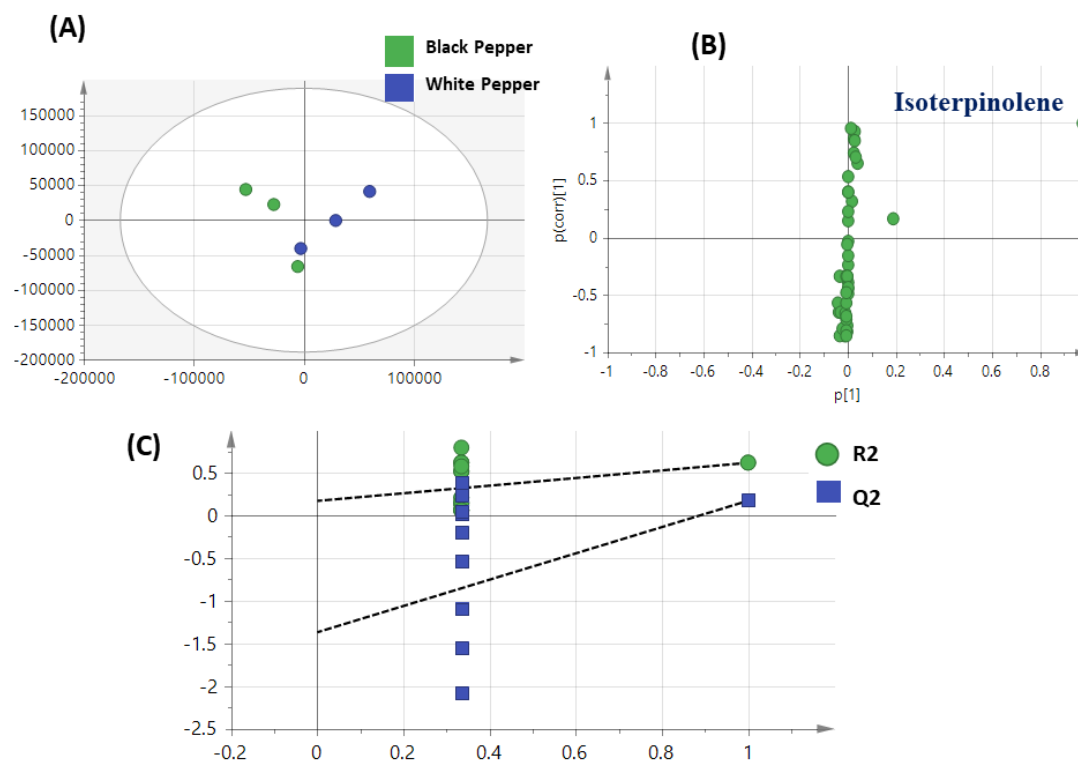
**Figure S8.**  $^1\text{H}$ - $^{13}\text{C}$  correlations observed in the HMBC spectrum for major primary metabolites



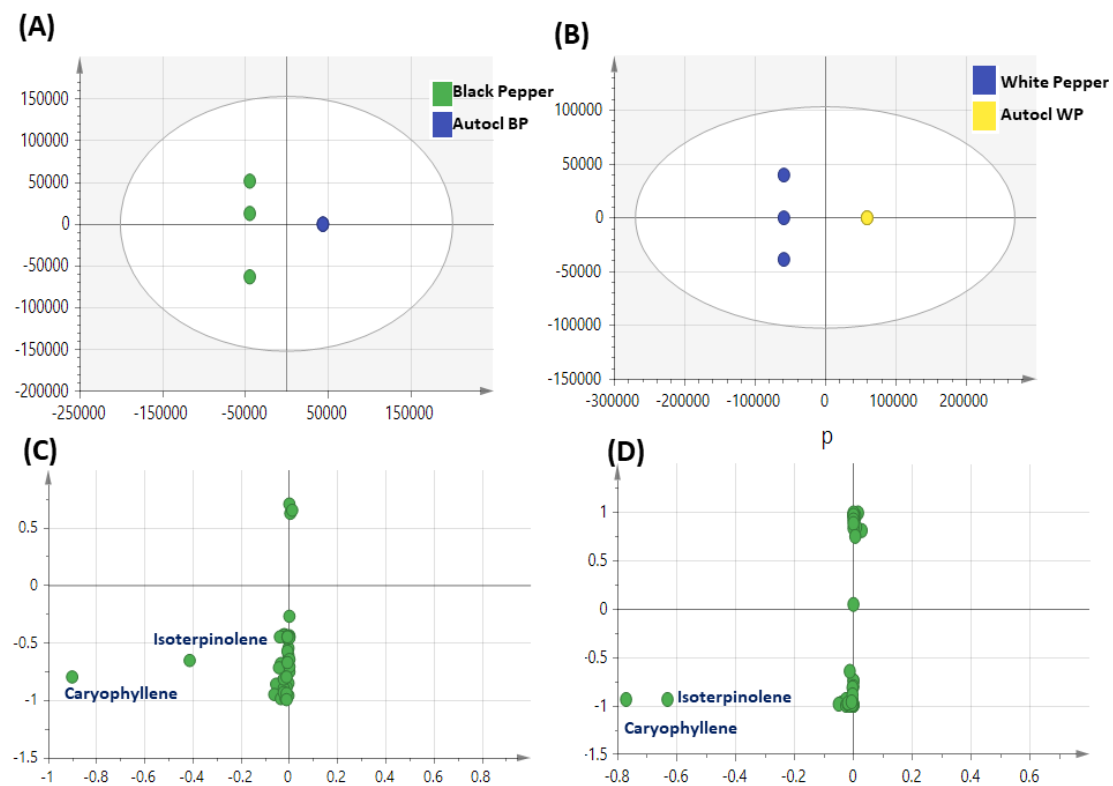
**Figure S9.** A heat map for identified metabolites in black and white pepper as extracted from NMR Table 1



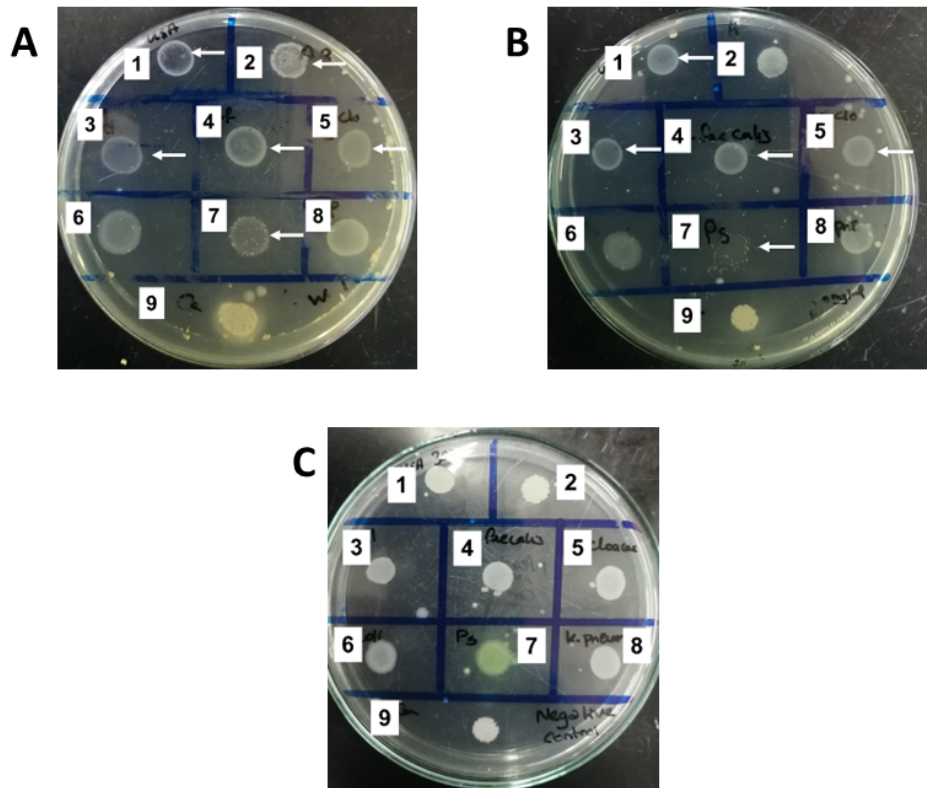
**Figure S10.** Representative GC–MS chromatograms of volatile constituents of black and white pepper.



**Figure S11.** A) OPLS-DA score plot. B) Loading S-plots derived from modeling black pepper against white pepper as analyzed using SPME GC-MS showing the covariance  $p(1)$  against the correlation  $p(\text{cor})(1)$  of the variables of the discriminating component of the OPLS-DA model. C) Permutation plot (n=20).



**Figure S12.** GC-MS-based OPLS-DA score plot derived from modeling volatile metabolites in raw and autoclaved black pepper (A), and raw and autoclaved white pepper (B) alongside their respective loading plots (C & D) Designated variables are highlighted and identifications are discussed in the text.



**Figure S13: Inverted petri dishes showing the vapor-phase MIC experiment for (A) ground white pepper, (B) ground Black pepper, (C) growth control.**

The cultures were spotted on MHA and numbered as follow: 1- MRSA *Staphylococcus aureus* USA 300, 2- *Acinetobacter baumannii* AB5075, 3- *Salmonella typhi* ATCC35664, 4- *Enterococcus faecalis* ATCC19433, 5- *Enterobacter cloacae*, 6- *Escherichia coli* ATCC87, 7- *Pseudomonas aeruginosa* PAO1, 8- *Klebsiella pneumoniae* ATCC13883, 9- *Candida albicans*. The cultured agar was upward while the ground pepper was placed downwards. The white arrows on the spots in (A) and (B) indicate a positive growth suppression. VP-MIC was determined as the least concentration of the tested sample that resulted in apparent growth inhibition of the tested microorganism when compared to the control.