Electronic Supplementary Materials

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Hydroxychavicol, a polyphenol from *Piper betle* leaf extract, induces cell cycle arrest and apoptosis in *TP53*-resistant HT-29 colon cancer cells

Aiysvariyah RAJEDADRAM[1], Kar Yong PIN[2], Sui Kiong LING[2], See Wan YAN[1], Mee Lee LOOI[3]✉

Table S1 The assignments of HC for the spectrum of ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$). The tabulated ^{1}H and ^{13}C NMR signals matched with the literature values (Kumar et al., 2015)

Position	Chemical shift value obtained		Chemical shift value from literature (Kumar, et al., 2015)	
	^{1}H	^{13}C	^{1}H	^{13}C
1	6.70 (*s*, 1H)	115.45	6.64 (*s*, H-1)	115.52
2		143.61		143.41
3		141.83		141.62
4	6.78 (*d*, *J*=8.02 Hz, 1H)	115.79	6.72 (*d*, *J* = 8.0 Hz, H-4)	115.85
5	6.62 (*d*, *J*=8.02 Hz, 1H)	115.63	6.55 (*dd*, *J* = 8.0, 1.6 Hz, H-5)	115.66
6		121.06		121.14
7	3.26 (*d*, *J*=5.73 Hz, 2H)	39.57	3.19 (*d*, *J*= 6.7 Hz, H-7)	39.51
8	5.92 (*m*, 1H)	133.29	5.03-4.91 (*m*, H-8)	133.37
9	5.04 (*d*, *J*=10.31 Hz, 2H)	137.74	5.84 (*td*, *J* =16.9, 6.7 Hz, H-9)	137.66

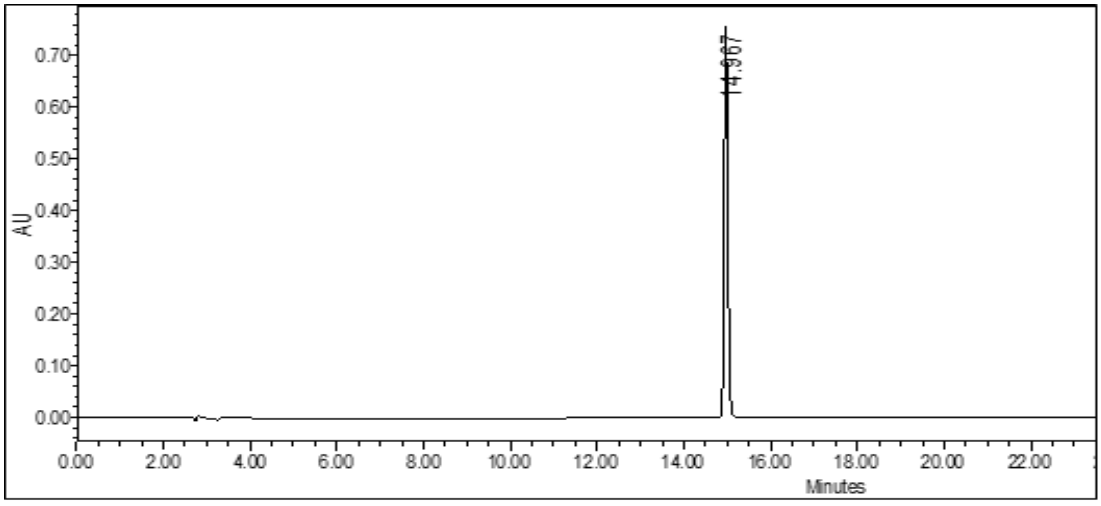


Fig. S1 HPLC chromatogram of isolated HC. HPLC chromatogram was acquired using Phenomenex Luna C18 100A (250 mm × 4.6 mm, 5 µm particle size) with a gradient system of 0.1 % formic acid (AR grade) in Milli-Q water and 100 % acetonitrile (AR grade) as mobile phase. The retention time of HC was acquired at ~ 15 minutes.

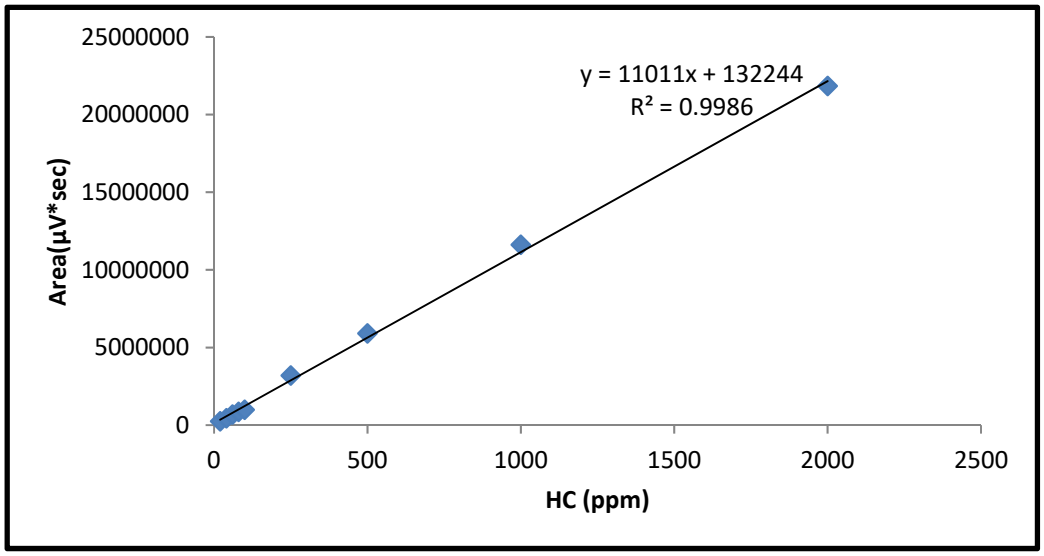
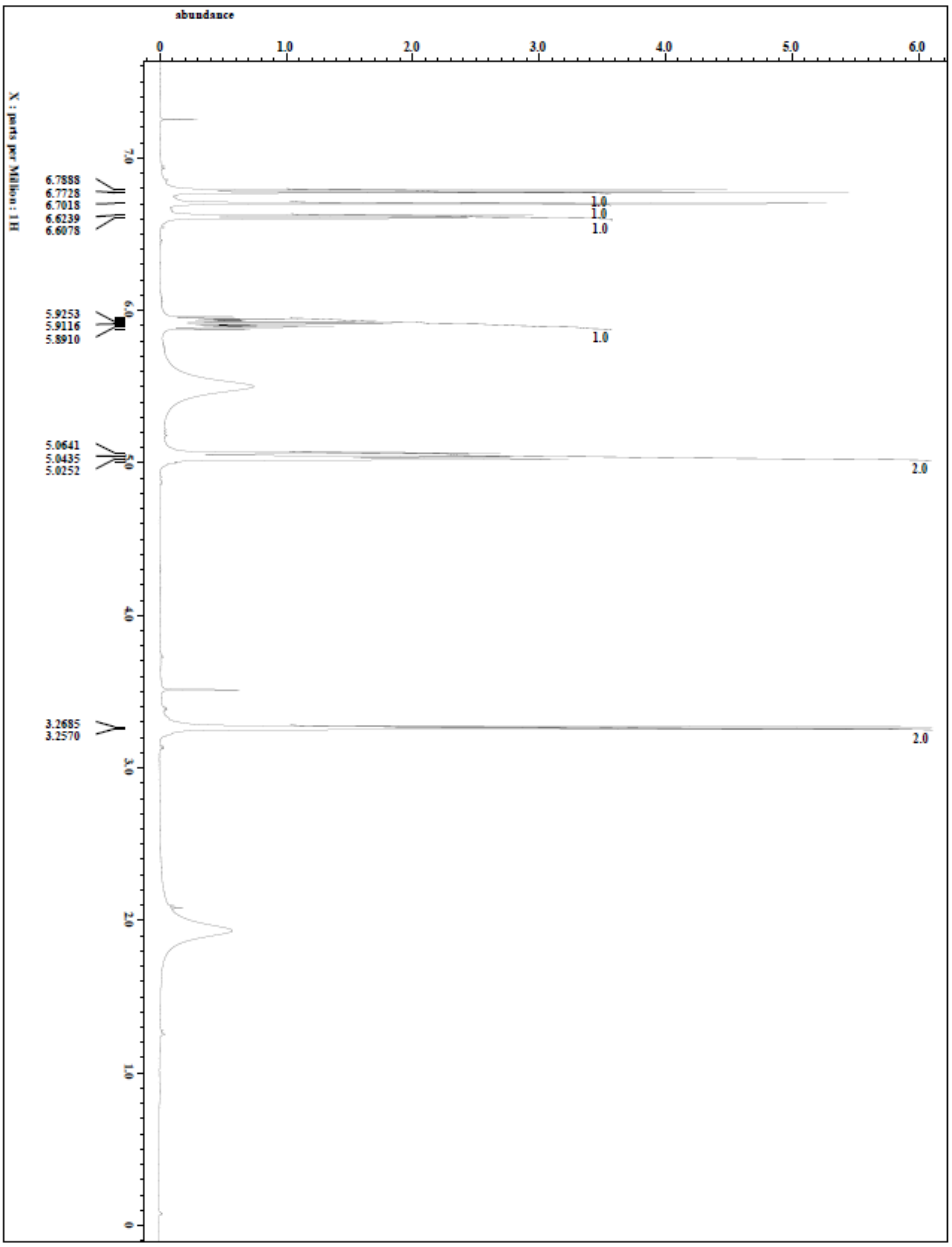


Fig. S2 HC standard curve for the concentration calculation.



Supplementary Figure 3A

Supplementary Figure 3B

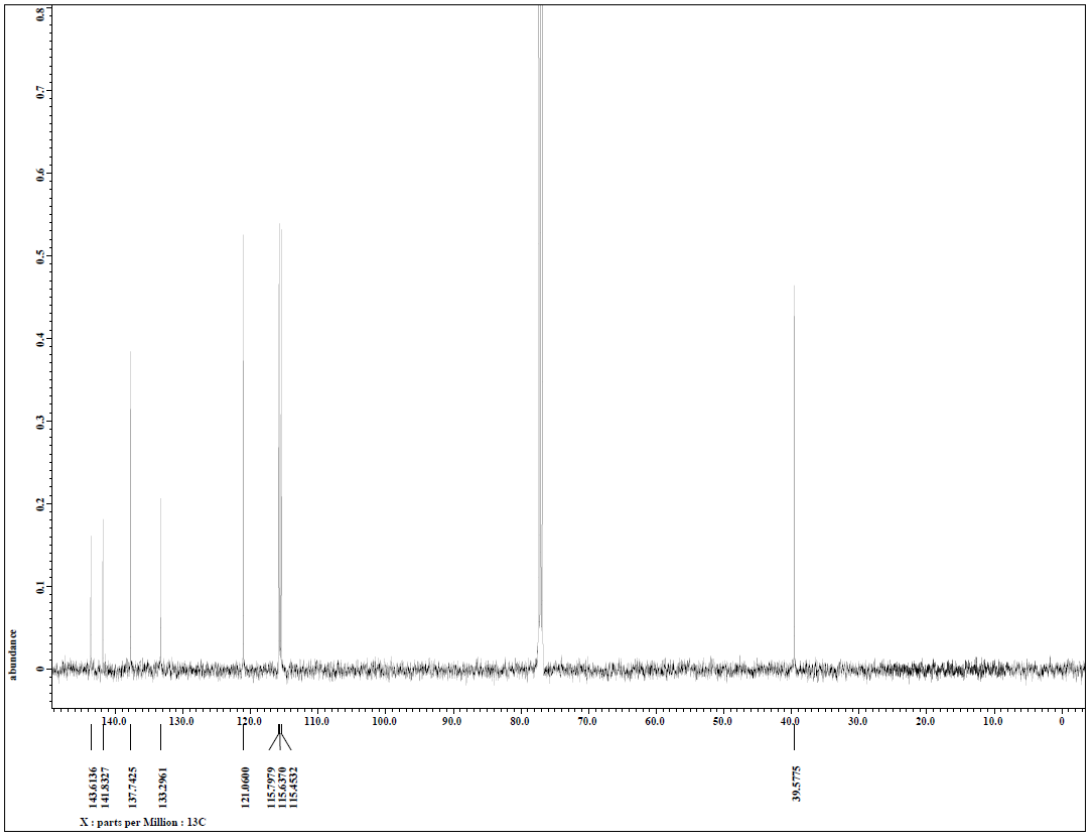


Fig. S3 NMR spectrum of isolated HC. NMR spectra of ^{1}H (A) and ^{13}C (B) recorded in $CDC1_3$ (deuterated chloroform) on a Varian FT-500 MHz instrument confirmed the structure of isolated HC. The ^{1}H NMR spectrum showed one singlet at δ 6.70 and two doublets signal δ_H 6.78 (J = 8.02) and δ_H 6.62 (J = 8.02) in the aromatic ring at position C-1, C-4 and C-5, respectively. There is an allyl group attached to C-6 of the aromatic ring. The ^{1}H NMR spectrum displayed a doublet signal δ_H 6.78 (J = 5.73) attached at C-7 which is the methylene bridge of the allyl group. One multiplet signal at δ 5.94 and one doublet signal at δ_H 5.06 (J = 10.31) was observed on ^{1}H NMR spectrum which corresponds to the vinyl group at postion C-8 and C-9. The ^{1}H and ^{13}C NMR signals is tabulated in Table S1.

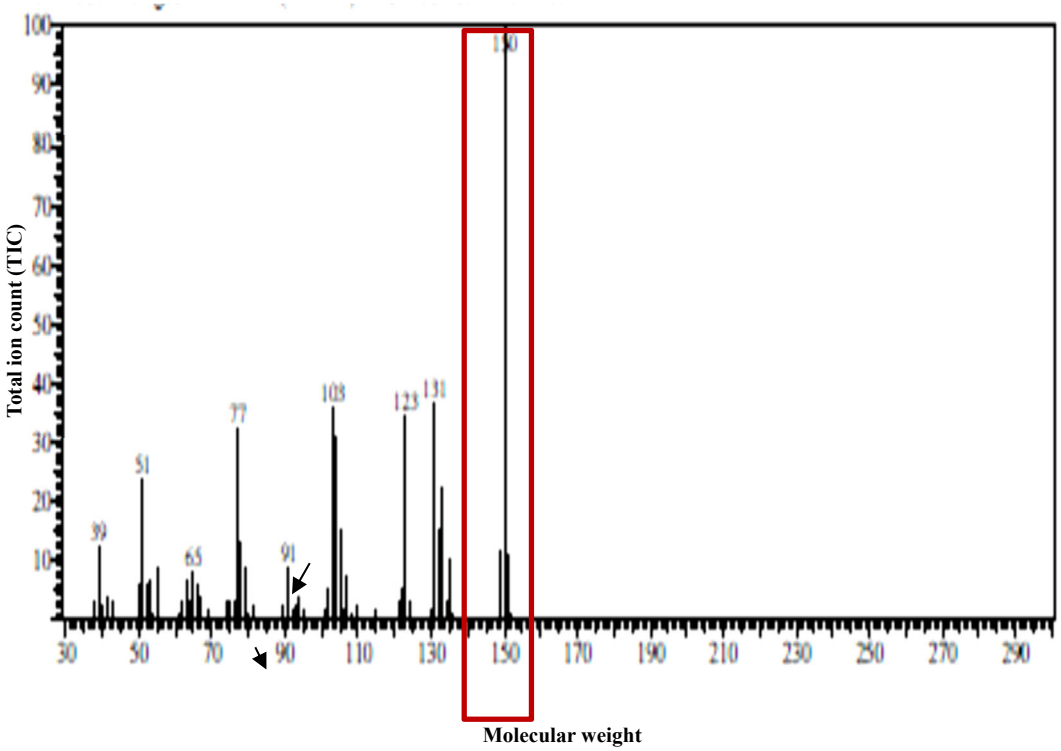


Fig. S4 Mass spectrum of isolated HC. GCMS chromatogram analysis recorded the molecular weight of isolated HC as *m/z* 150.2 which matched with the literature molecular weight of HC (Lin et al., 2013) and corresponds with molecular formula of HC; $C_9H_{10}O_2$.

References

Kumar S, Pathania AS, Satti NK, et al., 2015. Synthetic modification of hydroxychavicol by mannich reaction and alkyne-azide cycloaddition derivatives depicting cytotoxic potential. *Eur J Med Chem*, 92:236-245. https://doi.org/10.1016/j.ejmech.2014.12.047

Lin CF, Hwang TL, Chien CC, et al., 2013. A new hydroxychavicol dimer from the roots of piper betle. *Molecules*, 18(3):2563-2570. https://doi.org/10.3390/molecules18032563