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STUDY OF ANTI-PROLIFERATIVE ACITVITY OF NATURALLY OCCURRING ALLYL PHENOLS

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ABSTRACT

Various types of allyl phenols are biosynthesised by medicinal plants, herbs, shrubs, and micro-organism abundance in nature. Among them, some phenols are very important and useful in different aspects such as flavours and fragrances, condiment, folklore medicines to cure different kinds of diseases, used as botanical supplement for health care and health promoting purposes. In this regard, few medicinal plants, herbs, shrubs and spices are selected for chemical profiling to investigate major allyl phenols present in them and to study of their anti-proliferative activity against different cancer cell lines using SRB assay.

KEYWORD: Allyl phenols; isolation; structural characterization; cell cytotoxicity study; SRB assay.

INTRODUCTION

Plenty of medicinal plants, herbs and shrubs are available in nature those produce very selective allyl phenols. These allyl phenols are useful in food and confectionary industries due to their pleasant aroma and flavour. Among them, few most useful and important medicinal plants, herbs, shrubs and spices were chosen for analysis of their major chemical constituents. Majority of these selected plants biosynthesised very specific allyl phenols as major constituents. These allylic phenols are used for different purposes due to their specific biological activities or medicinal properties. Of course, these allyl phenols are biosynthesised by medicinal plants, herbs and shrubs for their own survival, but human being has utilized these phenols in different aspects for their own requirements and beneficial purposes from very ancient time. These phenols are used as flavours, fragrances, as condiment in cooking, as folklore medicines to cure different kinds of ailments, used as botanical supplement for health care and health promoting agents. In this connection, few medicinal plants, herbs and spices are frequently used by us and selected for phytochemical investigation to find the major allyl phenols present into them. In this regard, these plant materials were extracted with suitable solvents to obtain crude extracts. The major allyl phenols present in these crude extracts were isolated by column chromatography over silica gel with gradient solvent elution. The final purification of these respective allyl phenols was carried out by preparative thin layer chromatography (PTLC). The structural characterization of these allyl phenols was determined by spectroscopic and spectrometric methods. To evaluate anti-proliferative activity of these allyl phenols, they were tested for cell cytotoxicity against different cancer cell lines using SRB assay in comparison with therapeutically used authentic drug paclitaxel as control.

RESULTS AND DISCUSSION

The plenty of medicinal plants, herbs and shrubs are available in nature those produce selectively different kinds of allyl phenols without changing the basic skeletons of the main molecules. Among them, few plants, herbs and shrubs are chosen for their chemical profiling. Majority of the selected plants biosynthesised very specific allyl phenols for their own survival. These allylic phenols possessed very specific biological activity, selective medicinal properties and significant food values. These allyl phenols are produced by medicinal plants, herbs and shrubs for their own requirements, but human being has utilized these phenols for their own beneficial purposes. Most of them are highly fragrant and pungent substances. Some of the allyl phenols are used in perfuming agents in soap, toothpaste, mouthwash, skin creams and as aroma in incense, as flavours, condiment, fragrances in cuisines and confectionary. Besides these, crude products are used as reliable source of botanical supplement to promote health care in modern society. In this regard, chemical profiling of few plants, herbs and spices are targeted for segregation of the major active allyl phenols. The structural characterization of major allyl phenols isolated from various natural resources was carried out with help of spectroscopic and spectrometric study and their chemical structures were depicted below in figure 1.

Naturally occurring allyl phenols-isolated from resources

(i). R_1 = H, R_2 = OH & R_3 = OCH_3; Chevibetol (**1**)
(ii). R_1 = H & R_2 = R_3 = OH; Allyl pyrocatecol/APC (**2**)
(iii). R_1 = R_3 = H & R_2 = OCH_3; Anethol (**3**)
(iv). R_1 = R_3 = H & R_2 = OCH_3; Estragol (**4**)
(v). R_1 = H, R_2 = OCH_3 & R_3 = OH; Eugenol (**5**)
(vi). R_1 = H, R_2 = OH & R_3 = OCH_3; Eugenol acetate (**6**)
(vii). R_1 = R_2 = R_3 = OCH_3; Elemisin (**7**)
(viii). R_1, R_2 = -OCH_2O- & R_3 = H; Safrol (**8**)
Source of **1-2** / **3-4** / **5-6** / **7** / **8**: Leaves of
P. bettle / Star anise / Clove / Vach / Nutmeg.

Derivatives of naturally occurring allyl phenols-Chemically transformed/semi-synthetic products of them

ix). R_1 = H, R_2 = OH & R_3 = OCH_3; 2H-chevibetol (**9**)
(x). R_1 = H & R_2 = OH; 2H-allyl pyrocatecol (**10**)
(xi). R_1 = R_3 = H & R_2 = OCH_3; 2H-anethole (**11**)
(xii). R_1 = R_3 = H & R_2 = OCH_3; 2H-estragole (**12**)
(xiii). R_1 = H, R_2 = OCH_3 & R_3 = OH; 2H-eugenol (**13**)
(xiv). R_1 = H, R_2 = OH & R_3 = OCH_3; 2H-eug-acetate (**14**)
(xv). R_1 = R_2 = R_3 = OCH_3; 2H-Elemisin phenol (**15**)
Products **9-17** are hydrogenated and methylated products of respective isolated allyl phenols

Figure 1: Chemical structure of allyl phenols isolated from various natural resource.

This manuscript deals with the isolation of the major allyl phenols from different natural resources, their structural determination by spectroscopic methods and study of inhibitory activity of cell proliferation of respective allyl phenols on five different types of human cancer cell lines including urinary bladder (HT-1376), pancreatic (HPAC), prostate (DU-145), colon (HT-29) and thyroid (MDA-T32) using SRB assay in comparison with paclitaxel as control.

EXPERIMENTAL SECTION

General experimental procedures

Melting points were determined using a Buchi melting point apparatus (Model number M560). UV spectra were measured on Shimadzu UV-2100 UV-Vis spectrophotometer. NMR spectra were recorded in $CDCl_3$ (manufactured by Euro-Isotope, UK; CAS number 2206-27-1) on a Bruker Avance 200 and Varian 500 and 600 NMR spectrometer using residual $CHCl_3$/HDO as an internal standard. Chemical shifts are given in ppm (δ_H & δ_C), relative to residual $CHCl_3$ at 7.26 and 77.00 ppm. UHPLC/MS analysis of the samples was performed using a Vanquish UHPLC system coupled to a Q-Exactive quadrupole orbitrap mass spectrometer (Thermo Fisher Scientific, USA) at the Ohio State University, College of Pharmacy, Instrumentation Facility. Silica gel 60 (230-400 mesh, Merck) was used for analytical TLC. Silica gel 60 (70-230 mesh, Merck) was used for column chromatography. All compounds were visualized by TLC using vanillin-perchloric acid-EtOH followed by heating at 110° C for 5 min, DNP in EtOH and neutral $FeCl_3$ in MeOH.

BIO-ACTIVE ALLYL PHENOLS ISOLATED FROM DIFFERENT PLANT SOURCES

1. Leaf of Pan (*P. beetle* leaves)

The genus *piper* mostly tropical herbs, but some are shrub; herbs are creepers or climbers belongs to *Piperacae* family. It presented about nine genera and about 1400 species.[1,2] Some of them are economically important and widely cultivated all over the world. It includes several medicinal properties such as anti-fungal,[3] nematocidal,[3] anti-ulcer,[4-6] anti-leishmial,[7] anti-filarial,[8] anti-tumour,[9] immunomodulatory,[10,11] anti-oxidant,[11-13] anti-microbial,[12] anti-inflammatory[12], anti-fertility,[14] anti-bacterial,[12] disinfectant,[12] digestive,[13] antacid,[13] radio-protective,[15] anti-pyretic,[16] anti-platelet,[17] chemo preventive agent[18] etc. The betel leaves also act as breath-freshener against halitosis[19] and use as folklore medicine to cure different kinds of ailments from very ancient time has been described in Susruta Samhita a medico-scientific treatise on Indian traditional system of medicine, Ayurveda.[1] The ceremonial soporific daily beverage prepared from *Piper methysticum* Forst forms a part of social, economic and religious life of Polynesian and people in the Pacific islands.[20,21] Like *P. methysticum*, the leaf of *P. beetle* is well-known social habit-forming masticatory of Eastern World chewed in the form of morsels.[20,21] In order to find out major active ingredients, detailed phytochenical investigation was conducted. The good quality tendered leaf of *Piper beetle* (200 pieces) was purchased from local market Dadar, Mumbai, India on June 2021. These fresh tendered leaves of *Piper beetle* (500 gm) were dried on shade at 30 ºC for one week. The weight of dry leaves is around 64.0 gram. These dry leaves were used for extraction and isolation of ally phenols present into it.

2. Star anise (Dry fruit of *Illicium verum*)

It is a medium size evergreen tree native to North-East Vietnam and South China belonging to *Schisandraceae* family.[22] The main purpose to cultivate this tree in Vietnam and China is to fulfil the requirement of shikimic acid which is use as synthons for preparation of drug Tamiflu, a remedial agent for all kinds of influenzas.[23] The abundance of shikimic acid is 2 gm/100 gm of dry fruit. The botanical name of the tree is

Illicium verum. Its fruit are look like star/wheel. In local dialect in India, its dry fruit is known as chakra-phool and it called as star anise in English. Star anise oil is a highly fragrant oil used in cooking, perfumery, soap, toothpaste, mouthwash, skin creams and in aromatherapy.[24,25] In order to chemical analysis, the good quality dry star anise (100 gm) was procured from spice market Mumbai, India on year 2020. Fresh dry star anise (100 gm) was procured from spice market Mumbai, India in year 2020 for analysis of its chemical constituents. These fruits were powdered and used for extraction to separate allyl phenols.

3. Clove (Dry bud of *Syzygium aromaticum*)

It is an evergreen tree belongs to *Myrtaceae* family. The botanical name of the tree is *Syzygium aromaticum*.[26] The dry flower buds of *Syzygium aromaticum* tree is called clove. Clove is iconic condiment in the cuisine Asian, African, Mediterranean and Middle East Countries. Cloves are used in traditional medicine as an essential oil in aromatherapy[27] and acts as an anodyne for dental emergencies.[28] The chemical constituent responsible for medicinal performance is eugenol. The good quality dry cloves (100 gm) was procured from a hill station Mede Kari located in Corgi district in Karnataka State of India.

Very good quality of dry cloves (100 gm) was procured from a hill station Mede Kari located in Corgi district in Karnataka State of India for its chemical profiling. In local language, it is known as ′lavanga‵ or ′long‵. Macerated cloves were used for solvent extraction to yield crude extract. This crude extract was used for isolation of major allyl phenols present into it.

4. Vach (Rhizome of *Acorus calamus*)

Vach is tall perennial wetland and monocot scented leaves and rhizomes. It belongs to *Aceraceae* family which possesses medicinal properties and indigenous medicinal plant in India.[29] It is also available across the Europe, Russia, Northern Asia minor, Siberia, China, Japan Thailand, Myanmar, Australia etc. In ethnopharmacology, fresh quality tender of vach is used to improve, to enhance the memory power, increase the intellectuality of human being.[30] It is also use as folklore botanical medicine to prevent Alzheimer disease.[31]

The good quality rhizome of vach (100 gm) was procured from Jagdalpur forest located in Chhattisgarh, India on June 2021. These fresh quality rhizome of *A. calamus* (vach) were dried on shade at 30 ºC for one week. The weight of dry tender of vach was around 25.0 gram and used for extraction to isolate allyl phenol present as major chemical constituent.

5. Nut meg (Dry fruit of *M. fragrans*)

Myristica fragrans is an evergreen tree belongs to *Myristicaceae* family indigenous to Indonesia and Malaysia. It is also available in Eastern and Western Ghats Mountains of South India. The botanical name of the tree is *Myristica fragrans*. The fruit of tree is called nutmeg and jaiphal in regional/local language in India. The major potentialities of nutmeg are acts as stimulant for the brain and helps to reduce the stress,[32] oil of nutmeg is effect mouthwash in manging foul odour of breathing (halitosis),[33] possesses cardiovascular activity to enhance blood circulation and improve heat functions,[34] acts detoxifier for liver and kidney, effective remedy for insomnia.[35] Nutmeg is iconic condiment in the cuisine of Asian, African, Mediterranean and Middle East Countries. Nutmeg is used as folklore traditional medicine for bronchovesicular problem, neuro-disease[36] and also used as an iconic spice for preparation of ‘Malvani’ masala available in Arabian Sea shore in South Indian.[37] To investigate chemical constituent present in nutmeg, phytochemical analysis was carried out. In this connection, the good quality dry nutmeg (100 gm) was purchased from spice market, Mumbai, India on June 2021for isolation of allyl phenols present as major chemical ingredient in it.

EXTRACTION AND ISOLATION

Allyl phenols from leaves of *Piper beetle* (also known as pan/tambul)

Dry leaves of *P. beetle* crushed with a mechanical grinder. It was defatted with hexane (3 x 500 mL x 24h). These defatted powders of leaves were extracted with diethyl ether (3 x 500 mL x 24h). The ether extract was filtered to obtain supernatant solution appeared as dark green in colour due to presence of chloroplast. In order to remove chloroplast, the green coloured solution was treated with activated charcoal at 35-40 ^{0}C. It was filtered to obtain a solution appeared as pale yellow in colour. The solvent was removed using rota vapour at room temperature to yield a crude residue (2.0 gm; 5% of dry leaves by weight). The defatted diethyl ether extract of *P. bettle* was fractionated by column chromatography over silica gel with a gradient solvent elution using a mixture of *n*-hexane-ethyl acetate to afford twenty fractions. The volume of each aliquot was approximately 100 ml. The individual fractions were monitored by thin layer chromatography (TLC) to expose under UV radiation (254 nm) for visualization of spot appeared in micro plate using different combination of solvent system and sprayed with neutral $FeCl_3$ solution for classification of chemical constituents. Fractions with the similar TLC profile and final purification has been carried out by preparative TLC. The structural characterization of pure chevibetol (**1**) and allyl pyrocatechol (**2**) has been determined by means of spectroscopic and spectrometric methods. The structure of allyl phenols depicted in figure 1.

Allyl phenols from star anise (Dry fruit of *Illicium verum*) locally known as chakra phool

These dry fruits of star anise were powdered with a mechanical grinder and defatted with distilled hexane (3 x 300 mL x 24h). The defatted fruit powder was extracted with diethyl ether (3 x 300 mL x 24h). The diethyl ether extract was filtered to obtain supernatant as light pale yellow in colour. The solvent was removed using rota vapour at room temperature to yield a crude residue (1.0 gm; 1% of dry weight of the fruits). The crude extract of star anise, it was fractionated by column chromatography over silica gel with gradient elution with binary mixture of solvent ethyl acetate in hexane.

The individual fractions were monitored by thin layer chromatography (TLC) and visualized under UV radiation (254 nm), to expose in iodine vapour and spray with different reagents. The fractions having similar chemical profiles were combined. The major ingredients were present in fraction 4 and fraction 5 eluted with 5% ethyl acetate in hexane. The final purification of these allyl phenols was performed by means of preparative thin layer chromatography (PTLC). The structural characterization of major allyl phenols present into diethyl extract of star anise was assigned by spectrometric and spectroscopic methods and identified as anethole (**3**) and estragole (**4**) depicted in figure 1.

Allyl phenols from clove (Dry bud of *Sgzygium aromaticum*, in local language known as lovonga)

Dry cloves were macerated with a grinder. The wax and fatty substances were removed with hexane (3 x 300 mL x 24h). The defatted materials were extracted with (3 x 300 mL x 24h). The diethyl ether extract was filtered to obtain supernatant and appeared as light pale-yellow in colour. The solvent was removed using rota vapour at room temperature to yield a crude residue (1.0 gm; 1% of dry material by weight). The crude extract was fractionated by column chromatography over silica gel with gradient solvent elution. Fractions were monitored by TLC and visualized under UV radiation, to expose in iodine vapour and spray with different reagents. The fractions containing major chemical constituents with similar chemical profiling were combined. The final purification of major allyl phenols was carried out by means of repetitive chromatography followed by preparative thin layer chromatography. Their structural characterization was secured by spectroscopic and spectrometric methods and identified as eugenol (**5**) and eugenol acetate (**6**) as major component present into it as depicted in figure 1.

Allyl phenols from vach (Rhizome of *Acorus calamus*)

Dried tender was powdered with a mechanical grinder. This powdered of rhizome was defatted with hexane (3 x 100 mL x 24h), followed by extraction with diethyl ether (3 x 100 mL x 24h). The ether extract was filtered to obtain supernatant. This crude extract was monitored by micro plate. One major chemical constituent was visualized in TLC upon the exposure under UV radiation, exposure of iodine vapour in iodine chamber and sprayed with alkaline KMnO$_4$ solution. The solvent was removed using rota vapour at room temperature to yield a crude residue (~1.0 gm; 1.0% of tendered weight of the rhizome). The major allyl phenols present in the extract of rhizome was separated by column chromatography over silica gel with a gradient solvent elution followed by preparative thin layer chromatography (PTLC). The structural determination of this ingredient was carried out by means of spectroscopic and spectrometric methods and identified as trimethoxy allyl benzene (elemicin). Therefore, it may conclude that allyl phenol (1, 2, 3, trihydroxy-4-allyl benzene) present in rhizome in *Acorus calamus* (vach) is responsible to furnish its medicinal property. The structure of allyl phenol (**7**) depicted in figure 1.

Allyl phenols from nut meg (Dry fruit of *Myristica fragrans* locally known as Jaiphal)

Dry fruits of nutmeg were massacred with a mechanical grinder to yield fine powder owing pleasant aroma. This powder of fruit was defatted with hexane (3 x 100 mL x 24h), followed by extraction with diethyl ether (3 x 100 mL x 24h). The diethyl ether extract was passed through filter paper to obtain supernatant solution. The solvent was removed using rota vapour at room temperature to yield a crude buttery residue (1.0 gm; 1.0% of dry wt. of fruits). The residue was fractionated by column chromatography over silica gel with a gradient solvent elution. It was found that major chemical constituent present in diethyl ether extract was an allyl phenol known as myristicin as major constituent. Its structural characterization was determined by means of spectroscopic and spectrometric methods. The structure of allyl phenol identified as safrole (**8**) depicted in figure 1.

Spectral characterization of bioactive major allyl phenols from leaves of *P. beetle*

Chevibetol (1)

Faint yellow liquid; possessed peculiar aromatic pungent smell. ^{1}H NMR (CDCl$_3$, 500 MHz): δ_H 7.00 (d, 1H, J = 8.2 Hz, H-6), 6.90 (d, 1H, J = 2.8 Hz, H-5), 6.87 (d, 1H, J = 8.0 Hz, J = 8.0 Hz, H-3) 5.96-5.89 (m, 1H, H-2′), 5.09-5.05 (m, 2H, H-3′), 3.81 (s, 3H, -OCH$_3$), 3.32 (dd, 2H, J = 6.6 Hz, H-1′). ^{13}C NMR (CDCl$_3$, 50 MHz): δ_C 149.5 (C-1), 139.7 (C-2), 137.3 (C-4), 132.8 (C-3′), 126.8 (C-2′), 123.1 (C-5), 119.9 (C-6), 112.4 (C-3), 56.1 (-OCH$_3$), 39.3 (C-1′). EIMS: *m/z* (%) 206 [M$^+$] (not appeared), 175 (8.6), 164 (38.9), 149 (19.5), 132 (8.2), 121 (12.8), 107 (13.5), 103, 91 (40.9), 91 (100%, base peak), 77 (45.5), 65 (35.7), 51.0 (49.7).

Allyl pyrocatechol (2)

Pale yellow viscous liquid substance with pleasant aroma. ^{1}H NMR (CDCl$_3$, 500 MHz): δ_H 6.79 (d, 1H, J = 8.0 Hz, H-6), 6.71 (d, 1H, J = 2.2 Hz, H-5), 6.63 (d, 1H, J = 8.0 Hz, J = 8.0 Hz, H-3), 5.96-5.88 (m, 1H, H-2′), 5.08-5.03 (m, 2H, H-3′), 3.28 (dd, 2H, J = 6.6 Hz, H-1′). ^{13}C NMR (CDCl$_3$, 50 MHz): δ_C 143.49 (C-1), 141.68 (C-2), 137.68 (C-4), 133.39 (C-3′), 121.15 (C-2′), 116.01 (C-5), 115.70 (C-6), 115.62 (C-3), 39.6 (C-1′). EIMS: m/z (%) 206 [M$^+$], (10.5), 164 (bsae peak, 100%), 149 (40.3), 131 (15.9), 121 (13.50), 103, 91 (14.7), 91 (23.8) 77 (12.9), 65 (10.9), 55 (8.0).

Characterization of bioactive major allyl phenols from diethyl ether extract of star anise

Anethole (3)

It is a viscus yellow liquid with pleasant aroma having molecular formula $C_{10}H_{12}O$ established by mass spectrometry. It is present as major constituent in star anise. The spectral characterization of this component has been carried out spectroscopic and spectrometric methods. The spectral data of anethole is given below.

^{1}H NMR ($CDCl_3$, 200 MHz): δ_H 7.28 (dd, 2H, *J* = 8.2 & 2.2 Hz, H-6), 6.84 (dd, 2H, *J* = 8.2 & 2.2 Hz, H-5), 6.39 (dd, 1H, *J* = 8.2 Hz), 6.17-6.03 (m, 1H, H-2′), 3.80 (s, 3H, OCH_3), 1.87 (d, 2H, J = 6.6 Hz, H-1′). δ_C ^{13}C NMR ($CDCl_3$, 50 MHz): 158.5 (C-1), 130.6 (C-3′), 130.3 (C-4), 126.7 (C-2 & C-6), 123.1 (C-2′), 111.7 (C-2 & C-6), 54.9 (-OCH_3), 18.2 (C-1′). LRESI (positive mode): obs. *m/z* value 171.099 au; calc. m/z 171.079.

Estragole (4)

This ingredient is a viscus yellow liquid with pleasant aroma having molecular formula $C_{10}H_{12}O$ established by mass spectrometry. It is a viscus yellow liquid with pleasant aroma having molecular formula $C_{10}H_{12}O$ established by mass spectrometry. It is also present as major constituent in star anise. The spectral characterization of this component has been carried out spectroscopic and spectrometric methods. The spectral data estragole is given below.

^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.12 (dd, 2H, *J* = 8.2 & 2.2 Hz, H-6), 6.85 (dd, 2H, *J* = 8.2 & 2.2 Hz, H-3 & H-5), 5.98-5.92 (m, 1H, H-2′), 5.08-5.04 (m, 2H, H-3′), 3.80 (s, 3H, -OCH_3), 3.34 (dd, 2H, *J* = 6.6 Hz, H-1′). ^{13}C NMR ($CDCl_3$, 50 MHz): δ_C 158.1 (C-1), 138.0 (C-2′), 132.2 (C-4), 129.6 (C-3 & C-5), 115.5 (C-1′), 113.9 (C-2 & C-6), 55.3 (-OCH_3), 39.4 (benzylic C-3′).

IR spectrum (neat), ν_{max}: 3365 (brs peak, OH), 3010, 2931, 2857, 1713, 1605, 1522, 1445, 1282, 1196, 916, 818, 793. LRESI (positive mode): obs. *m/z* 17.099 au; calc. *m/z* 171.079 au.

Characterization of bioactive allyl phenols isolated from diethyl ether extract of clove

Eugenol (5)

Viscus dark yellow liquid owing pleasant aroma; molecular formula $C_{10}H_{12}O$ established by mass spectrometry. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 6.84 (d, 1H, *J* = 8. Hz, H-6), 6.69 (dd, 2H, *J* = 8.2 & 2.2 Hz, H-5 & H-3), 5.98-5.92 (m, 1H, H-2′), 5.10 - 5.04 (m, 2H, H-1ˋ), 3.87 (s, 3H, -OCH_3), 3.32 (d, 2H, H-3′). δ_C ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 146.5 (C-1), 144.0 (C-2), 137.9 (C-4), 122.6 (C-2′), 121.3 (C-1), 115.6 (C-5), 114.3 (C-6), 111.2 (C-3), 56.9 (-OCH_3), 40.0 (C-3′). EIMS *m/z* (%): 51 (5.2), 66 (3.4), 77.00 (6.4), 91(3.1), 109 (1.4), 122 (12.2), 137 (100, base peak), 166.1 (23.5) $[M^+]$.

Eugenol acetate (6)

pale yellow liquid; owing plesant aroma; present substaintial amount in clove. ^{1}H NMR ($CDCl_3$, 200 MHz): δ_H 6.95 (d, 2H, *J* = 8.2 Hz, H-6), 6.78 (d, 1H, *J* = 2.2 Hz, H-5), 6.76 (d, 1H, *J* = 2.2 Hz, H-3), 5.99-5.91 (m, 1H, H-2′), 5.12-5.07 (m, 2H, H-2′), 3.81 (s, 3H, -OCH_3), 3.37 (d, 2H, H-3′), 2.30 (s, 3H, $OCCH_3$). ^{13}C NMR ($CDCl_3$, 50 MHz): δ_C 169.2 (-$COCH_3$), 150.8 (C-1), 138.9 (C-2), 137.9 (C-4), 122.4 (C-1′), 120.6 (C-6), 116.1 (C-5), 112.6 (C-3), 55.7 (-OCH_3), 40.0 (C-3′), 20.6 ($OCCH_3$). HRESI-MS (positive mode): obs. *m/z* 193.3188 au; cal. *m/z* 193.086 au

Characterization of allyl phenol isolated from rhizome of *A. calamus*

Elemicin (7)

It is a peculiar sented pale yeollow liquid; molecula formula $C_{12}H_{16}O_3$ and mass 208.110 au determined by HR-ESI-MS study. ^{1}H NMR ($CDCl_3$, 200 MHz): δ_H 6.84 (d, 1H, J = 2.2 Hz, H-4), 6.53 (d, 1H, J = 2.2 Hz, H-6), 5.80-5.735 (m, 1H, H-3′), 5.54-5.33 (m, 1H, H-2′), 3.90 (s, 3H, OCH_3), 3.84 (s, OCH_3, 3H), 3.81 (s, OCH_3, 3H), 1.85 (d, 2H, J = 6.6 Hz, H-1′). ^{13}C NMR ($CDCl_3$, 50 MHz): δ_C 150.79 (C-2), 147.87 (C-1), 141.55 (C-3), 124.3 (C-2′), 124.1 (C-5), 117.1 (C-1′), 113.1 (C-4), 96.6 (C-6), 55.6 (C_2-OCH_3), 55.1 (C_1-OCH_3), 54.9 (C_3-OCH_3), 13.7 (C-3′).

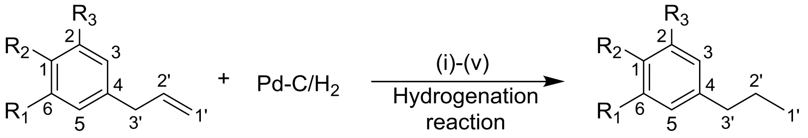
Characterization of allyl phenol isolated from nut meg

Safrole (8)

It is a peculiar sented pale yeollow liquid; molecula formula $C_{12}H_{16}O_3$ and mass 208.110 au determined by HR-ESI-MS study. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 6.39 (d, 1H, J = 2.2 Hz, H-4), 6.35 (d, 1H, J = 2.2 Hz, H-6), 6.00-5.35 (m, 1H, H-3′), 5.10-5.05 (m, 1H, H-2′), 3.89 (s, 3H, OCH_3), 3.30 (d, 2H, J = 6.6 Hz, H-1′). ^{13}C NMR ($CDCl_3$, 50 MHz): δ_C 150.79 (C-2), 147.87 (C-1), 141.55 (C-3), 124.3 (C-2′), 124.1 (C-5), 117.1 (C-1′), 113.1 (C-4), 96.6 (C-6), 55.6 (C_2-OCH_3), 55.1 (C_1-OCH_3), 54.9 (C_3-OCH_3), 13.7 (C-3′).

Hydrogenation of different allyl phenols isolated from various natural resources

About 10.0 mg of each isolates were dissolved in distilled ethanol in small round bottom fitted with a small magnetic stirrer. A pinch of Pd-C was added into these ethanolic solutions. It was sealed with two neck joint and degassing with vacuum pump and connected with H_2 gas. These reaction mixtures were stirring with help of magnetic stirrer over a span of 12 h. These reaction products were filtered through a small glass column over silica gel to obtain supernatant solution of them. Solvent was removed with the help of rota vapour at reduced pressure to yield respective hydrogenated products of these isolates. The structural characterization of the hydrogenated products has been secured by spectroscopic and spectrometric methods. The hydrogenation reaction of allyl phenols was depicted in scheme 1.



(i). C_2H_5OH (ii). Degasing (iii) Stirring (iv) 12 h (v) Room temp

(ix). R_1 = H, R_2 = OH & R_3 = OCH_3; 2H-chevibetol (**9**)
(x). R_1 = H & R_2 = OH; 2H-allyl pyrocatecol (**10**)
(xi). R_1 = H & R_2 = R_3 = OCH_3; 2H-methyl chevibetol (**11**)
(xii). R_1 = H & R_2 = R_3 = OCH_3; 2H-dimethyl APC (**12**)
(xiii). R_1 = R_3 = H & R_2 = OCH_3; 2H-anethole (**13**)
(xiv). R_1 = R_3 = H & R_2 = OCH_3; 2H-estragole (**14**)
(xv). R_1 = H, R_2 = OCH_3 & R_3 = OH; 2H-eugenol (**15**)
(xvi). R_1 = H, R_2 = OH & R_3 = OCH_3; 2H-eug. acetate (**16**)
(xvii). R_1 = R_2 = R_3 = OCH_3; 2H-Elemicin (**17**)
Products **9**-**17** are hydrogenated products

Scheme 1: Hydrogenation of allylic double bond using Pd-C/H_2 in ethanol at room temperature.

Spectral characterization of hydrogenated product of respective allyl phenols has been characterized by means of spectral methods. The spectral data are given below.

Dihydro chevibetol (9)

It is a very light yellow liquid; possessed peculiar aromatic pungent odor. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.00 (dd, 2H, *J* = 8.2 & 2.2 Hz, H-6), 6.88 (dd, 2H, J = 8.2 & 2.2 Hz, H-5), 6.85 (dd, 2H, *J* = 8.2 Hz, H-3), 2.52 (t, 1H, H-3′), 1.63-1.58 (m, 2H, H-2′), 1.2 (t, 3H, *J* = 6.6 Hz, H-1′). δ_C ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 146.3 (C-1), 143.6 (C-2), 134.8 (C-4), 114.1 (C-5), 114.88 (C-6), 111.1 (C-3), 56.0 (-OCH_3), 37.8 (C-3′), 25.0 (C-2′), 13.9 (C-1′). EIMS m/z (%): 51 (4.3), 65 (3.7), 77 (5.8), 9 3 (6.6), 109 (2.7), 122 (11.6), 137 (100%, base peak), 166 (25) [M^+].

Dihydro allyl pyrocatecol (10)

Light yellow liquid; possessed peculiar aromatic odor; ^{1}H NMR ($CDCl_3$, 200 MHz): δ_H 7.07 (dd, 1H, *J* = 8.2 & 2.2 Hz, H-6), 7.03 (dd, 1H, *J* = 8.2 & 2.2 Hz, H-5), 6.98 (d, 1H, *J* = 2.2 Hz, H-5) 2.59-2.56 (m, 1H, H-2′), 1.67-1.60 (m, 2H, H-3′), 0.92 (t, 3H, J = 6.6 Hz, H-1′). ^{13}C NMR ($CDCl_3$, 50 MHz): δ_C 143.5 (C-1), 141.4 (C-2), 136.9 (C-4), 120.9 (C-6), 115.6 (C-5), 115.3 (C-3), 37.4 (C-3′), 24.7 (C-2′), 13.8 (C-1′). Obs. *m/z*: 181.0888 [M+ Si]$^+$ au; calc. *m/z*: 181.061 au.

Structural characterization of dihydro methyl chevibetol (11) or dihydro dimethyl allyl pyrocatecol (12)

Colourless liquid substance; possessed pungent aromatic odor; ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 6.80 (d, 1H, *J* = 8.2 Hz, H-6), 6.78 (dd, 1H, *J* = 2.2 Hz, H-3), 6.72 (dd, 1H, H-5, *J* = 8.8 & 2.2 Hz), 3.87 (s, 3H, -OCH_3), 3.86 (s, 3H, -OCH_3), 2.55-2.52 (m, 1H, H-2′), 1.65-1.60 (m, 2H, H-1′), 0.94 (t, 3H, H-3′). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 148.5 (C-1), 146.9 (C-2), 135.2 (C-4), 120.0 (C-6), 111.6 (C-3), 110.9 (C-5), 55.7 (-OCH_3), 55.5 (-OCH_3), 37.5 (C-3′), 24.6 (C-2′), 13.9 (C-1′).

Dihydro anethole (13)/dihydro estragole (14)

Light pale yellow in colour; possessed pungent aromatic odor;. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.10 (dd, 2H, *J* = 8.2 & 2.2 Hz, H-3 & H-5), 6.83 (dd, 2H, *J* = 8.2 & 2.2 Hz, H-2 & H-6), 3.79 (s, 3H, -OCH_3), 2.53 (t, 2H, H-3′), 1.63-1.57 (m, 2H, H-2′), 0.93 (t, 3H, *J* = 6.6 Hz, H-1′).^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 157.7 (C-1), 134.8 (C-4), 129.3 (C-3 & C-4), 113.6 (C-6 & C-2), 55.2 (C1-OCH_3), 37.2 (C-3′), 24.9 (C-2′), 13.8 (C-1′).

Dihydro-eugenol (15)

Light yellow liquid substance. Possessed aroma. ^{1}H NMR ($CDCl_3$, 200 MHz): δ_H 6.84 (d, 1H, J = 8.2 Hz, H-6), 6.68 (d, 1H, *J* = 8.2, H-5), 6.66 (d, 1H, *J* = 2.2, H-3), 3.87(s, 3H, -OCH_3), 2.53 (t, 2H, H-3′), 1.64-1.59 (m, 2H, H-2′), 0.94 (t, 3H, *J* = 6.6 Hz, H-1′). ^{13}C NMR ($CDCl_3$, 50 MHz): δ_C 146.3 (C-1), 143.6 (C-2), 134.8 (C-4), 132.7 (C-6), 128.7 (C-3), 121.0 (C-3), 114.1 (C-6), 111.1 (C-5), 56.9 (-OCH_3), 37.8 (C-3′), 25.0 (C-2′), 13.9 (C-1′).

Dihydro-eugenol acetate (16)

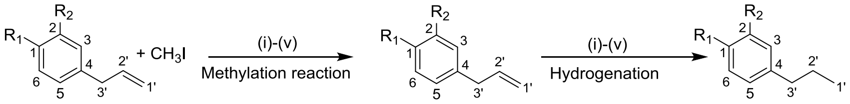
Pale yellow in colour; possessed pungent odour. ^{1}H NMR ($CDCl_3$, 200 MHz): δ_H 6.92 (d, 1H, J = 8.2 Hz, H-6), 6.78 (d, 1H, *J* = 2.2 Hz, H-3), 6.75 (dd, 1H, *J* = 8.2 & 2.2 Hz, H-5), 3.82 (s, 3H, -OCH_3), 2.57 (m, 2H, H-3′), 2.30 (s, 3H, -OAc), 1,66-1.62 (m, 2H, H-2′), 1.2 (t, 3H, *J* = 6.6 Hz, H-1′). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 169.5 (ester carbonyl,-$COCH_3$), 150.7 (C-1), 141.8 (C-2), 137.7 (C-4), 122. 4 (C-5), 120.6 (C-6), 112.7 (C-3), 55.9 (-OCH_3), 38.1 (C-3′), 24.8 (C-2′), 14.0 (C-1′).

Dihydro-elemicin (17)

Colourless liquid substance; possessed peculiar aroma. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 6.69 (s, 1H, H-6), 6.51 (s, 1H, H-5), 3.89 (s, 3H, -OCH_3), 3.83 (s, 3H, -OCH_3), 3.79 (s, 3H, -OCH_3), 2.53-2.50 (m, 2H, H-2′), 1.59-1.55 (m, 2H, H-2′), 0.94 (t, 3H, J = 5.60 Hz, H-1′). ^{13}C NMR ($CDCl_3$, 50 MHz): δ_C 151.5 (C-2), 147.5 (C-1), 142.58 (C-3), 122.9 (C-5), 114.2 (C-3), 98.0 (C-6), 56.7 (-OCH_3), 56.6 (-OCH_3), 56.3 (-OCH_3), 31.8 (C-3′), 23.6 (C-2′), 14.1 (C-1′).

Methylation of chevibetol (1) and allyl pyrocatechol (2) followed by hydrogenation

About 15.0 mg of each phenols were dissolved in distilled acetone in small 10 mL round bottom fitted with a small magnetic stirrer. A pinch of freshly prepared silver oxide was added into these aforesaid solutions. These reaction mixtures were stirring with help of magnetic stirrer over a span of 12 h at room temperature. These reaction products were filtered through a small glass column over silica gel to obtain supernatant solution. Solvent was removed from supernatant solution with the help of rota vapour at reduced pressure to yield respective methylated products of these phenols. The methylation reaction of allyl phenols was depicted in scheme 2.



(i). Acetone (ii). Catalyst AgO (iii). Stirring (iv).12 h (v). Room temp (RT). (i). Ethanol (ii). Catalyst Pd-C/H$_2$ (iii). Stirring (iv).12 h (v).RT.

R$_1$ = OH & R$_2$ = OCH$_3$; Chevibetol (**1**) R$_1$ = R$_2$ = OCH$_3$; Methyl chevibetol (**16**) R$_1$ = R$_2$ = OCH$_3$; 2H-methyl chevibetol (**18**)

R$_1$ = R$_2$ = OH; Allyl pyrocatechol (**2**) R$_1$ = R$_2$ = OCH$_3$; Dimethyl APC (**17**) R$_1$ = R$_2$ = OCH$_3$; 2H-dimethyl allyl pyrocatecol (**19**)

Scheme 2: Methylation naturally occurring isolated phenols from various plant sources followed by hydrogenation

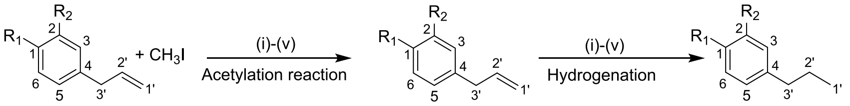
The structural characterization of the methylated phenols has been secured by spectroscopic and spectrometric methods.

Structural characterization of methyl chevibetol (18) or dimethyl allyl pyrocatecol (19)

Colourless liquid substance; possessed pungent aromatic odor; ^{1}H NMR (CDCl$_3$, 500 MHz): δ_H 6.80 (d, 1H, J = 8.2 Hz, H-6), 6.78 (dd, 1H, J = 2.2 Hz, H-3), 6.72 (dd, 1H, H-5, J = 8.8 & 2.2 Hz), 6.00-5.92 (m, 1H, H-2′), 5.10 (m, 2H, H-1′), 3.87 (s, 3H, -OCH$_3$), 3.86 (s, 3H, -OCH$_3$), 3.34 (d, 2H, H-3′). ^{13}C NMR (CDCl$_3$, 125 MHz): δ_C 148.6 (C-1), 147.3 (C-2), 137.6 (C-4), 132.5 (C-2′), 120.0 (C-1′), 115.5 (C-3), 111.7 (C-6), 111.7 (C-5), 111.2 (C-3), 55.7 (C1-OCH$_3$), 55.5 (C2-OCH$_3$), 39.7 (C-3′).

Acetylation reaction of of chevibetol (1) or allyl pyrocatechol (2)

About 10.0 mg of each phenols were dissolved in distilled pyridine in small round bottom fitted with a small magnetic stirrer. A pinch of freshly prepared DMAP and freshly distilled acetic anhydride was added into these aforesaid solutions. These reaction mixtures were stirring with help of magnetic stirrer over a span of 12 h at room temperature. These reaction products were filtered through a small glass column over silica gel to obtain supernatant solution of them. Solvent was removed with the help of rota vapour at reduced pressure to yield respective acetylated products of these phenols. The acetylation reaction of allyl phenols was depicted in scheme 3.



(i). Acetone (ii). Catalyst AgO (iii). Stirring (iv).12 h (v). Room temp. (i). Ethanol (ii). Catalyst Pd-C/H$_2$ (iii). Stirring (iv).12 h (v). Room temp.

R$_1$ = OH & R$_2$ = OCH$_3$; Chevibetol (**1**) R$_1$ = OAc & R$_2$ = OCH$_3$; Acetoxy chevibetol R$_1$ = OAc & R$_2$ = OCH$_3$; 2H-acetoxy chevibetol (**20**)

R$_1$ = R$_2$ = OH; Allyl pyrocatechol (**2**) R$_1$ = R$_2$ = OAc; Diacetoxy allyl pyrocatechol R$_1$ = R$_2$ = OAc; 2H-diacetoxy allyl pyrocatechol (**21**)

Scheme 3: Acetylation reaction of naturally occurring isolated phenols from various plant sources fdollowed by hydrogenation

The structural characterization of the acetylated phenols has been determined by spectroscopic and spectrometric methods.

Structural analysis of dihydro chevibetol acetate (20)

Colourless liquid substance; possessed pungent aromatic odor;. ^{1}H NMR (CDCl$_3$, 500 MHz): δ_H 7.00 (d, 1H, J = 8.2 Hz, H-6), 6.98 (dd, 1H, J = 2.2 Hz, H-3), 6.87 (dd, 1H, H-5, J = 8.8 & 2.2 Hz), 3.81 (s, 3H, -OCH$_3$), 2.52 (t, 2H, H-2′), 1.63-1.58 (m, 2H, H-2′), 0.92 (t, 3H, H-1′).

Structural analysis of dihydro diacetoxy allyl pyrocatecol (21)

Colourless liquid substance; possessed pungent aromatic odor; ^{1}H NMR (CDCl$_3$, 500 MHz): δ_H 7.07 (d, 1H, J = 8.2 Hz, H-6), 7.03 (dd, 1H, J = 2.2 Hz, H-3), 6.99 (dd, 1H, H-5, J = 8.8 & 2.2 Hz), 2.57 (t, 2H, H-2′), 1.67-1.60 (m, 2H, H-2′), 0.94 (t, 3H, H-1′).

EVALUATION OF CELL CYTOYOXICITY AGAINST DIFFERENT HUMAN CANCER CELL LINES

The efficacy of cell cytotoxicity of isolated naturally occurring allyl phenols from has been examined on five different types of human cancer cell lines including urinary bladder (HT-1376), pancreatic (HPAC), prostate (DU-145), colon (HT-29) and thyroid (MDA-T32) using SRB assay in comparison with therapeutically prescribed authentic drug paclitaxel as control.

Anti-proliferative bioassay

The study of cell cytotoxicity of allyl phenols and their derivatives against different cancer cell lines by using sulfo-rhodamine B assay was performed at College of Pharmacy, The Ohio State University, Ohio, Columbus, USA.[37-38]

Sample preparation. Test samples (allyl phenols and their derivatives) and controls (paclitaxel) were dissolved in 100% DMSO to prepare stock solutions of 10 mg/ml. Dilutions were prepared using 10% DMSO in water and 100% water.

Cell culture. Cancer cells [urinary bladder (HT-1376), pancreatic (HPAC), prostate (DU-145), colon (HT-29) and thyroid (MDA-T32)] were obtained from American Type Culture Collection, Manassas, VA, USA. Monolayer cells were cultured using T75 tissue culture flasks in Roswell Park Memorial Institute medium (RPMI) or Dulbecco's Modified Eagle Medium (DMEM), containing 10% fetal bovine serum and 1% anti-biotic-anti-mycotic from Gibco. Cells were kept for incubation at 37 °C and in an atmosphere with 5% of CO_2.

Anti-proliferative assays. The inhibition of cell proliferation activity of test samples allyl phenols and their derivatives was evaluated in triplicate on cancer cells using a sulforhodamine B (SRB) assay as reported previously, in three independent experiments.[38,39]

Sample[a]	**Five types cancer cell lines used to evaluate anti-proliferative activity in comparison with paclitaxel as control using (SRB) assay**									
	Uri. bladder (HT-1376)		**Thyroid (MDA-T32)**		**Pancreatic (HPAC)**		**Prostate (DU-145)**		**Colon (HT-29)**	
	% Inhibit.[b]	**IC_{50}**[c]	**% Inhibit.**[b]	**IC_{50}**[c]	**% Inhibit.**[b]	**IC_{50}**[c]	**% Inhibit.**[b]	**IC_{50}**[c]	**% Inhibit.**[b]	**IC_{50}**[c]
1	<50		<50		<50		<50		<50	
2	<50		<50		<50		<50		<50	
3	<50		<50		<50		<50		<50	
4	<50		<50		**52 ± 1.10**	**18.66± 0.80**	<50		<50	
5	<50		<50		<50		<50		<50	
6	<50		<50		<50		<50		<50	
7	<50		<50		<50		<50		<50	
8	<50		<50		<50		<50		<50	
9	<50		<50		<50		<50		<50	
10	<50		<50		<50		<50		<50	
11	<50		<50		<50		<50		<50	
12	<50		<50		<50		<50		<50	
13	<50		<50		<50		<50		<50	
14	<50		<50		<50		<50		<50	
15	<50		<50		<50		<50		<50	
16	<50		<50		<50		<50		<50	
17	<50		<50		<50		<50		<50	
18	<50		<50		<50		<50		<50	

[a]Sample tested in triplicate and in two separate experiments at 20µg/mL. [b]% Inhibition <50 deemed inactive. [c]$IC_{50} \geq 20$ deemed inactive.

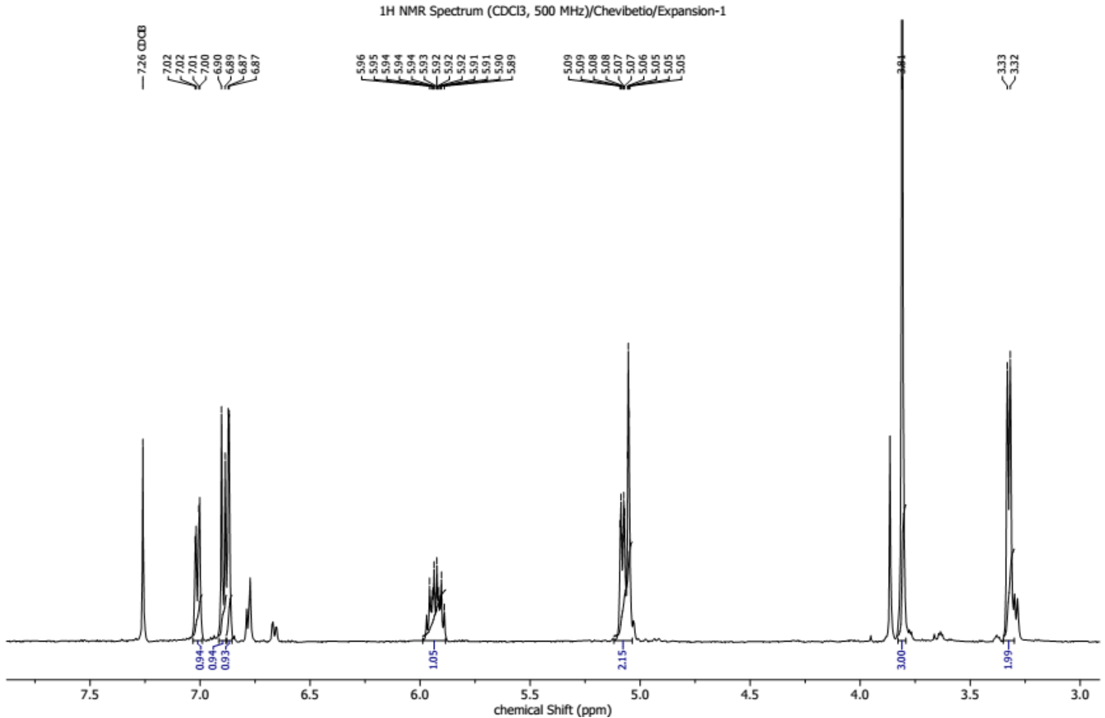


Figure S1: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of chevibetol (1).

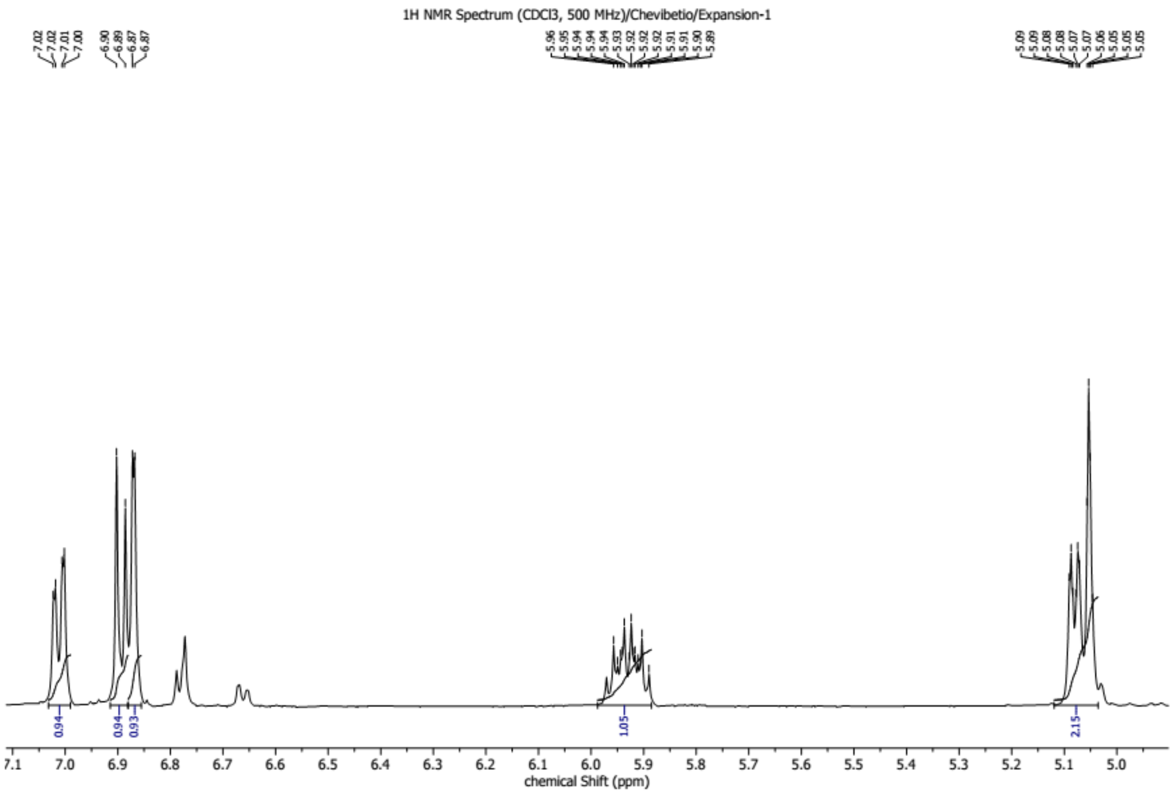


Figure S2: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of chevibetol (1).

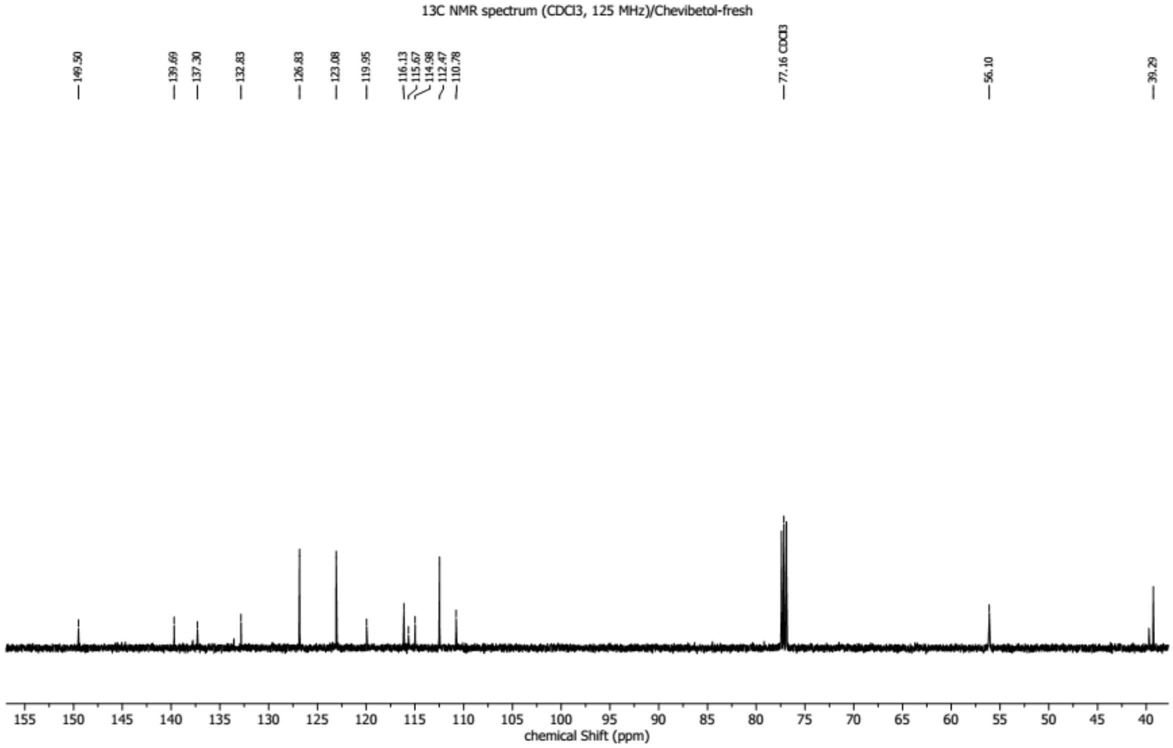


Figure S3: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of chevibetol (1).

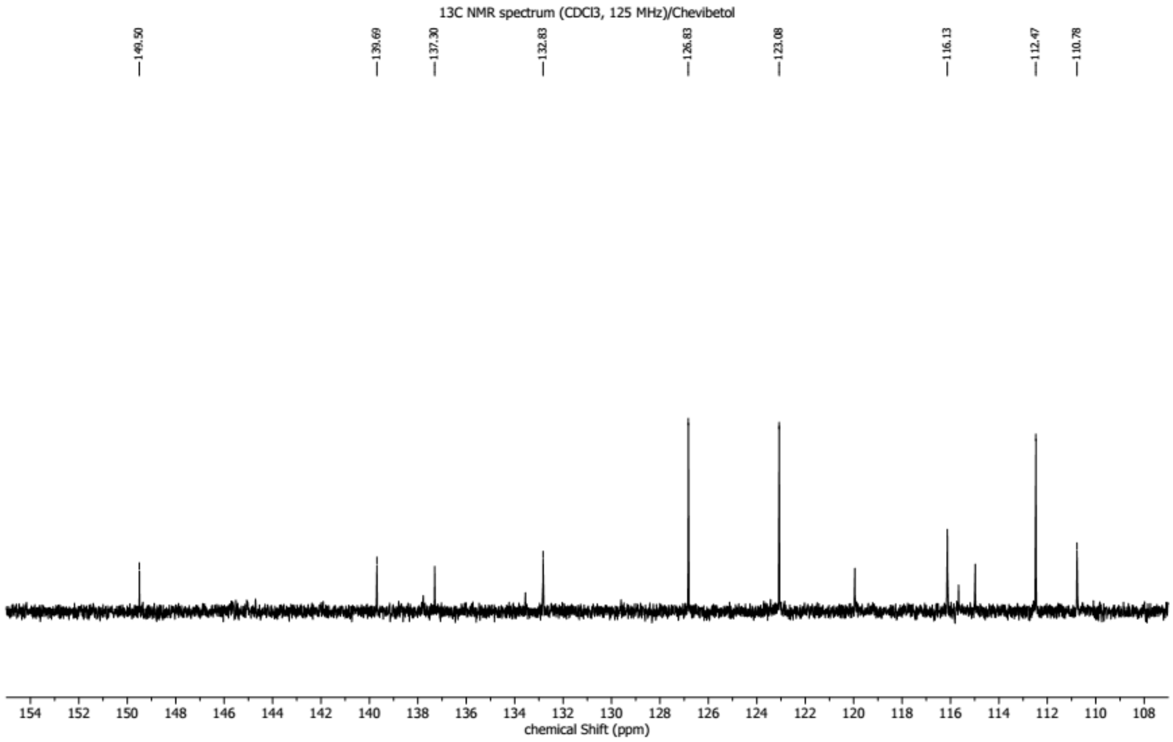


Figure S4: Expansion of ^{13}C NMR spectrum (CDCl$_3$, 125 MHz) of chevibetol (1).

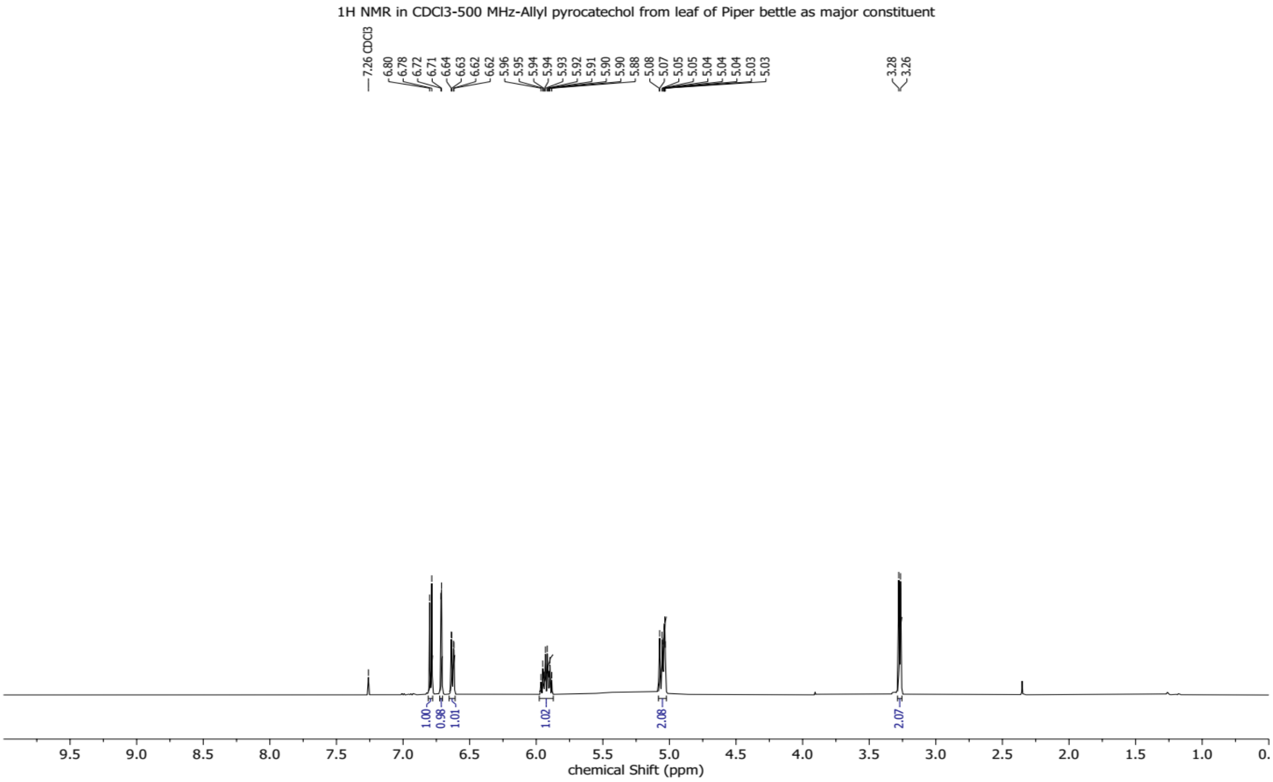


Figure S5: ^{1}H NMR spectrum (CDCl$_3$, 500 MHz) of allyl pyrocatechol isolated from *P. beetle* leaves (2).

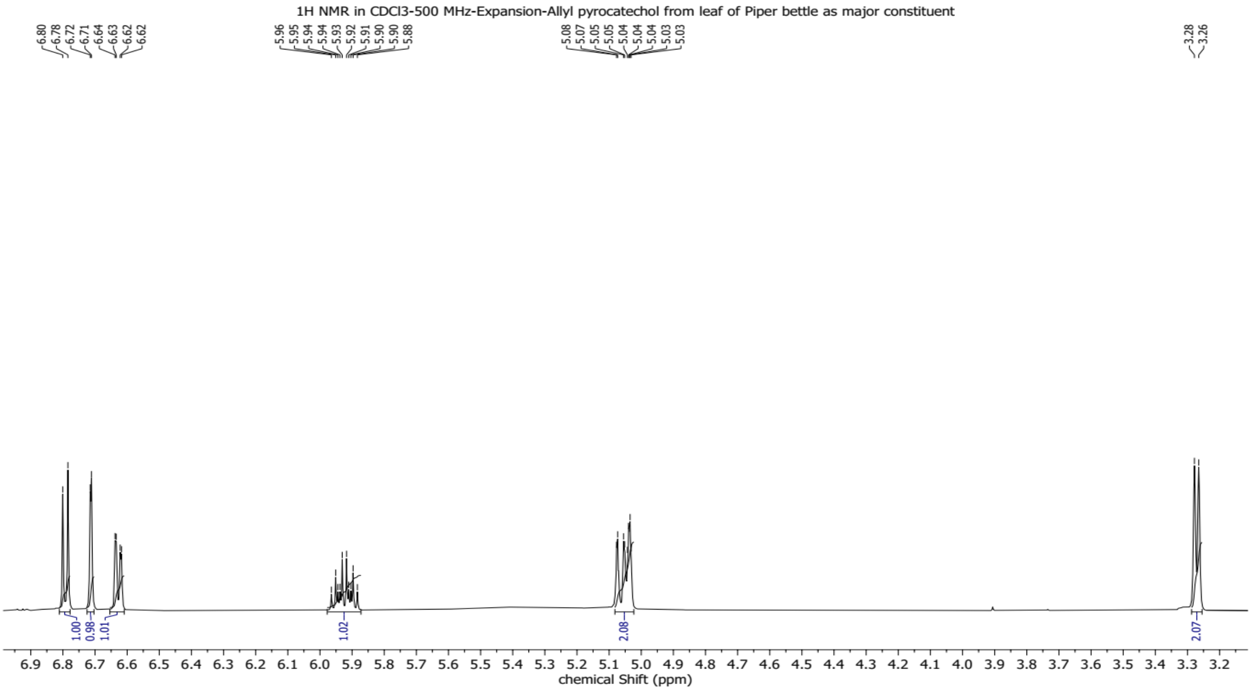


Figure S6: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of allyl pyrocatechol (2).

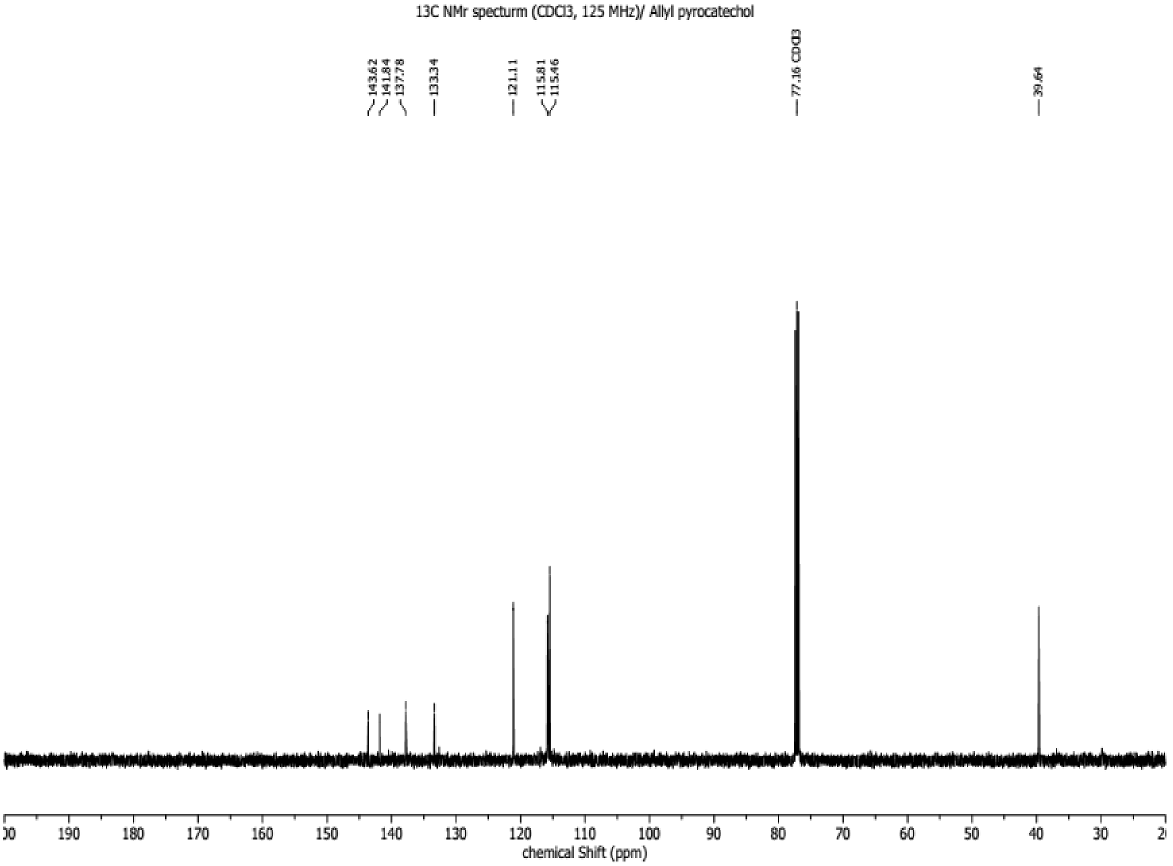


Figure S7: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of allyl pyrocatechol (2).

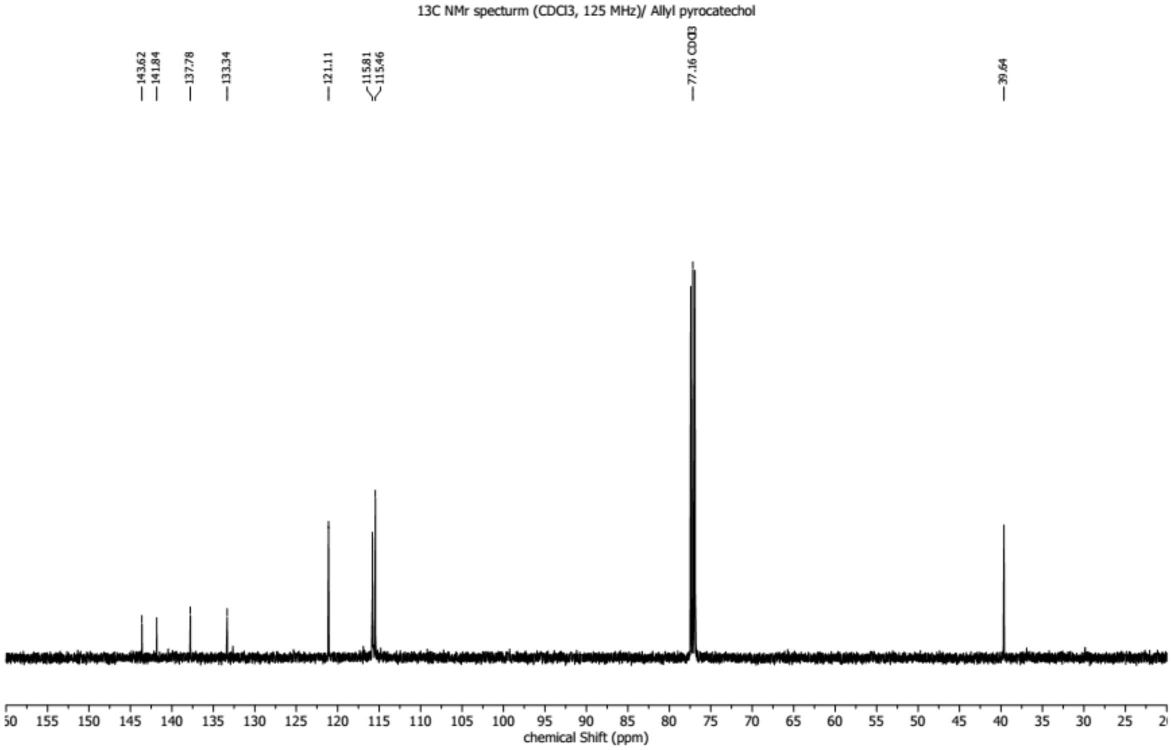


Figure S8: Expansion of ^{13}C NMR spectrum (CDCl$_3$, 125 MHz) of allyl pyrocatechol (2).

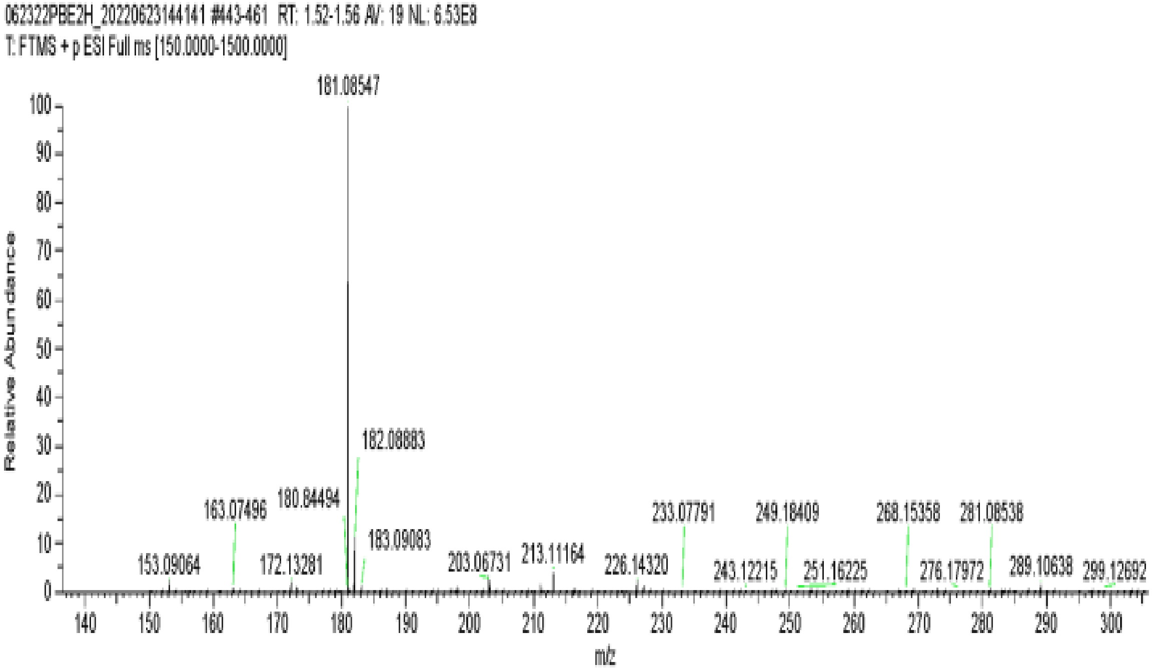


Figure S9: HRESI-MS of allyl pyrocatechol (2).

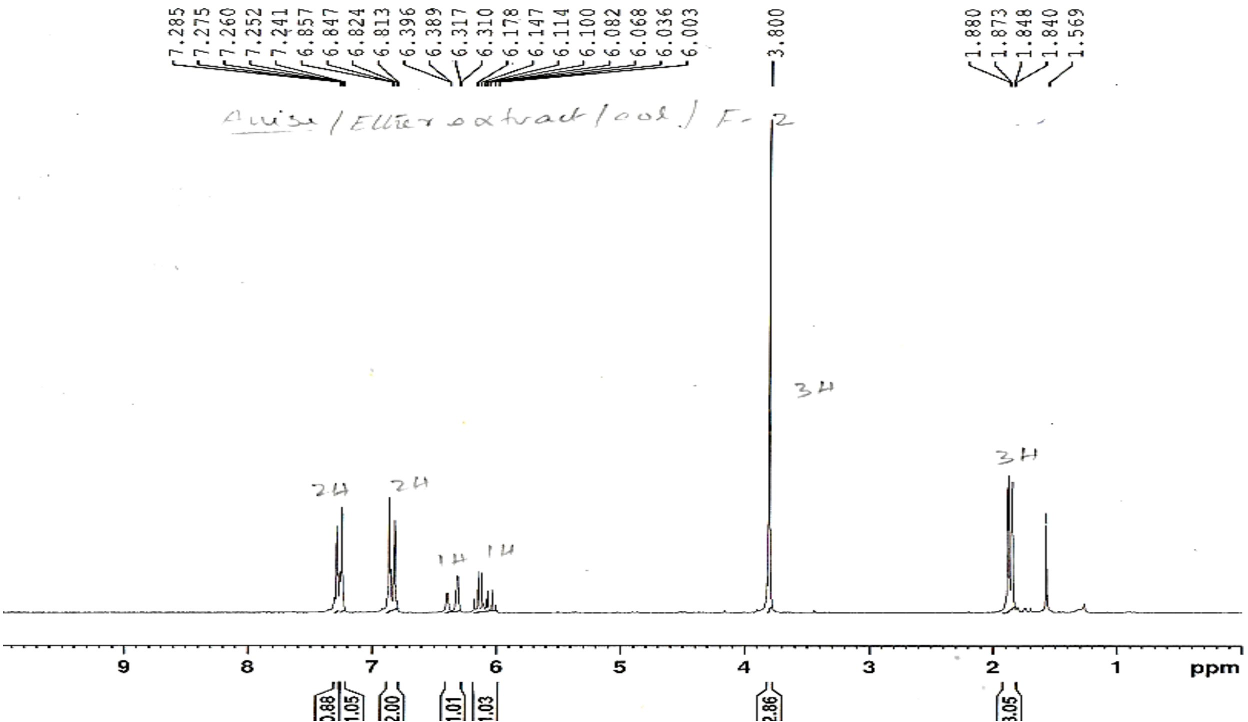


Figure S10: ^{1}H NMR spectrum ($CDCl_3$, 200 MHz) of anethol isolated from fruits of *I. verum* (3).

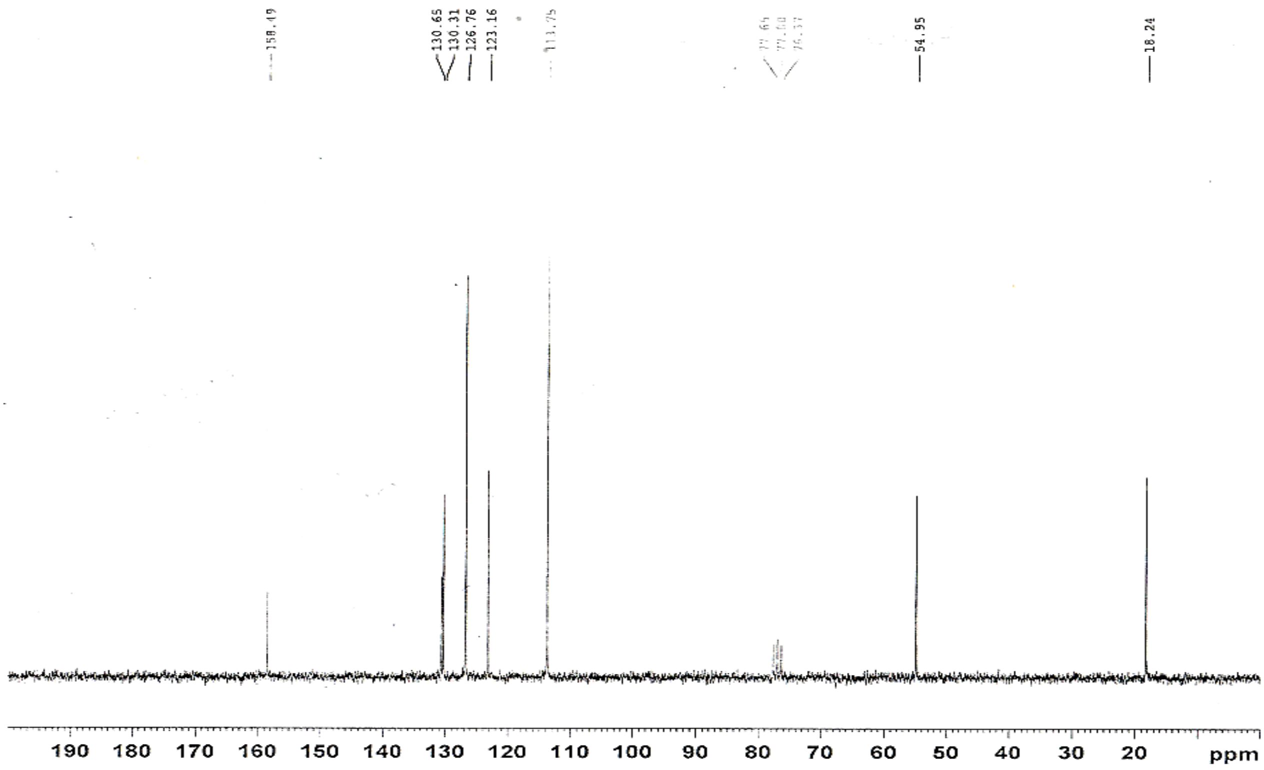


Figure S11: ^{13}C NMR spectrum ($CDCl_3$, 50 MHz) of anethole (3).

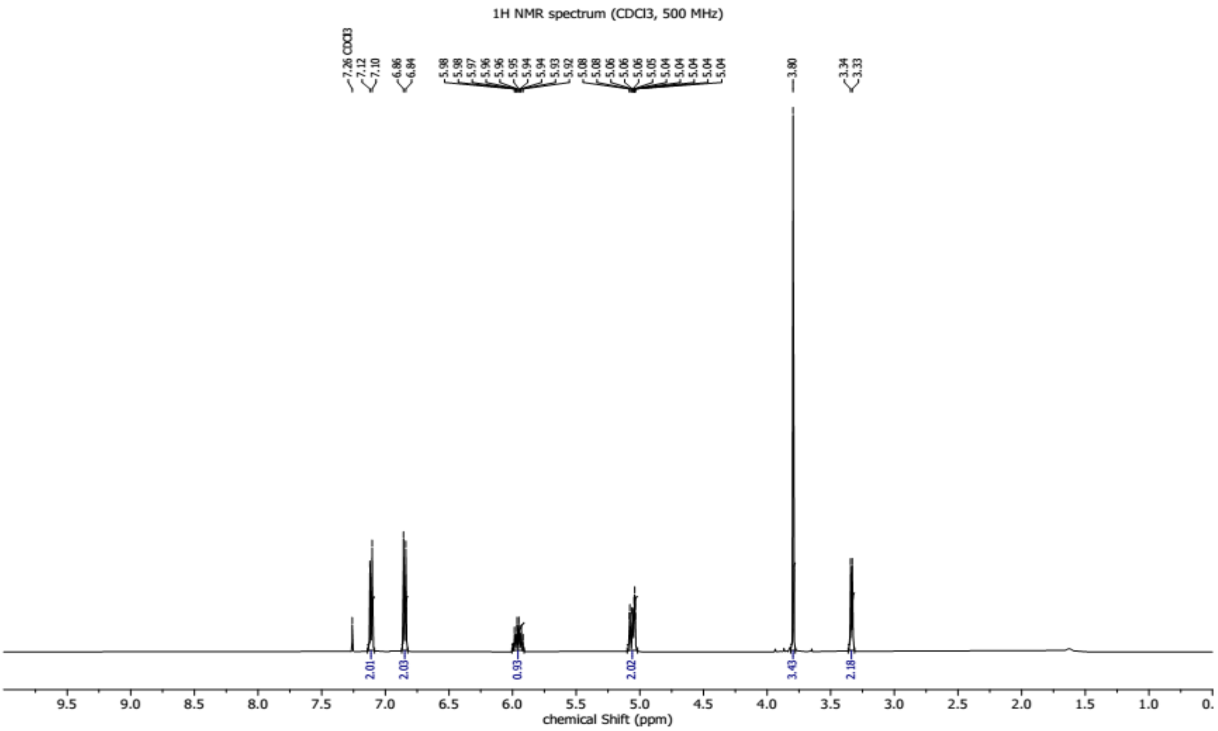


Figure S12: ^{1}H NMR spectrum (CDCl$_3$, 500 MHz) of estragole isolated from fruits of *I. verum* (4).

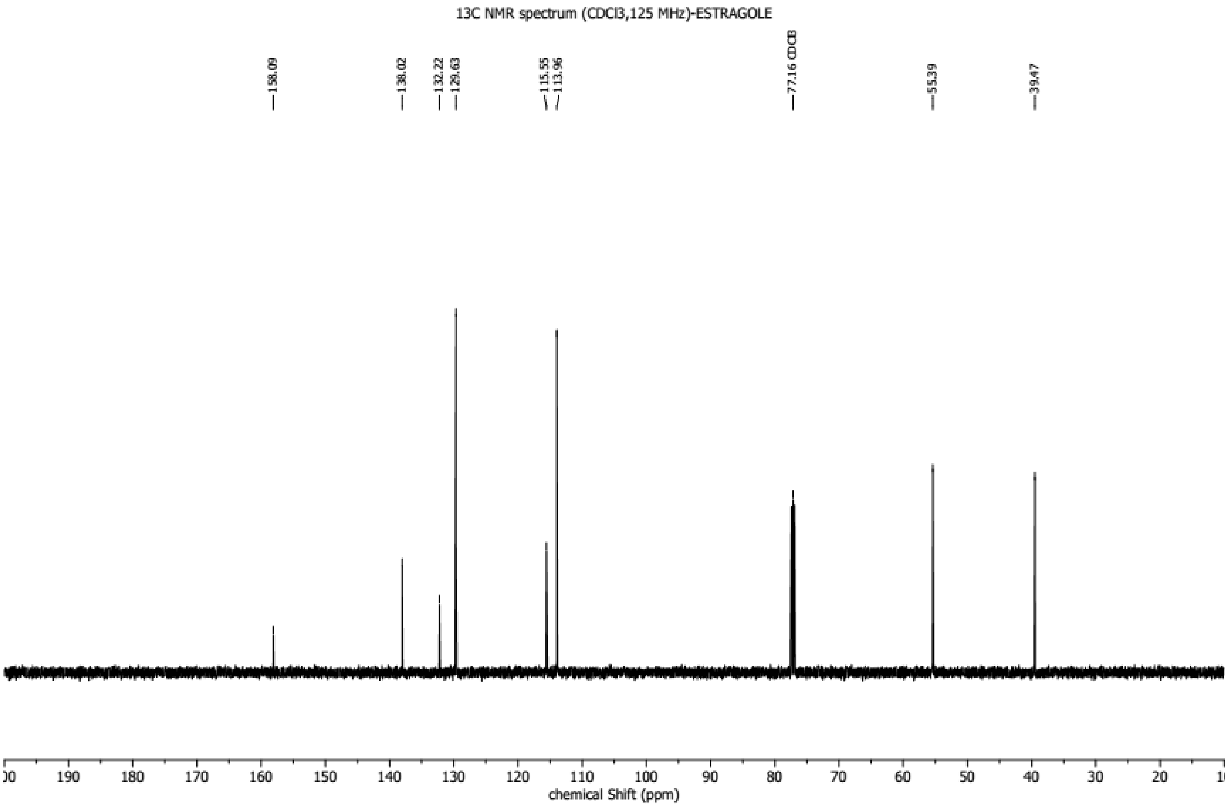


Figure S13: ^{13}C NMR spectrum (CDCl$_3$, 125 MHz) of estragole (4).

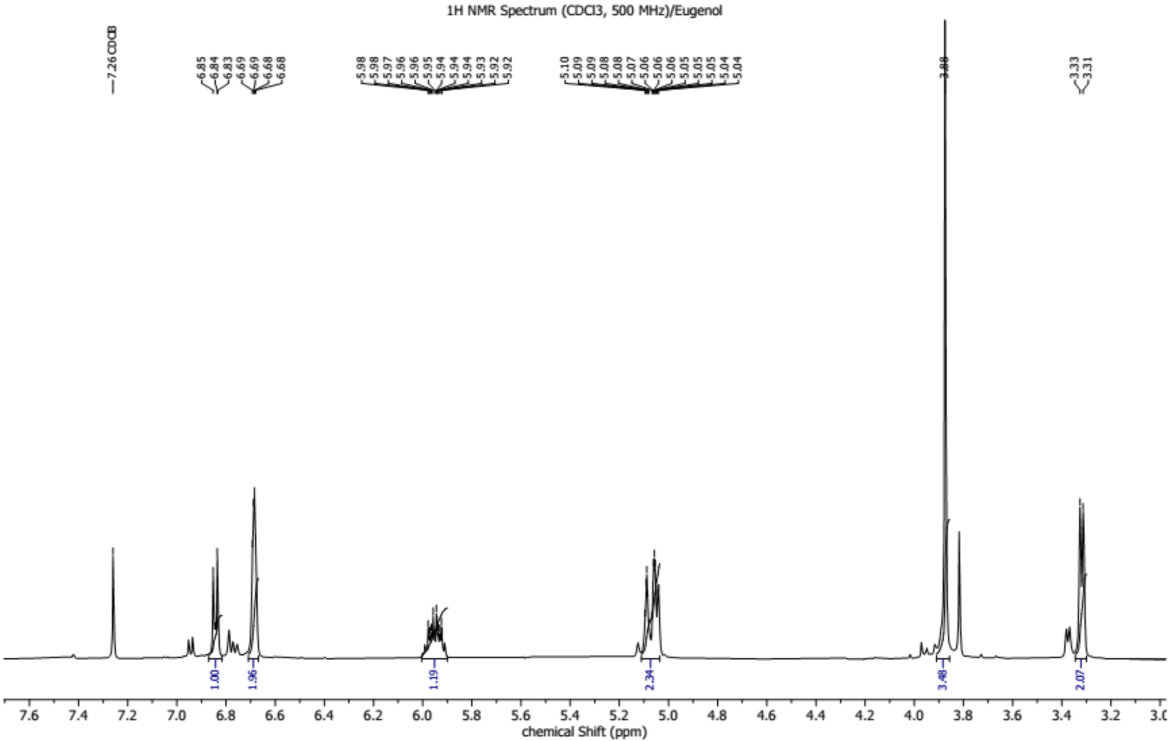


Figure S14: ^{1}H NMR spectrum (CDCl$_3$, 500 MHz) of eugenol isolated from clove (5).

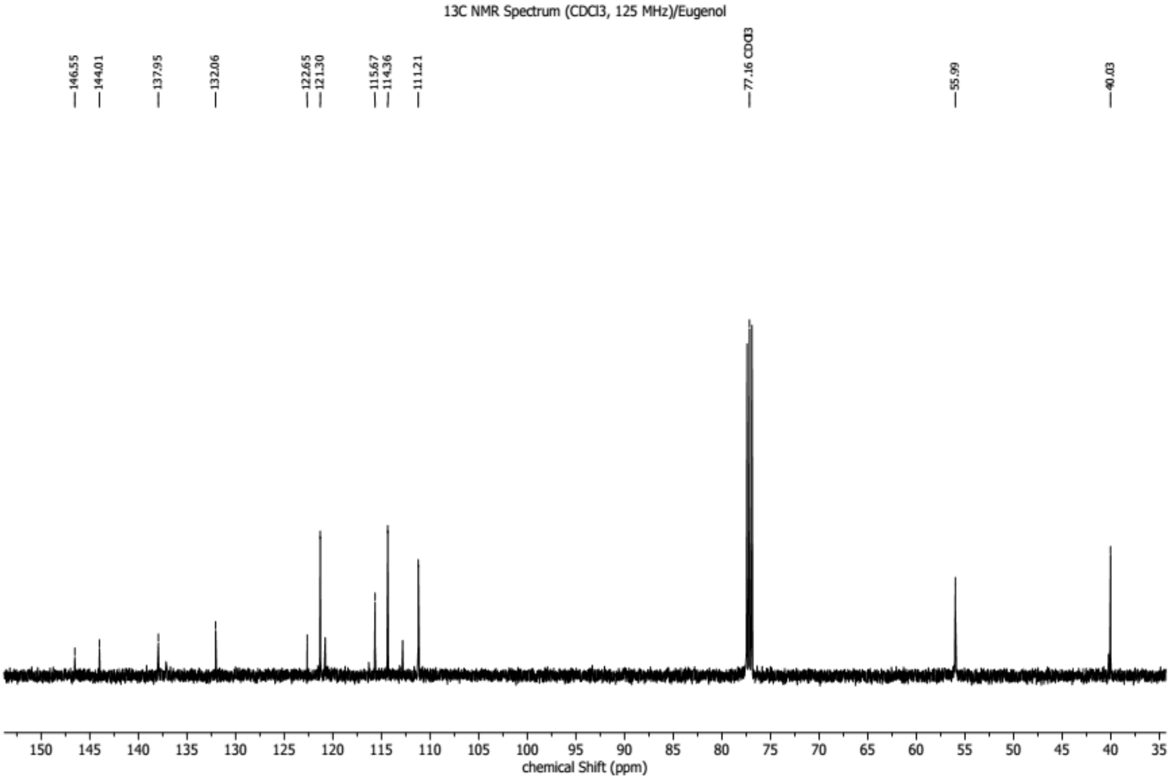


Figure S15: ^{13}C NMR spectrum (CDCl$_3$, 125 MHz) of eugenol isolated from clove (5).

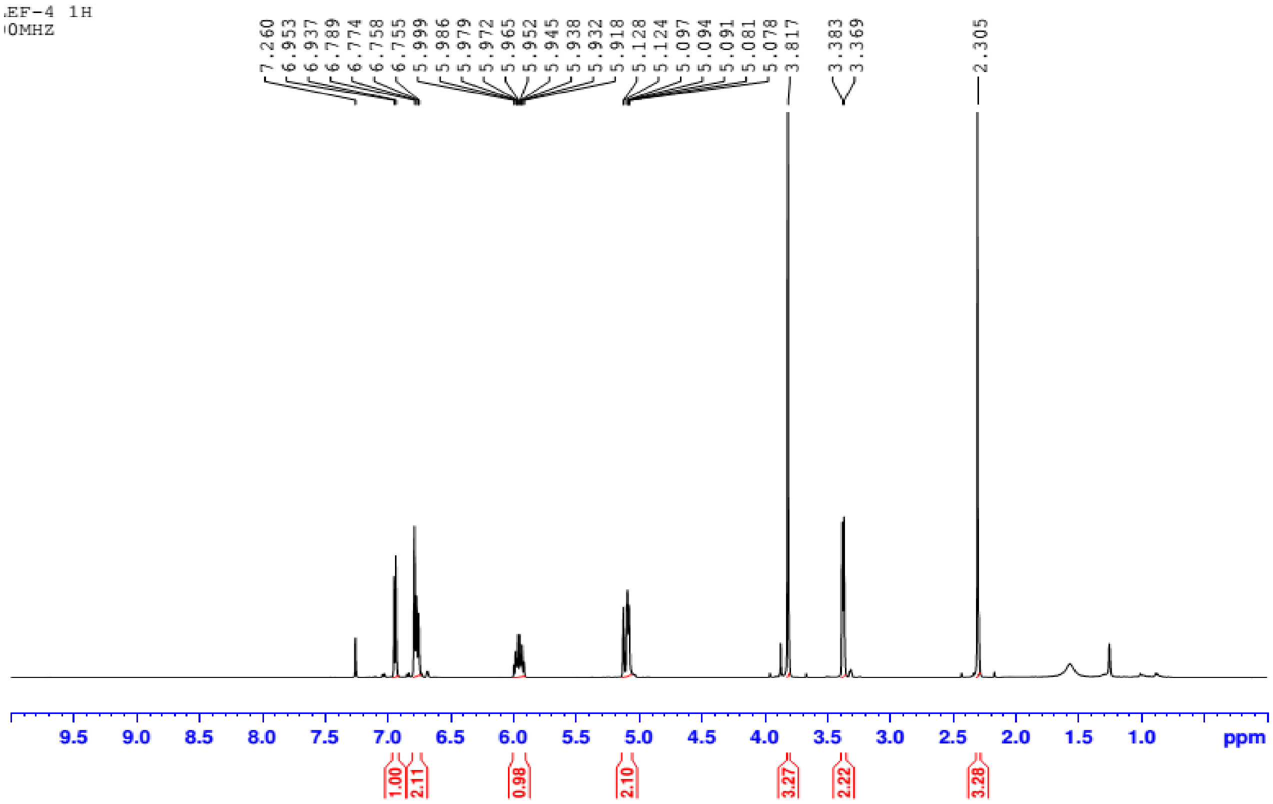


Figure S16: ^{1}H NMR spectrum ($CDCl_3$, 200 MHz) of eugenol acetate isolated from clove (6).

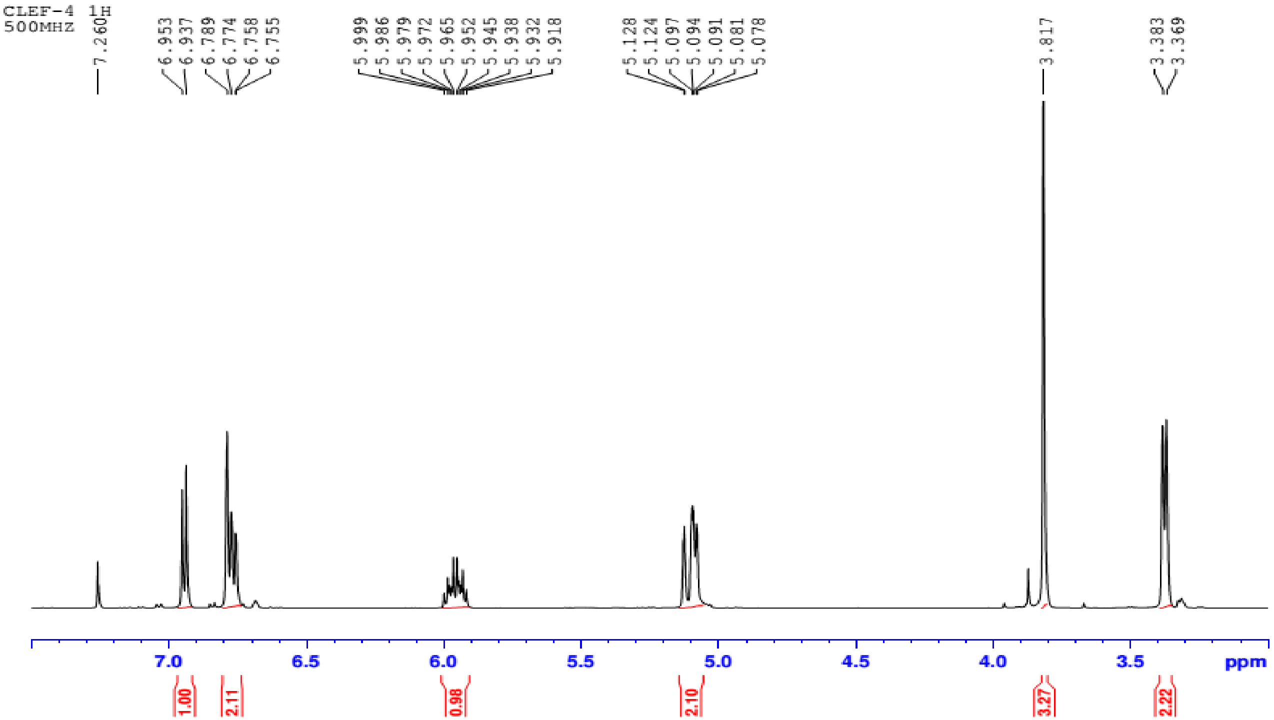


Figure S17: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 200 MHz) of eugenol acetate (6).

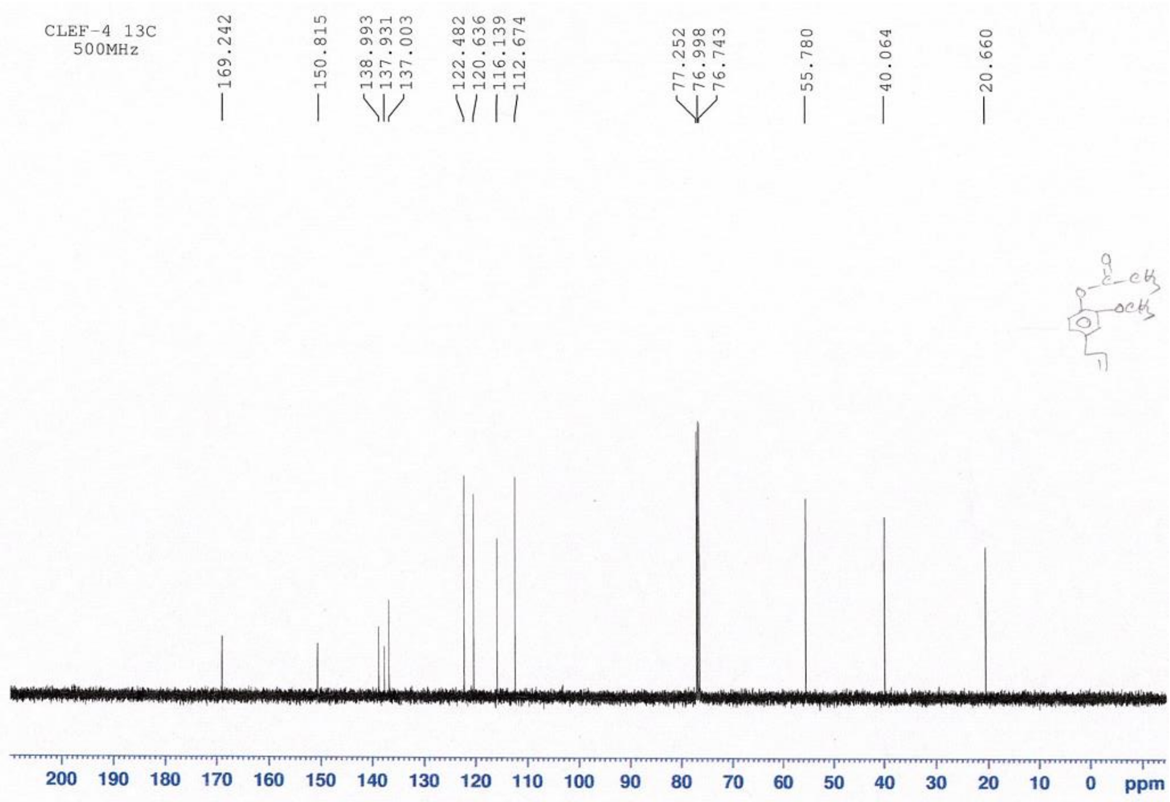


Figure S18: ^{13}C NMR spectrum ($CDCl_3$, 50 MHz) of eugenol acetate isolated from clove (6).

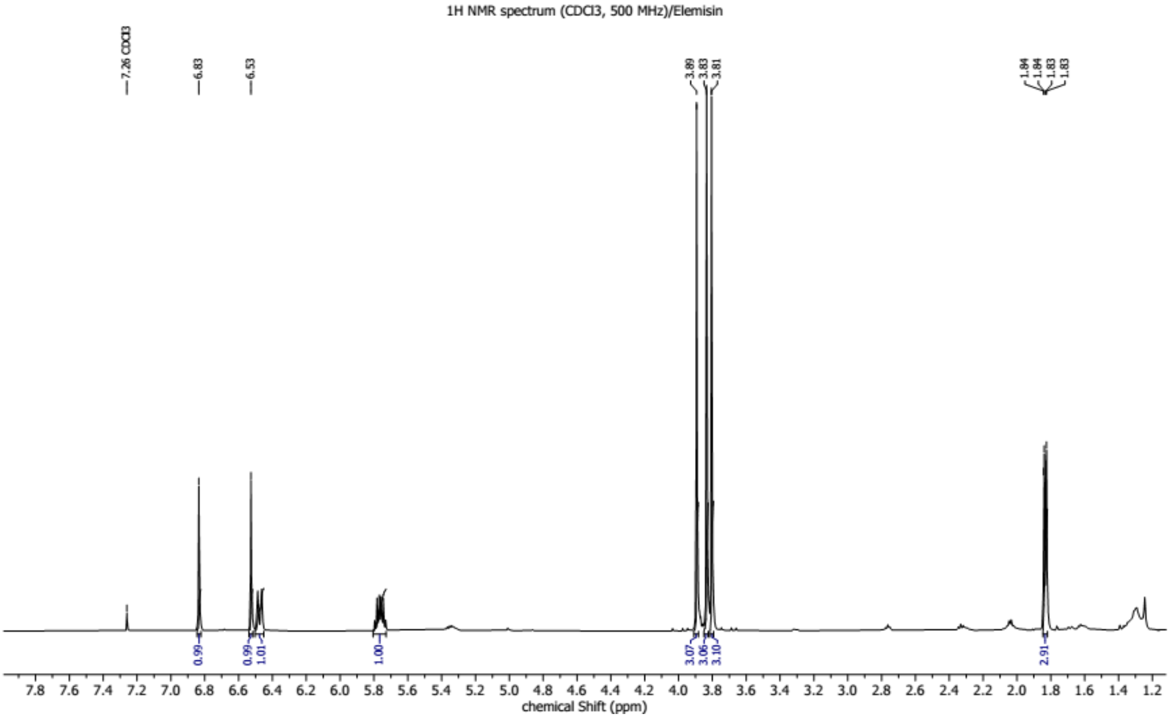


Figure S19: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of elemicin isolated from rhizome of *A. calamus* (7).

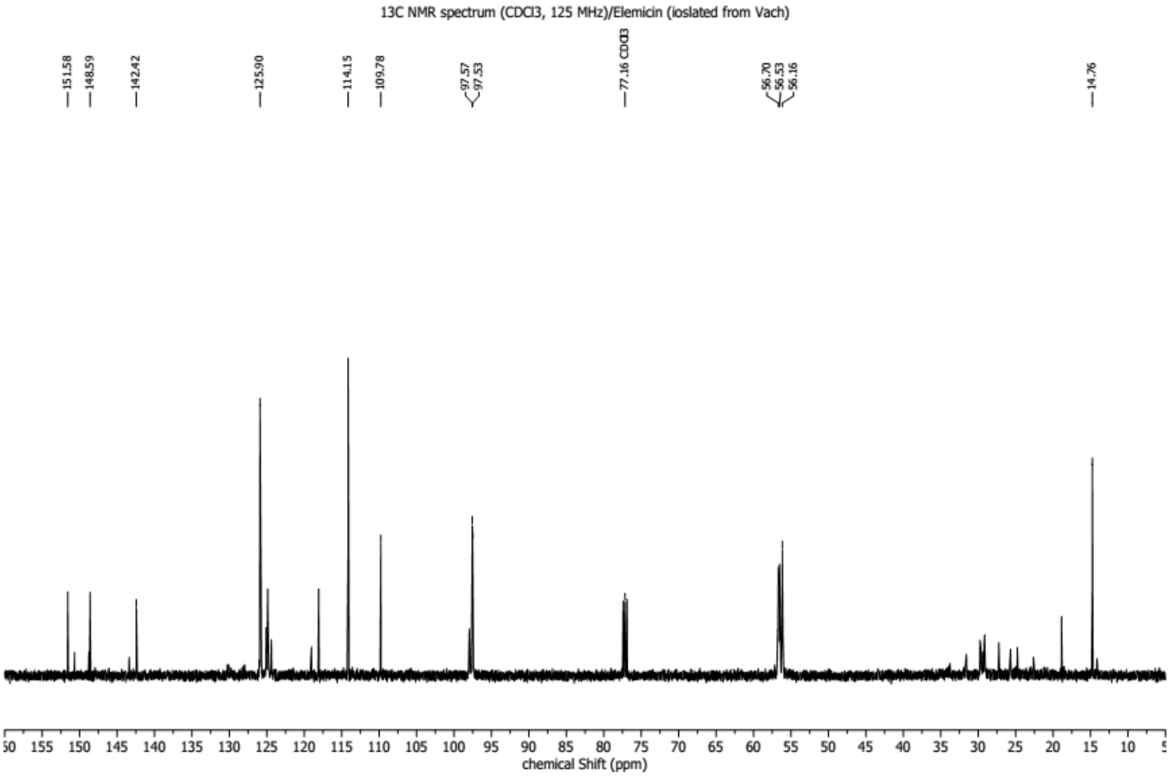


Figure S20: ^{13}C NMR spectrum (CDCl$_3$, 50 MHz) of elemicin isolated from rhizome of *A. calamus* (7).

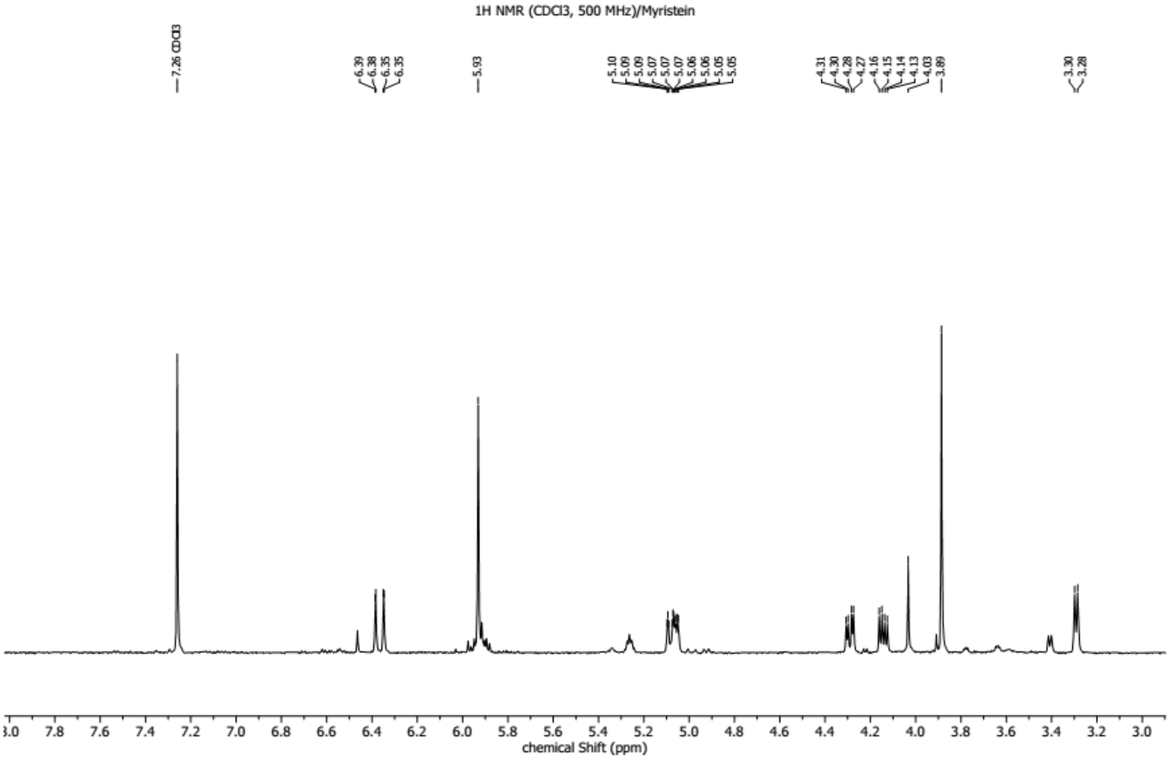


Figure S21: ^{1}H NMR spectrum (CDCl$_3$, 500 MHz) of safrole isolated from nutmeg (8)

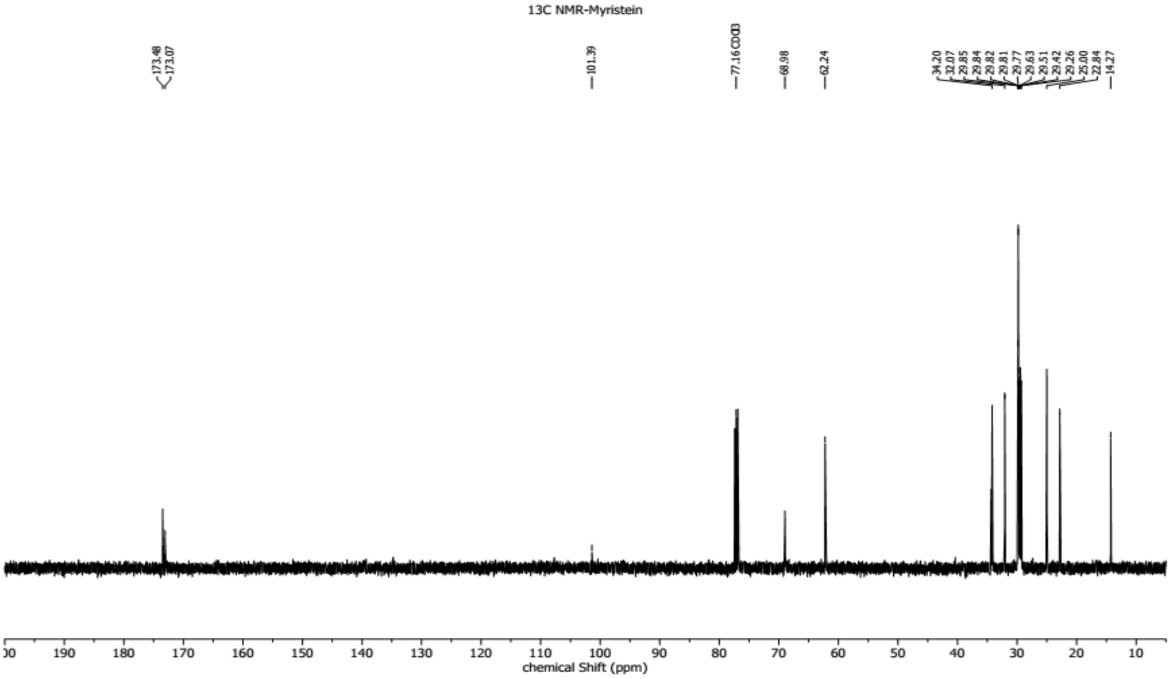


Figure S22: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of safrole isolated from nutmeg (8). Not decent spectrum due to insufficient of the compound.

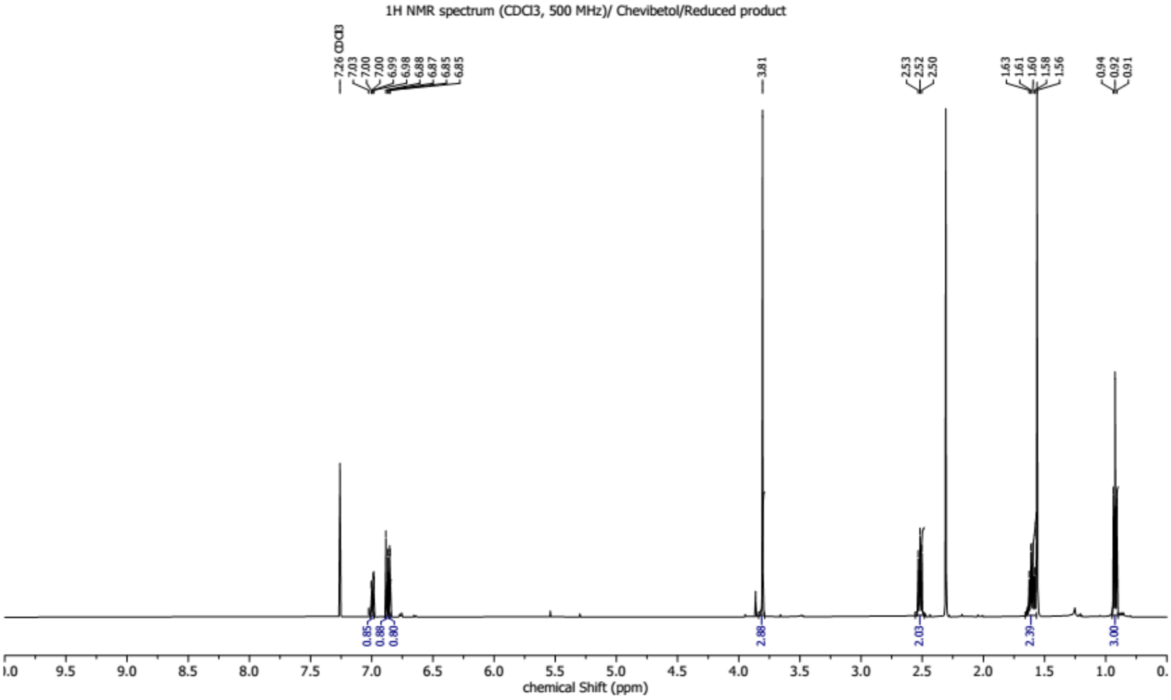


Figure S23: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of dihydro-chevibetol (9).

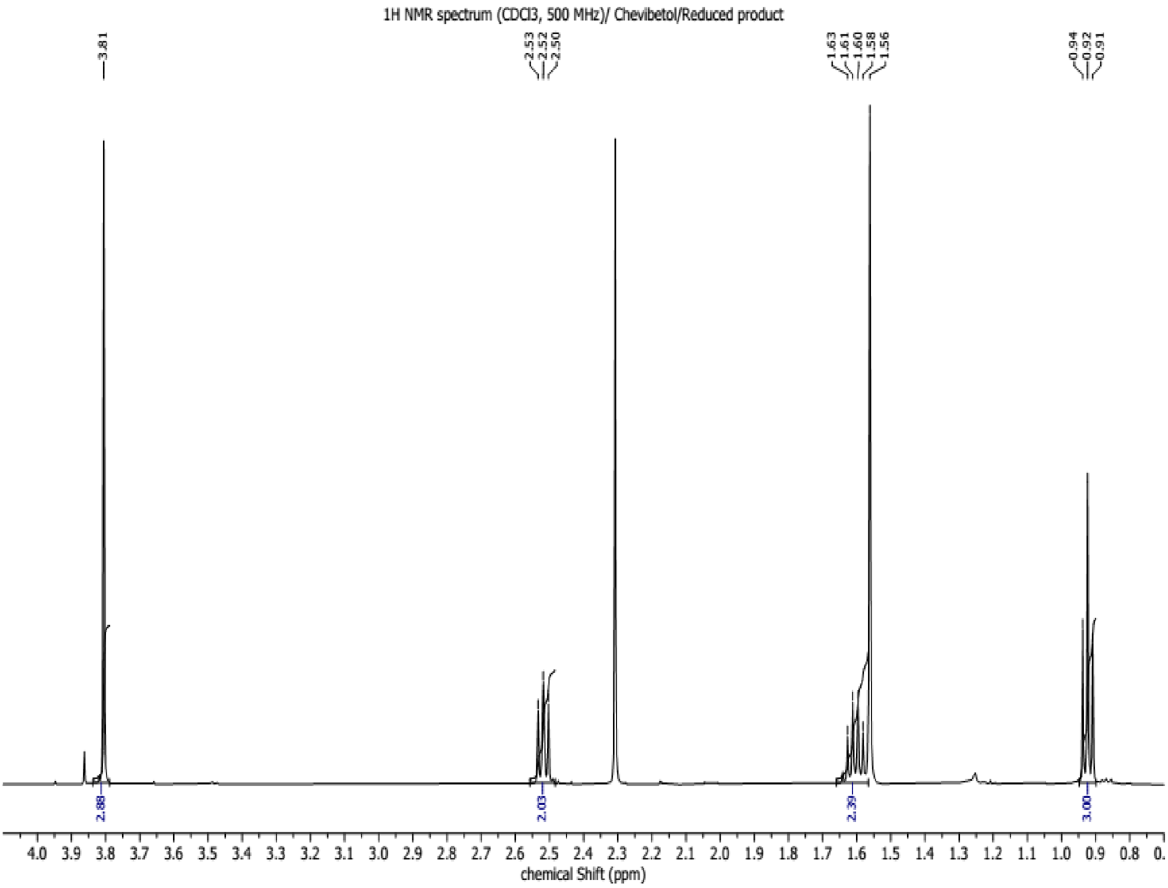


Figure S24: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of dihydro-chevibetol (9).

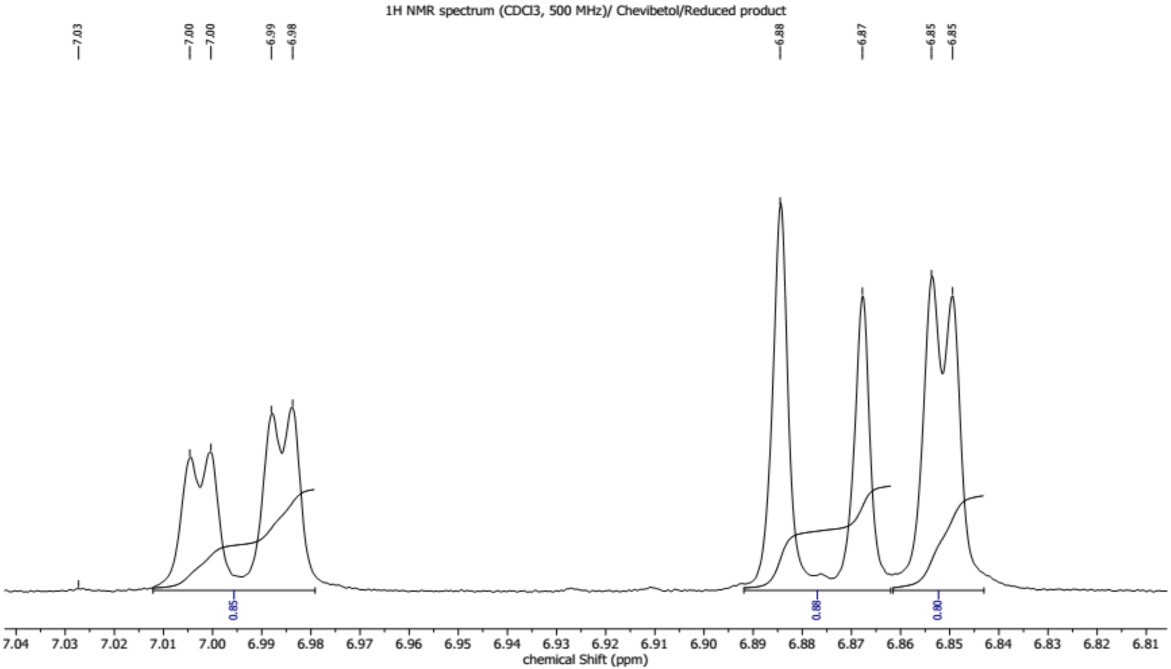


Figure S25: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of dihydro-chevibetol (9).

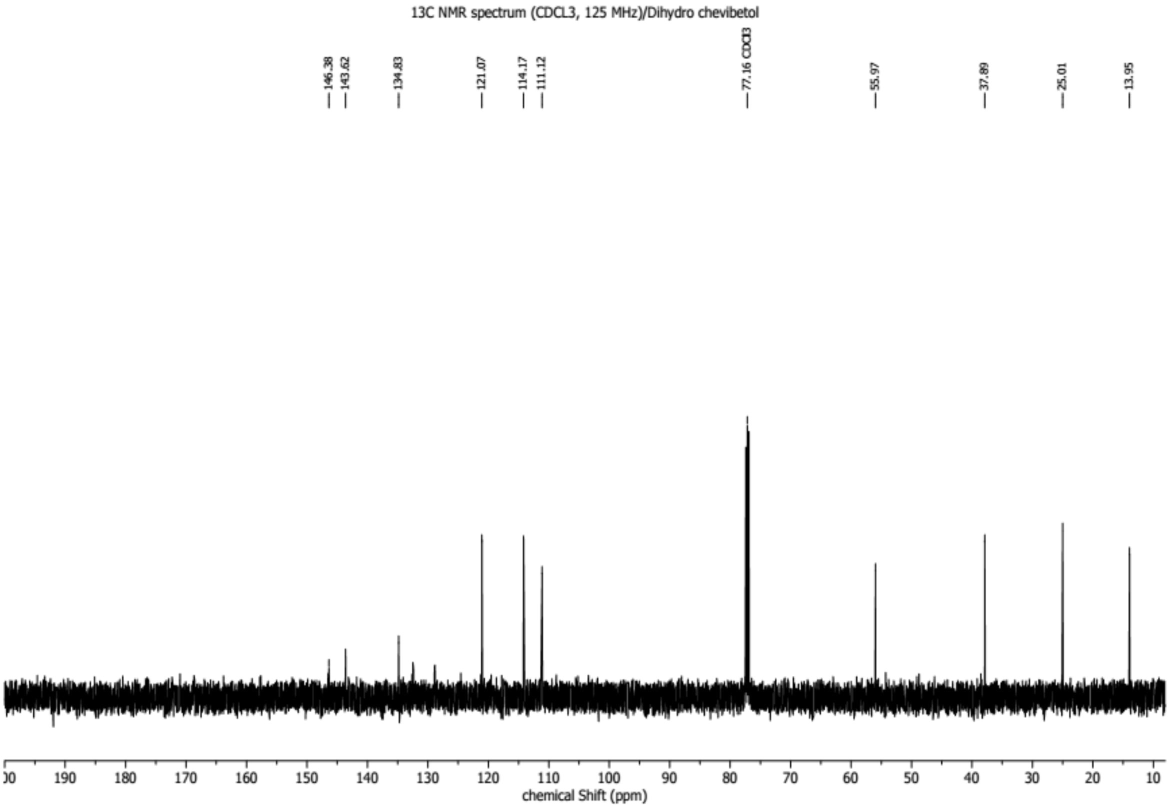


Figure S26: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of dihydro-chevibetol (9).

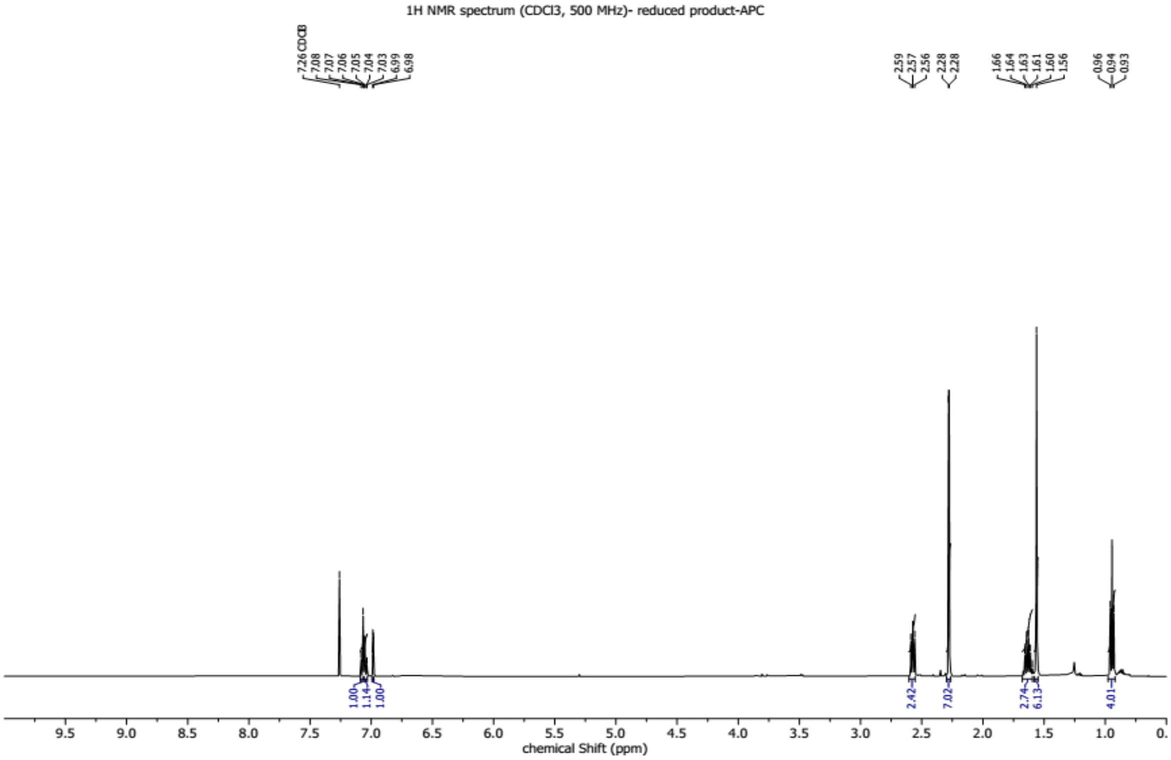


Figure S27: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of dihydro allyl pyrocatechol (10).

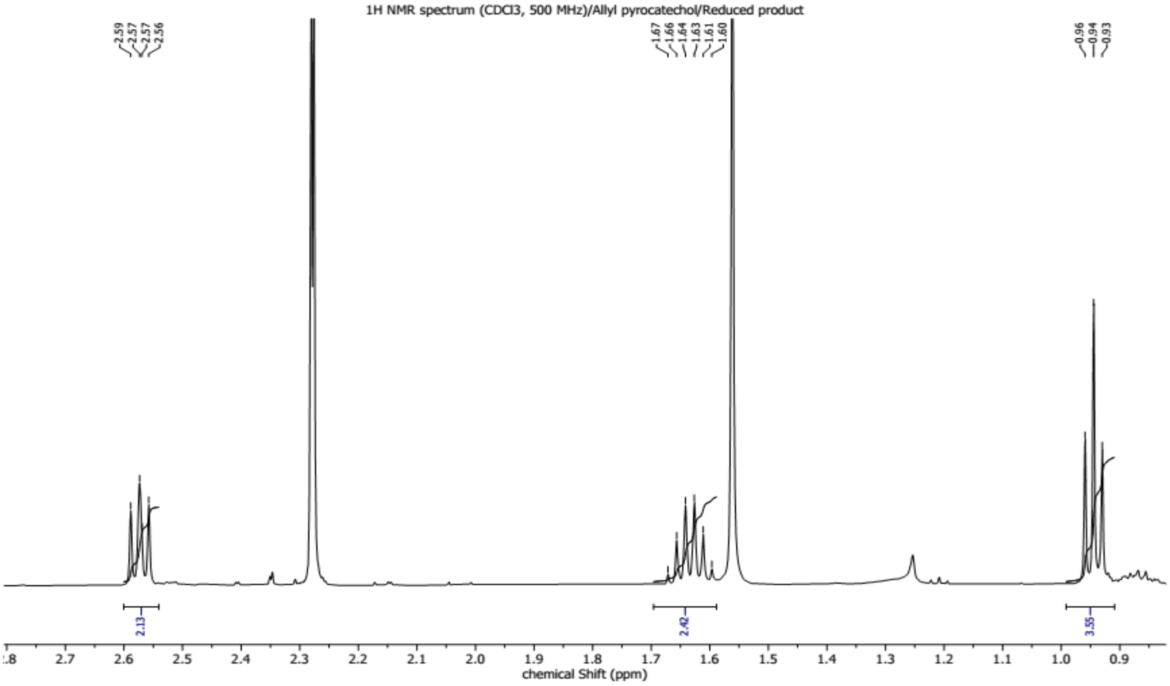


Figure S28: Expansion of ^{1}H NMR spectrum (CDCl$_3$, 500 MHz) of dihydro allyl pyrocatechol (10).

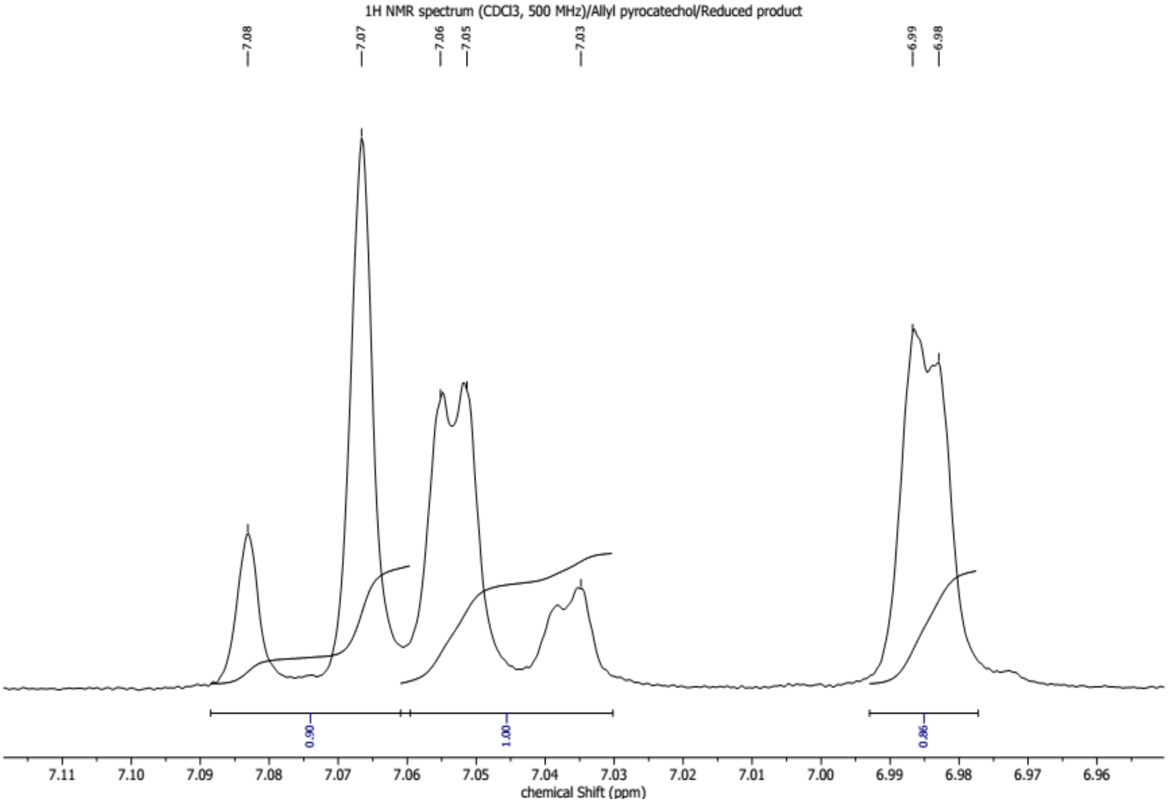


Figure S29: Expansion of ^{1}H NMR spectrum (CDCl$_3$, 500 MHz) of dihydro-allyl pyrocatechol (10).

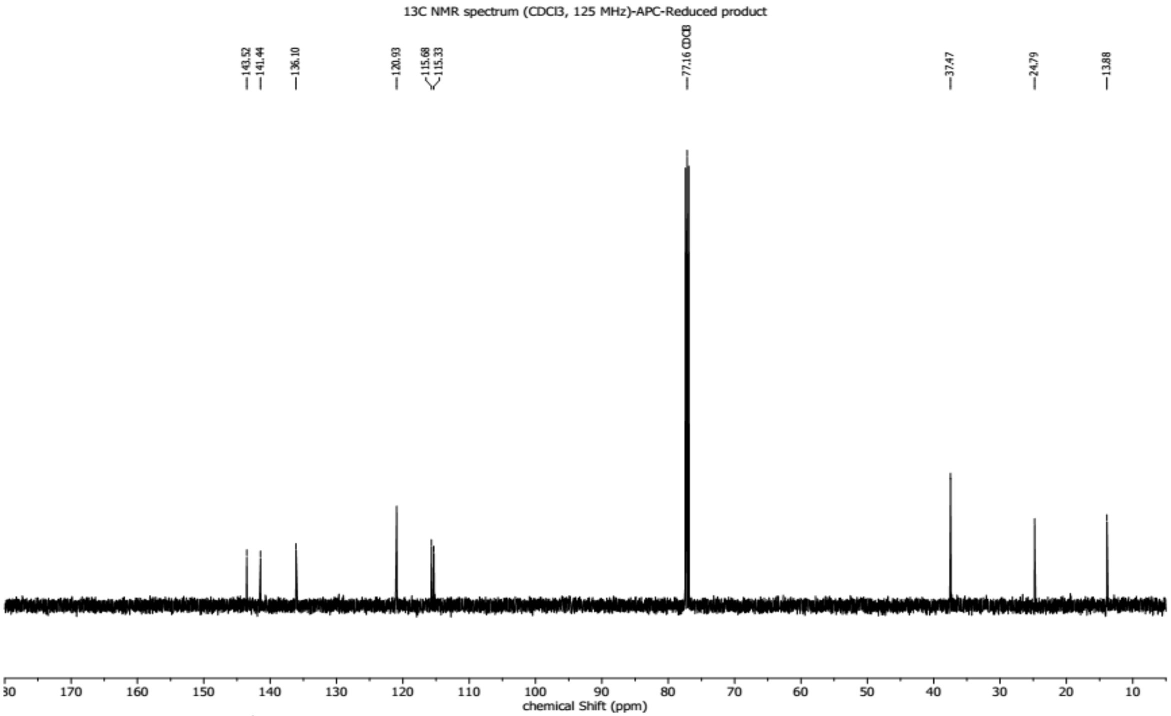


Figure S30: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of dihydro-allyl pyrocatechol (10).

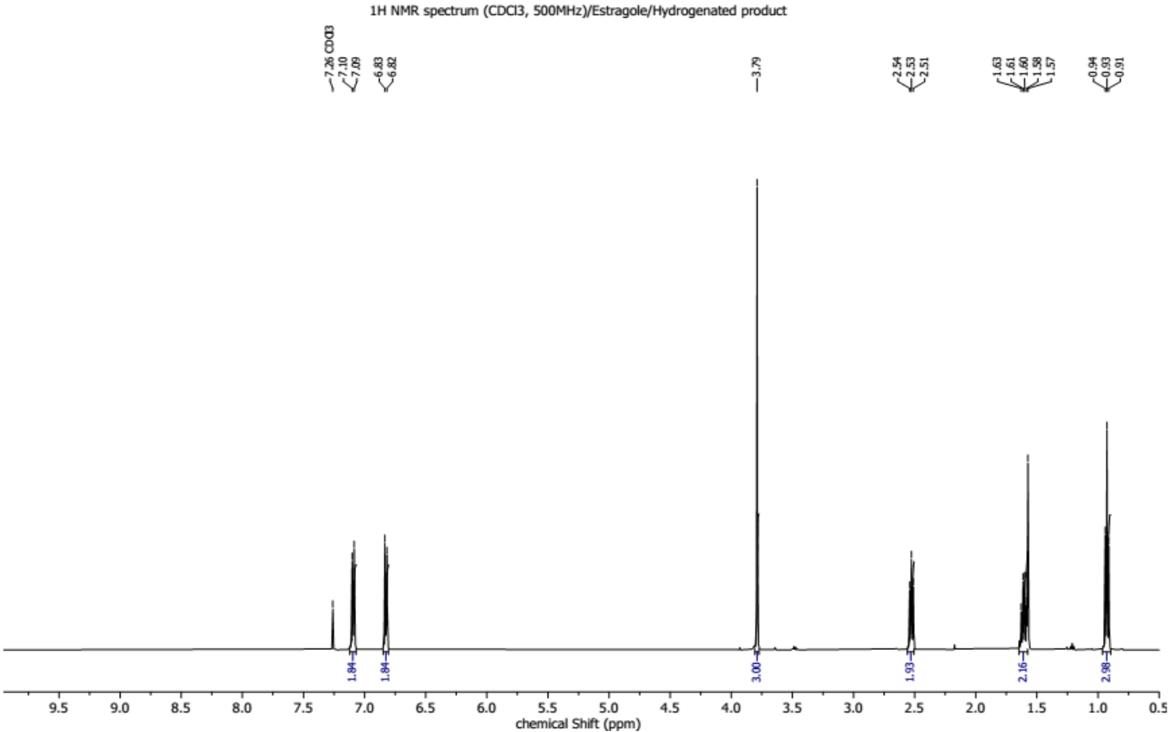


Figure S31: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of dihydro-anethole (11) or dihydro-estragole (12).

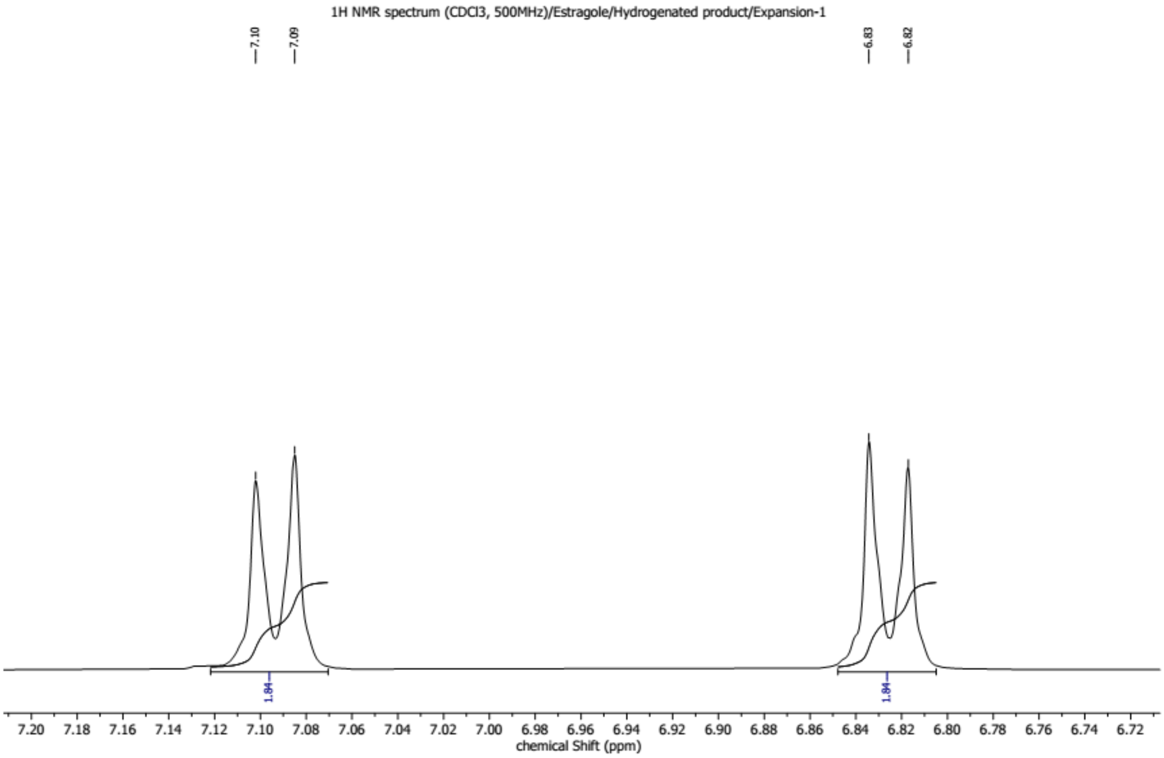


Figure S32: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of dihydro-anethole (11) or dihydro-estragole (12).

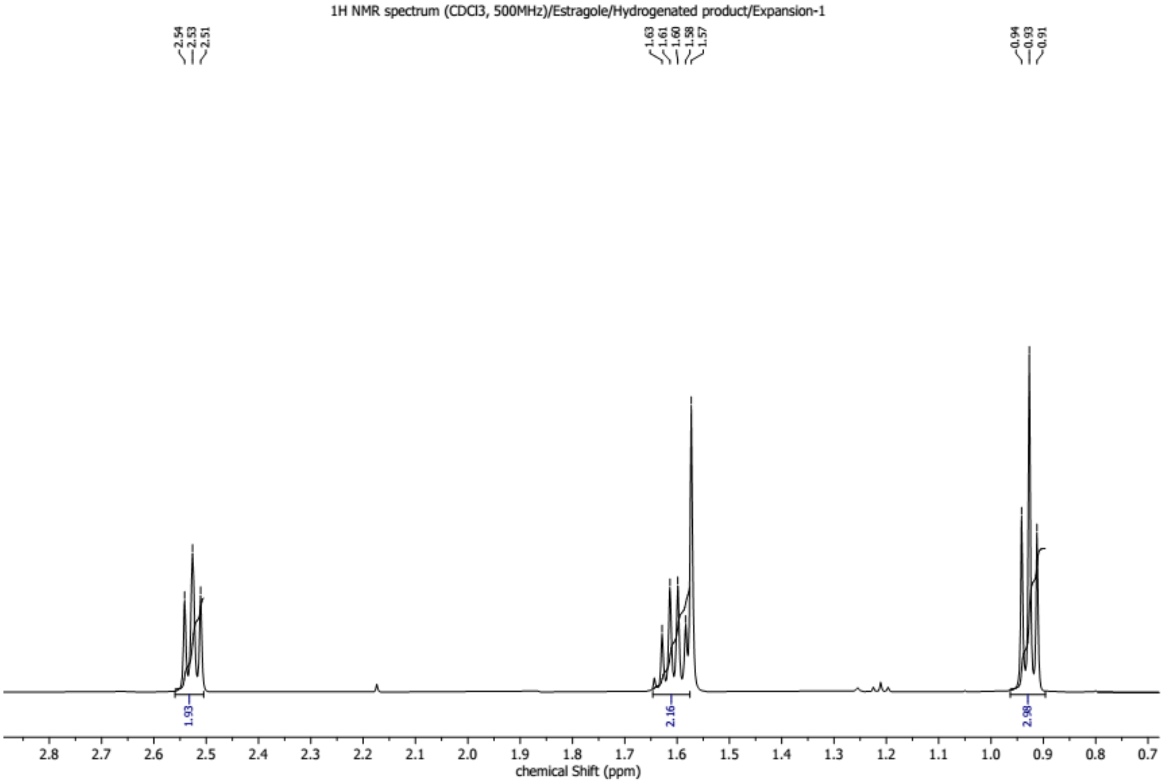


Figure S33: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of dihydro-anethole (11) or dihydro-estragole (12).

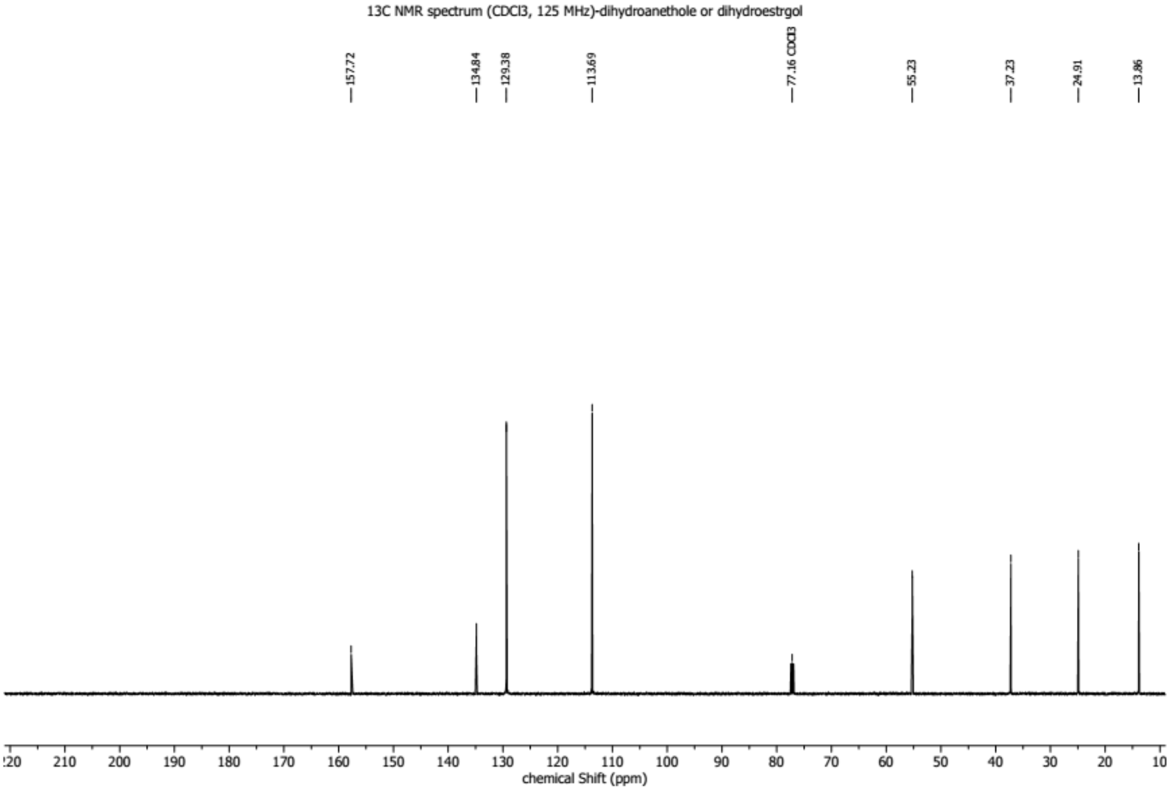


Figure S34: ^{13}C NMR spectrum (CDCl$_3$, 125 MHz) of dihydro-anethole (11) or dihydro-estragole (12).

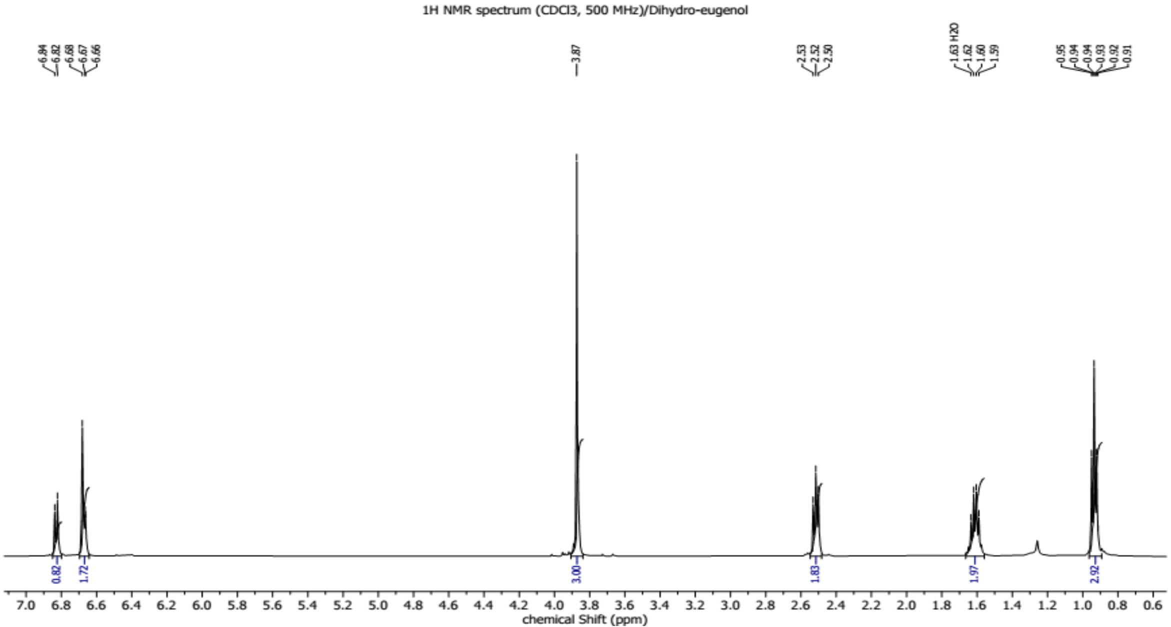


Figure S35: ^{1}H NMR spectrum (CDCl$_3$, 500 MHz) of dihydro-eugenol (13).

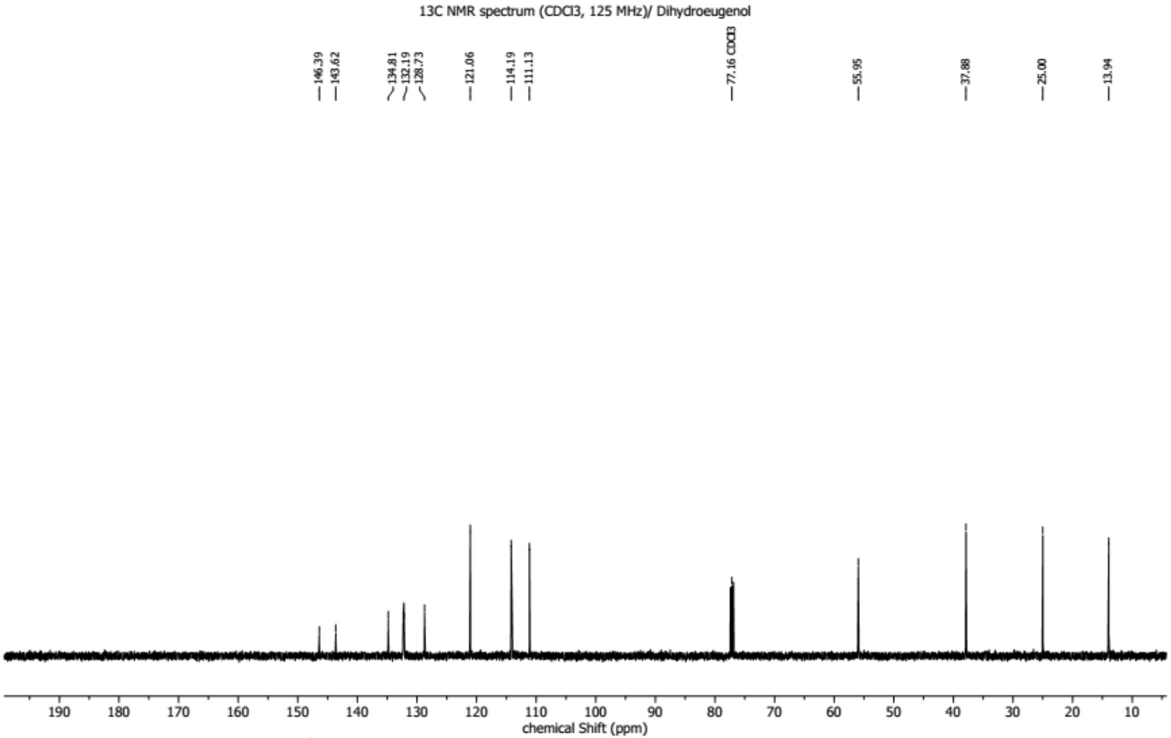


Figure S36: ^{13}C NMR spectrum (CDCl$_3$, 125 MHz) of dihydro-eugenol (13).

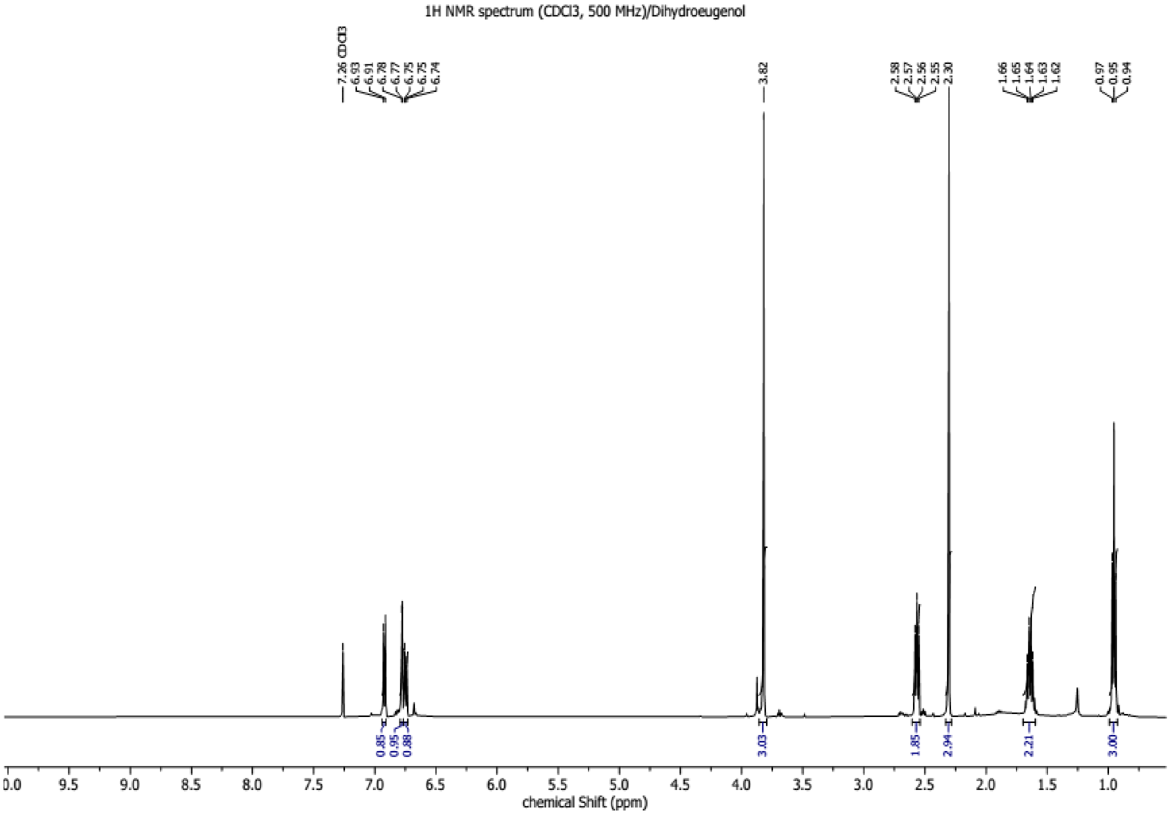


Figure S37: ^{1}H NMR spectrum (CDCl$_3$, 500 MHz) of dihydro-acetoxy eugenol (14).

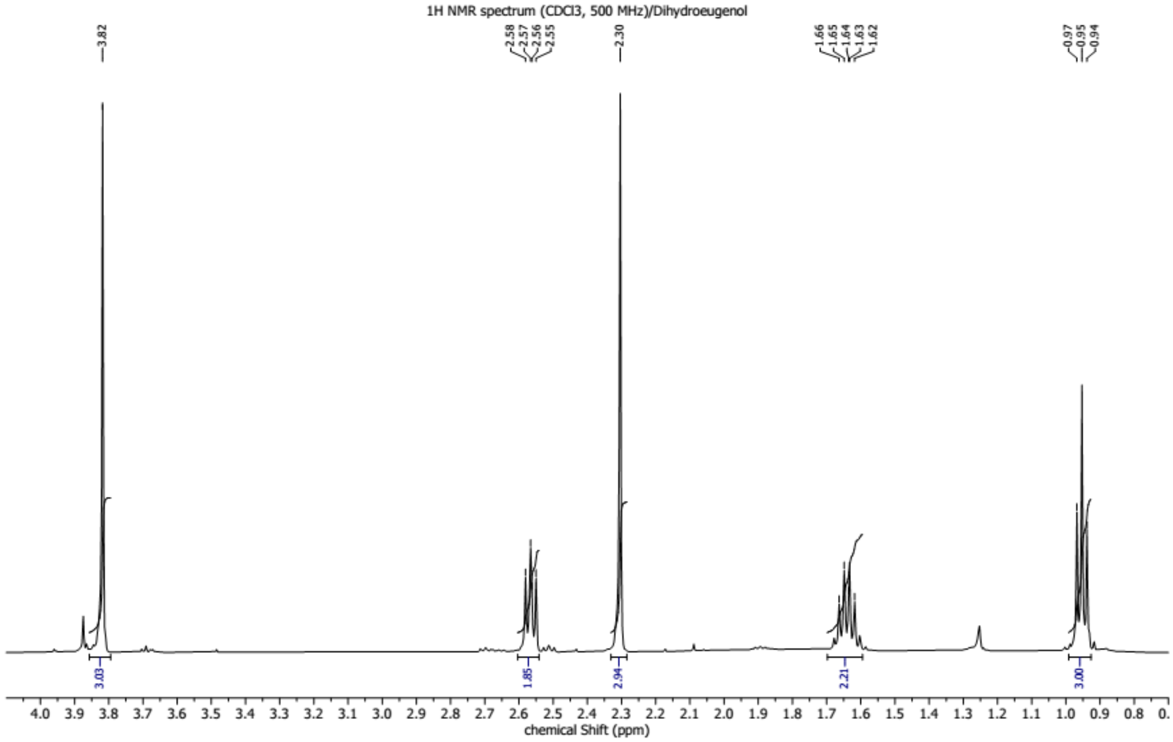


Figure S38: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of dihydro-acetoxy eugenol (14).

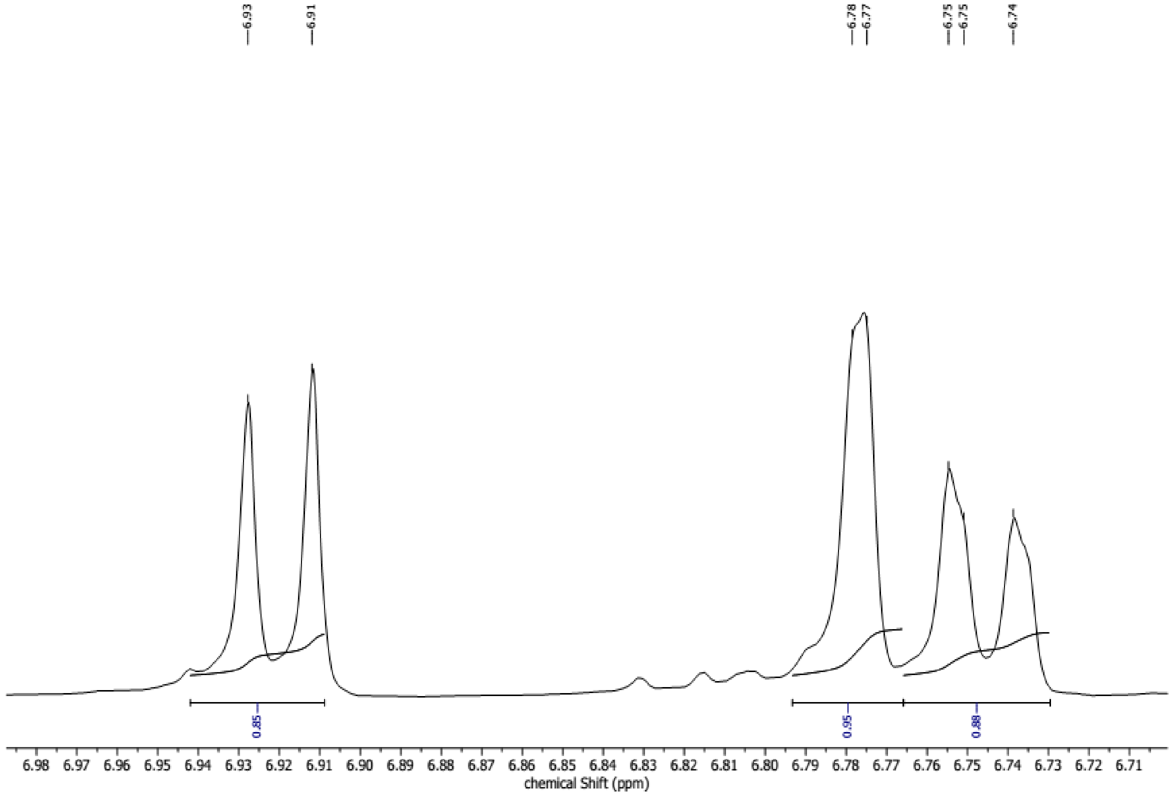


Figure S39: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of dihydro-acetoxy eugenol (14).

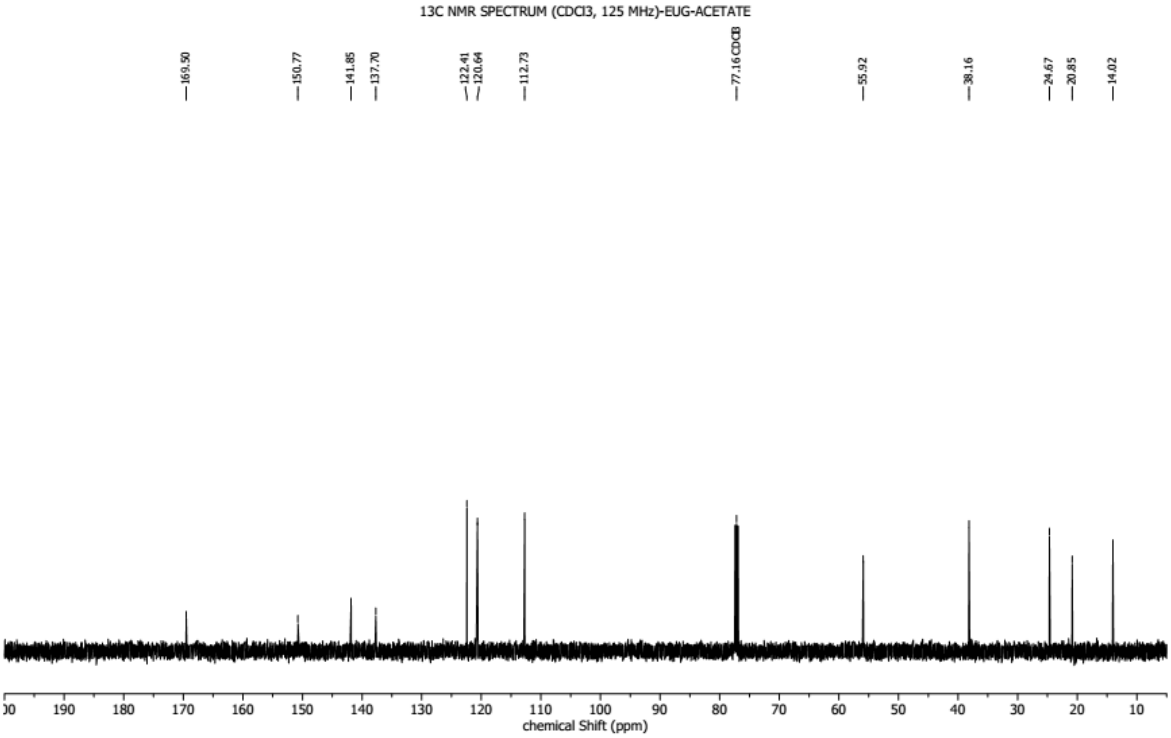


Figure S40: ^{13}**C NMR spectrum (CDCl$_3$, 125 MHz) of dihydro-acetoxy eugenol (14).**

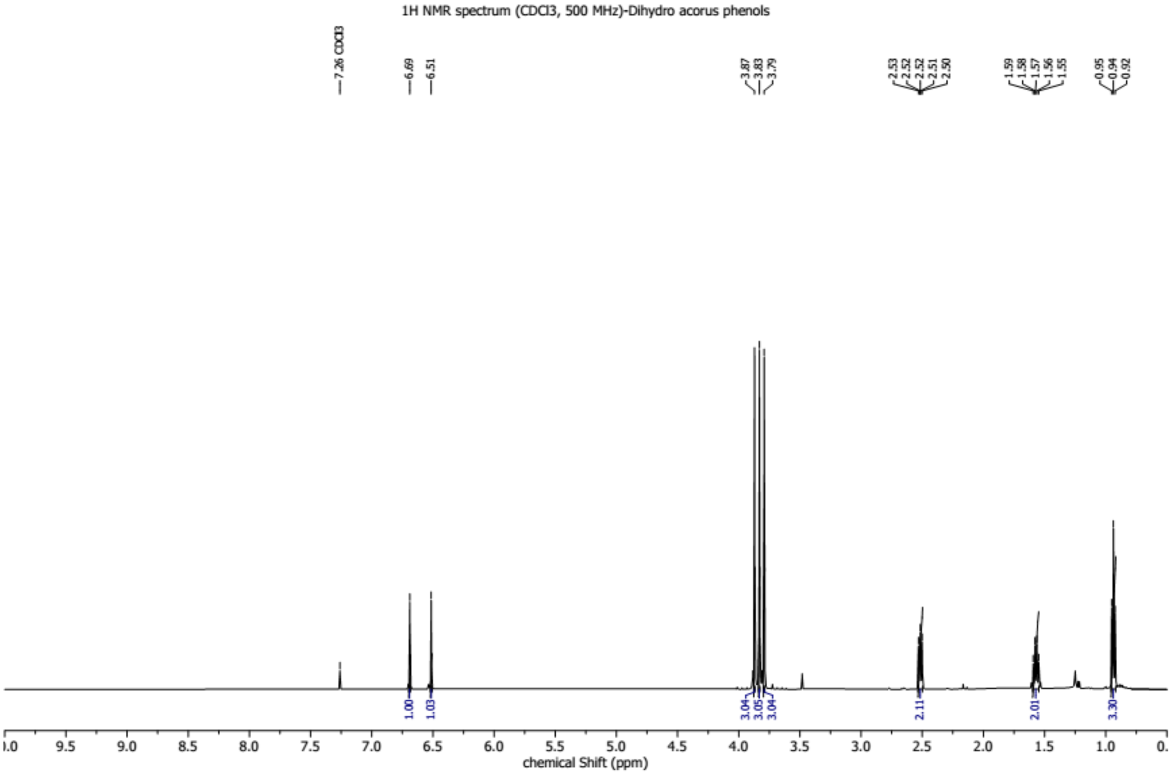


Figure S41: ^{1}**H NMR spectrum (CDCl$_3$, 500 MHz) of dihydro-elemicin (15).**

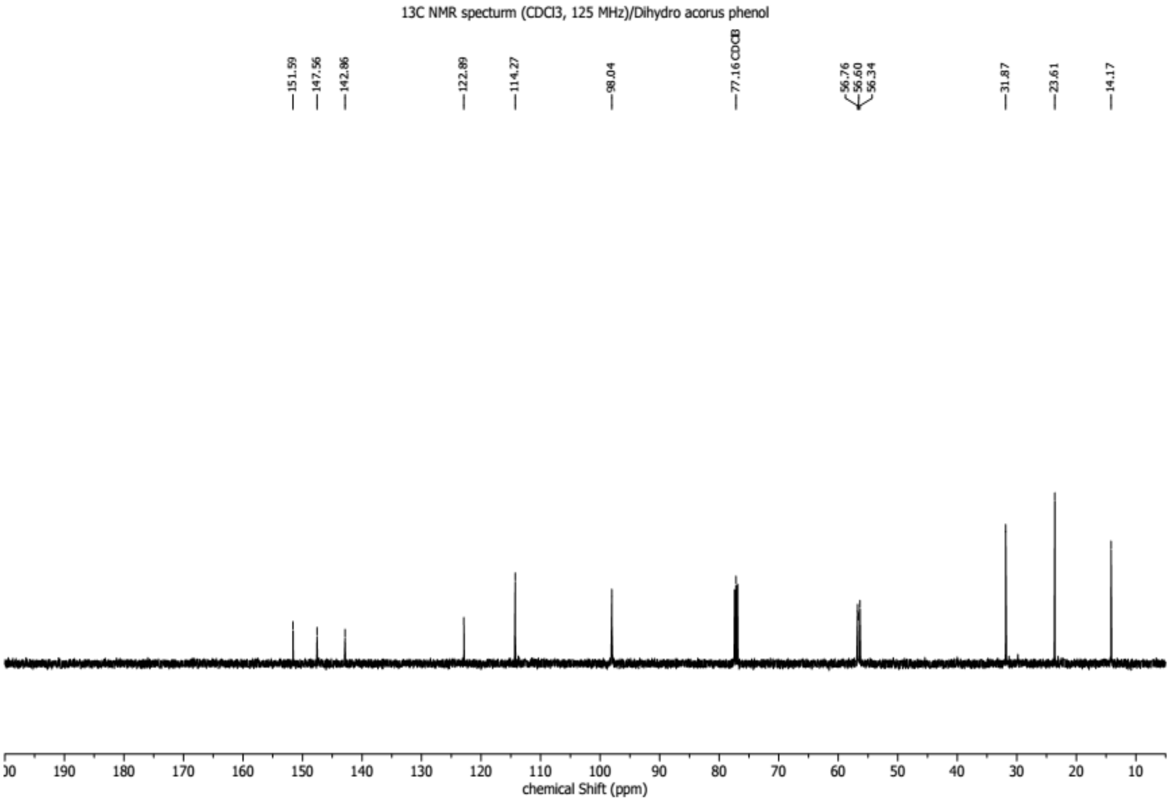


Figure S42: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of dihydro-elemicin (15).

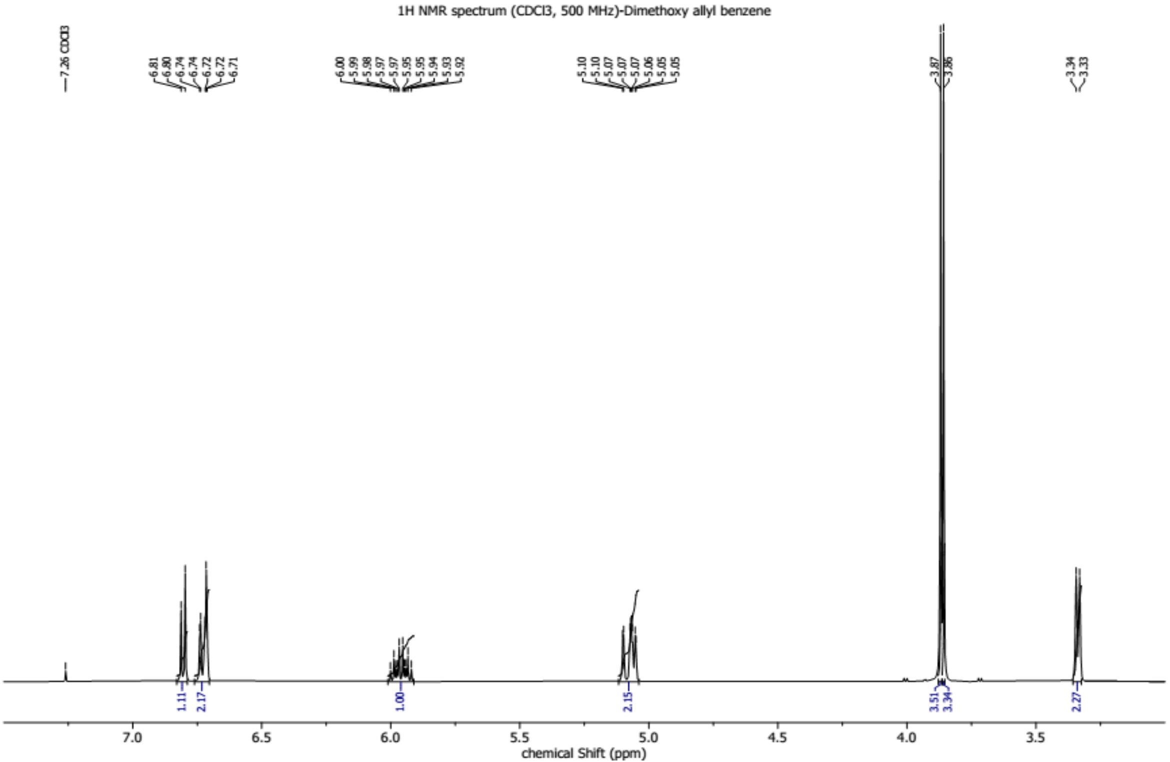


Figure S43: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of dimethyl ally pyrocatechol (16/17).

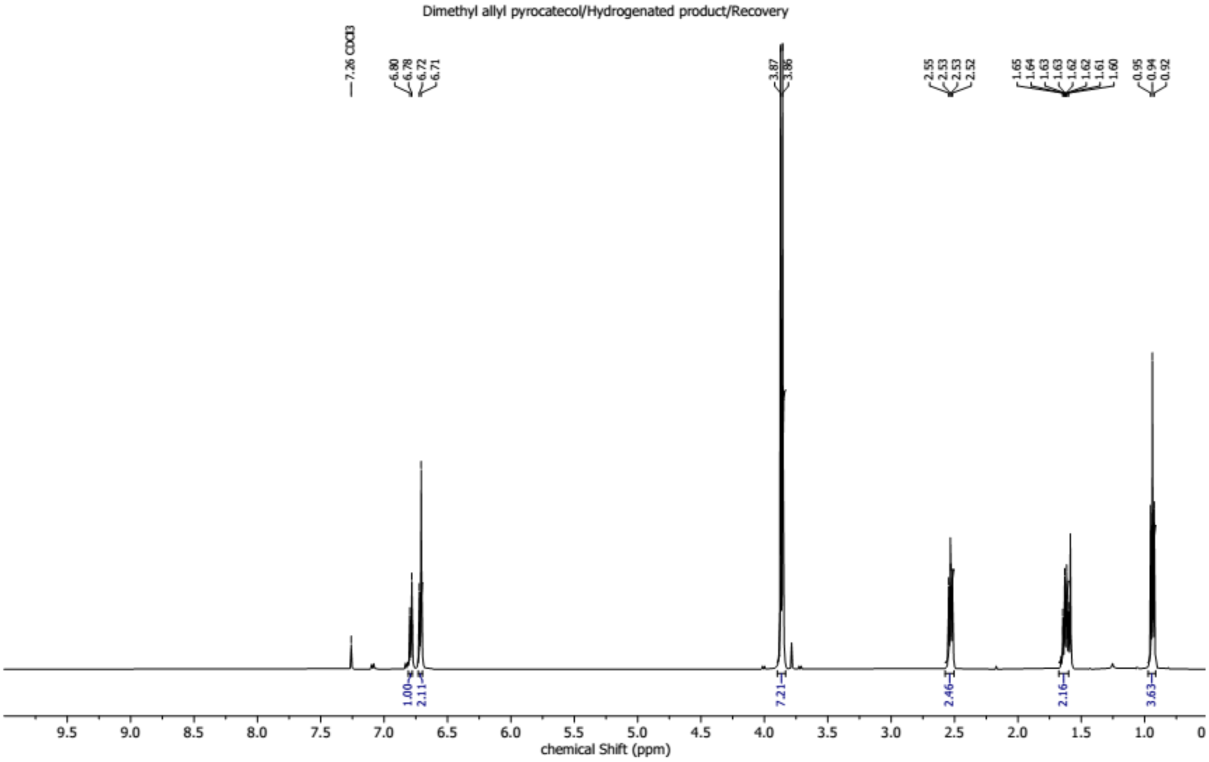


Figure S44: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of dihydro dimethoxy propyl benzene (derivative of pyrocatechol 16/17).

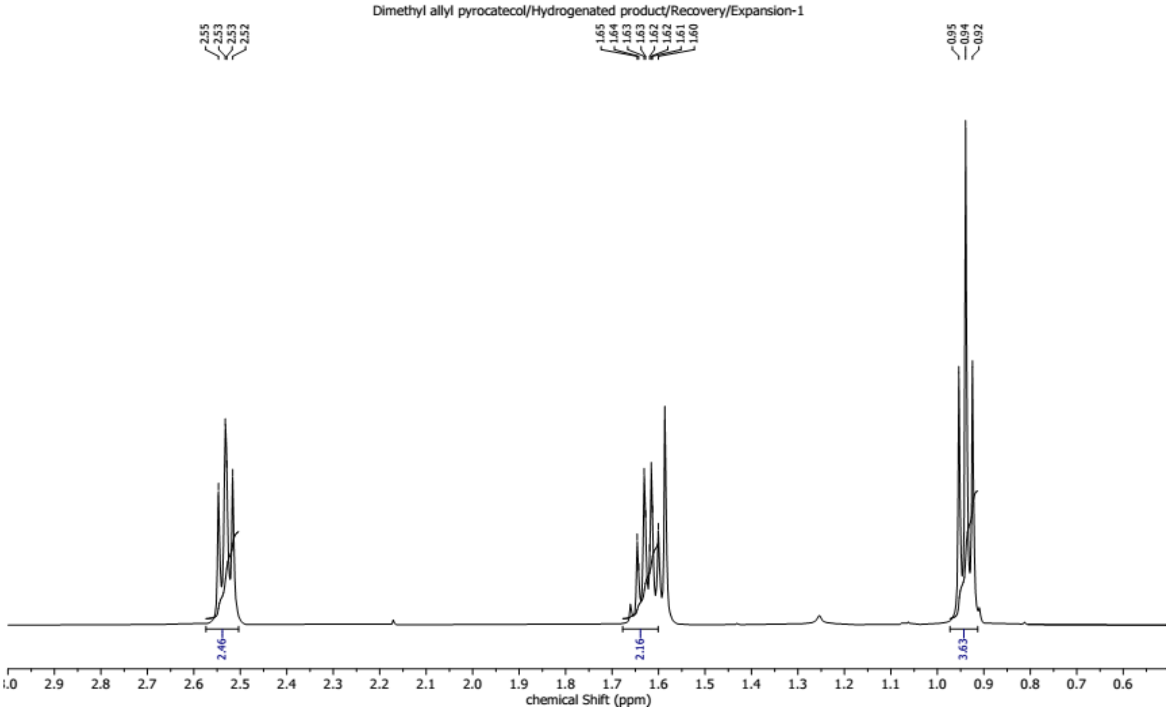


Figure S45: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of dihydro dimethyl propyl benzene (derivative of pyrocatechol, 16/17).

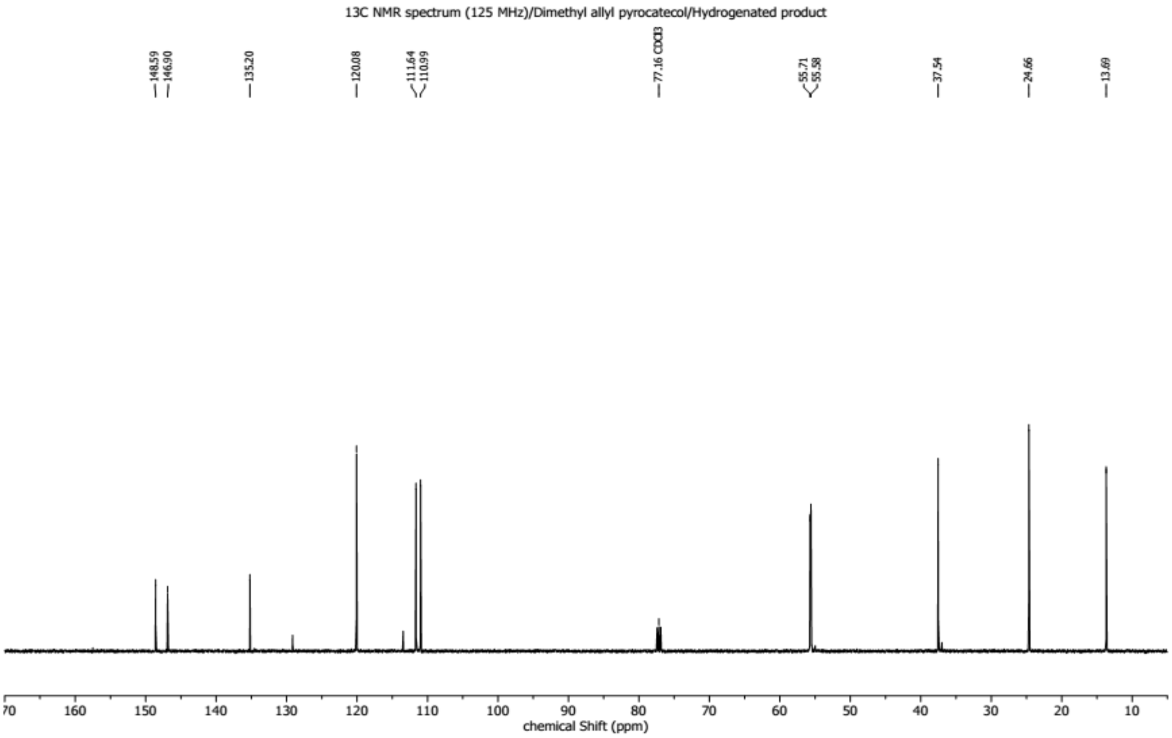


Figure S46: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of dihydro-dimethoxy allyl pyrocatechol (16/17).

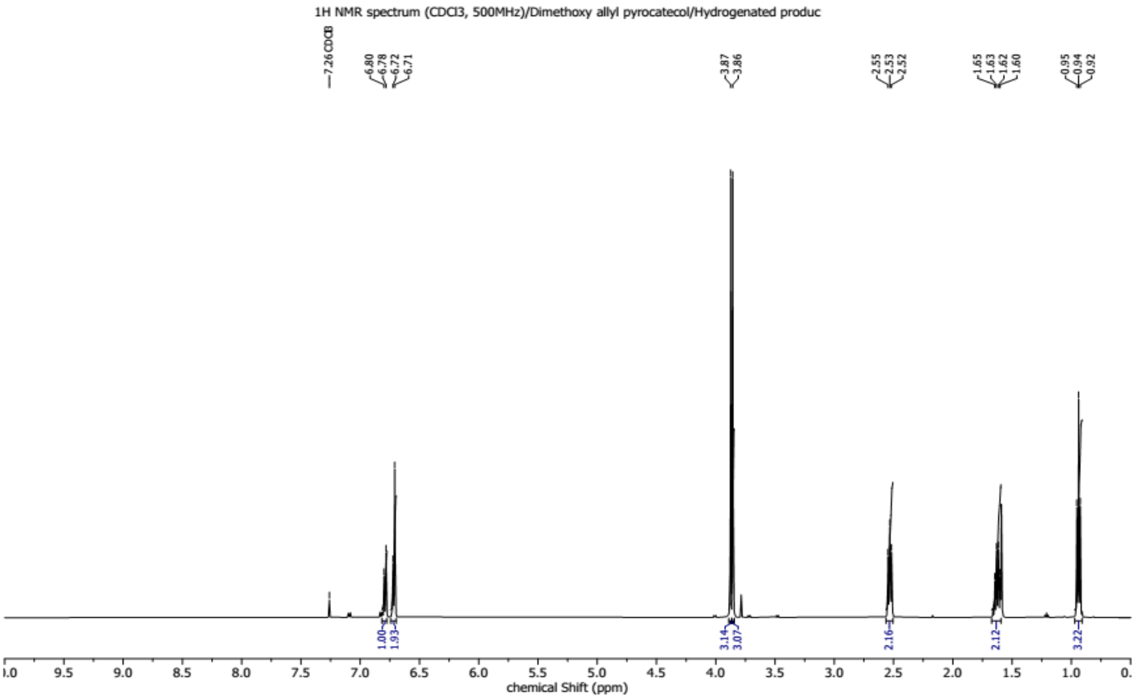


Figure S47: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of dihydro-dimethoxy allyl pyrocatechol (18/19).

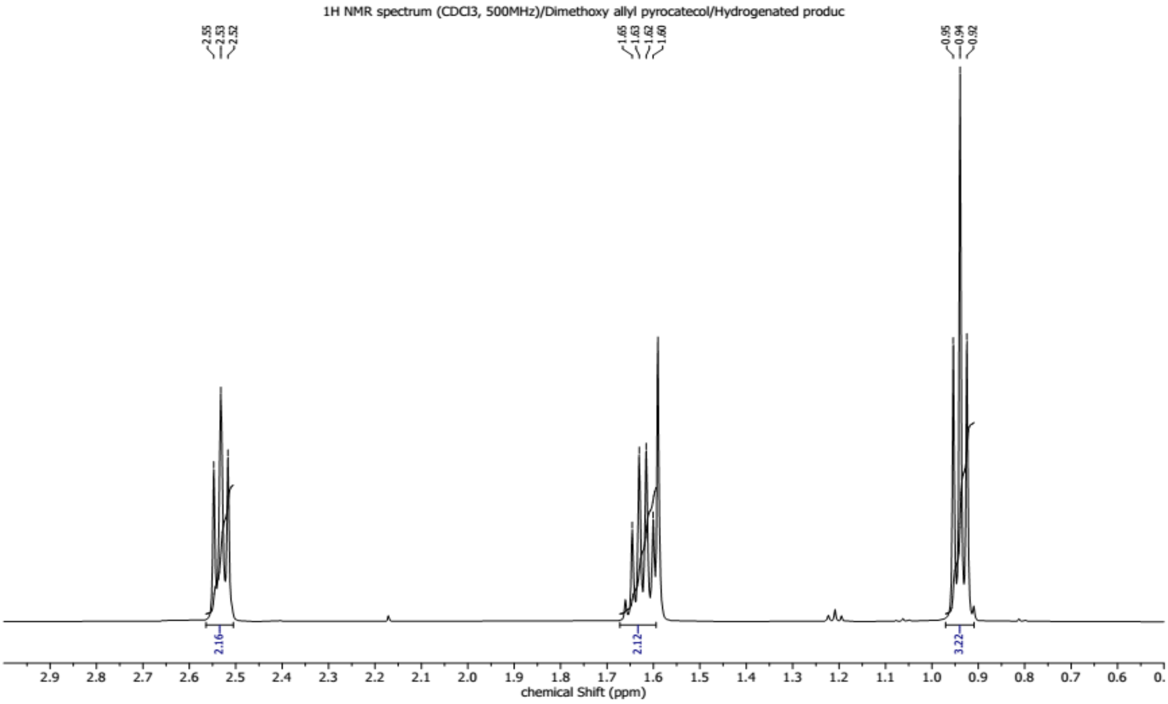


Figure S48: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of dihydro dimethoxy allyl pyrocatechol (18/19).

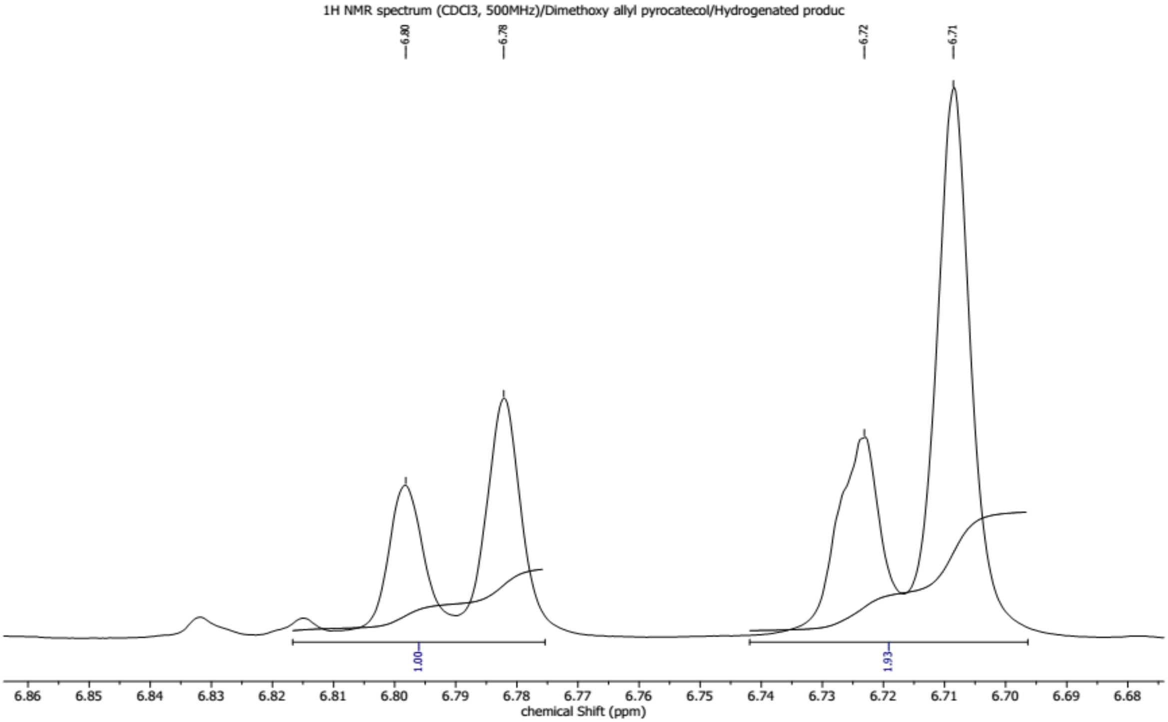


Figure S49: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of dihydro-dimethoxy allyl pyrocatechol (18/19).

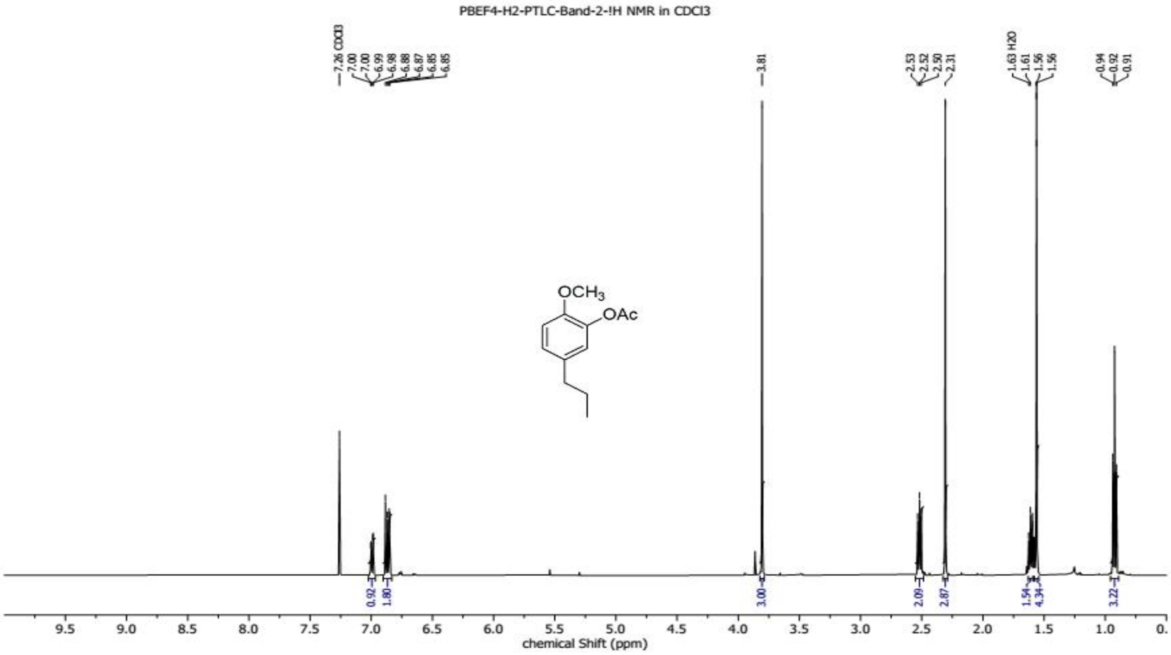


Figure S50: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of acetoxyl chevibetol (20).

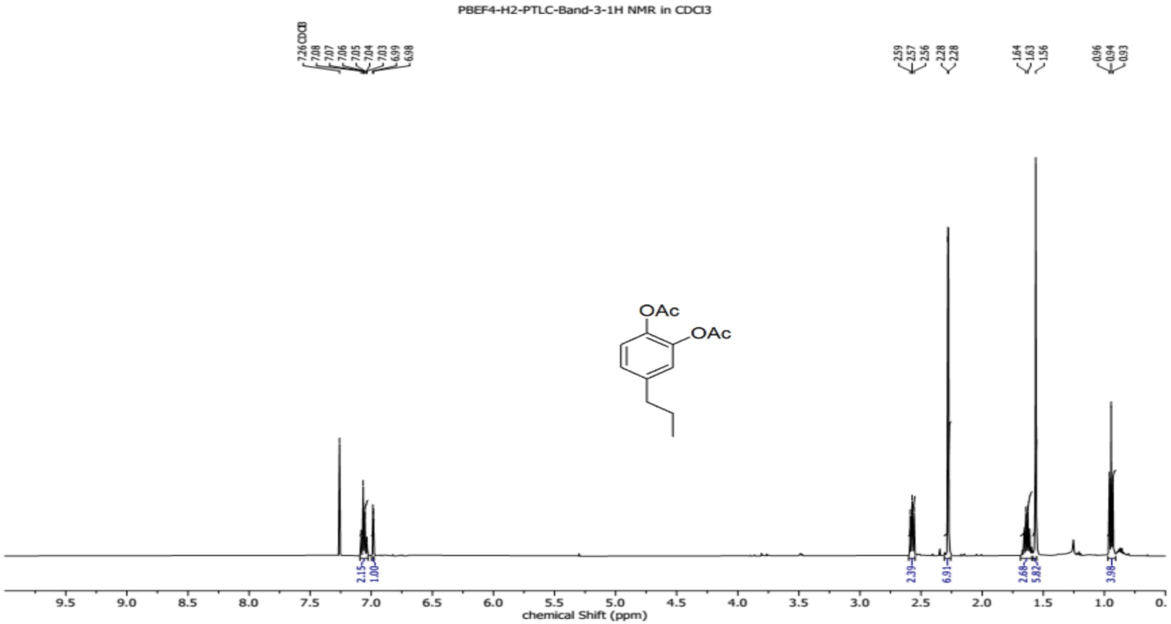


Figure S51: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of dihydro-allyl pyrocatechol (21).

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SUPPLIMENTARY INFORMATION

Supplementary information is available for this paper in ANNEXURE 1.

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AUTHOR CONTRIBUTION STATEMENT

AKB performed isolation, structural characterization, chemical transformation/reaction and drafted manuscript. IC and ESA contributed anti-proliferative assay on different carcinoma cell lines. SF contributed analysis tools and analyzed the XRD data of crystals, structure analysis and computation. EJCB supervised biological experimental, analysed anti-proliferative assay data, reviewed the manuscript, and manage acquiring HRESI-MS and LR ESI-MS data.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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