Biological Activity of Chemical Constituents from *Cinnamomum porrectum* (Roxb.) Kosterm

Pornpimoll Muanchaichum

A Thesis Submitted in Partial Fulfillment of the Requirements for the

Degree of Master of Science in Applied Chemistry

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Author Miss Pornpimoll Muanchaichum

Major Program Applied Chemistry

Major Advisor

Parinuch Chumkaew

(Assoc. Prof. Dr. Parinuch Chumkaew)

Co-advisor

Saranyoo Klaiklay

(Asst. Prof. Dr. Saranyoo Klaiklay)

Co-advisor

Jaraslak Pechwang

(Asst. Prof. Dr. Jaraslak Pechwang)

Examining Committee :

.....Chairperson

(Asst. Prof. Dr. Sopa Chewchanwuttiwong)

Parinuch Chumkaew .....Committee

(Assoc. Prof. Dr. Parinuch Chumkaew)

Saranyoo Klaiklay .....Committee

(Asst. Prof. Dr. Saranyoo Klaiklay)

Jaraslak Pechwang .....Committee

(Asst. Prof. Dr. Jaraslak Pechwang)

Nisakorn Saewan .....Committee

(Assoc. Prof. Dr. Nisakorn Saewan)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Science Degree in Applied Chemistry

.....

(Asst. Prof. Dr. Kawinbhat Sirikantisophon)

Acting Dean of Graduate School

This is to certify that the work here submitted is the result of the candidate's own investigation. Due acknowledgement has been made of any assistance received.

Parinuch Chumkaew Signature

(Assoc. Prof. Dr. Parinuch Chumkaew)

Major Advisor

Pornpimoll Muanchaichum Signature

(Miss Pornpimoll Muanchaichum)

Candidate

I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

Pornpimoll Muanchaichum Signature

(Miss Pornpimoll Muanchaichum)

Candidate

ชื่อวิทยานิพนธ์ ฤทธิ์ทางชีวภาพขององค์ประกอบทางเคมีจากต้นเทพทาโร
ผู้เขียน นางสาวพรพิมล หมื่นไชยชุม
สาขา เคมีประยุกต์
ปีการศึกษา 2566

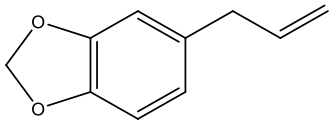
บทคัดย่อ

เทพทาโร มีชื่อทางวิทยาศาสตร์ *Cinnamomum porrectum* (Roxb.) Kosterm. จัดเป็นสมุนไพรที่มีกลิ่นหอม ส่วนใหญ่กระจายพันธุ์อยู่ทางภาคใต้ของประเทศไทย และมีสรรพคุณทางยา เช่น แก้ท้องร่วง ท้องอืด ท้องเฟ้อ แก้วิงเวียน แก้อาเจียน การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อแยกสารบริสุทธิ์จากส่วนรากและเปลือกของเทพทาโร และการยับยั้งเชื้อแบคทีเรีย เชื้อราก่อโรคในพืช และฤทธิ์ต้านอนุมูลอิสระของสารสกัดหยาบและสารบริสุทธิ์จากรากและเปลือกของเทพทาโร โดยนำส่วนรากและเปลือกเทพทาโรมาหมักด้วยตัวทำละลายไดคลอโรมีเทนและอะซิโตน ผลการศึกษาพบว่าสามารถแยกสารบริสุทธิ์จากสารสกัดหยาบรากและเปลือก ประกอบด้วยสารอนุพันธ์ฟีนิลโพรพานอยด์ จำนวน 6 สารคือ Safrole (**CP1**), 3,4 Methylenedioxy cinnamaldehyde (**CP2**), n-Butyl ferulate (**CP3**), 3-(3,4-Methylenedioxyphenyl)-1,2-propanediol (**CP4**), Elemicin (**CP5**) และ 3,4-(Methylenedioxy) cinnamyl Alcohol (**CP6**) การวิเคราะห์โครงสร้างของสารประกอบเหล่านี้โดยอาศัยข้อมูล ^{1}H และ ^{13}C NMR สเปกโทรสโกปีและเปรียบเทียบกับข้อมูลของสารประกอบที่มีการรายงานแล้ว

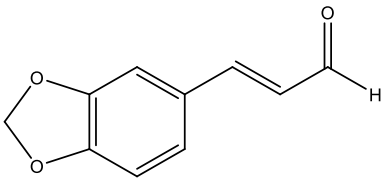
สารสกัดหยาบไดคลอโรมีเทน อะซิโตน และสารประกอบทั้ง 6 สาร ได้รับการยับยั้งเชื้อแบคทีเรีย 4 สายพันธุ์ คือ *Staphylococcus aureus* (ATCC25923), *Bacillus cereus* (ATCC11778) *Pseudomonas aeruginosa* (ATCC27853) และ *Escherichia coli* (ATCC25922) พบว่า สารสกัดหยาบไดคลอโรมีเทนจากรากและเปลือก แสดงบริเวณการยับยั้งเชื้อ *B. cereus* ได้ดีที่ค่า 22.33 และ 17.00 มิลลิเมตร ตามลำดับ และ CP6 แสดงฤทธิ์ในการยับยั้งเชื้อ *S. aureus* และ *B. cereus* ที่มีค่า MIC เท่ากับ 1,000 และ 2,000 ไมโครกรัม/มิลลิลิตร ตามลำดับ และรองลงมาคือ CP4 แสดงศักยภาพในการยับยั้งเชื้อ *S. aureus* และ *B. cereus* ที่มีค่า MIC เท่ากันที่ 2,000 ไมโครกรัม/มิลลิลิตร สำหรับการยับยั้งเชื้อราสาเหตุโรคพืช พบว่า สารสกัดหยาบไดคลอโรมีเทนจากรากเทพโรที่ความเข้มข้น 10, 5, 1 มิลลิกรัม/มิลลิลิตร สามารถยับยั้งเชื้อรา *A. alternata* ได้ดีที่สุด

ที่ 48.86 – 100.00 เปอร์เซ็นต์ และสารประกอบทั้ง 6 ชนิด แสดงฤทธิ์ต้านเชื้อราที่ต่ำ โดยมีค่า MIC สูงกว่า 100 ไมโครกรัม/มิลลิลิตร สำหรับการทดสอบฤทธิ์ต้านอนุมูลอิสระด้วยวิธี DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity assay ผลการทดสอบพบว่า CP3 มีฤทธิ์ต้านอนุมูลอิสระได้ดีที่สุด โดยมีค่า IC_{50} เท่ากับ 55.52 ไมโครกรัม/มิลลิลิตร

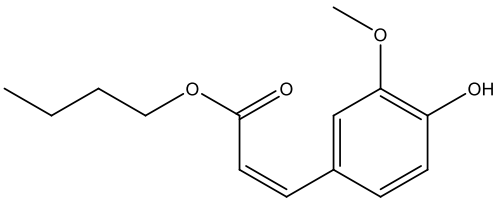
คำสำคัญ : ต้นเทพทาโร ฤทธิ์ต้านเชื้อแบคทีเรีย ฤทธิ์ต้านอนุมูลอิสระ อนุพันธ์ฟีนิลโพรพานอยด์ เชื้อราสาเหตุโรคพืช



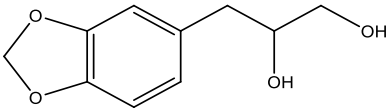
Safrole (**CP1**)



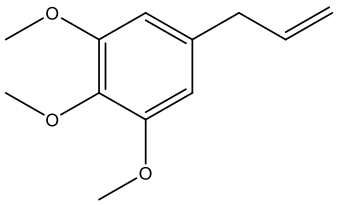
3,4 Methylenedioxy cinnamaldehyde (**CP2**)



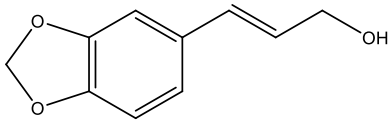
n-Butyl ferulate (**CP3**)



3-(3,4-Methylenedioxyphenyl)-1,2-propanediol (**CP4**)



Elemicin (**CP5**)



3,4-(Methylenedioxy) cinnamyl Alcohol (**CP6**)

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Major Program Applied Chemistry

Academic Year 2023

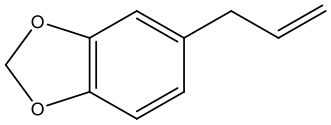
ABSTRACT

Cinnamomum porrectum (Roxb.) Kosterm (Theptaro) is a aromatic tree. Mostly of the species are in the throughout southern Thailand and have medicinal properties such as helping to resolve diarrhea, flatulence, dizziness and vomiting. The purpose of this study was to isolate pure compounds from crude extracts the roots and barks of *C. porrectum* and assess for antibacterial activities, plant pathogenic fungal and antioxidant activity of crude extracts and pure compounds from the roots and barks. Air-dried roots and bark of *C. porrectum* to maceratied with dichloromethane and acetone. The results revealed that the pure compounds could be isolated from crude extracts the roots and barks and contains phenylpropanoid derivatives. Six compounds were isolated including Safrole (**CP1**), 3,4 Methylenedioxy cinnamaldehyde (**CP2**), n-Butyl ferulate (**CP3**), 3-(3,4 Methylenedioxy-phenyl)-1,2-propanediol (**CP4**), Elemicin (**CP5**) and 3,4-(Methylenedioxy) cinnamyl Alcohol (**CP6**). Their structures were characterized of these compounds based on ^{1}H and ^{13}C NMR spectroscopy data and compared with data of the previously reported.

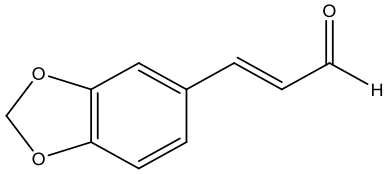
The crude extracts of dichloromethane, acetone and all six compounds have been evaluated for antibacterial activity of 4 bacteria species: *Staphylococcus aureus* (ATCC25923), *Bacillus cereus* (ATCC11778), *Pseudomonas aeruginosa* (ATCC27853) and *Escherichia coli* (ATCC25922). The results revealed that dichloromethane extracts of the roots and barks were showing a good inhibition in the region of *B. cereus* (ATCC11778) values of 22.33 and 17.00 mm respectively. CP6 showed activity in inhibiting *S. aureus* and *B. cereus* with MIC values of 1,000 and 2,000 µg/ml respectively. more effectively than CP4 which showed inhibition potential

against *S. aureus* and *B. cereus* with equal MIC values of 2000 µg/ml for antifungal activity. To investigate the plant pathogenic fungal, The results were found that dichloromethane root extracts from *C. porrectum* at concentrations of 10, 5, 1 mg/ml showed best activities to inhibit the growth of *A. alternata* at 48.86 – 100.00 percent. All compounds exhibited weak activity against *A. alternata* with MIC values of greater than 100 µg/ml. Antioxidant activity assays by using DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity assay method, test results found that n-Butyl ferulate has the best antioxidant activity. The inhibitory concentration at 50%, IC_{50} values of CP3 were 55.52 mg/ml.

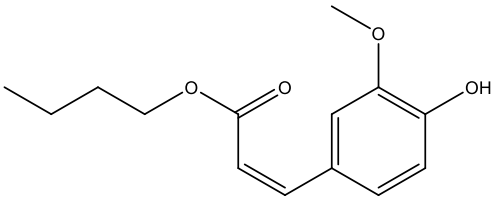
Keywords: *Cinnamomum porrectum* (Roxb.) Kosterm, Antibacterial activity Antioxidant activity, Phenylpropanoids, Plant pathogenic fungal



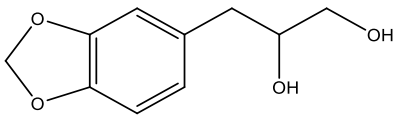
Safrole (**CP1**)



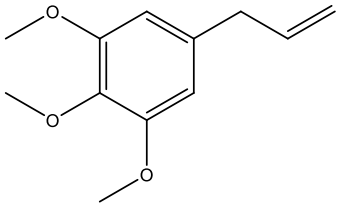
3,4 Methylenedioxy cinnamaldehyde (**CP2**)



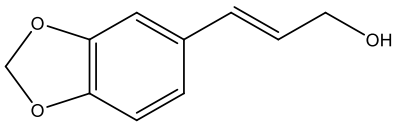
n-Butyl ferulate (**CP3**)



3-(3,4-Methylenedioxyphenyl)-1,2-propanediol (**CP4**)



Elemicin (**CP5**)



3,4-(Methylenedioxy) cinnamyl Alcohol (**CP6**)

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LIST OF ABBREVIATIONS AND SYMBOLS

s	=	singlet
d	=	doublet
t	=	triplet
q	=	quartet
m	=	multiplet
brs	=	broad singlet
brd	=	broad doublet
brt	=	broad triplet
brqn	=	broad quintet
brm	=	broad multiplet
dd	=	doublet of doublet
dt	=	doublet of triplet
dq	=	doublet of quartet
dm	=	doublet of multiplet
td	=	triplet of doublet
tm	=	triplet of multiplet
tq	=	triplet of quartet

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

J	=	coupling constant
R_f	=	retention factor
g	=	gram
mg	=	milligram
mL	=	milliliter
L	=	liter
cm^{-1}	=	reciprocal centimeter (wavenumber)
nm	=	nanometer
ppm	=	part per million
Hz	=	Hertz
MHz	=	megahertz
[α]	=	specific rotation
Conc.	=	concentration
TLC	=	thin-layer chromatography
UV	=	Ultraviolet wavelength
FT-IR	=	Fourier Transform Infrared
NMR	=	Nuclear Magnetic Resonance
1D NMR	=	One Dimensional Nuclear Magnetic Resonance

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

2D NMR	=	Two Dimensional Nuclear Magnetic Resonance
HMQC	=	Heteronuclear Mutiple Quantum Coherence
HMBC	=	Heteronuclear Mutilple Bond Correlation
DEPT	=	Distortionless Enhancement by Polarization Transfer
COSY	=	Correlation Spectroscopy
Acetone-d_6	=	hexadeuteroacetone
$CDCl_3$	=	deuterochloroform
CD_3OD	=	tetradeuteromethanol
$CHCl_3$	=	chloroform
CH_2Cl_2	=	dichloromethane
%	=	percentage (pencentage rate)
MeOH	=	methanol
EtOH	=	ethanol
EtOAc	=	ethyl acetate
IC_{50}	=	half-maximal inhibitory concentration
MICs	=	Minimal inhibitory concentration
MHA	=	Mueller Hinton agar
MHB	=	Mueller Hinton broth

CHAPTER 1

INTRODUCTION

1.1 Introduction

Infectious diseases are one of the major public health issues in Thailand. These infectious diseases include: infectious diseases of the skin, gastrointestinal infections, respiratory infections, urinary tract infections and more these diseases are caused by various microorganisms such as *Escherichia coli* and *Staphylococcus aureus* etc. Due to the increasing number of antimicrobial resistance that are a major cause of death make the opportunity to cure these infections a priority. This made the search for natural products to cure these infections more urgent. Because natural products are biodiversity, the chance of germs becoming resistant to the drugs are lower than the use of synthetic chemical drugs. In the past, modern synthetic chemical drugs were introduced Thai people used a lot of medicinal herbs. Medicinal herbs produce a large number of secondary metabolites that have Antibacterial activity.

Cinnamomum porrectum (Roxb.) Kostem., (common name Thep tharo) (The Forest Herbarium, Royal Forest Department, 2001), is a rare herbal and aromatic tree mostly intersperse throughout southern Thailand. All parts of the plant were used alone or in combination and have antimicrobial properties. A variety of chemicals that plants produce are likely to play a crucial role to protect from infectious diseases, the composition of these antimicrobial compounds are found in various parts of the plant: bark, leaves, fruit, roots, flowers and seeds.

Many researchers interested in the *C. porrectum* is classified as a fragrant herb and has high medicinal properties and has been used by local people by boiling wood or bark with drinking water and is used as a remedy for diarrhea, flatulence, indigestion, dizziness, vomiting, and curing dysentery, also the oil squeezed from the fruit can be applied during to relieve pain, sprains, relieve rash, swelling, cure inflammation, cure insect bites, the oil can also be applied on burns, hot water scalds. In the generic drug list in the year 2013, *C. porrectum* has been placed into the

Carminative drug group. The medicine uses the bark or wood boiled in water to relieve flatulence, and to help strengthen the body it is also in the group of laxatives as well as for pain relieve and the relief of Hemorrhoids.

C. porrectum has been reported to biological activity including antimicrobial, antioxidant, antileukemic and antiproliferative activity (Palanuvej et al., 2006 Uthairatsamee et al., 2011 Adfa et al., 2015 Villegas et al., 2011)

There are studies aiming to research and continue to understand about medicinal plants that inhibit microorganisms and antioxidant to find natural extracts to replace and prevent microorganisms and antioxidant. It is another important option in the search for new drugs derived from natural plants to replace the production of drugs from synthetic chemicals.

1.2 Objective

1.2.1 To extract, separate and purify chemical constituents from the *Cinnamomum porrectum* (Roxb.) Kosterm roots and analyze the structure of the pure compounds

1.2.2 To test the antimicrobial activity of crude extracts and pure compounds against pathogenic bacteria and plant pathogenic fungi.

1.2.3 To test the antioxidant of pure compounds.

1.3 Expected Benefits

1.3.1 The pure compounds can be extracted from the root of the *C. porrectum* and the structure of the pure compounds can be known.

1.3.2 Expect to find natural products that inhibit bacteria and fungi. developed to be It is beneficial to public health and agriculture in the future.

1.3.3 In the study, investigation of pure compounds from *C. porrectum* with antioxidant.

CHAPTER 2

LITERATURE REVIEW

2.1 *Cinnamomum porrectum* (Roxb.) Kosterm.

Botanical Name:	*Cinnamomum porrectum* (Roxb.) Kosterm, *Cinnamomum parthenoxylon* Meissn
Family:	Lauraceae
Common name:	Citronella laurel, True laurel
Local Thai name:	Thep-tha-ro (Chantaburi, Suratthani), Chuang, Chuang hom (Southern), Cha khai ton (Northeastern), Phlu ton khoa (Chiang Mai), Cha khai hom (Northern), Mue-dae-ka-ma-nging (Malay-Pattani), Karabun (Nong Khai) (Uthairatsamee, et al.,2011).
Synonyms	*Cinnamomum glanduliferum C. Nees, Camphora inodora Blume ex Miq. Camphora parthenoxy (Jack) Nees, Cinnamomum parthenoxylon (Jack.), Camphora pseudosassafras Miq., Cinnamomum inunctum (*Nees*) Meisn, Cinnamomum neesianum Meisn, Cinnamomum sumatranum (Miq.)* Meissner*, Cinnamomum penninervium Kosterm, Cinnamomum pseudosassafras Meisn, Laurus porrecta Roxb. Laurus pruinosa Reinw. ex Blume, Cinnamomum camphora (L.)* J.Presl*, Parthenoxylon porrectim (Roxb.) Blume, Phoebe larifolia Champ. ex Benth, Sassafras loureiroi Kostel,*

2.2 Botanical and characteristic of *C. porrectum*

In Thailand, C. porrectum is widely intersperse throughout the southern Thailand (The forest Association of Thailand, 1984; Chayamarit, 1997; Forest Research and Development Bureau, 2009), This genus contains small, evergreen trees and shrubs of 10–45 m tall and up to 105 cm in diameter. The bark is either smooth or slightly fissured and is usually reported to be fragrant (Figure 1 (a)). Leaves are sub-opposite to spiral; oval shaped or ovate-oblong, 2.5 – 6.5 cm x 5.5 – 20.5 cm; surface is smooth; base cuneate to rounded; curved leaf veins with 3 - 8 pairs of lateral veins, older leaves are red, tertiary venation reticulate, faint on both surfaces, with aromatic when crushed; petiole long 1.2- 3.5 cm (Figure 1 (b)). Flower glabrous is white or light yellow, aromatic (Figure 1 (c)). Fruit is globose is small size, across 0.8-1 cm, one-seeded drupe, and purple-black at maturity (Figure 1 (d)) (Lemmens et al., 1995; Chayamarit,1997) It is extensively intersperse and locally common in flatlands to mountain forest

In Thai folk medicine, the roots of *C. porrectum* is boiled, and its decoction is given after childbirth and also for treating fever. seed oil can also be used for arthritis. The bark and fruits are also used in perfumery. (Kumar et al., 2019) For treating inflammation gastritis, blood circulation, liver and spleen disorders. (Lee and Balick, 2005)

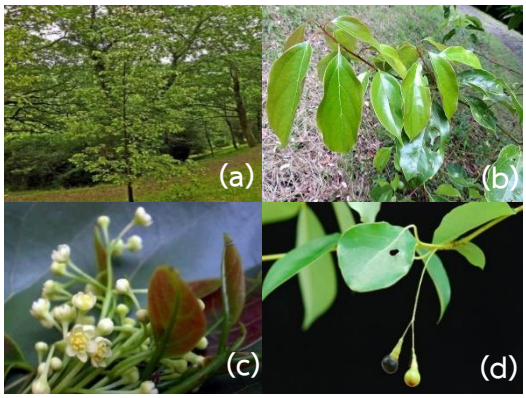


Figure 1. *Cinnamomum porrectum* stem (a), leaves (b), flowers(c), and fruits (d)

2.3 Chemical Constituents from *Cinnamomum* species

Cinnamomum belong to the Lauraceae family. The previous reports were demonstrated of the various substance such as flavonoids (Yang et al., 2017; Izadpanah et al., 2016; Huang et al., 2018) phenylpropanoids (Chen et al., 2015; Li et al., 2018; Chen et al., 2007) terpenes (Huang et al., 2018; Hao et al., 2015; Feng et al., 2016) lignans (Chen et al., 2015; Xu et al., 2016; Xu et al., 2015) aromatic compound (Zhou et al., 2016; Huang et al., 2018) in **Table 1.**

Table 1. Chemical Constituents from *Cinnamomum* Species

Species Resource	Investigated parts	Compounds	Bibliography
C. cassia	Bark	7,4′-Di-*O*-methyl-(+)-catechin, **F1**	Yang et al., 2017
		5,7,3′-Tri-*O*-methyl-(−)-epicatechin, **F2**	Xu et al., 2015
		Cassiferaldehyde, **D6**	Xu et al., 2016
		Pinoresinol, **E3**	Chen et al., 2015
		Cinncassiol A, **A4**	He, et al., 2016
		Cinncassins B, **G28** Coniferaldehyde, **D10** Syringic acid, **G11** Styrene glycol, **G14** Glycerin-1-benzoatel benzoate, **G22** Methyl homovanillate, **G23** (−)-Gynuraone, **G25** 3ζ-(1ζ-Hydroxyethyl)-7-hydroxy-1-isobenzofuranone, **G26**	Yang et al.,2017
		cis-4-Hydroxymellein, **H4**	Chen et al., 2015
		β-Sitosterol, **I1**	

Table 1. Chemical Constituents from *Cinnamomum* Species (Continued)

Species Resource	Investigated parts	Compounds	Bibliography
		Cinnzeylanine, **A5** Cinnacasside A **G20**	Yan et al., 2015
		Sinapaldehyde, **D11** Vanillin, **G2** ,225 Protocatechualdehyde, **G3** Vanillic acid, **G7** Protocatechuic acid, **G8** Isovanillic acid, **G9** *p*-Hydroxybenzoic acid, **G10**	Chen et al.,2015
		Picrasmalignan A, **E10** Anhydrocinnzeylanol, **A1** Anhydrocinnzeylanine, **A2** Epianhydrocinnzeylanol, **A3** Kelampayoside A, **G17** 3,4-Dimethoxyphenol- β-D-apiofuranosyl (1 $\longrightarrow$ 6)-*O*-β-D-glucopyranoside, **G19** 1-Hydroxy-3,6-dimethoxy-8-methylanthraquinone, **G29**	He et al., 2016
		Cinnacasside B, **G21** Glycerin-1-benzoatel benzoate, **G22**	Zeng et al.,2017
	Bark, Twig	Cinnamyl alcohol, **D17**	Chen et al.,2015
		Cinnacasiol H, **A7**	He et al., 2016
		Cinnamoid D, **B1** Mustakone, **B2**	Yan et al.,2015

Table 1. Chemical Constituents from *Cinnamomum* Species (Continued)

Species Resource	Investigated parts	Compounds	Bibliography
		Cinnamaldehyde, **D5**	Chen et al.,2015
		Cinnamic acid, **D7**	Chen et al., 2015
		2-Methoxycinnamaldehyde, **D8**	Kim et al., 2017
C. cassia	Twig	2-Hydroxycinnamaldehyde, **D9** Cinnamomulactone, **D15**	Kim et al., 2017
		Cinnacassin N, **D16** Cinnacassin I, **E9** 3′,4′,5,7-Tetrahydroxyflavanone, **F5**	Liu et al., 2018
		Benzoic acid, **G6**	Kim et al., 2017
		Icariside DC, **G13**	Guo et al., 2017
		3-glyceroylindole, **J1** Indole-3-carboxaldehyde, **J2**	Liu et al., 2018
	Leaves	Cinncassiol C, **A6** Tachioside**, G18** Dimethanol, **C1**	Zhou et al., 2016
C. camphora	Aerial part	(2*S*,3*S*)-3'-Hydroxy-5,7,4′-trimethoxy-flavan-3-ol, **F3** Quercetin, **F4** 6,7,4′-Trimethoxyflavone, **F6** Dihydrokaempferol, **F7** 3*S*-(+)-9-Oxonerolidol, **B8** Syringaldehyde, **G1** 5-Hydroxyethyl salicylate, **G12** Scopoletin, **H1** 6,7-Dimethoxycoumarin, **H2** *p*-Hydroxybenzoic acid, **G10**	Feng et al., 2016

Table 1. Chemical Constituents from *Cinnamomum* Species (Continued)

Species Resource	Investigated parts	Compounds	Bibliography
		Vanillin, **G2** Protocatechualdehyde, **G3**	Chen et al., 2015
		Protocatechuic acid, **G8**	Xu, 2015
	Bark, Leaves	Eugenol, **D1**	Li et al., 2018
		Magnolone, **E6**	Feng et al., 2016
	Bark	Paulownin, **E4**	Xu et al., 2016
		5,7-Dimethoxychromone**, G24**	Xu et al., 2015
		β -Sitosterol, **I1**	Feng et al., 2016
	Leaves	4,3-Dihydroxy- 4′methoxysesamin, **E2** (+)-Kusunokinin, **E5**	Xu et al., 2015
C. subavenim	Bark	Eugenol, **D1** Safrole, **D2** 3,4- Dimethoxycinnamyl alcohol **D18** Carvacrol, **C2** Thymol, **C3** β-Sitosterol, **I1** Isovanillin**, G4** Veratraldehyde**, G5** Methyleugenol, **D3** Methyl cinnamate, **D13** 2,2,7a,7a,7b,7b’ Hexamethyldiphenyl ether, **G27**	Huang et al., 2018
		(±)-Subaveniumins A, **E7** (±)-Subaveniumins B, **E8**	Lai et al., 2015

Table 1. Chemical Constituents from *Cinnamomum* Species (Continued)

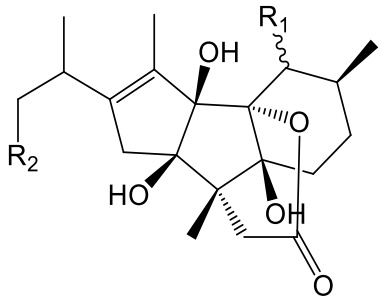
Species Resource	Investigated parts	Compounds	Bibliography
		3,4-Methylenedioxy cinnamaldehyde, **D12**	Feng et al., 2016
		(–)-Sesamin, **E1**	Xu et al., 2016
		Vanillin, **G2** Vanillic acid, **G7**	Chen et al., 2015
	Leaves	Wilsonol H, **B6** Asicariside B1 **B7** D-threo-guaiacylglycerol 7-*O*-β-D-glucopyranoside, **D14** 3-Hydroxy-4,5-dinethoxyphenyl-β-Dglucopyranoside, **G15** 3,4,5-Trimethoxyphynol-1-*O*-β-D-glucoside, **G16** Aristophyll C, **J3**	Hao et al., 2019
	Root	Cinnamyl alcohol, **D17**	Chen et al., 2007
C.glaucescen	Leaf	Limonene, **C7**	Baruah & Nath, 2006
		Geraniol, **C8**	Chinh et al.,2017
	Fruit and pericarp	1,8-Cineole, **C9**	Prakash et al., 2013
	Root	Ascaridole, **C10**	Satyal et al.,2013
C.longipanicu latum	Leaves	β-Eudesmol, **B3** *cis*-Caryophyllene, **B4** β-Phellandrene, **C4**	Cong, 2016
C. japonicum Siebold	Leaves	Guaiol, **B5** Terpinen-4-ol, **C5**	Zhao et al., 2018

Table 1. Chemical Constituents from *Cinnamomum* Species (Continued)

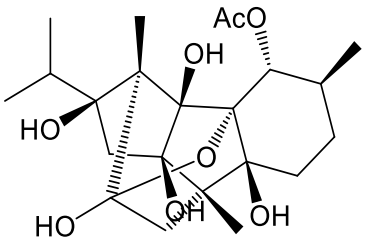
Species Resource	Investigated parts	Compounds	Bibliography
C.osmophloeum	Leaves	Camphor, **C6** Estragole, **D4** Coumarin, **H3**	Lee et al., 2018

Structure some isolated compounds from the plant of *Cinnamomum* species

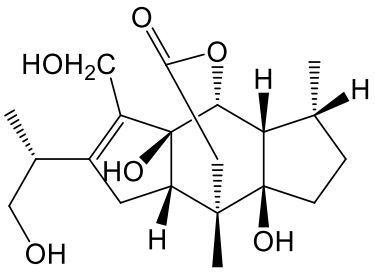
A. Diterpenes



	R_1	R_2	
A1:	α-OH	H	: Eugenol
A2:	OAc	H	: Anhydrocinnzeylanine
A3:	β -OH	H	: Epianhydrocinnzeylanol
A4:	α-OH	OH	: Cinncassiol A

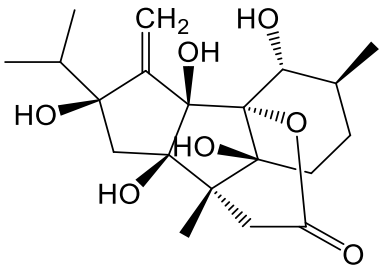


A5: Cinnzeylanine



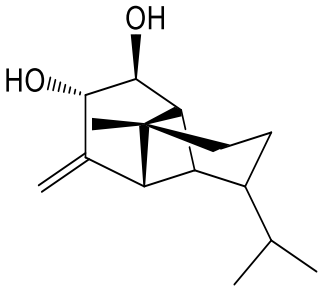
A6: Cinncassiol C

Figure 2. Chemical structure of isolated compounds from *Cinnamomum* Species

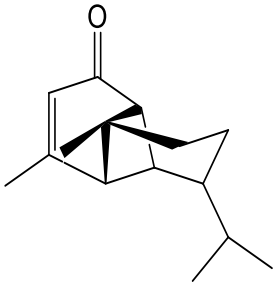


A7: Cinnacasiol H

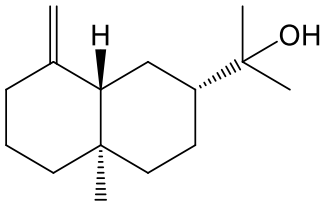
B. Sesquiterpenes



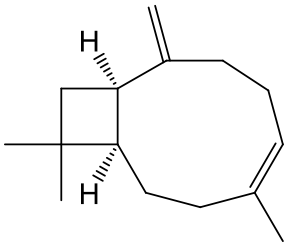
B1: Cinnamoid D



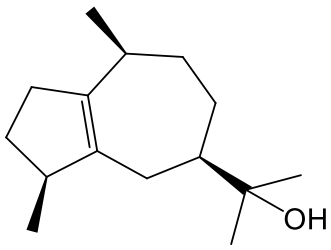
B2: Mustakone



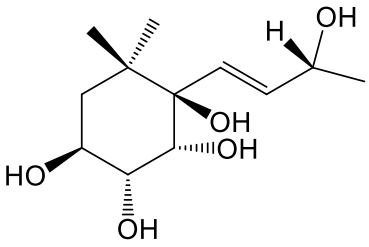
B3: *β*-Eudesmol



B4: *cis*-Caryophyllene

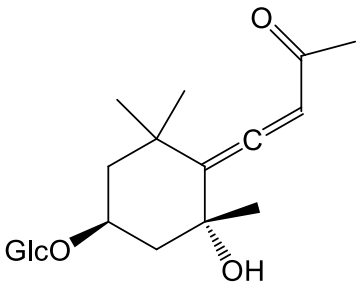


B5: Guaiol

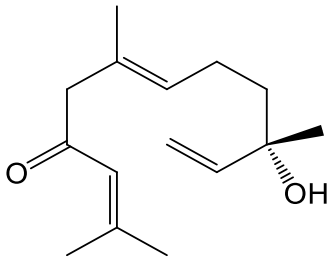


B6: Wilsonol H

Figure 2. Chemical structure of isolated compounds from *Cinnamomum* species (continued)

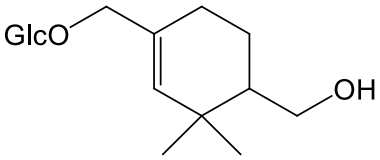


B7: Asicariside B1

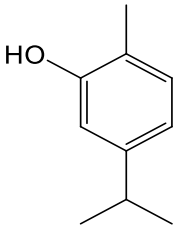


B8: 3*S*-(+)-9-Oxonerolidol

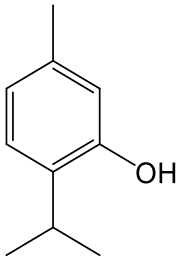
C. Monoterpenes



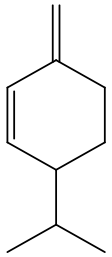
C1: Dimethanol



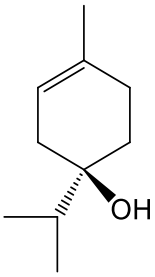
C2: Carvacrol



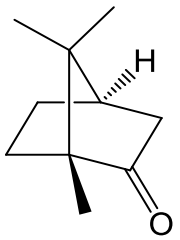
C3: Thymol



C4: β -Phellandrene

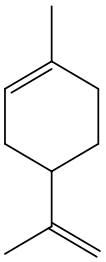


C5: Terpinen-4-ol

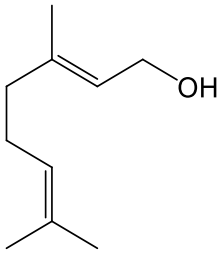


C6: Camphor

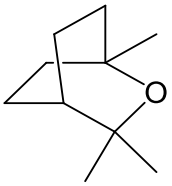
Figure 2. Chemical structure of isolated compounds from *Cinnamomum* species (continued)



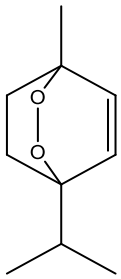
C7: Limonene



C8: Geraniol

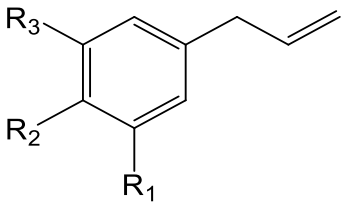


C9: 1,8-Cineole



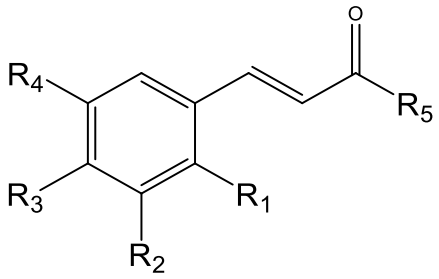
C10: Ascaridole

D. Phenylpropanoids

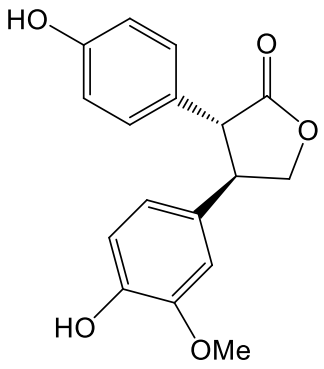


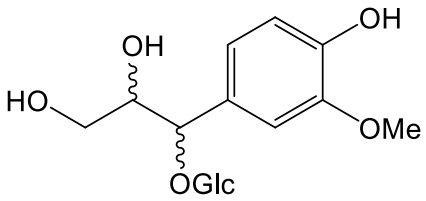
	R_1	R_2	R_3	
D1:	OMe	OH	H	: Eugenol
D2:	H	OCH_2O		: Safrole
D3:	H	OMe	OMe	: Methyleugenol
D4:	H	OMe	H	: Estragole

Figure 2. Chemical structure of isolated compounds from *Cinnamomum* species (continued)



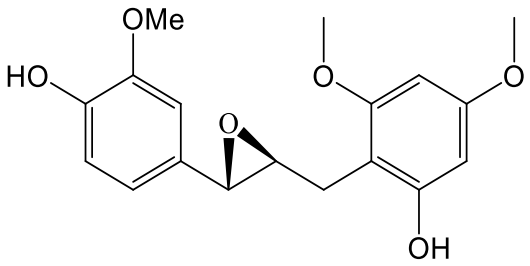
	R_1	R_2	R_3	R_4	R_5	
D5:	H	H	H	H	H	: Cinnamaldehyde
D6:	OMe	OH	H	H	H	: Cassiferaldehyde
D7:	H	H	H	H	OH	: Cinnamic acid
D8:	OMe	H	H	H	H	: 2-Methoxycinnamaldehyde
D9:	OH	H	H	H	H	: 2-Hydroxycinnamaldehyde
D10:	H	H	OH	OMe	H	: Coniferaldehyde
D11:	H	OMe	OH	OMe	H	: Sinapaldehyde
D12:	H	OCH_2O		H	H	: 3,4Methylenedioxy



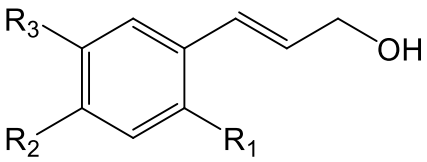


D14: D-threo-guaiacylglycerol -7-*O*- β -D-glucopyranoside **D15**: Cinnamomulactone

Figure 2. Chemical structure of isolated compounds from *Cinnamomum* species (continued)

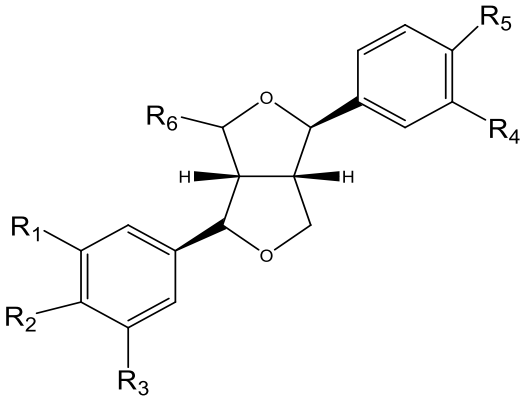


D16: Cinnacassin N



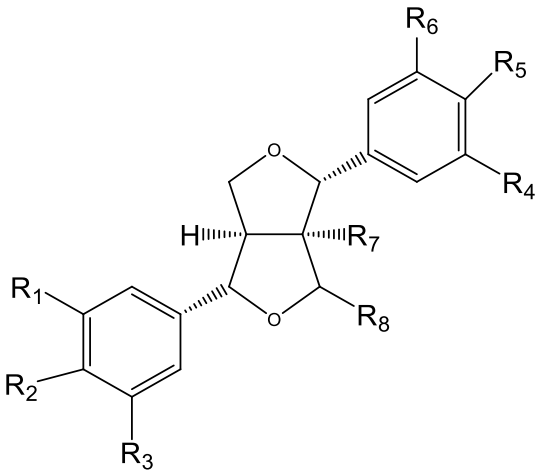
	R_1	R_2	R_3	
D17:	H	H	H	: Cinnamyl alcohol
D18:	H	OMe	OMe	: 3,4-Dimethoxycinnamyl alcohol

E. Lignans

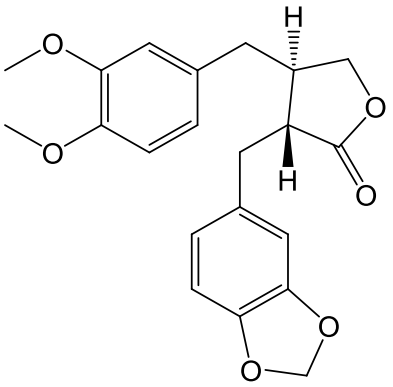


	R_1	R_2	R_3	R_4	R_5	R_6	
E1:	OCH_2O		H	OCH_2O		H	: (–)-Sesamin
E2:	OCH_2O		H	OH	OMe	OH	: 4,3′-Dihydroxy-4′-methoxysesamin

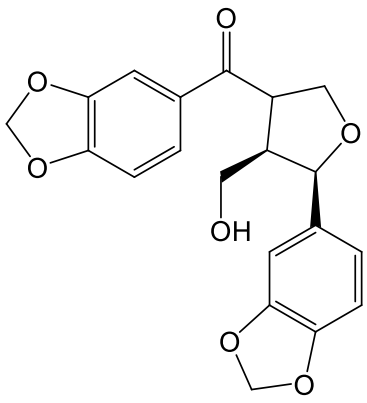
Figure 2. Chemical structure of isolated compounds from *Cinnamomum* species (continued)



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	
E3:	OMe	OH	H	H	OH	OMe	H	H	: (–)-Sesamin
E4:	H	OCH_2O	H		OCH_2O	OH	H		: Paulownin

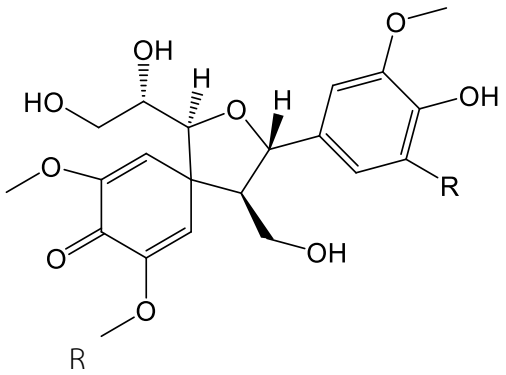


E5: (+)-Kusunokinin



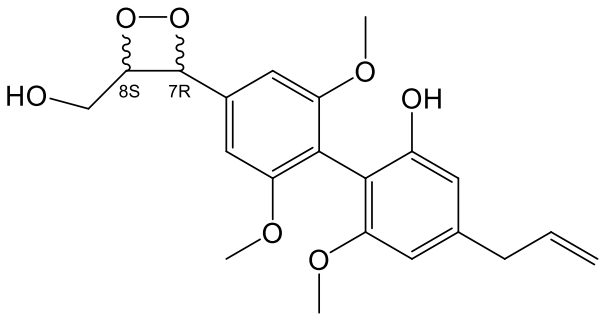
E6: Magnolone

Figure 2. Chemical structure of isolated compounds from *Cinnamomum* Species (continued)

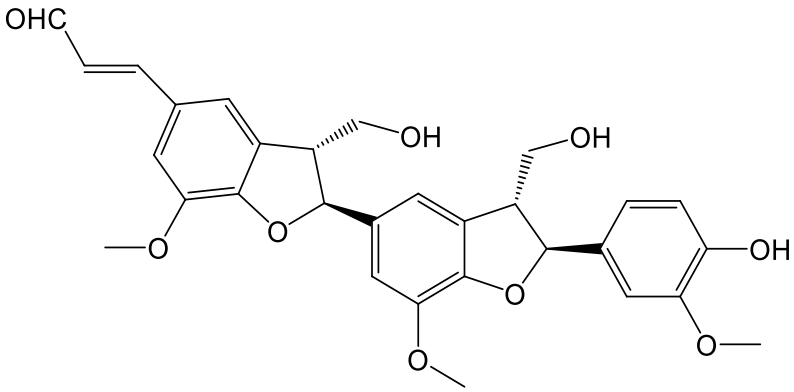


E7: H : (±)-Subaveniumins A

E8: OMe : (±)-Subaveniumins B



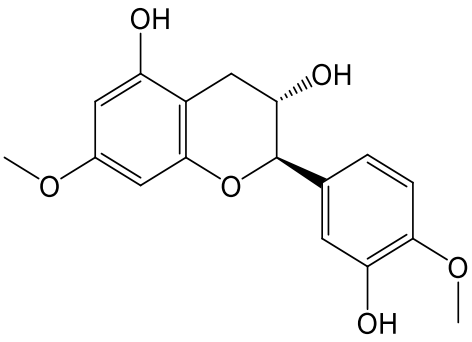
E9: Cinnacassin I



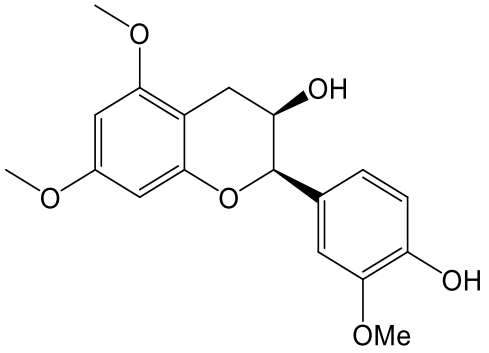
E10: Picrasmalignan A

Figure 2. Chemical structure of isolated compounds from *Cinnamomum* species (continued)

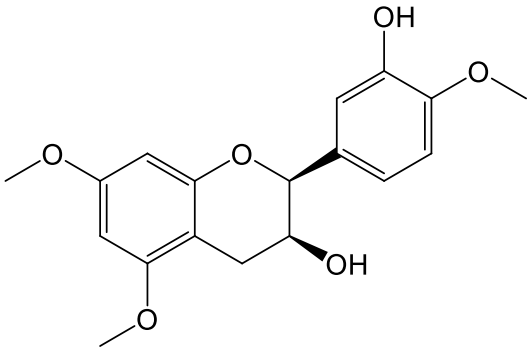
F. Flavonoids



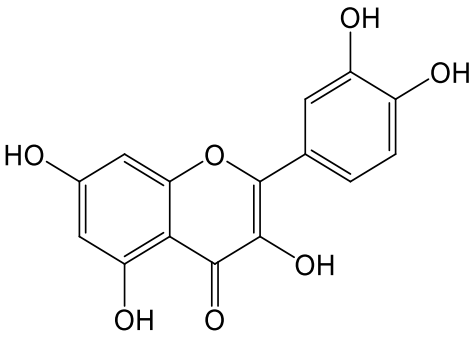
F1: 7,4′-Di-*O*-methyl-(+)-catechin



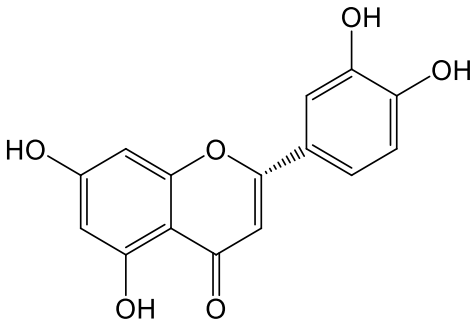
F2 :5,7,3′-Tri-*O*-methyl-(−)-epicatechin



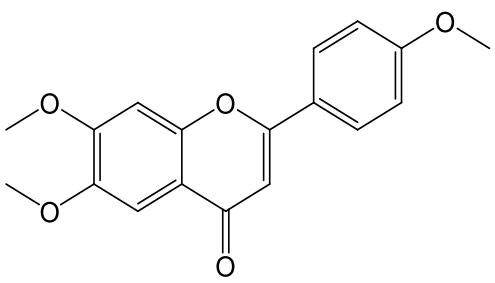
F3 :(2*S*,3*S*)-3’-Hydroxy-5,7,4′ -trimethoxy-flavan-3-ol



F4: Quercetin

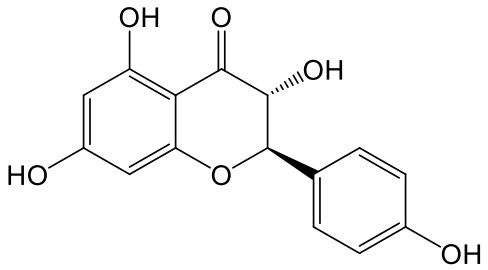


F5: 3′,4′,5,7-Tetrahydroxyflavanone



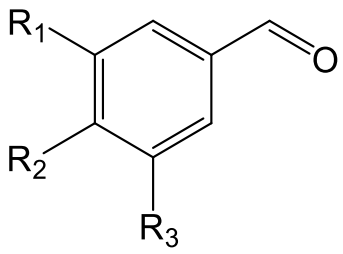
F6: 6,7,4′-Trimethoxyflavone

Figure 2. Chemical structure of isolated compounds from *Cinnamomum* species (continued)



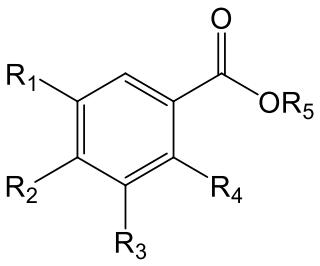
F7: Dihydrokaempferol

G. Aromatic compounds

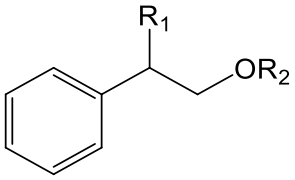


	R_1	R_2	R_3	
G1:	OMe	OH	OMe	: Syringaldehyde
G2:	OMe	OH	H	: Vanillin
G3:	OH	OH	H	: Protocatechualdehyde
G4:	OH	OMe	H	: Isovanillin
G5:	OMe	OMe	H	: Veratraldehyde

Figure 2. Chemical structure of isolated compounds from *Cinnamomum* Species (continued)

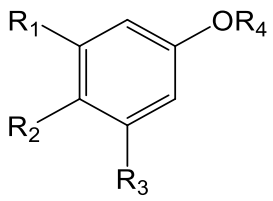


	R_1	R_2	R_3	R_4	R_5	
G6:	H	H	H	H	H	: Benzoic acid
G7:	OMe	OH	H	H	H	: Vanillic acid
G8:	H	OH	OH	H	H	: Protocatechuic acid
G9:	OH	OMe	H	H	H	: Isovanillic acid
G10:	H	OMe	H	H	H	: *p*-Hydroxybenzoic acid
G11:	OMe	OH	OMe	H	H	: Syringic acid
G12:	OH	H	H	OH	Et	: 5-Hydroxyethyl salicylate

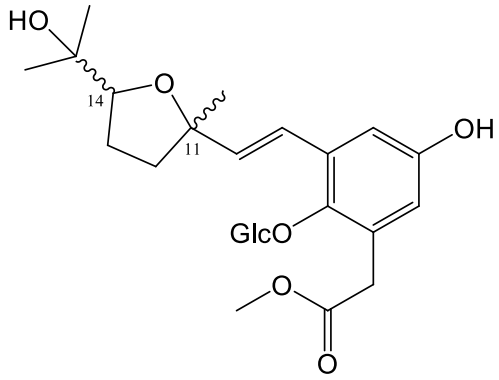


	R_1	R_2	
G13:	H	Api (1”→ 4’)Glc	: Icariside DC
G14:	OH	H	: Styrene glycol

Figure 2. Chemical structure of isolated compounds from *Cinnamomum* Species (continued)

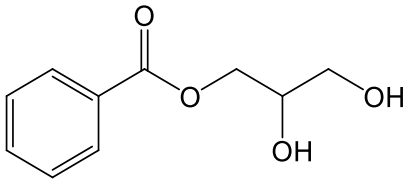


	R_1	R_2	R_3	R_4	
G15:	OH	OMe	OMe	Glc	: 3-Hydroxy-4,5-dinethoxyphenyl- β -Dglucopyranoside
G16:	OMe	OMe	OMe	Glc	: 3,4,5-Trimethoxyphynol-1-*O*- β -D-glucoside
G17:	OMe	OMe	OMe	Api(1"→ 4')Glc	: Kelampayoside A
G18:	OMe	Glc	H	H	: Tachioside
G19:	OMe	OMe	H	Api(1"→ 4')Glc	: (1 ⟶ 6)-*O*- β -D-glucopyranoside

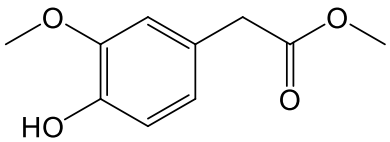


G20: (11*S*,14*S*) Cinnacasside A

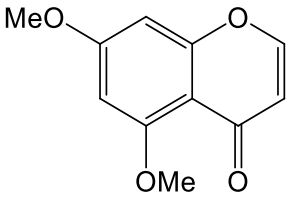
G21: (11*R*,14*R*) Cinnacasside B



G22: Glycerin-1-benzoatel benzoate

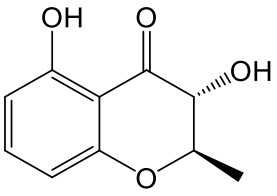


G23: Methyl homovanillate

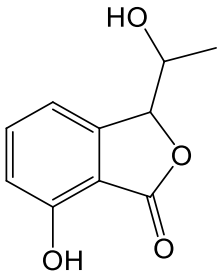


G24: 5,7-Dimethoxychromone

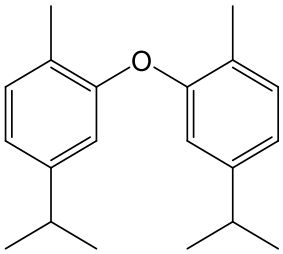
Figure 2. Chemical structure of isolated compounds from *Cinnamomum* species (continued)



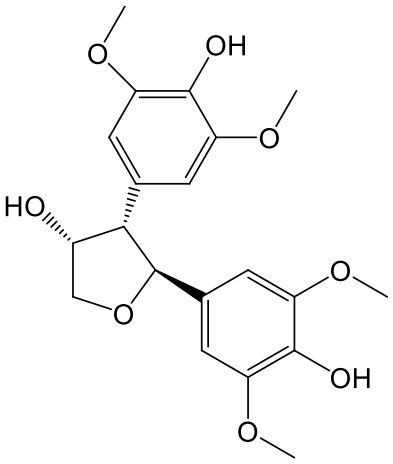
G25: (–)-Gynuraone



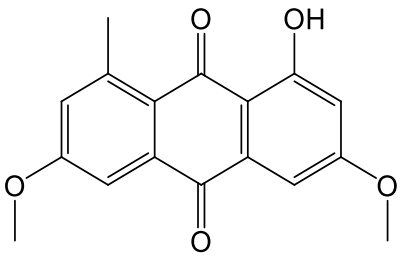
G26: 3ζ-(1ζ-Hydroxyethyl)-7-hydroxy-1-isobenzofuranone



G27: 2,2′,7a,7a’,7b,7b’-Hexamethyldiphenyl ether



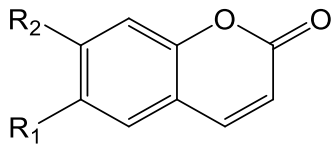
G28: Cinncassins B



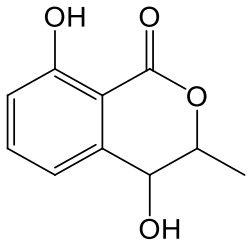
G29: 1-Hydroxy-3,6-dimethoxy-8-methylanthraquinone

Figure 2. Chemical structure of isolated compounds from *Cinnamomum* species (continued)

H. Coumarins

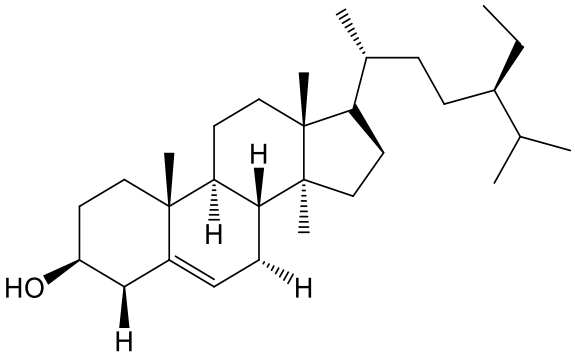


	R_1	R_2	
H1:	OMe	OH	: Scopoletin
H2:	OMe	OMe	: 6,7-Dimethoxycoumarin
H3:	H	H	: Coumarin



H4: *cis*-4-Hydroxymellein

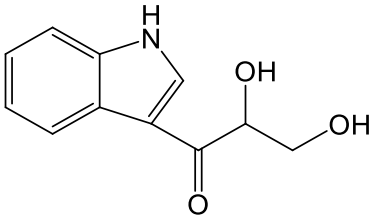
I. Steroids



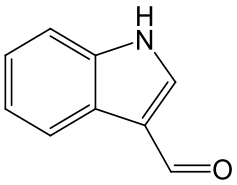
I1: β -Sitosterol

Figure 2. Chemical structure of isolated compounds from *Cinnamomum* species (continued)

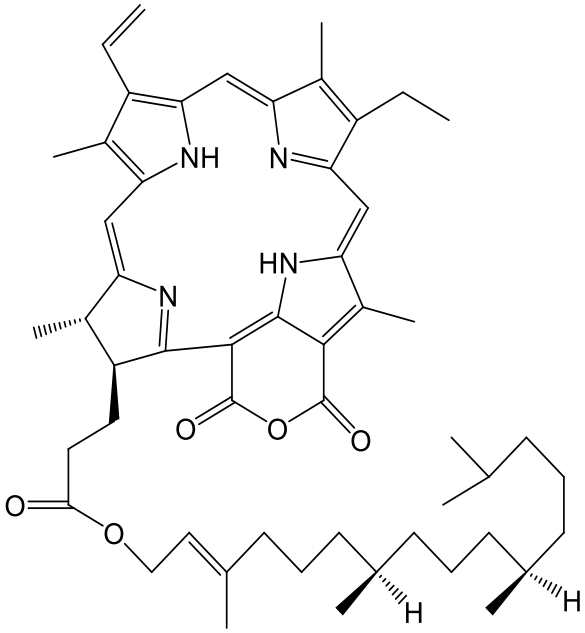
J. Alkaloids



J1: 3-glyceroylindole



J2: Indole-3-carboxaldehyde



J3: Aristophyll C

Figure 2. Chemical structure of isolated compounds from *Cinnamomum* species (continued)

2.4 Chemical Constituents from the *Cinnamomum porrectum* (Roxb.) Kosterm

The antecedently reports was display that the root, wood, leaves, Stem bark, and bark of *C. porrectum* composed various types of substance such as Phenylpropanoids, benzophenones and anthocyanins. The compile data were showed in **Table 2.**

Table 2. Chemical Constituents from the *Cinnamomum porrectum* (Roxb.) Kosterm

Phytochemical	Investigated parts	Compounds	Bibliography
Terpenes	Fruit	5-(2,3-Dihydroxy-3-methylbutyl)-4-methylfuran-2(5*H*)-one, **1** 8-Hydroxy-4,7,7-trimethyl-1,6-dioxaspiro[4,4] non- 3- en- 2-one, **2** 8-Hydroxy-4,7,7-trimethyl-1,6-dioxaspiro[4,4]non-3-en-2-one, **3**	Fuchino et al., 2015
	Woods	Torreyol, **4**	Dũng et al.,1995
	Stem bark	1,8-Cineole, **5** Borneol, **6** Bicyclogermacrene, **7** Camphene, **8** Camphor, **9** Caryophyllene, **10** Linalool, **11** Myrcene, **12**	Zhao et al., 2018

Table 2. Chemical Constituents from the *Cinnamomum porrectum* (Roxb.) Kosterm (continued)

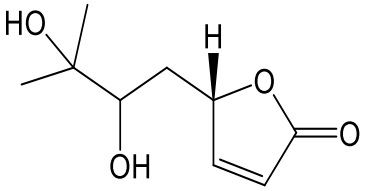
Phytochemical	Investigated parts	Compounds	Bibliography
		Terpinen-4-ol, **13** Terpinolene, **14** Cymene, **15** β-phellandrene, **16** β-pinene, **17** α-humulene, **18** α-farnesene, **19**	Cong et al., 2016
Phenylpropanoids	Woods	Safrole, **20**	Adfa et al., 2016
		3-(3,4-methylenedioxyphenyl)-1,2-Propanediol, **21**	
		Piperonal, **22**	Dũng et al., 1995
Flavonoids	Leaves	Scopoletin, **23** Quercetin- 3- *O*- α- L-rhamnoside, **24** Kaempferol- 3- *O*- α- L-rhamnoside, **25**	Chen et al., 2015
		Herbacetin, **26**	Wei et al., 2017
		Eepicatechin, **27** Isorhoifolin, **28** Rutin, **29** Nicotiflorin, **30**	Pardede et al., 2017

Table 2. Chemical Constituents from the *Cinnamomum porrectum* (Roxb.) Kosterm (continued)

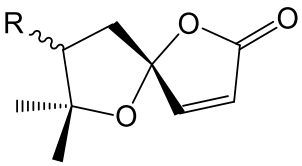
Phytochemical	Investigated parts	Compounds	Bibliography
Lignans	Woods	Dehydroxycubebin, **31** Cubebin, **32** Hinokinin, **33**	Adfa et al., 2016
Aromatic compounds	Leaves	4-Hydroxybenzaldehyde, **34** 1,2,4- Trihydroxybenzene, **35**	Wei et al., 2017
		(3*R*,4*R*,3′*R*,4′*R*)-6,6′-Dimethoxy-3,4,3′,4′-tetrahydro-2H,2′H-[3,3′]bichromenyl-4,4′-diol, **36**	Chen et al., 2015
		4-Hydroxybenzoic acid, **37**	Pardede et al., 2017
	Woods	Benzaldehyde, **38**	Dũng et al., 1995

Structure of isolated compound from *Cinnamomum porrectum* (Roxb.) Kosterm

A. Terpenes

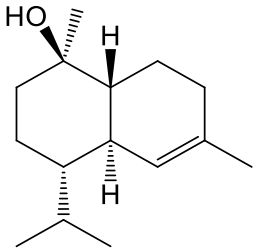


1: 5-(2,3-Dihydroxy-3-methylbutyl)-4-methylfuran-2(5*H*)-one

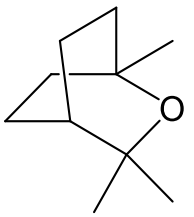


8-Hydroxy-4,7,7-trimethyl-1,6-dioxaspiro [4.4]non-3-en-2-one **2**: R= β-OH

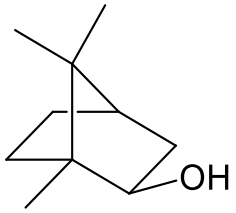
8-Hydroxy-4,7,7-trimethyl-1,6-dioxaspiro [4.4]non-3-en-2-one **3**: R=α-OH



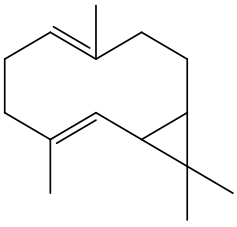
4: Torreyol



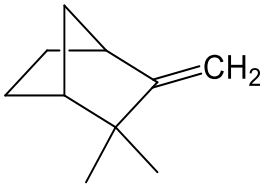
5: 1,8-Cineole



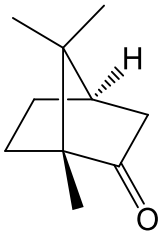
6: Borneol



7: Bicyclogermacrene

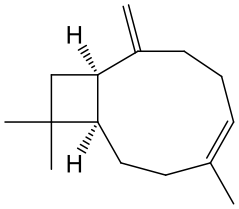


8: Camphene

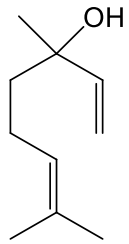


9: Camphor

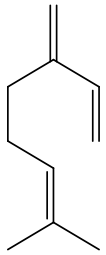
Figure 3. Chemical structure of isolated compounds from *Cinnamomum porrectum* (Roxb.) Kosterm



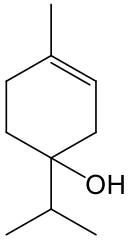
10: Caryophyllene



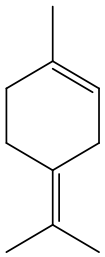
11: Linalool



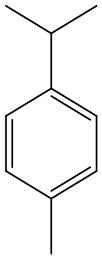
12: Myrcene



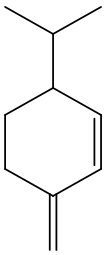
13: Terpinen-4-ol



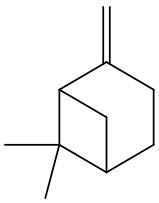
14: Terpinolene



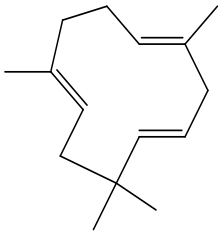
15: Cymene



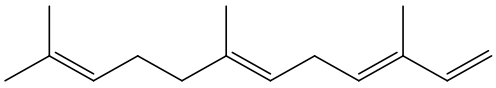
16: β -phellandrene



17: β -pinene



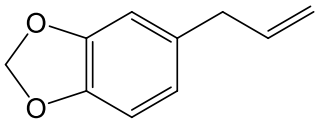
18: α-humulene



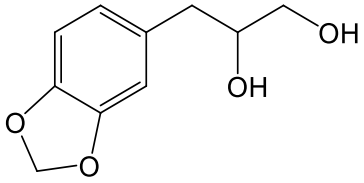
19: α-farnesene

Figure 3. Chemical structure of isolated compounds from *Cinnamomum porrectum* (Roxb.) Kosterm (continued)

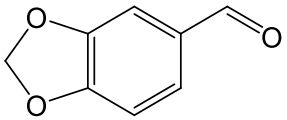
B. Phenylpropanoids



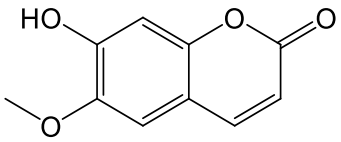
20: Safrole



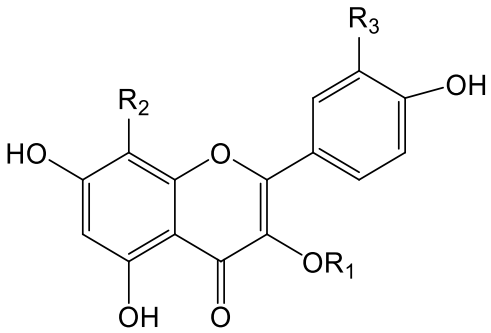
21: 3-(3,4-methylenedioxyphenyl)-1,2-Propanediol

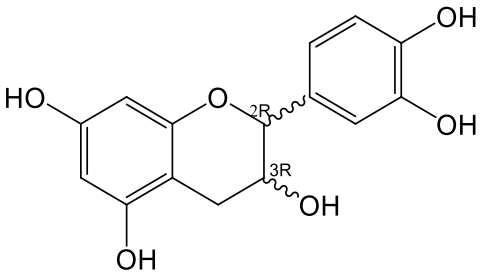


22: Piperonal



23: Scopoletin

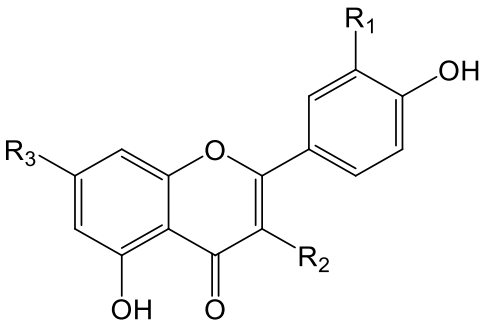




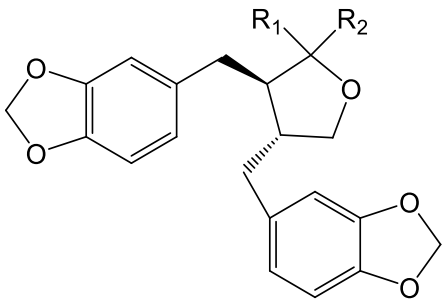
27: Eepicatechin

	R_1	R_2	R_3	
24:	α-L-Rha	H	OH	: Quercetin-3-*O*-α-L-rhamnoside
25:	α-L-Rha	H	H	: Kaempferol-3-*O*-α-L-rhamnoside
26:	H	OH	OH	: Herbacetin

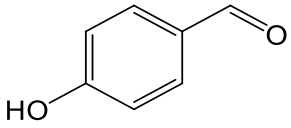
Figure 3. Chemical structure of isolated compounds from *Cinnamomum porrectum* (Roxb.) Kosterm (continued)



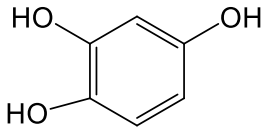
	R_1	R_2	R_3	
28:	H	H	*O*-Rhutinoside	: Isorhoifolin
29:	OH	*O*-Rhutinoside	OH	: Rutin
30:	H	*O*-Rhutinoside	OH	: Nicotiflorin



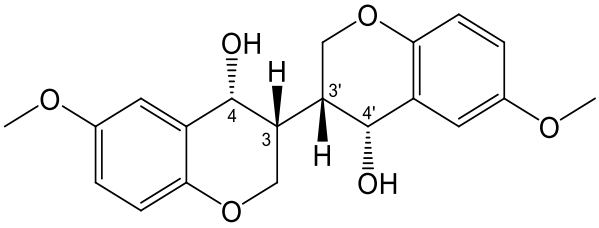
	R_1	R_2	
31:	H	H	: Dehydroxycubebin
32:	OH	H	: Cubebin
33:	=O		: Hinokinin



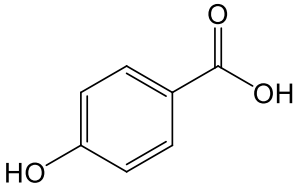
34: 4-Hydroxybenzaldehyde



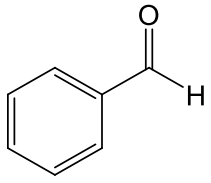
35: 1,2,4-Trihydroxybenzene



36: (3*R*,4*R*,3′*R*,4′*R*)-6,6′-Dimethoxy-3,4,3′,4′-tetrahydro -2H,2′H-[3,3′] bichromenyl-4,4′-diol



37: 4-Hydroxybenzoic acid



38: Benzaldehyde

Figure 3. Chemical structure of isolated compounds from *Cinnamomum porrectum* (Roxb.) Kosterm (continued)

2.5 Antibacterial activity of crude extract from *Cinnamomum* species

2.5.1 Antibacterial activity of *Cinnamomum* species

In the literature review, (Uthairatsamee et al., 2011) and coworker was to investigate the antibacterial activities of hexane, dichloromethane and methanolic extracts from the leaves, inner bark, outer bark, wood and roots of *C. porrectum.* To assess antibacterial activity, the methanolic extracts from the inner bark and outer bark were excellent in their activity against *B. cereus*, *S. aureus*, *S. flexneri*, and *V. parahaemolyticus*, with MBC values ranging from 0.24 to 7.81 mg/ml

CHAPTER 3

EXPERIMENTAL

This research is divided into two parts, each of which includes a chemical and biological test that includes the extraction and isolation of crude extract from *Cinnamomum porrectum* roots, and purification of chemicals constituents from crude extracts. Finally, elucidate the structures of isolated chemicals and conduct biological tests.

3.1 Instruments and chemicals

3.1.1 Instruments

3.1.1.1 Bruker FTNMR Ultra Shield 400 MHz spectrometers

3.1.1.2 Column chromatography (CC)

3.1.1.3 Perkin-Elmer 783 FTS165 FT-IR spectrometers

3.1.1.4 SHIMADZU UV-160A spectrometers

3.1.2 Chemicals

3.1.2.1 Acetone

3.1.2.2 Barium chloride

3.1.2.3 Dichloromethane or Methylene chloride

3.1.2.4 Dimethyl Sulfoxide

3.1.2.5 Ethanol

3.1.2.6 Ethyl acetate

3.1.2.7 Hexane

3.1.2.8 Malt Extract Agar (MEA)

3.1.2.9 Malt Extract Broth (MEB)

3.1.2.10 Methanol

3.1.2.11 Potato Dextrose Agar (PDA)Silica gel

3.1.2.12 Potato Dextrose Broth (PDB)

3.1.2.13 Resazurin

3.1.2.14 Silica gel

3.1.2.15 Sulfuric acid

3.2 Plant materials

The air–dried roots and barks of *Cinnamomum porrectum* were collected from Moo 8, Khao Phanom District, Krabi Province, Thailand in October, 2019.

3.3 Extraction

3.3.1 Extraction of the roots and barks of *Cinnamomum porrectum* (Roxb.) Kosterm.

The roots and barks of *C. porrectum* (1.0 kg) were extracted with Dichloromethane then Acetone (each 5 L x 2, 120 hours) at room temperature (25 ± 1°C) The filtration was done using a filter paper (Whatman No.1) giving filtrate and residue in which to the next maceration stage. Solvent was made to evaporate using Rotavapor R-210 with Vacuum Pump V-700 (Buchi) to collect crude the four crude extracts were obtained as shown in **Figure 4.**

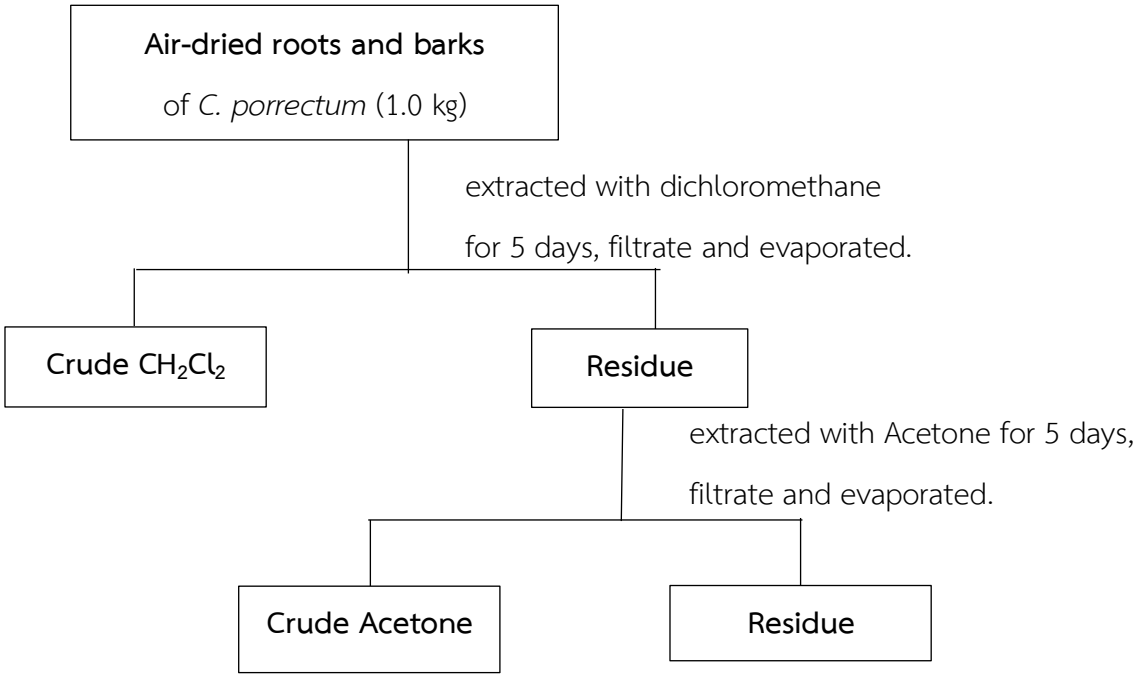


Figure 4. Extraction of the roots of *Cinnamomum porrectum*

Table 3. Fractions of dry weight and extraction yield of different parts of *Cinnamomum porrectum.*

Fraction	Solvent	Dry weight (g)	Yield after extraction (%)	Physical appearance
Root	Dichloromethane	25.41	2.54	brown gum
	Acetone	35.50	3.55	dark-brown gum
Bark	Dichloromethane	27.76	2.78	yellow gum
	Acetone	27.91	2.79	brown gum

3.3.2 Isolation from roots of extraction Dichloromethane

The Dichloromethane extract (6.00 g) was chromatographed on normal phase TLC with 100 % dichloromethane as a mobile phase. Chromatogram characteristics showed four spots under UV light, show purple coloration with anisaldehyde–H_2SO_4 reagent on TLC. Further separation to column chromatography (CC) over silica gel eluting with gradient system of 100% CH_2Cl_2 and increasing polarity with ethyl acetate give one hundred. sixty-two main fractions. Combined by their TLC characteristic, the similar fraction were combined to give sixteen main fractions (D1 – D16) as shown in **Table 4**.

Table 4. Fractions obtained from extraction dichloromethane by column chromatography

Fractions	Eluent	Weight (mg)	Physical appearance
D1	100% C	39.2	yellow viscous liquid
D2	100% C	1,763	dark-yellow viscous liquid
D3	100% C	39.3	light-yellow viscous liquid
D4	100% C	78.8	light-yellow viscous liquid
D5	100% C	72	dark-yellow viscous liquid
D6	100% C	425.3	dark-brown viscous liquid
D7	10% E : C	92.6	yellow viscous liquid
D8	10% E : C	246.3	dark-brown viscous liquid
D9	10% E : C	192.1	dark-brown viscous liquid
D10	10% E : C	234.3	dark-brown viscous liquid
D11	30% E : C	269.5	dark-brown viscous liquid
D12	30% E : C	400	dark-brown viscous liquid
D13	30% E : C	76	dark-brown viscous liquid
D14	50% E : C	114.9	dark-brown viscous liquid
D15	50% E : C	120.4	dark-brown viscous liquid
D16	70-100% E : C	188.2	dark-brown viscous liquid

Following that, the chosen fraction was purified using CC and PLC.

Fraction D1 (yellow viscous liquid; 39.2 mg) was chromatographed on normal phase TLC with 10% acetone-hexane as a mobile phase. These fractions showed mostly fatty many spots overlap under UV light, Fraction D1 appears like wax. Thus, was no further investigation.

Fraction D2 (dark-yellow viscous liquid; 1,763 mg) was chromatographed over on normal phase TLC using gradient of 10 % CH_2Cl_2 in n–hexane as a mobile phase Chromatogram characteristics revealed showed two spots under UV light. Further separation was chromatographed over silica gel column using gradient of 10 % CH_2Cl_2-hexane. The similar subfraction on TLC characteristic were combined and evaporated under reduced pressure to give five fractions (D2A-D2E) as shown in **Table 5**.

Table 5. Subfractions obtained from **Fraction D2** by column chromatography

Fractions	Eluent	Weight (mg)	Physical appearance
D2A	10% C : H	16.0	light-yellow viscous liquid
D2B	10% C : H	1098.1	light-yellow viscous liquid
D2C	10% C : H	298.2	light-yellow viscous liquid
D2D	10% C : H	37.3	light-yellow viscous liquid
D2E	10% C : H	8.6	light-yellow viscous liquid

Subfraction D2A D2C to **D2E** Chromatogram characteristics on normal phase TLC with 10% CH_2Cl_2-hexane as a mobile phase. Chromatogram characteristics revealed showed two spots under UV light. were contained mostly fatty material and was not further investigated.

Subfraction D2B (light-yellow viscous liquid; 1098.1mg) were chromatographed by two condition on normal phase TLC with 1% ethyl acetate-hexane and 10% CH_2Cl_2-hexane as a mobile phase. Thin layer chromatography (TLC) analysis showed a major spot under UV light. Followed by purification through preparative thin layer chromatography subfraction D2B with 10% CH_2Cl_2-hexane afforded two bands.

Band 1 (D2B1) Chromatogram characteristics on normal phase TLC with a mobile phase of 10% CH_2Cl_2-hexane demonstrated a long tail under UV light and was not further investigated.

Band 2 (D2B2) was obtained as a light-yellow viscous liquid (**CP1**, 33.0 mg). Chromatogram characteristics on normal phase TLC with a mobile phase of 30% acetone-hexane. shown a one spot with the R_f value of 0.68 under UV light, When the TLC plate was dipped in vanillin- Sulfuric acid reagent, one purple spot.

Fraction D3 (393.0 mg) was chromatographed over on normal phase TLC using gradient of 10% ethyl acetate-hexane as a mobile phase. chromatogram characteristics revealed showed three spots under UV light. When the TLC plate was dipped in vanillin sulfuric acid reagent, multicolored spot emerged. Followed by purification through Sephadex LH-20 gel column chromatography (with a gradient of methanol). All subfraction were examined by TLC, then evaporated to dryness under reduced pressure to seven fractions as shown in **Table 6**.

Table 6. Subfractions obtained from **Fraction D3** by column chromatography over Sephadex LH-20

Fractions	Weight (mg)	Physical appearance
D3A	6.2	yellow gum
D3B	18.4	dark-yellow gum
D3C	45.8	dark-yellow gum
D3D	44.5	dark-yellow gum
D3E	60.7	light-yellow gum
D3F	3.2	yellow gum
D3G	2.2	yellow gum

Subfraction D3A to D3C Chromatogram characteristics on normal phase TLC with a mobile phase of 10% ethyl acetate-hexane. These fractions showed mostly fatty many spots overlap under UV light, Fraction D1 appears like wax. Thus, was not further investigated.

Subfraction D3D (**44.5** mg) was chromatographed over on normal phase TLC using gradient of 10% ethyl acetate-hexane as a mobile phase (2 times). chromatogram characteristics revealed showed three spots under UV light. One orange spot was found on the TLC plate after dipping with the vanillin sulfuric acid reagent. Thin layer chromatography (TLC) analysis showed a major spot under UV light. Further separation by preparative thin layer chromatography subfraction D3D with 10% ethyl acetate-hexane (2 times) afforded three bands.

Band 1 and 3 (D3D1 and D3D3) Chromatogram characteristics on normal phase TLC with a mobile phase of 10% ethyl acetate-hexane (2 times) demonstrated a long tail under UV light and was not further investigated.

Band 2 (D3D2) was obtained as a white solid (**CP5**, 29.7 mg). Chromatogram characteristics on normal phase TLC with a mobile phase of 30 %

acetone-hexane. as a mobile phase (2 times). Under UV light in the dark box with the R_f value of 0.51. One orange spot was found on the TLC plate after dipping with the vanillin sulfuric acid reagent.

Subfraction D3E (**60.7** mg) were chromatographed by two condition on normal phase TLC with 80% CH_2Cl_2-hexane and 30% ethyl acetate-hexane as a mobile phase. Chromatogram characteristics showed a major two spot under UV light. One green spot was found on the TLC plate after dipping with the vanillin sulfuric acid reagent. Further separation by preparative thin layer chromatography subfraction D3E with 30% ethyl acetate-hexane afforded one bands.

Band 1 (D3E1) was obtained as a white solid (**CP2**, 14.8 mg). Chromatogram characteristics on normal phase TLC with a mobile phase of 30 % acetone-hexane. under shortwave UV light in the dark box with the R_f value of 0.41, One green spot was found on the TLC plate after dipping with the vanillin sulfuric acid reagent.

Subfraction D3F and D3G Chromatogram characteristics on normal phase TLC with a mobile phase of 10% ethyl acetate-hexane. Under UV light, demonstrated a long tail under UV light and was not further studied.

Fraction D3 (195.0 mg) insoluble fraction of methanol was chromatographed on normal phase TLC using gradient of 80 % CH_2Cl_2 in n–hexane as a mobile phase. chromatogram characteristics revealed showed two spots under UV light. One brown spot was found on the TLC plate after dipping with the vanillin sulfuric acid reagent. Followed by purification through preparative thin layer chromatography eluting with gradient system of 80% CH_2Cl_2-hexane afforded two bands.

Band 1 (D31) Chromatogram characteristics on normal phase TLC with a mobile phase of 80% CH_2Cl_2-hexane demonstrated a long tail under UV light and was not further studied.

Band 2 (D32) was obtained as a white solid (**CP3**, 14.5 mg). Chromatogram characteristics on normal phase TLC with a mobile phase of 30 % acetone-hexane. under shortwave UV light in the dark box with the R_f value of 0.51.

Fraction D4 (78.8 mg) Chromatogram characteristics on normal phase TLC with 30% acetone-hexane as a mobile phase demonstrated a long tail under UV light and was not further studied.

Fraction D5 (72.0 mg) Chromatogram characteristics on normal phase TLC with 30% acetone-hexane as a mobile phase demonstrated a long tail under UV light and was not further investigated.

Fraction D6 (425.3 mg) was chromatographed over on normal phase TLC using gradient of 30% ethyl acetate-hexane as a mobile phase. chromatogram characteristics revealed showed three spots under UV light. Many spot was found on the TLC plate after dipping with the vanillin sulfuric acid reagent. Further separation by Sephadex LH-20 column chromatography using 100 % methanol as a mobile phase. All subfraction were inspect by TLC, then evaporated to dryness under reduced pressure to five fractions as shown in **Table 7**.

Table 7. Subfractions obtained from **Fraction D6** by column chromatography over Sephadex LH-20

Fractions	Weight (mg)	Physical appearance
D6A	9.6	yellow gum
D6B	12.0	brown gum
D6C	7.5	brown gum
D6D	4.1	yellow gum
D6E	20.9	light-yellow gum

Subfraction D6A to D6D Chromatogram characteristics on normal phase TLC with 30 % acetone-hexane as a mobile phase demonstrated a long tail under UV light and was not further investigated.

Subfraction D6E (**20.9** mg) was chromatographed over on normal phase TLC using gradient of 30% ethyl acetate-hexane as a mobile phase. Chromatogram characteristics revealed showed one spot was found on the TLC plate after dipping with the vanillin sulfuric acid reagent. Further separation by preparative thin layer chromatography subfraction D6E with 30% ethyl acetate-hexane afforded one bands.

Band 1 (D6E1) was obtained as a yellow solid (**CP6**, 17.6 mg). Chromatogram characteristics on normal phase TLC with a mobile phase of 30 % acetone-hexane. Under UV light in the dark box with the R_f value of 0.29. One yellow spot was found on the TLC plate after dipping with the vanillin sulfuric acid reagent.

Fraction D8 (246.3 mg) Chromatogram characteristics on normal phase TLC with 30% acetone-hexane as a mobile phase demonstrated a long tail under UV light and was not further studied.

Fraction D12 (400.0 mg) Chromatogram characteristics on normal phase TLC using gradient system of 50 % acetone-hexane as a mobile phase. Chromatogram characteristics revealed showed two spots under UV light. Which gave a purple coloration with anisaldehyde–H_2SO_4 reagent on TLC. The fraction D12 was subjected to Sephadex LH-20 column chromatography over silica gel eluting with gradient system of 100 % methanol. All subfraction were examined by TLC, then evaporated to dryness under reduced pressure to six fractions as described in **Table 8**.

Table 8. Subfractions obtained from **Fraction D12** by column chromatography over Sephadex LH-20

Fractions	Weight (mg)	Physical appearance
D12A	35.9	dark-brown gum
D12B	110.8	dark-brown gum
D12C	24.2	brown gum
D12D	39.1	yellow gum
D12E	7.2	brown gum
D12F	0.4	brown gum

Subfraction D12A to D12C and D12E to D12F Chromatogram characteristics on normal phase TLC with 50% acetone-hexane as a mobile phase demonstrated a long tail under UV light and was not further investigated.

Subfraction D12D (39.1mg) crystallized in methanol was chromatographed over on normal phase TLC using gradient of 50% acetone-hexane as a mobile phase. Thin layer chromatography (TLC) analysis showed a major spot under UV light. Which gave a purple coloration with anisaldehyde–H_2SO_4 reagent on TLC. Further separation by dissolving with hexane and then dissolving with CH_2Cl_2, a colorless crystal (**CP4**, 14.0 mg) was obtained as a major compound from insoluble part.

3.3.3 Isolation of acetone extract from roots.

The acetone extract (6.00 g) was chromatographed on normal phase TLC with 100% dichloromethane as a mobile phase. Chromatogram characteristics showed four spots under UV light, Which gave a purple coloration with anisaldehyde–H_2SO_4 reagent on TLC. Further separation to column chromatography (CC) over silica gel eluting with gradient system of 100% CH_2Cl_2 and increasing polarity with ethyl acetate to give one hundred percent. twelve main fractions. Combined by their TLC characteristic, were qualitatively the same to give twelve main fractions (D1–D16) as shown in **Table 9**.

Table 9. Fractions obtained from **Extraction acetone**

Fractions	Eluent	Weight (mg)	Physical appearance
A1	100% C	570.8	light-yellow viscous liquid
A2	100% C	59.4	light-yellow gum
A3	100% C	189.9	dark-brown gum
A4	10% E : C	134.5	light-brown gum
A5	10-20% E : C	331.2	dark-brown gum
A6	20% E : C	60.3	light- yellow gum
A7	20% E : C	75.9	dark -brown gum
A8	30% E : C	283.7	dark -brown gum
A9	30% E : C	58.9	yellow gum
A10	40% E : C	321.3	dark -brown gum
A11	50% E : C	394.9	brown gum
A12	60-100% E : C	922.9	dark -brown gum

The selected fractions were further purified by CC and PLC as followings**.**

Fraction A1 (light-yellow viscous liquid; 570.8 mg) was chromatographed on normal phase TLC with a mobile phase of 10 % CH_2Cl_2 in n–hexane. Under UV light, Thin layer chromatography (TLC) analysis showed a major spot under UV light. Fraction A1 is similar in composition to fraction D1 in dichloromethane crude extract. Which gave a purple coloration with anisaldehyde–H_2SO_4 reagent on TLC. Further was subjected to preparative thin layer chromatography (PLC) (10% CH_2Cl_2 in n–hexane) to furnish compound **1**, a light-yellow viscous liquid (**CP1,** 25.6 mg).

Fraction A2 (light-yellow gum; 59.4 mg) was chromatographed on normal phase TLC using gradient of 10 % ethyl acetate-hexane as a mobile phase. chromatogram characteristics revealed showed two spots under UV light. Followed was subjected preparative thin layer chromatography (PLC) eluting with gradient system of 10% ethyl acetate-hexane. a white solid (**CP3**, 16.1 mg)

Fraction A7 (dark-brown gum; 75.9 mg) crystallized in dichloromethane was chromatographed over on normal phase TLC using gradient of 30% acetone-hexane as a mobile phase. Thin layer chromatography (TLC) analysis showed a major spot under UV light. Which gave a purple coloration with anisaldehyde–H_2SO_4 reagent on TLC. Further separation by dissolving with hexane and then dissolving with CH_2Cl_2, a colorless crystal (**CP4**, 25.0 mg) was obtained as a major compound from insoluble part.

3.3.4 Isolation of dichloromethane extract from barks

The dichloromethane extract (6.00 g) was chromatographed on normal phase TLC with 10%acetone-hexane as a mobile phase. Chromatogram characteristics showed three spots under UV light, Which gave a purple coloration with anisaldehyde–H_2SO_4 reagent on TLC. Further separation to column chromatography (CC) over silica gel eluting with gradient system of 10 % acetone-hexane and increasing polarity with acetone to give one hundred forty-one main fractions. Combined by their TLC characteristic, the similar fraction were combined to give fifteen main fractions (B1 – B15) as shown in **Table 10**.

Table 10. Fractions obtained from **Extraction dichloromethane**

Fractions	Eluent	Weight (mg)	Physical appearance
B1	10% A : H	448.5	light-yellow viscous liquid
B2	10% A : H	84.7	dark-yellow viscous liquid
B3	10% A : H	263.9	light-yellow viscous liquid
B4	20% A : H	150.8	light-yellow viscous liquid
B5	20% A : H	79.1	dark-yellow viscous liquid
B6	20% A : H	81.7	dark-brown viscous liquid

Table 10. Fractions obtained from **Extraction dichloromethane** (continued)

Fractions	Eluent	Weight (mg)	Physical appearance
B7	20% A : H	126.3	yellow viscous liquid
B8	20% A : H	55.2	dark-brown viscous liquid
B9	20% A : H	66.5	dark-brown viscous liquid
B10	30% A : H	42.7	dark-brown viscous liquid
B11	30% A : H	45.5	dark-brown viscous liquid
B12	30% A : H	102.9	dark-brown viscous liquid
B13	40% A : H	112.4	dark-brown viscous liquid
B14	40% A : H	32.5	dark-brown viscous liquid
B15	50-80% A : H	55.5	dark-brown viscous liquid

Fraction B1 (light-yellow viscous liquid; 448.5 mg) was chromatographed on normal phase TLC with a mobile phase of 10% acetone-hexane. Under UV light, Thin layer chromatography (TLC) analysis showed a major spot under UV light. Fraction B1 is similar in composition to fraction A1 and fraction D1. Which gave a purple coloration with anisaldehyde–H_2SO_4 reagent on TLC. to furnish compound **1**, a light-yellow viscous liquid (**CP1,** 22.8 mg).

Fraction B2 to B15 Chromatogram characteristics on normal phase TLC with 50% acetone-hexane as a mobile phase demonstrated a long tail under UV light and was not further studied.

3.4 Biological assays

3.4.1 Antibacterial activities

The microorganisms used in this study where *E. coli* (ATCC25922) *P. aeruginosa* (ATCC27853) *S. aureus* (ATCC25923) *B. cereus* (ATCC11778) The working media used for susceptibility assays were Mueller–Hinton Agar and Mueller–Hinton Broth by agar well diffusion method and broth microdilution method.

3.4.1.1 Bacteria test preparation method

Four different microorganisms including *E. coli*, *P. aeruginosa*, *S. aureus* and *B. cereus* were cultured in Mueller–Hinton Broth (MHB) medium and colonies were separated by streak plate method on Mueller–Hinton Agar (MHA) medium plates were incubated for 18-24 hours at 37°C. After picking a colony from plate was cultured in Mueller–Hinton Broth and incubated at 37 °C for 10-18 hours. The cultures were adjusted for turbidity using Mueller–Hinton Broth. The turbidity was equal to 0.5 McFarland (to have approximately 1.5 x 10^8 CFU/ml) of bacteria for testing for antibacterial activity. (Omoregie et al.,2010)

3.4.1.2 Agar well diffusion method

Test the antibacterial activity of the extract by agar disc diffusion method according to the method by the standard method of (Bauer et al.1966) Stock solutions of test samples were prepared in 20%v/v aqueous dimethyl-sulfoxide (DMSO) solution serial dilutions were performed out of initial at concentrations 1000 mg/ml (for crude extract). The inoculum of microorganisms were prepared from 18-24 hours. Prepared MHA medium in a culture dish. After solidification of the agar, Use a sterile cotton swab

moistened with microorganisms. 1.5 x 10^8 cells/ml were obtained with turbidity-adjusted medium on the surface at sterile of nutrient agar medium. a circular cavity of 6 mm diameter were made, at equidistant from the plate, The extract to be tested was dropped into the wells. 50 µL of the extracts at different concentration (1000.0, 500.0, 250.0, 125.0 mg/ml) per well. This Petri dish was cooled at 4 °C for 2 hours and then incubated for 18-24 hours at 37 °C, Chloramphenicol at a concentration of 25 µg/mL was used as positive control and 20% dimethyl sulfoxide (DMSO) as solvent of the samples and negative control. The test was performed for a total of 3 replicates and then the line size was measured in millimeters through the center of the inhibition zone and the results were reported as Mean ± standard deviation (SD). (Munazir et al., 2012)

3.4.1.3 Broth microdilution method

MIC (Minimum inhibitory concentration) test, the lowest concentration of pure compound used for testing that can completely inhibit the growth of the test bacteria (Paula et al., 2009). In this study, a broth microdilution test was performed on a 96- well plate. The antimicrobial was dissolved in 100% DMSO, then diluted to a concentration of 4000 mg/ml in 20% DMSO and tested against 4 strains: *E. coli*, *S. aureus*, *B. cereus* and *P. aeruginosa* by preparing a test sample. The sample was diluted by two-fold serial dilution to a concentration of 4000 to 7.81 µL/ml Aspirate 50 µL of the sample solution into the microplate well. Then bacteria with 50 µL of dilution was added to the wells, incubated at 37 °C for 18–24 hours, then 30 µL of 0.015% resazurin solution was added to all wells and mixed well. Continue incubating at 37 °C for 2-4 hours observing the change in color. Wells that are purple indicate that they can inhibit bacteria, while those that turn pink indicate that they cannot inhibit bacteria. The minimum antibacterial concentration was then recorded as the MIC (3 replicates of the experiment) and the antibiotic chloramphenicol was used at a

concentration of 0.781 µL/ml as a positive control and 20% DMSO, MHB as a negative control. (Tangjitjaroenkun et al., 2020)

3.4.2 Anti plant pathogenic fungi test

3.4.2.1 Isolation of fungi

Collected selected leaf chilli, rubber, rice and banana fragments with disease lesions infected parts was cut into small pieces (1 × 1 cm) surface sterilized with 95% ethanol for 3 minutes and was sterilized by using 0.5% sodium hypochlorite for 1-2 minute to remove the dirt attached in it then washed in sterilized water three times and then blotted with clean paper to dry. The samples were placed on Potato Dextrose Agar and incubated for 7 days at 30°C to be able the mycelium to growth. (Durgeshlal et al., 2019)

3.4.2.2 Preparation of fungi

The fungus contains *Colletotrichum sp*., *Fusarium sp*., *Alternaria sp*., and *Rhizoctonia solani*. The fungi were subculture into potato dextrose agar (PDA) slant and incubated at room temperature for 7 days before being stored at 4 °C.

3.4.2.3 Poisoned food technique

Antifungal activity test by poisoned food technique. Test the antifungal activity in plants of extracts by poisoned food technique by preparing potato dextrose agar (PDA) medium and plant extracts were added at concentrations of 10, 5, 1 mg/ml. Petri dish In the control part, the extract was not mixed and a cork borer with a diameter of 5 mm was used to puncture the apical end of the mycelium of the 7-day-old fungi, then transfer the agar slices onto the PDA medium with a needle. Center of petri dish was incubated at room temperature for 7 days, 3 replicates of the

experiment were performed to observe fungal growth. After that, the results were recorded by measuring the colony diameter of the fungi and comparing it with Captan fungicide at a concentration of 1 mg/ml and the percentage inhibition was calculated. Growth of fungal hyphae according to the following formula (Durgeshlal et al., 2019)

$$\text{Growth inhibition percentage} = \frac{C - T}{C} \times 100$$

C = mean diameter of mycelium growth in control set

T = mean diameter of fungal growth in the experimental set

3.4.2.4 Microplate dilution method

Alternaria are cultured on fungal culture dish PDA medium for 72 h. Then the surface of the colonies are gently scraped. The spores are then suspended in sterilized beakers. Spores are counted using haemacytometer and adjusted to get concentration of $1x10^6$ spores/ml.

The antifungal activity of the compounds using the microplate dilution method with 24-well microplates from (Seepe et al., 2021) Exactly 100 µL of sterile potato dextrose broth was added in each well. Isolated compounds were dissolved in 20% DMSO at 200 µg/mL. The sample was diluted by two-fold serial dilution to a concentration of 100 to 6.25 µL/ml and added 50 µL of spore suspensions (1.0×10^6 spores/mL) with three replicates. The microplate was sealed and incubated at 27°C for 72 hours. Therefore, the lower concentration at which visible inhibition of fungal mycelium was observed treaed was defined as the MIC value (µL/ml). Captan was positive control and 20% DMSO, sterile potato dextrose broth and spore suspension were the negative controls. (Mamarabadi et al., 2018)

3.4.3. Antioxidants activity assays

Study on the antioxidant activity of pure compounds from *Cinnamomum porrectum* by DPPH method. (2,2-diphenyl-1- picrylhydrazyl) radical scavenging activity assay modified from (Khatua et al., 2017), Preparation of 0.002% DPPH solution using 2 mg of DPPH, dissolve with 100 mL of methanol, shake well and set aside at room temperature in the dark for 2 hours.

Prepare the concentration of the purified compound and the ascorbic acid solution in methanol at the concentrations of 25, 50, 100, 200 and 400 and 0.0033 to 100 µg/ml respectively. 100 µl of the solution to be tested is mixed with100 µl of DPPH solution were added to 96-well plates, then stored in the dark for 30 min at room temperature. Each treatment was three replicates. The absorbance was measured at a wavelength of 518 nm using a SPECTROstar NANO Microplate Reader (BMG LABTECH) and ascorbic acid as a positive control (antioxidant standard) and methanol was used as a negative control test (blank solution). Calculate the percentage inhibition of free radical DPPH from the equation (Nascimento et al., 2020)

$$\text{Scavenging of DPPH (\%)} = [(A0 - A1) / A0] \times 100$$

A0 is the absorbance of the DPPH solution (control).

A1 is the absorbance value of pure compound used in tests and standard compound.

Graph the relationship between Scavenging of DPPH (%) and pure compound concentration. Reported as IC_{50} (50 % Inhibitory concentration) compared to the Ascorbic acid standard.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Extraction from the roots and barks of *C. porrectum*

The roots of *C. porrectum* (1.0 kg) were extracted with dichloromethane then acetone (each 5 L x 2). Each extract was combined and concentrated under vacuum to yield Dichloromethane and Acetone extracts in 25.41 g (2.54 %, based on dried plant material) and 35.50 g (3.55 %), respectively

Table 11. Fractions of dry weight and extraction yield of different parts of *Cinnamomum porrectum.*

Fraction	Solvent	Dry weight (g)	Yield after extraction (%)
Root	Dichloromethane	25.41	2.54
	Acetone	35.50	3.55
Bark	Dichloromethane	27.76	2.78
	Acetone	27.91	2.79

4.2 Structure elucidation of compounds the roots and of *C. porrectum.*

Structure elucidation of all compounds (**CP1-CP6**) was based on NMR spectroscopic method.

4.2.1 Compound CP1

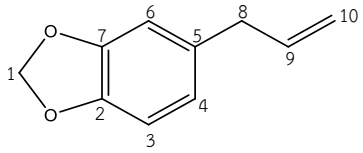


Figure 5. Chemical structure of **CP1**

Compound **CP1** was isolated as a light yellow oil. The UV (MeOH) spectrum exhibited absorption bands λ_{max} 234 and 286 nm characteristic of conjugated aromatic system. The IR (KBr) spectrum showed absorption bands at ν_{max} 2900 (=C-H), 2700 (C-H), 1500 (C=C), 1250 (C-O), 1050 (=C-H) and 900 (=C-H) cm^{-1}. The ^{1}H NMR spectrum (**Table12**.) showed signals for a singlet signal at δ_H 5.91 (O-CH_2-O) revealed a methylenedioxy group attached to the benzene ring, a signals of aromatic proton at δ_H 6.67 (1H, d, *J*=1.4 Hz), δ_H 6.62 (1H, dd, *J*=10,1.5 Hz) and δ_H 6.73 (1H, d, *J*=8 Hz) were defined to H-3, H-4 and H-6 respectively. a doublet signals. The down field shift of methylene protons H-8" at δ_H 3.29 (2H, d, *J*=10 Hz) showed that the prenyl group was attached to C-5 carbon, a multiplet signals of methine protons at δ_H 5.94 (1H, m) (H-9) and doublet signals of methylene protons at δ_H 5.03 (2H, d) (H-10) (**Figure 5**). The ^{13}C NMR and DEPT spectrum of showed 10 resonances comprising of a methylenedioxy group appeared at δ_c 100.77 ppm, two signals of C-2 and C-7 were observed at δ_c 145.78 and 147.59 ppm for a pair of overlapping quaternary carbons, four methine carbons (δ_c 109.07, 121.27, 108.14 and 137.58 ppm), two methylene carbons (δ_c39.89, 115.66 ppm) and a shielded aromatic quaternary carbon (δ_c 133.83 ppm).The HMBC correlations of methylenedioxy was attached to C-2, C-7 and C-8 between H-1, and HMBC correlation from H-3 to C-2, as well as C-4, C-5, C-6, C-7, C-8 and H-4 to C-8. and CH_2-10 to C-9 and H-9 to C-5, C-7 and C-8, suggesting that the $CHCH_2$ fragment bearing on C-8. Comparison NMR data for **CP1** with **Safrole** (Mohottalage et al., 2007), it

displayed similarity data. Thus, the entire structure of **CP1** was confirmed and named Safrole.

Table 12. ^{1}H (500 MHz) and ^{13}C NMR (500 MHz) spectral data of compound **CP1** and Safrole in $CDCl_3$

Positions	**CP1**		**Safrole***	
	δ_C (type)	δ_H (Multi, *J* in Hz)	δ_C (type)	δ_H (Multi, *J* in Hz)
1	100.77 (O-CH_2-O)	5.91(2H, s)	101.21 (O-CH_2-O)	5.95(2H, s, O-CH_2-O)
2	145.78 (qC)	-	146.23 (qC)	-
3	109.07 (CH)	6.67 (1H, d, *J*=1.4 Hz)	109.52 (CH)	6.72 (1H, d, *J*=1.4 Hz)
4	121.27 (CH)	6.62 (1H, dd, *J*=10,1.5 Hz)	121.72 (CH)	6.67(1H, dd, *J*=7.9,1.7 Hz)
5	133.83 (qC)	-	134.28 (qC)	-
6	108.14 (CH)	6.73 (1H, d, *J*=8 Hz)	108.58 (CH)	6.78(1H, d, *J*=7.9 Hz)
7	147.59 (qC)	-	148.04 (qC)	-
8	39.89 (CH_2)	3.29 (2H, d, *J*=10 Hz)	40.33 (CH_2)	3.34(2H, d, *J*=6.7 Hz)
9	137.58 (CH)	5.94 (1H, m)	138.02 (CH)	5.96(1H, m)
10	115.66 (CH_2)	5.03 (2H, d)	116.20 (CH_2)	5.10(2H, m)

*Mohottalage *et al*., 2007

Table 13. NMR data of **CP1** in $CDCl_3$

Positions	δ_H (ppm)	^{13}C HSQC (δ_C, ppm)	DEPT 90	DEPT 135	HMBC correlations
1	5.907	100.771 ($O\text{-}CH_2\text{-}O$)	-	100.771	-
2	-	145.776 (qC)	-	-	-
3	6.673	109.071 (CH)	109.071	-	C-2, C-4, C-5, C-6, C-7, C-8
4	6.620	121.272 (CH)	121.272	-	C-2, C-3, C-5 C-6, C-7, C-8,
5	-	133.832 (qC)	-	-	-
6	6.729	108.141 (CH)	108.141	-	C-2, C-3, C-4, C-5, C-6, C-7
7	-	147.589 (qC)	-	-	-
8	3.291	39.889 (CH_2)	-	39.889	C-3, C-4, C-5, C-6, C-9, C-10
9	5.935	137.575 (CH)	137.575	-	C-5, C-7, C-8
10	5.033	115.664 (CH_2)	-	115.664	C-8, C-9

4.2.2 Compound CP2

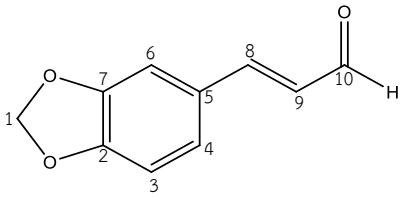


Figure 6. Chemical structure of **CP2**

Compound **CP2** was obtained as a yellow viscous oil, UV (MeOH) λ_{max} 337.5 (nm): FT-IR (KBr) ν_{cm-1}: 2913 (C-H); 2825 (C-H); 1725 (C=O); 1664 (C=C-C=O); 1613 (C=C); 1492 (C=C); 1254 (C-O-C); 937 (-C-O-C-). The ^{1}H NMR spectrum of **CP2** (**Table 14**.) was similar those of CP1, except the presence of an additional an aldehyde carbonyl group (a doublet signals at δ_H 9.67 (d, 1H, *J*=10 Hz). was related to the signals at δ_H 7.40 (1H, d, *J*=20 Hz) and δ_H 6.58 (1H, dd *J*=10,17.5 Hz) associating to an α, β unsaturated system (H-9 and H-8). signals of aromatic proton at δ_H 6.88 (1H, d, *J*=10 Hz), δ_H 7.09 (1H, d, *J*=1.8 Hz) and δ_H 7.10 (2H, dd, *J*=2.5,7.5Hz) were assigned to H-3, H-4 and H-6 respectively (**Figure 6**). The ^{13}C NMR and DEPT spectrum of showed 10 resonances comprising of an aldehyde carbonyl group at 193.59 ppm. The coupling pattern between H-9 and H-10 and the COSY correlation represents the existence of an aldehyde carbonyl group fragment. which was located at alkene chain due to the HMBC correlation from H-10 to C-8 (δc 152.61) and C-9 (δc 126.87). Comparison NMR data for **CP2** with **3-(3',4'-methylenedioxi) phenyl acrylaldehyde** (Catalan et al., 2010), it displayed similarity data. Thus, the entire structure of **CP2** was confirmed and named 3-(3',4'-methylenedioxi) phenyl acrylaldehyde**.**

Table 14. ^{1}H (500 MHz) and ^{13}C NMR (500 MHz) spectral data of compound **CP2** and **(2*E*)-3-(3',4'-methylenedioxi) phenyl acrylaldehyde** in $CDCl_3$

Positions	**CP2**		**3-(3',4'-methylenedioxi) phenyl acrylaldehyde***	
	δ_c (type)	δ_H (Multi, *J* in Hz)	δ_c (type)	δ_H (Multi, *J* in Hz)
1	101.83 ($O\text{-}CH_2\text{-}O$)	6.06 (2H, s, $O\text{-}CH_2\text{-}O$)	101.0 ($O\text{-}CH_2\text{-}O$)	6.04 (2H, s, $O\text{-}CH_2\text{-}O$)
2	150.52(qC)	-	150.10 (qC)	-
3	108.69(CH)	6.88(1H, d, *J*=10 Hz)	107.70 (CH)	6.86 (1H, d, *J*=8.6 Hz)
4	125.29(CH)	7.09(1H, d, *J*=1.8 Hz)	124.70 (CH)	7.06 (1H, d, *J*=1.7 Hz)
5	128.53(qC)	-	128.30 (qC)	-
6	106.72(CH)	7.10 (2H, dd, *J*=2.5,7.5Hz)	107.30 (CH)	7.08 (2H, dd, *J*=1.7, 8.6Hz)
7	148.60(qC)	-	148.50 (qC)	-
8	152.61(CH)	7.40(1H, d, *J*=20 Hz)	152.90 (CH)	7.38(1H, d, *J*=15.8 Hz)
9	126.87(CH)	6.58 (1H, dd *J*=10,17.5 Hz)	127.0 (CH)	6.56 (1H, dd *J*=7.7,15.8 Hz)
10	193.59(CHO)	9.67(1H, d, *J*=10 Hz)	193.90 (CHO)	9.65(1H, d, *J*=7.7 Hz)

* Catalan et al., 2010

Table 15. The ^{1}H-^{1}H COSY and HMBC correlations data of **CP2** in $CDCl_3$

Protons	COSY	HMBC correlations
H-1	-	C-2, C-7
H-3	H-4, H-6	C-2, C-4, C-5, C-6, C-7
H-4	H-3, H-6	C-2, C-3, C-5, C-6, C-7, C-8
H-6	H-3, H-4	C-2, C-3, C-4, C-5, C-7, C-8
H-8	H-9	C-4, C-5, C-6, C-7, C-9, C-10
H-9	H-8, H-10	C-5, C-10
H-10	H-8, H-9	C-8, C-9

4.2.3 Compound CP3

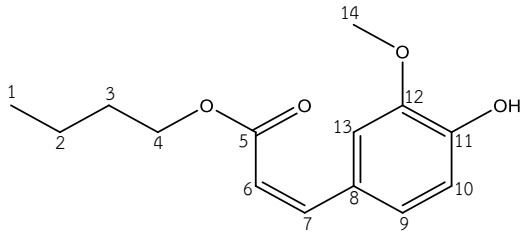


Figure 7. Chemical structure of **CP3**

Compound **CP3** was obtained as a light brown oil, FT-IR (KBr) ν_{cm-1}: 3559, 2849, 2331, 1955, 1859, 1063. The ^{1}H NMR data (**Table16**.) displayed typical signals methyl protons (δ_H 0.90 (3H, t, *J*=7.2 Hz)), three methylene protons (δ_H 1.72 (2H, m), 1.30 (2H, m) and 4.21 (2H, t, *J*=10 Hz)) and two methine protons (δ_H 6.32 (1H, d, *J*=15.0 Hz and 7.64 (1H, d, *J*=15.0 Hz)), three aromatic protons (δ_H 7.09, 6.95 and 7.29), hydroxyl proton (δ_H 5.88 (1H, s, OH)) and methoxy group (δ_H 3.95 (3H, s, OCH_3)). This was confirmed by the ^{13}C NMR and DEPT spectrum which showed signals of alkane chain at δ_C 14.14 (C-1), 29.38 (C-2), 28.79 (C-3) and 64.64 (C-4), signal of a typical ester group at δ_C 167.41 (C-5), signals of two methine carbons δ_C 115.69 (C-6) and 144.64 (C-7), signals of aromatic carbons at δ_C 127.07 (C-8), δ_C 123.06 (C-9), δ_C 114.70 (C-10), δ_C 147.90 (C-11), δ_C 146.76 (C-12) and δ_C 109.29 (C-13) and signal of methoxy group at δ_C 55.94 (C-14) (**Figure 7**). The HMBC correlations of ester group was attached to C-2, C-3, C-5, C-7 and C-8 between H-4 and H-6 and HMBC correlation from H-10 to C-8, as well as C-9, C-11, C-12, C-13 and H-14 to C-12, C-13. suggesting that the OCH_3 fragment bearing on C-12. Comparison NMR data for **CP3** with **n-Butyl ferulate** (Vafiadi *et al*., 2005), it displayed similarity data. Thus, the entire structure of **CP3** was confirmed and named n-Butyl ferulate.

Table 16. ^{1}H (500 MHz) and ^{13}C NMR (500 MHz) spectral data of compound **CP3** and **n-Butyl ferulate** in $CDCl_3$

Positions	**CP3**		**n-Butyl ferulate***	
	δ_C (type)	δ_H (Multi, *J* in Hz)	δ_C (type)	δ_H (Multi, *J* in Hz)
1	14.14(CH_3)	0.90 (3H, t, *J*=7.2 Hz)	14.1 ($CH_2CH_2CH_2CH_3$)	0.94 (3H, t, *J*=7.4 Hz, $CH_2CH_2CH_2CH_3$)
2	29.38(CH_2)	1.72 (2H, m)	31.0 ($CH_2CH_2CH_2CH_3$)	1.65(2H, m, $CH_2CH_2CH_2CH_3$)
3	28.79(CH_2)	1.30 (2H, m)	19.4 ($CH_2CH_2CH_2CH_3$)	1.40 (2H, m, $CH_2CH_2CH_2CH_3$)
4	64.64(CH_2)	4.21(2H, t, *J*=10 Hz, $CH_2CH_2CH_2CH_3$)	64.7 ($CH_2CH_2CH_2CH_3$)	4.18(2H, t, *J*=6.7 Hz, $CH_2CH_2CH_2CH_3$)
5	167.41(CHCHCOO)	-	167.9 (CHCHCOO)	_
6	115.69(CH)	6.32(1H, d, *J*=15.0 Hz, CHCHCOO)	109.8-148.4 (aromatic and CHCHCOO)	6.27(1H, d, *J*=15.7 Hz, CHCHCOO)
7	144.64(CH)	7.64(1H, d, *J*=15.0 Hz, CHCHCOO)	109.8-148.4 (aromatic and CHCHCOO)	7.59(1H, d, *J*=15.9 Hz, CHCHCOO)
8	127.07(qC)	-	109.8-148.4 (aromatic and CHCHCOO)	_
9	123.06(CH)	7.09	109.8-148.4 (aromatic and CHCHCOO)	6.88-7.05 (3H, m, aromatic)
10	114.70(CH)	6.95	109.8-148.4 (aromatic and CHCHCOO)	6.88-7.05 (3H, m, aromatic)

Table 16. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compound **CP3** and **n-Butyl ferulate** in $CDCl_3$ (continued)

Positions	**CP3**		**n-Butyl ferulate***	
	δ_C (type)	δ_H (Multi, *J* in Hz)	δ_C (type)	δ_H (Multi, *J* in Hz)
11	147.90(qC)	5.88(1H, s, OH)	109.8-148.4(aromatic and CHCHCOO)	6.23(1H, s, OH)
12	146.76(qC)	-	109.8-148.4(aromatic and CHCHCOO)	-
13	109.29(CH)	7.29	109.8-148.4(aromatic and CHCHCOO)	6.88-7.05 (3H, m, aromatic)
14	55.94 (OCH_3)	3.95(3H, s, OCH_3)	56.2 (OCH_3)	3.88(3H, s, OCH_3)

* Vafiadi *et al*., 2005

Table 17. The ^{1}H-^{1}H COSY and HMBC correlations data of **CP3** in $CDCl_3$

Protons	COSY	HMBC correlations
H-1	H-3	C-2, C-3
H-2	H-4, H-3	C-3, C-4
H-3	H-1, H-2	C-1, C-2
H-4	H-2	C-2, C-3, C-5
H-6	H-7	C-5, C-7, C-8
H-7	H-6	C-5, C-6, C-8, C-9, C-13
H-9	H-10	C-7, C-8, C10, C-11, C-12, C-13
H-10	H-9	C-8, C-9, C-11, C-12, C-13
H-13	-	C-9
H-14	-	C-12, C-13

4.2.4 Compound CP4

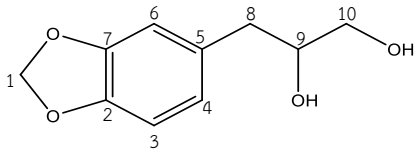


Figure 8. Chemical structure of **CP4**

Compound **CP4** was obtained as a colorless solid, m. p. 97–99 °C; UV (EtOH) λ_{max} (nm): FT-IR (KBr): 3369, 2922, 1596, 1448, 1442, 1245, 1037. The ^{1}H NMR spectrum of **CP4** (**Table 18**.) was similar those of **CP1**, except the presence of an additional hydroxyl group (a multiplet signals of two hydroxyl group at δ_H 2.01 (9-OH and 10-OH). This was related to the signals at δ_H 3.87 (1H, m) and δ_H 3.68-3.47 (2H, m) instead one of allylic moiety of **CP1**. The ^{13}C NMR spectrum display 10-carbon signals identities comprising of hydroxyl group at 73.00 and 65.95 ppm. Comparison NMR data for **CP4** with **3-(3′,4′-methylenedioxyphenyl)-prop-1,2-diol** (Mohottalage et al., 2007), it displayed similarity data. Thus, the entire structure of **CP4** was confirmed and named 3-(3′,4′-methylenedioxyphenyl)-prop-1,2-diol. (**Figure 8**).

Table 18. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compound **CP4** and ***3-(3′,4′-methylenedioxyphenyl)-prop-1,2-diol*** in $CDCl_3$

Positions	**CP4**		**3-(3′,4′-methylenedioxyphenyl)-prop-1,2-diol***	
	δ_C (type)	δ_H (Multi, *J* in Hz)	δ_C (type)	δ_H (Multi, *J* in Hz)
1	100.93 (O-CH_2-O)	5.92(2H, s, O-CH_2-O)	100.84 (O-CH_2-O)	5.90(2H, s, O-CH_2-O)
2	146.30(C)	-	146.14 (qC)	_
3	108.38(CH)	6.75(1H, d, *J*=8 Hz)	109.57 (CH)	6.73(1H, d, *J*=8.1 Hz)
4	122.20(CH)	6.70(1H, d, *J*=1.5 Hz)	122.14 (CH)	6.69(1H, d, *J*=1.5 Hz)
5	131.28(C)	-	131.39 (qC)	_
6	109.60(CH)	6.64(1H, dd, *J*=8, 1.5 Hz)	108.26 (CH)	6.62 (1H, dd, *J=8*.1,1.7 Hz)
7	147.82(C)	-	147.67 (qC)	_
8	39.45(CH_2)	2.72-2.62 (2H, m)	39.30 (CH_2)	2.74-2.56 (2H, m)
9	73.00(CH)	3.87 (1H, m)	73.06 (CH)	3.85 (1H, m)
10	65.95(CH_2)	3.68-3.47 (2H, m)	65.79 (CH_2)	3.79-3.41 (2H, m)
11	-	2.01 (2OH)	-	2.60 (2OH, br.s)

* Mohottalage et al., 2007

4.2.5 Compound CP5

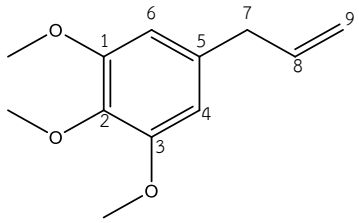


Figure 9. Chemical structure of **CP5**

Compound **CP5** was obtained as a colorless oil, FT-IR (KBr): 2937.1, 1590, 1507, 1240, 1128. The ^{1}H NMR spectrum of **CP5** (**Table 19**.) was similar that of **CP1**. The ^{1}H NMR showed an additional trimethoxyphenyl group (two singlet signals of methoxy group at δ_H 3.88 (3H, s, OCH_3 -1) and δ_H 3.84 (3H, s, OCH_3 -2). instead one of methylenedioxy group in **CP1**. Thus one of methylenedioxy group in **CP1** was replaced methoxy group in **CP5**. Moreover, **CP5** showed the presence of an aromatic methoxy group (δ_H 3.88 (3H, s, OCH_3 -3) and a terminal olefin group (δ_H 3.34 (2H, d, *J*=6.5Hz, H-7), δ_H 5.96 (1H, m, H-8) and δ_H 5.077 (1H, m, H-9), δ_H 5.13(1H, m, H-9). The ^{13}C NMR spectra of showed 12 resonances comprising of methoxy group at 56.06 (OCH_3-1,3) and 60.86 (OCH_3-2) ppm. Comparison NMR data for **CP5** with **Elemicin** (Lane & Kubanek, 2006), it displayed similarity data. Thus, the entire structure of **CP5** was confirmed and named Elemicin (**Figure 9**).

Table 19. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compound **CP5** and **Elemicin** in $CDCl_3$

Positions	**CP5**		**Elemicin***	
	δ_C (type)	δ_H (Multi, *J* in Hz)	δ_C (type)	δ_H (Multi, *J* in Hz)
1	153.18(qC)	-	153.10(qC)	-
2	135.81(qC)	-	135.80(qC)	-
3	153.18(qC)	-	153.10(qC)	-
4	105.41 (CH)	6.41(2H, s)	105.30 (CH)	6.41(2H, s)
5	136.28(qC)	-	136.20(qC)	3.84(6H, s, 3- OCH_3 and 5- OCH_3)
6	105.41 (CH)	6.41(2H, s)	105.30 (CH)	6.41(2H, s)
7	40.55(CH_2)	3.34(2H, d, *J*=6.5Hz)	40.50(CH_2)	3.32(2H, d, *J*=6.5Hz)
8	137.22(CH)	5.96(1H, m, CH=)	137.20(CH)	5.95(1H, m)
9	116.03(CH_2)	5.08 (1H, m) 5.13(1H, m)	116.00(CH_2)	5.08(1H, m) 5.12(1H, m)
1- OCH_3 and 3- OCH_3	56.06 (OCH_3-1,3)	3.88 (6H, s, OCH_3 -1 and -3)	56.10 (OCH_3-1,3)	3.85 (6H, s, OCH_3-1 and -3)
2- OCH_3	60.86 (OCH_3-2)	3.84(3H, s, OCH_3 -2)	60.80(OCH_3-2)	3.82(3H, s, OCH_3-2)

*(Lane & Kubanek, 2006)

4.2.6 Compound CP6

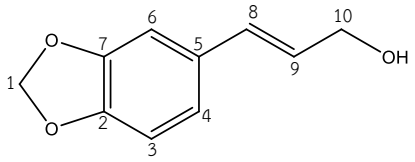


Figure 10. Chemical structure of **CP6**

Compound **CP6** was obtained as a white solid, m. p. 71-72 °C; UV (MeOH) λ_{max} 265, 304 (nm): FT-IR (KBr): 3400 (br), 2960, 1670, 1600, 1510, 1450, 970, 800. The ^{1}H NMR spectrum of CP6 (**Table 20**.) was similar those of **CP1**, except the presence of an additional hydroxyl group (a doublet signals of methylene protons at δ_H 4.29 (2H, dd, *J*=6.0, 1.0Hz) was related to the signals of an olefinic methine proton at δ_H 6.53 (1H, d, *J*=16.0Hz) and δ_H 6.23-6.17 (1H, m) associating to an α, β unsaturated system (H-8 and H-9). The ^{13}C NMR spectrum display 10-carbon signals identities comprising of hydroxyl group at 64.23 ppm. Comparison NMR data for **CP6** with **3-hydroxyisosafrole** (Angle et al., 2008), it displayed similarity data. Thus, the entire structure of **CP6** was confirmed and named 3-hydroxyisosafrole (**Figure 10**).

Table 20. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compound **CP6** and **3'-hydroxyisosafrole** in $CDCl_3$

Positions	**CP6**		**3'-hydroxyisosafrole**	
	δ_C (type)	δ_H (Multi, *J* in Hz)	δ_C (type)	δ_H (Multi, *J* in Hz)
1	101.26 (O-CH_2-O)	5.96(2H, s, O-CH_2-O)	100.9(O-CH_2-O)	5.92(2H, s, O-CH_2-O)
2	147.34(qC)	-	147.0(qC)	_
3	108.06(CH)	6.76 (1H, d, *J*=8.0Hz)	108.1(CH)	6.72(1H, d, *J*=8.4Hz)
4	121.19(CH)	6.82(1H, dd, *J*=8.0, 1.5Hz)	120.9(CH)	6.77(1H, d, *J*=8.4Hz)
5	133.91(qC)	-	131.0(qC)	_
6	105.89(CH)	6.93(1H, d, *J*=1.5Hz)	105.6(CH)	6.89(1H, s)
7	148.05(qC)	-	147.7(qC)	_
8	131.05(CH)	6.53(1H, d, *J*=16.0Hz)	130.6(CH)	6.47(1H, d, *J*=15.9Hz)
9	126.67(CH)	6.17-6.23 (1H, m)	126.6(CH)	6.15(1H, dt, *J*=15.9,5.4Hz)
10	64.23(CH_2)	4.29(2H, dd, *J*=6.0, 1.0Hz)	63.4(CH_2)	4.24(2H, d, *J*=5.4Hz)
- OH				2.51(1H, br s)

* Angle et al., 2008

4.3 Antimicrobial activities

4.3.1 Antibacterial activities

4.3.1.1 Agar well diffusion method

The antibacterial activity of dichloromethane and acetone crude extracts from the roots and bark of *C. porrectum* were investigated by agar diffusion test from crude extracts were assayed for antibacterial activity against *S. aures*, *B. cereus*, *P. aeruginosa* and *E. coli*. The results were evaluated by inhibition zone using a standard Chloramphenicol as positive control and 20% DMSO as negative control.

The results of 20%DMSO (Negative control) showed no inhibitory effect and Chloramphenicol (Positive control) showed the inhibition zones ranging from 15 to 28 mm. The largest zone of inhibition was found against *S. aureus* (ATCC25923) and *B. cereus* (ATCC11778), while *E. coli* (ATCC25922) and *P. aeruginosa* (ATCC27853) were not inhibited. The inhibition zone of dichloromethane extracts from root with a mean inhibition zone at 22.33 ± 1.45 mm. The inhibition zone of dichloromethane extracts of bark with inhibition zone value of 17.00 ± 1.73 mm and the most effective inhibition was observed at 22.33±1.45 mm are presented in **Table 21.**

Table 21. The inhibition growth of crude extracts from *Cinnamomum porrectum* against bacteria (Inhibition zone)

Test	Inhibition zone diameter (mm)				
	E. coli (ATCC25922)	*P. aeruginosa* (ATCC27853)	*S. aureus* (ATCC25923)	*B. cereus* (ATCC11778)	p - value
Root extract					
Crude Dichloromethane	N	N	12.00 ± 0.57^{Cb}	22.33 ± 1.45^{Ba}	*
Crude Acetone	N	N	11.16 ± 0.88^{Ca}	7.66 ± 0.33^{Db}	*
Bark extract					
Crude Dichloromethane	N	N	N	17.00 ± 1.73^{C}	
Crude Acetone	N	N	17.33 ± 0.33^{Ba}	16.00 ± 0.58^{Cb}	*
Positive control					
Chloramphenicol	25.33 ± 0.88^{b}	15.66 ± 0.33^{d}	22.00 ± 0.58^{Ac}	28.33 ± 0.33^{Aa}	*
Negative control					
20% DMSO	N	N	N	N	
p - value			*	*	

Remark Data represent with mean ± standard error, n=3

The different superscripts in the same column (A>B>C>D) and row (a>b>c>d) indicate a significant difference at $p<0.05$

N = no inhibition zone

4.3.1.2 Minimum inhibitory concentration (MIC)

The results of antibacterial activity of the pure compounds (**CP1 - CP6**) were shown in **Table 22**. Broth microdilution method was used to find the minimum concentration that can inhibit the growth of bacteria. The results showed that **CP1-CP6** were able to inhibit growth of *S. aureus* and *B. cereus* with MIC values in the range of 1,000 and 4,000 µg/ml. but *E. coli* and *P. aeruginosa* were not inhibited by **CP1-CP6**. The most effective MIC value at 1,000 µg/ml. were found in **CP6** against *S. aureus* and *B. cereus*, respectively.

Table 22. Minimal inhibitory concentration (MIC) of *C. porrectum* root extract and bark extract

Test	MIC (µg/ml)			
	E. coli (ATCC25922)	*P. aeruginosa* (ATCC27853)	*S. aureus* (ATCC25923)	*B. cereus* (ATCC11778)
Compounds				
CP1	-	-	4000	4000
CP2	-	-	4000	4000
CP3	-	-	4000	2000
CP4	-	-	2000	2000
CP5	-	-	4000	4000
CP6	-	-	1000	2000
Positive control				
Chloramphenicol	1000	500	62.5	62.5
Negative control				
20% DMSO	N	N	N	N

Remark N= inactive.

4.3.2 Inhibition plant pathogenic fungi

4.3.2.1 Poisoned food technique

Test of antifungal activity by poisoned food technique using crude extracts from Dichloromethane and Acetone at concentration of 10, 5, 1 mg/ml. The results found the dichloromethane crude extract from the root of *C. porrectum* at a concentration of 10, 5, 1 mg/ml showed the highest inhibition against the mycelial growth of *A. alternata* at 42.86 – 97.56 %, followed by *R. solani* at 33.33 – 96.84 %, *Colletotrichum* sp. 37.35 – 90.77% and crude dichloromethane extract from *C. porrectum* bark showed the against antifungal activity *Colletotrichum* sp. at 33.73 – 86.75 %. The crude acetone from the root and bark showed the highest mycelial growth inhibition of *R. solani* and *Fusarium sp*. at 2.30-95.40%, 13.64-81.82% and 1.15-90.45%, 18.18-86.36% respectively. The inhibition of fungal growth is still lower than Captan antifungal at concentration 1 mg/ml (Table 22.) showed the high inhibition on mycelial growth of *R. solani*, *Fusarium* sp., *A. alternata* And *Colletotrichum* sp. at 100.00, 100.00, 97.14 and 91.57 %, respectively.

Table 23. In vitro growth inhibitory effect of crude extracts from *Cinnamomum porrectum Kosterm* on the mycelial growth of plant pathogenic fungi at room temperature for 7 day.

Test	Conc. (mg/ml)	% mycelial growth Inhibition of plant pathogenic fungi			
		Colletotrichum sp.	*Alternaria alternate*	*Rhizoctonia solani*	*Fusarium sp.*
Root extract					
Crude Dichloromethane	10	90.77	97.56	96.84	95.45
	5	44.58	48.57	57.47	29.55
	1	37.35	42.86	33.33	27.27
Crude Acetone	10	59.04	65.71	95.40	81.82
	5	48.19	45.71	26.44	27.27
	1	36.14	44.29	2.30	13.64
Bark extract					
Crude Dichloromethane	10	86.75	72.86	49.43	72.72
	5	43.37	45.71	39.08	31.81
	1	33.73	32.86	17.24	20.45
Crude Acetone	10	61.45	57.14	90.45	86.36
	5	39.76	48.57	42.53	31.82
	1	36.14	38.57	1.15	18.18
Positive control					
Captan	1	91.57	97.14	100.00	100.00
Negative control					
DMSO	20%	N	N	N	N

Remark N= inactive.

4.3.2.2 Microplate dilution method

Selection by the Poisoned food technique, crude dichloromethane extract from *C. porrectum* roots should might inhibitory effect on the growth of *A. alternata* than acetone crude extract. The minimum antifungal concentration (MIC) of pure compounds **CP1-CP6** isolated from crude dichloromethane extract from *C. porrectum* roots were evaluated for inhibit the mycelial growth of *A. alternata* by microplate dilution method technique and antifungal drug Captan was used as a positive control. The result revealed that the pure compounds **CP1-CP6** showed low antifungal activity with MIC values above 100 µg/ml. The minimum (MIC) level of fungicide Captan shows a MIC value of 12.5 µg/ml as summarized in **Table 24**.

Table 24. Minimum inhibitory concentrations of pure compounds from *C. porrectum* against the growth of *A. alternata* at room temperature for 72 hours.

Compounds	*Alternaria alternata*
	MIC value (µg/ml)
CP1	>100
CP2	>100
CP3	>100
CP4	>100
CP5	>100
CP6	>100
Captan	12.5

4.3.3 Antioxidants activity assays

Test results of antioxidant activity of pure compounds **CP1-CP6** by using DPPH (2,2-diphenyl-1- picrylhydrazyl) assay. is obtained from the graph of a standard solution (Ascorbic acid) with the equation. The equation of a straight line is y=0.6938x +33.159 R^2 = 0.973 as shown in Figure 11, representing Y with 50 in the equation to find out the IC_{50} value for total antioxidant activities. The results demonstrated that the compounds **CP1 - CP6** has a different ability to inhibit free radicals, with compound **CP3** were found to be good antioxidant activity with IC_{50} values of 55.52 µg/ml, followed by CP5 compounds, where the IC_{50} value is 61.17 µg/ml. Compound **CP1** has the lowest antioxidant activity, with IC_{50} values of 98.93 µg/ml, which has antioxidant activity low free radicals compared to the standard ascorbic acid at 24.27 µg/ml as shown in **Table 25**. It was found to be consistent with the research of Venkateswarlu et al., 2006 to study the antioxidant activity. The independent n-Butyl ferulate by DPPH radical scavenging activity method showed an IC_{50} value of 51 µM, similar and also consistent with the research of Teixeira et al., 2013. A comparative study on the antioxidant activity of hydroxycinnamic acid and its ester derivatives by DPPH radical scavenging activity method. Butyl ferulate shows an IC_{50} value at 56.3 µmol L^{-1}. While hydroxycinnamic acid is a group of compounds found in food, it is a potent antioxidant in the group of hydroxycinnamic acids. These compounds act to scavenge free radicals related to the ability to electron donor.

Table 25. The IC_{50} values of DPPH scavenging effect of pure compounds **CP1-CP6** (µg/mL).

Compounds	DPPH IC_{50}(µg/mL)
CP1	98.93
CP2	73.63
CP3	55.52
CP4	79.72
CP5	61.17
CP6	86.22
Ascorbic acid	24.27

CHAPTER 5

CONCLUSION

Six phenylpropanoid compounds were isolated from crude Dichloromethane extract of *C. porrectum* root and bark. This study intended to examine the structure and compare the data of the reported compounds. ^{1}H and ^{13}C NMR is Safrole **(CP1)**, 3,4 Methylenedioxy cinnamaldehyde **(CP2)**, n-Butyl ferulate **(CP3)**, 3-(3,4-Methylenedioxyphenyl)-1,2-propanediol **(CP4)**, Elemicin **(CP5)** and 3,4-(Methylenedioxy) cinnamyl alcohol **(CP6)**, dichloromethane crude extract from the root and bark of the *C. porrectum* showed the inhibition zone of *B. cereus* well in the range of 12 - 22.33 and 10.33 - 17 mm, respectively, and the crude dichloromethane extract from roots and acetone extract from *C. porrectum* bark shows the inhibition zone of *S. aureus* well in the range of 10 -12 and 10 - 17.33 mm, respectively. compounds **CP6** and **CP4** showed activity against *S. aureus* with MIC values of 1,000 and 2,000 µg/ml respectively. and compounds **CP3**, **CP4** and **CP6** showed inhibitory activity against *B. cereus* containing MIC values were 2,000 µg/ml as well. dichloromethane extract from the root of the *C. porrectum* at the concentration of 10, 5, 1 mg/ml was the most effective inhibition on mycelial growth of *A. alternata* of 42.86 – 100.00% and the pure compound **CP1-CP6** showed antifungal activity against *A. alternata* is lowest, with MIC value above 100 µg/ml. These results indicate that the crude extracts and pure compounds from the root and bark of *C. porrectum* can be used as a source of natural health products due to its antioxidant and antimicrobial activities and might be potential to develop new bio fungicidal and antioxidant products.

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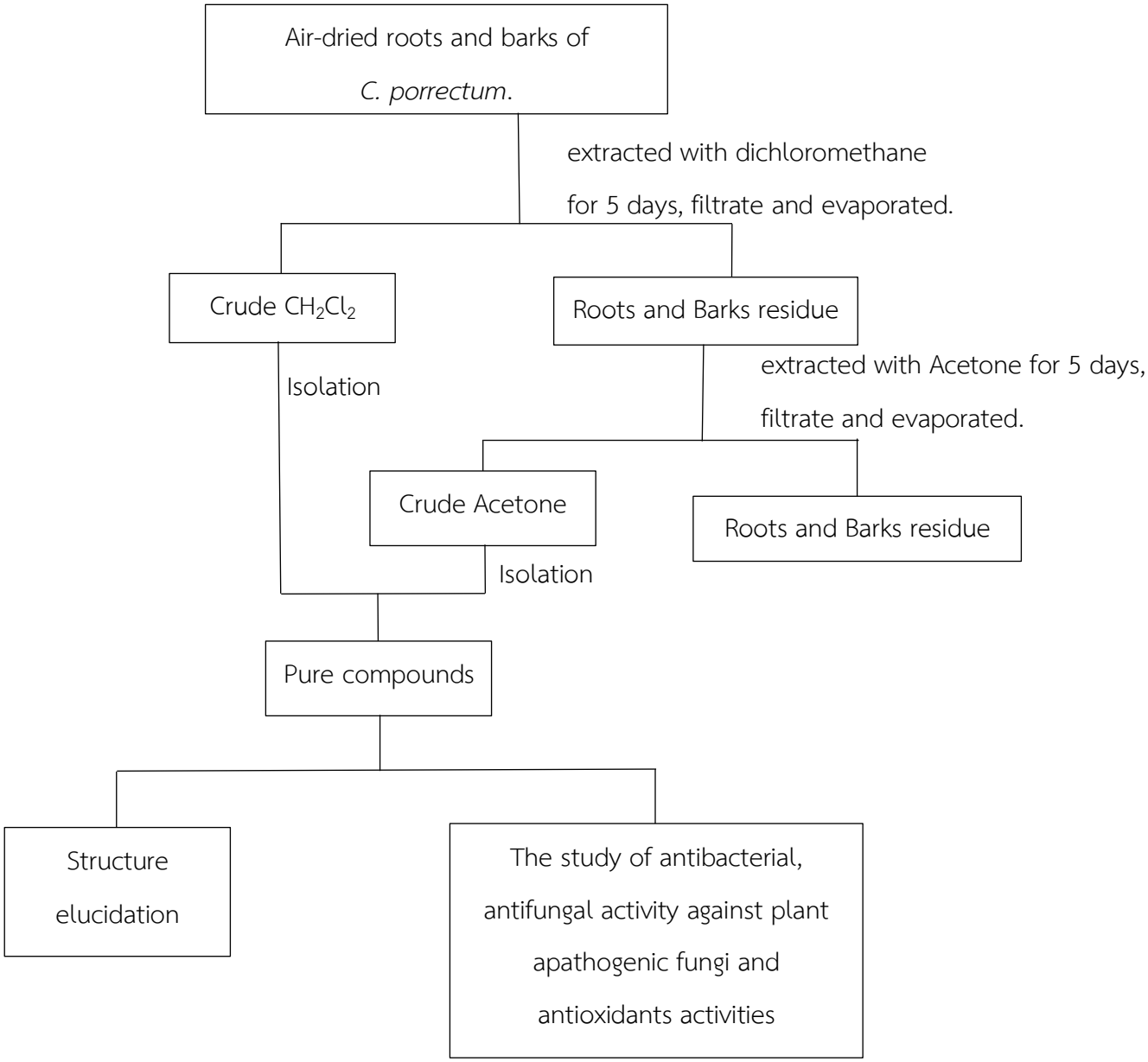
APPENDIX

APPENDIX A

Schematic diagram for experimental

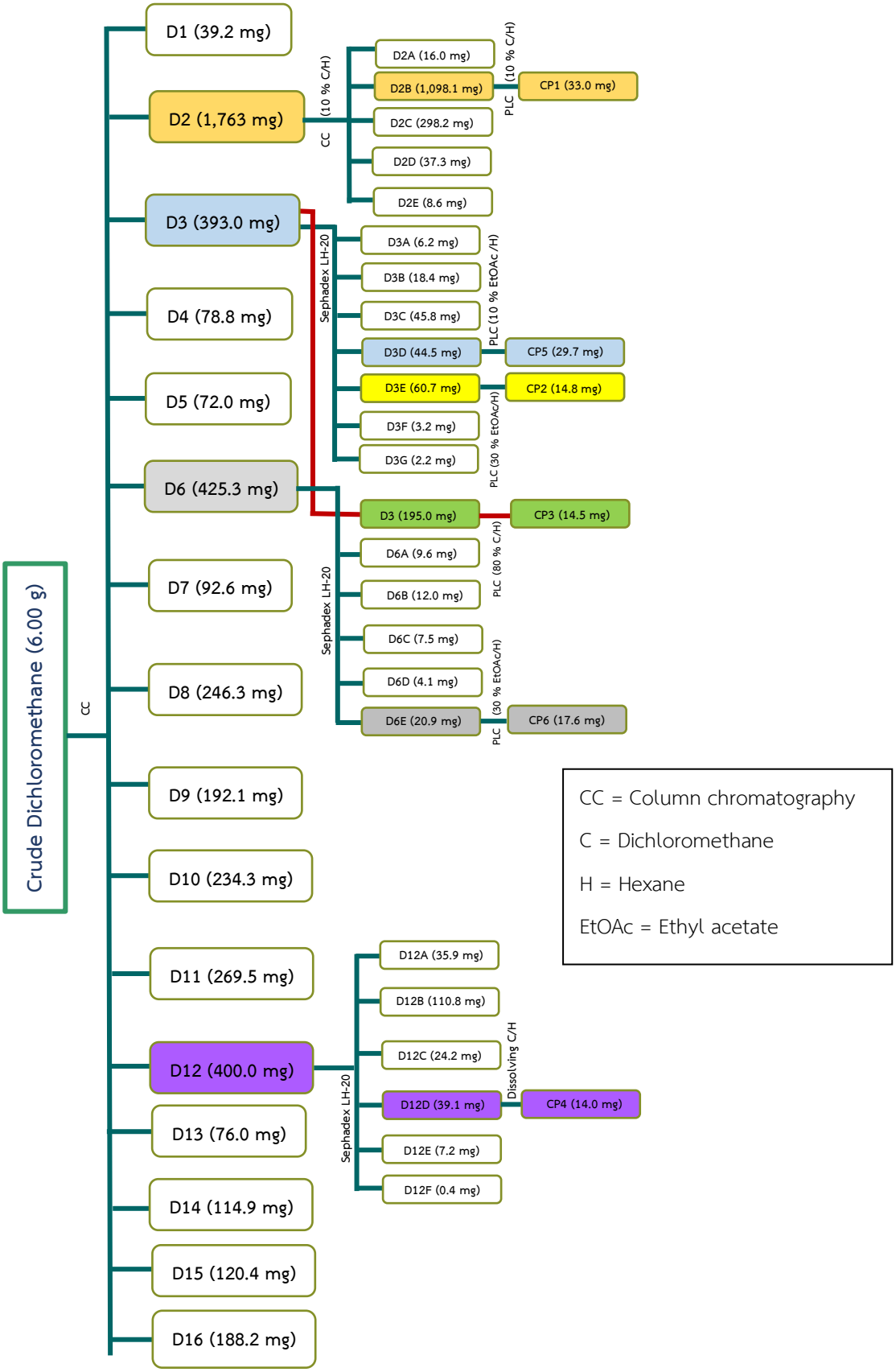
Schematic diagram for experimental



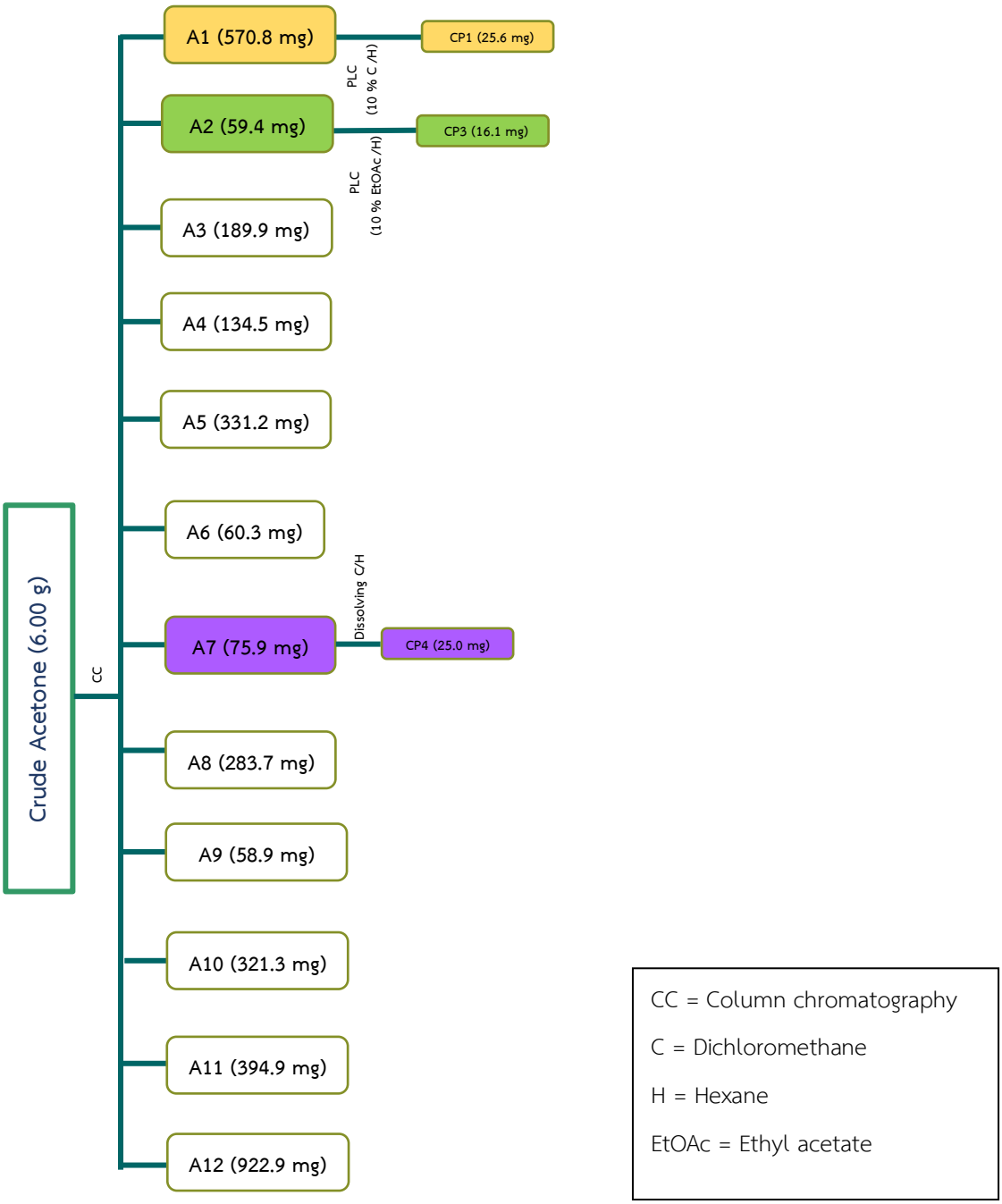
APPENDIX B

Schematic diagram for Isolation of compounds

Schematic diagram for Isolation of Crude Dichloromethane from roots



Schematic diagram for Isolation of Crude Acetone from roots



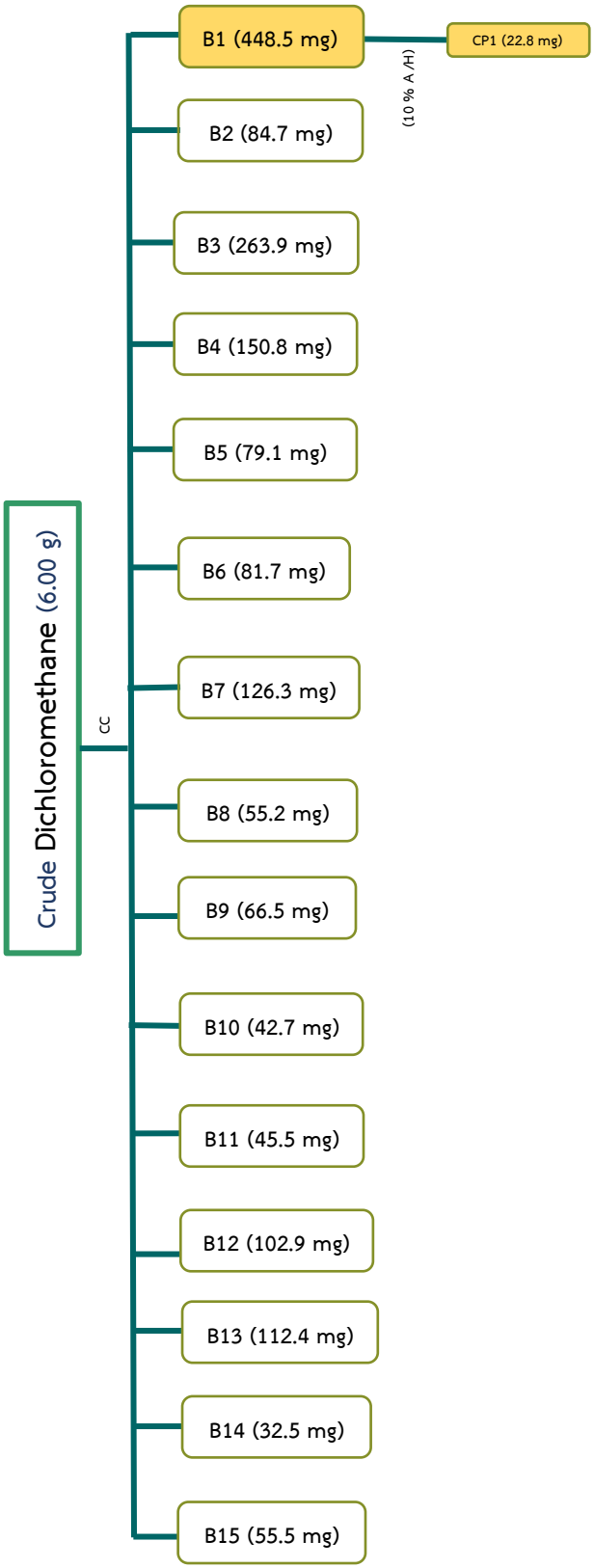
CC = Column chromatography

C = Dichloromethane

H = Hexane

EtOAc = Ethyl acetate

Schematic diagram for Isolation of Crude Dichloromethane from barks



CC = Column chromatography

C = Dichloromethane

H = Hexane

EtOAc = Ethyl acetate

A = Acetone

APPENDIX C

Preparation of chemical and culture media

1. Preparation of vanillin reagent

Vanillin	1	g
Concentrated sulfuric acid	100	mL

The mixture of components and packaging in reagent bottle brown amber glass.

2. Preparation of 2,2-diphenyl-1-picrylhydrazyl (DPPH)

2,2-diphenyl-1-picrylhydrazyl	**2**	**g**
Methanol	100	mL

3. Preparation of dimethyl sulfoxide (DMSO)

Dimethyl sulfoxide	60	mL
DI water	40	mL

These components were provided sterilized with an autoclave for 15 minutes at 121 °C. The mixture of components and stored at 4 °C.

4. Preparation of resazurin

7-Hydroxy-3H-phenoxazin-3-one 10 oxide	30	mg
DI water	200	mL

APPENDIX D

Spectral data of compounds CP1-CP6

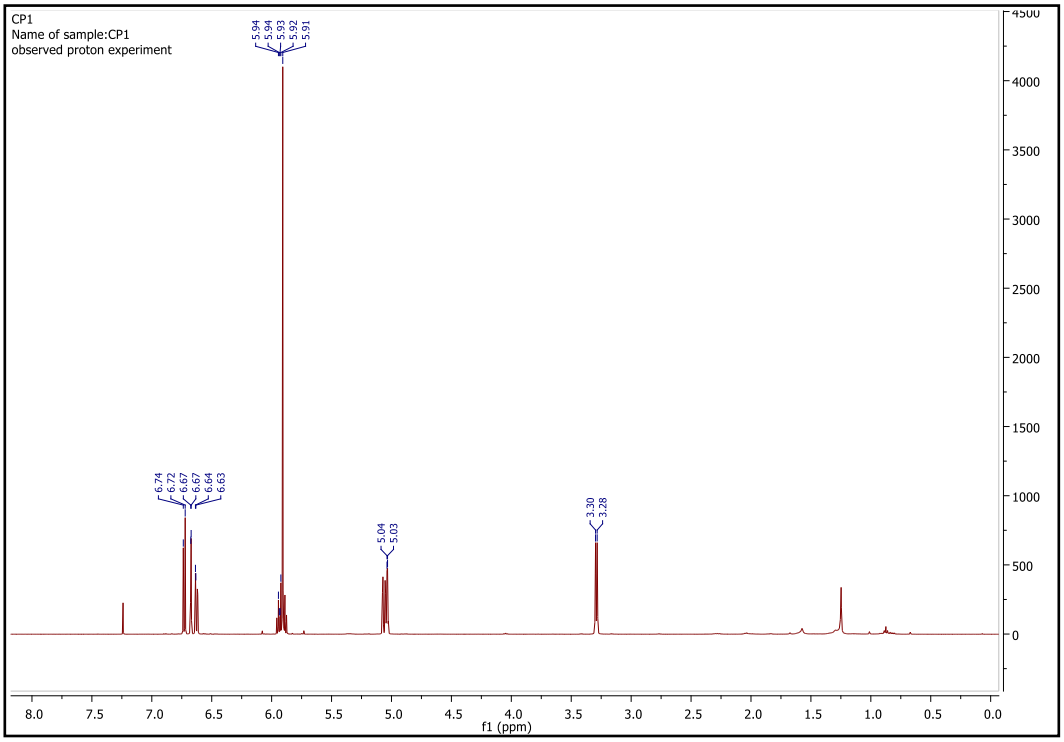


Figure 11. The 500 MHz ^{1}H NMR spectrum of compound **CP1** in $CDCl_3$

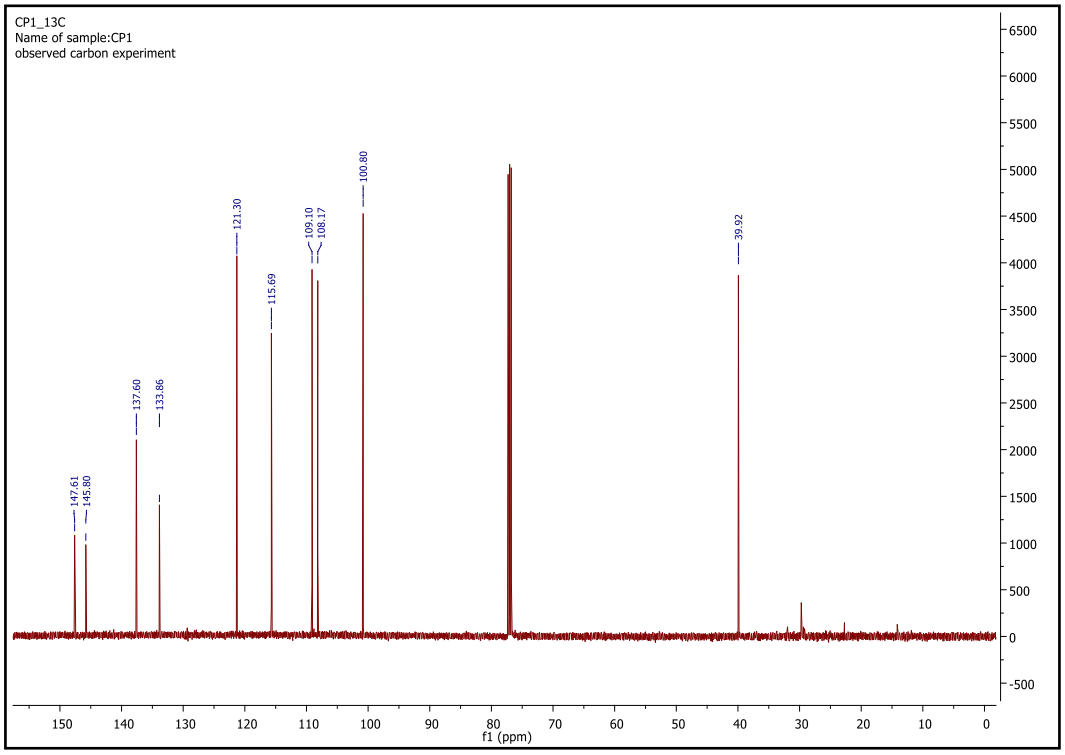


Figure 12. The 125 MHz ^{13}C NMR spectrum of compound **CP1** in $CDCl_3$

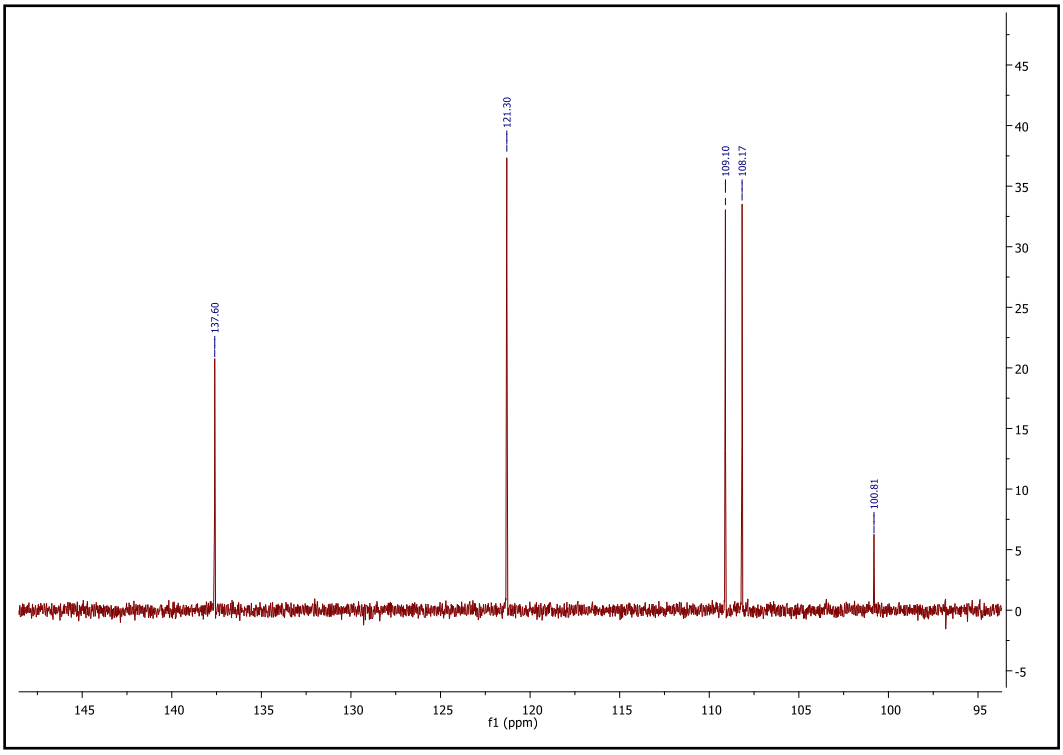


Figure 13. The 125 MHz DEPT90 spectrum of compound **CP1** in $CDCl_3$

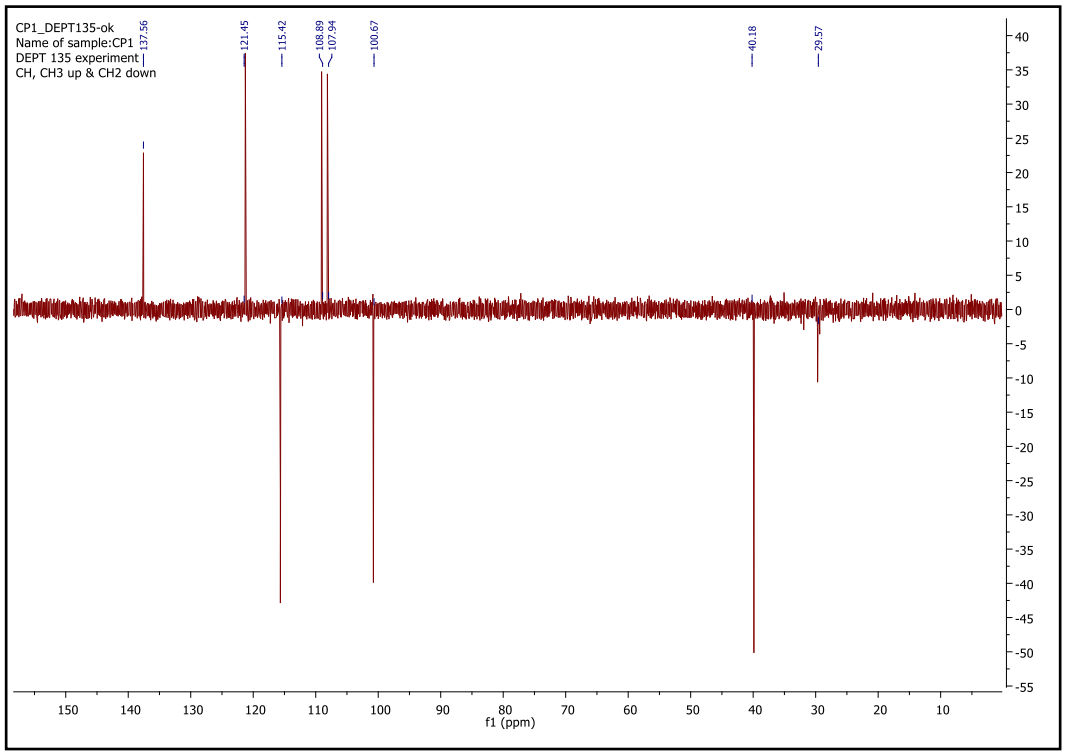


Figure 14. The 125 MHz DEPT135 spectrum of compound **CP1** in $CDCl_3$

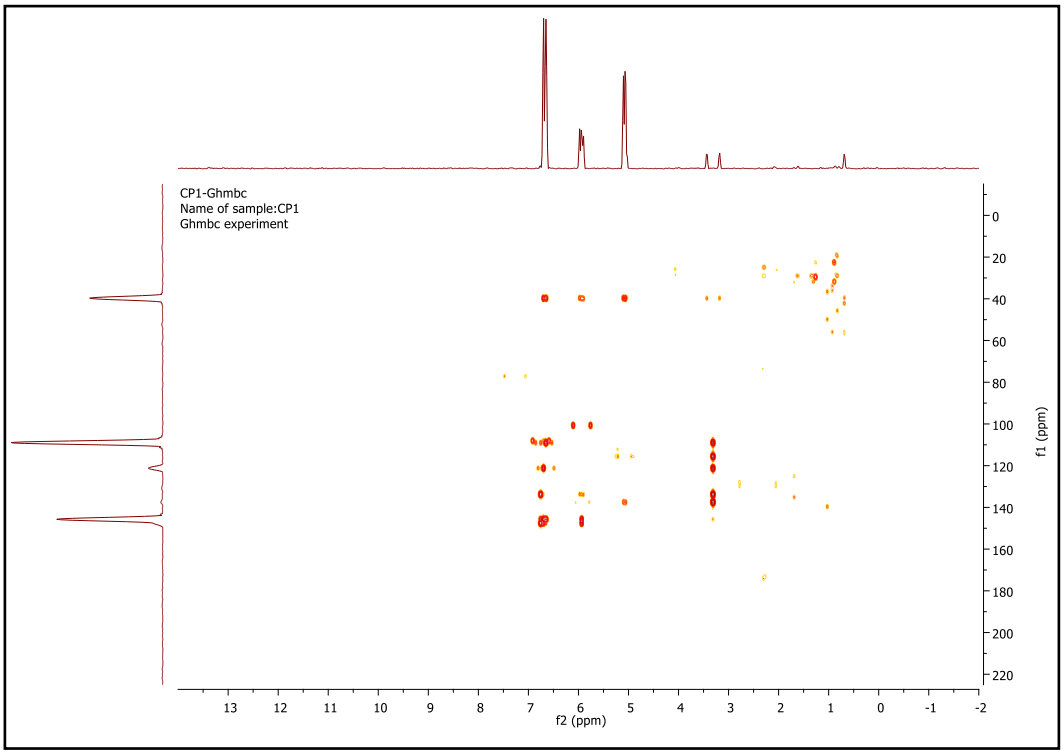


Figure 15. The HMBC correlations data of compound **CP1** in $CDCl_3$

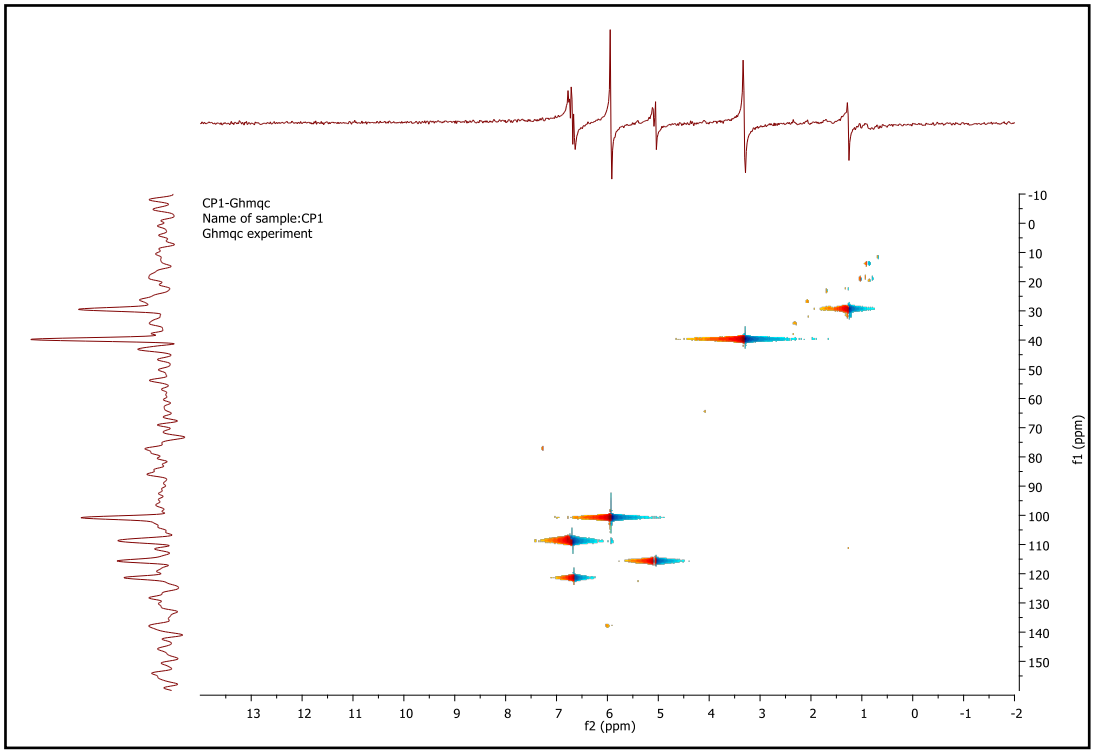


Figure 16. The HMQC correlations data of compound **CP1** in $CDCl_3$

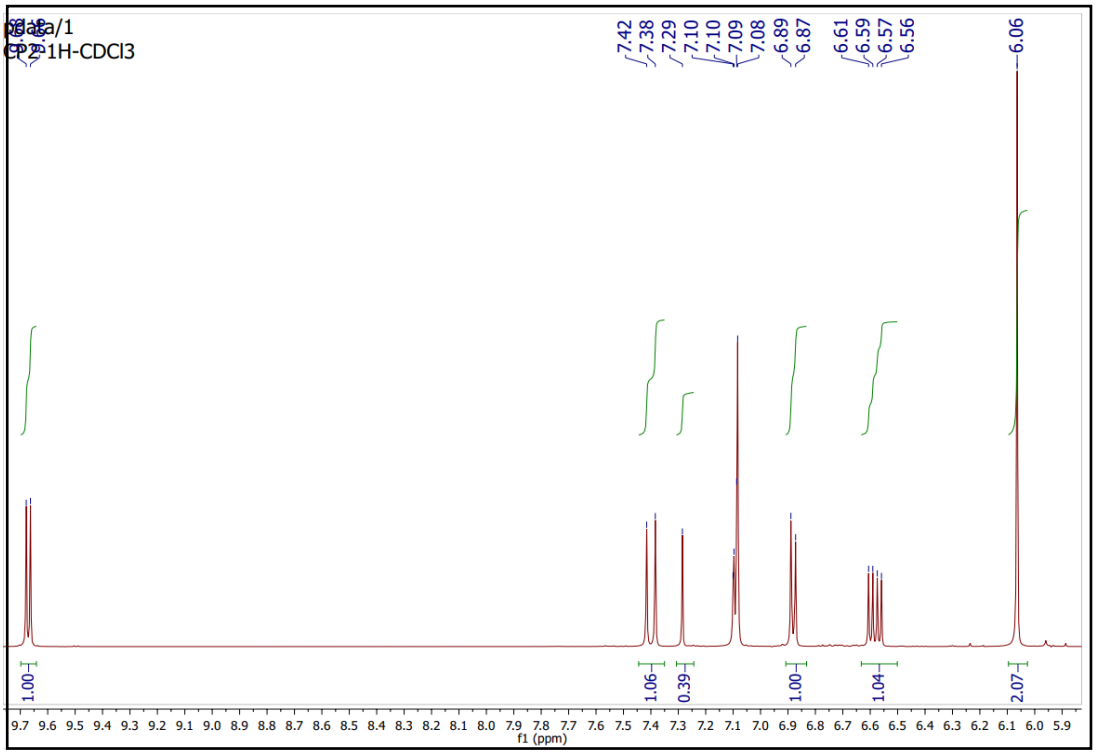


Figure 17. The 500 MHz ^{1}H NMR spectrum of compound **CP2** in $CDCl_3$

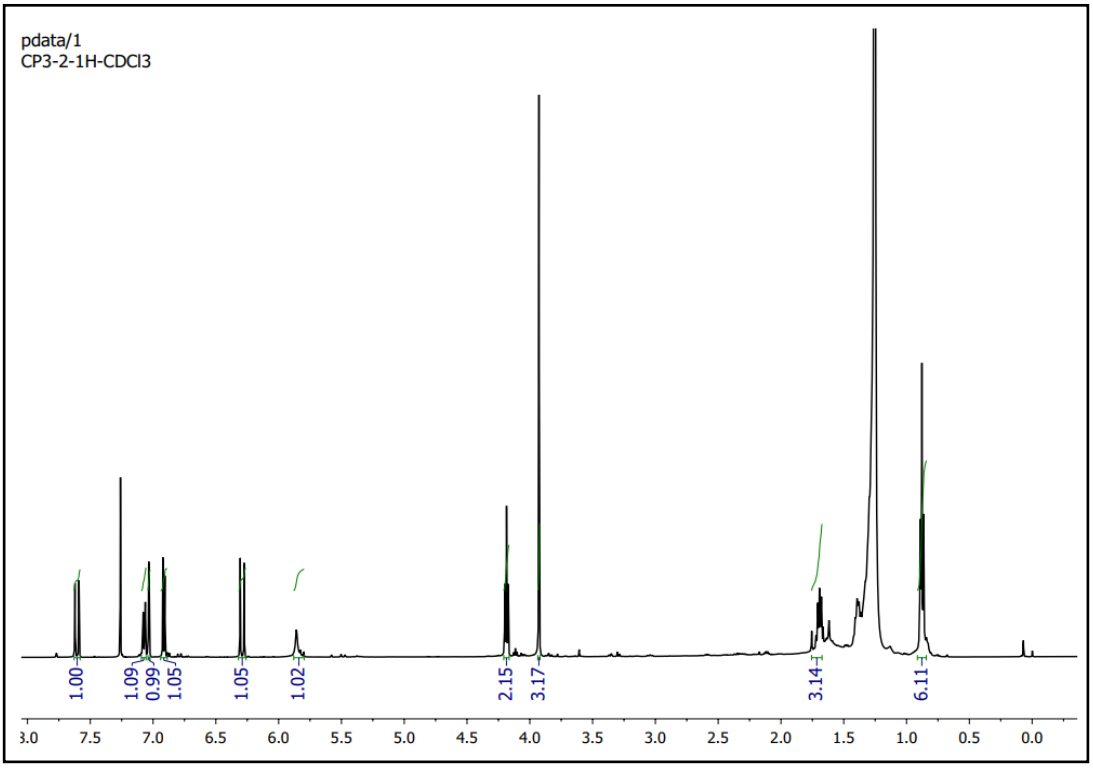


Figure 18. The 500 MHz ^{1}H NMR spectrum of compound **CP3** in $CDCl_3$

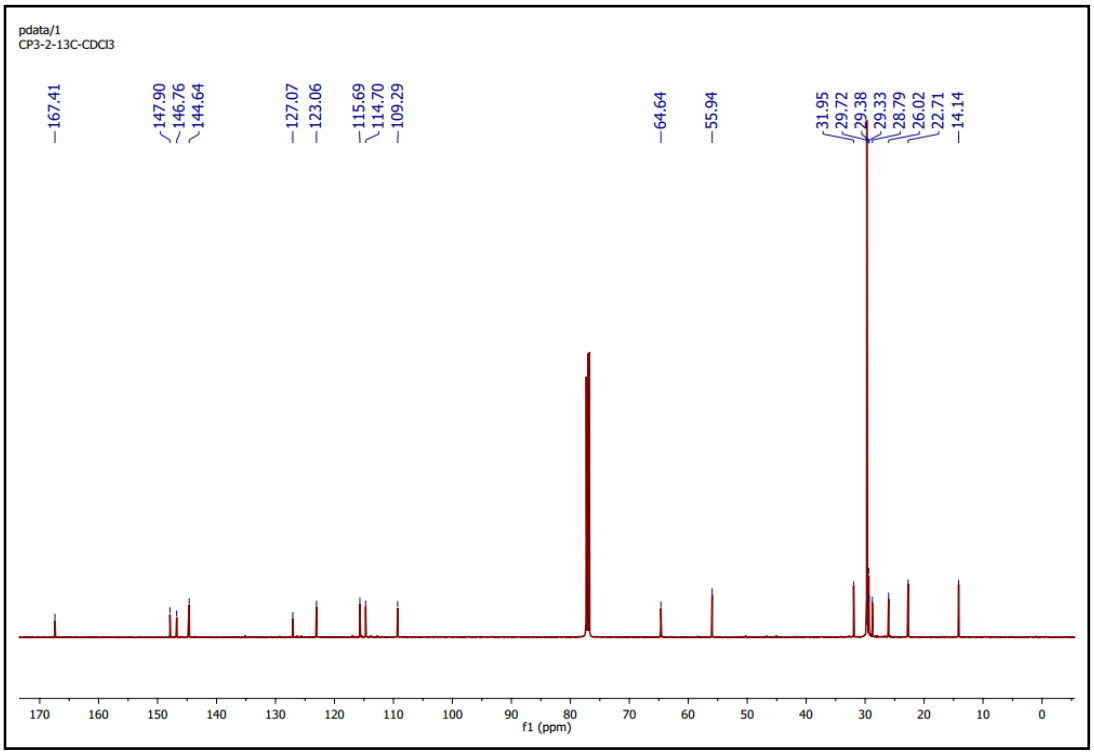


Figure 19. The 125 MHz ^{13}C NMR spectrum of compound **CP3** in $CDCl_3$

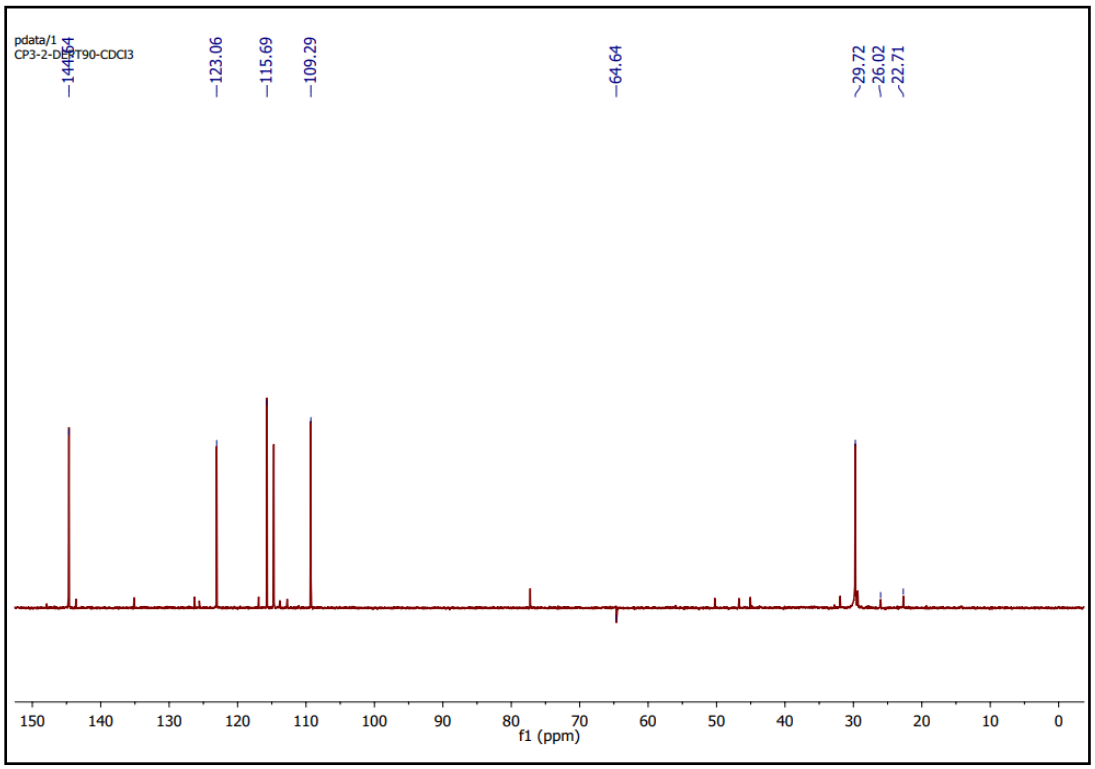


Figure 20. The 125 MHz DEPT90 spectrum of compound **CP3** in $CDCl_3$

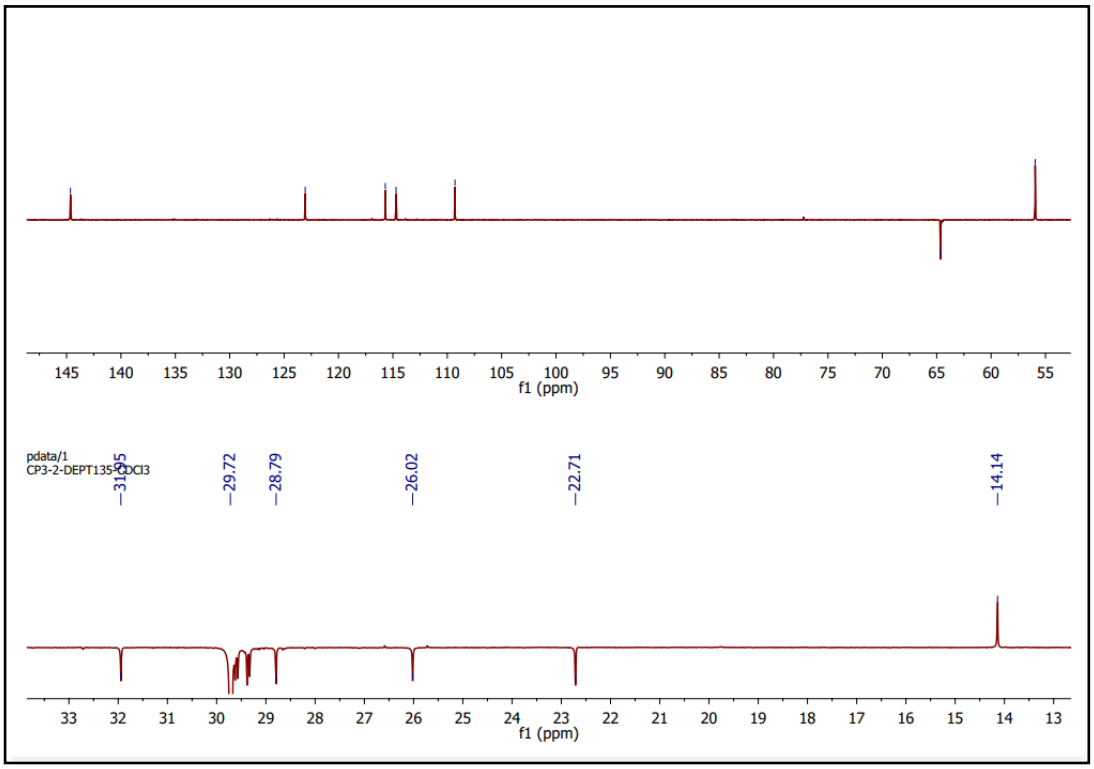


Figure 21. The 125 MHz DEPT135 spectrum of compound **CP3** in $CDCl_3$

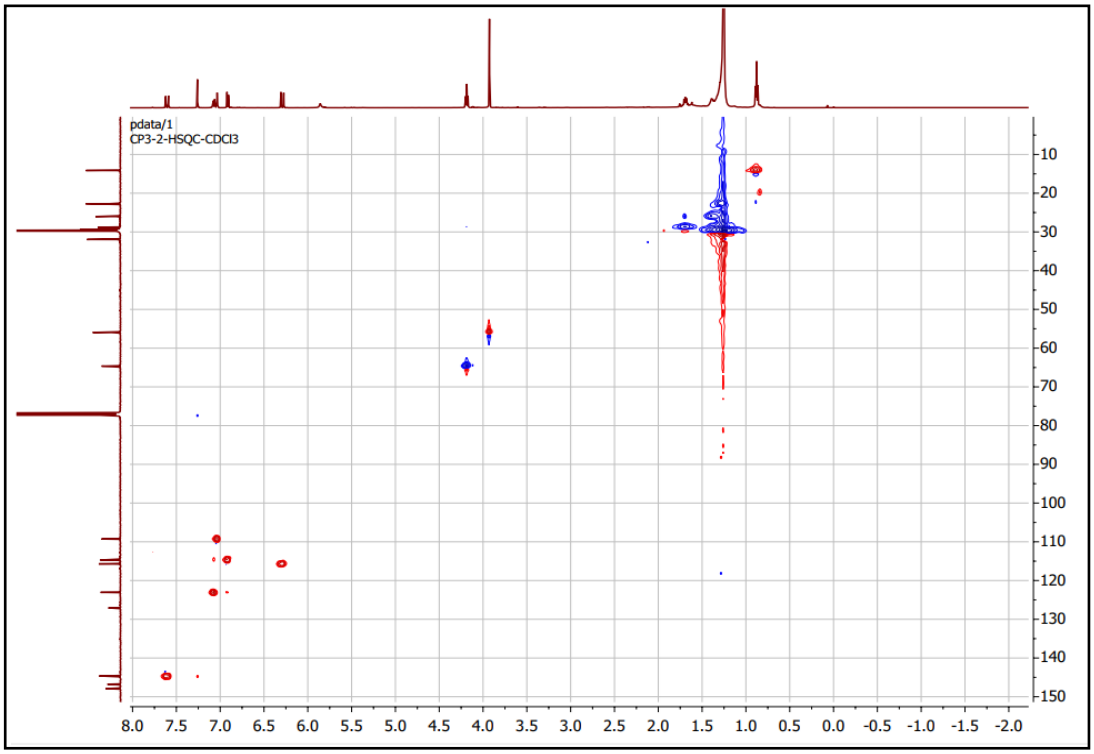


Figure 22. The HSQC correlations data of compound **CP3** in $CDCl_3$

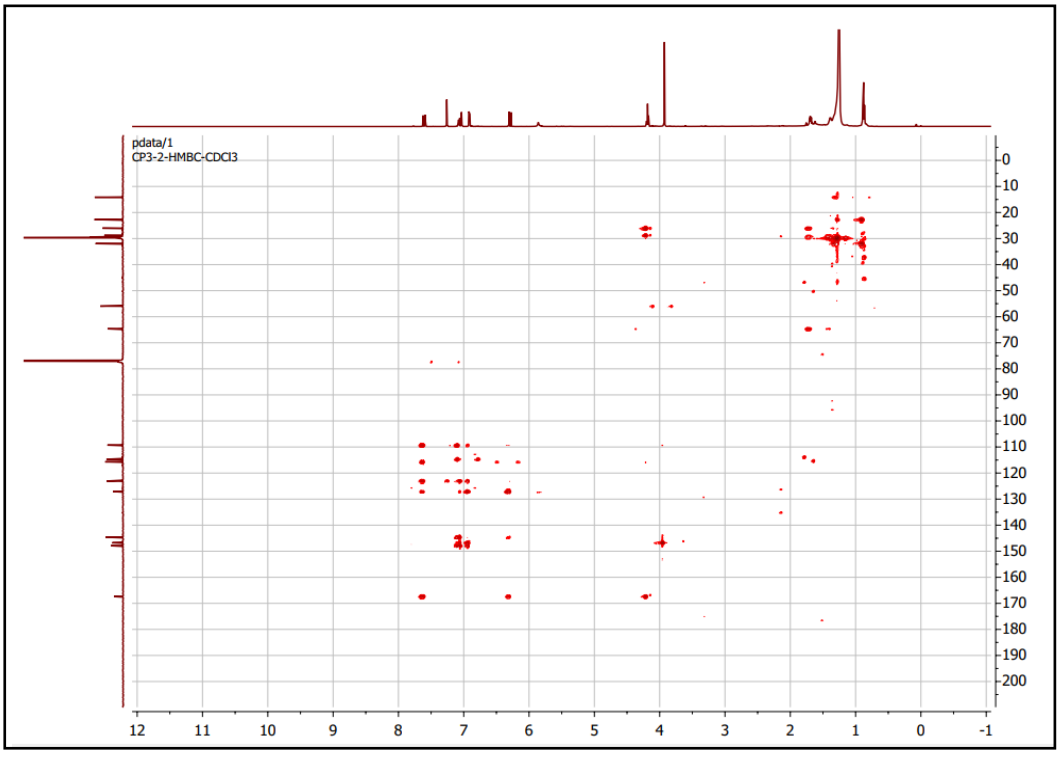


Figure 23. The HMBC correlations data of compound **CP3** in $CDCl_3$

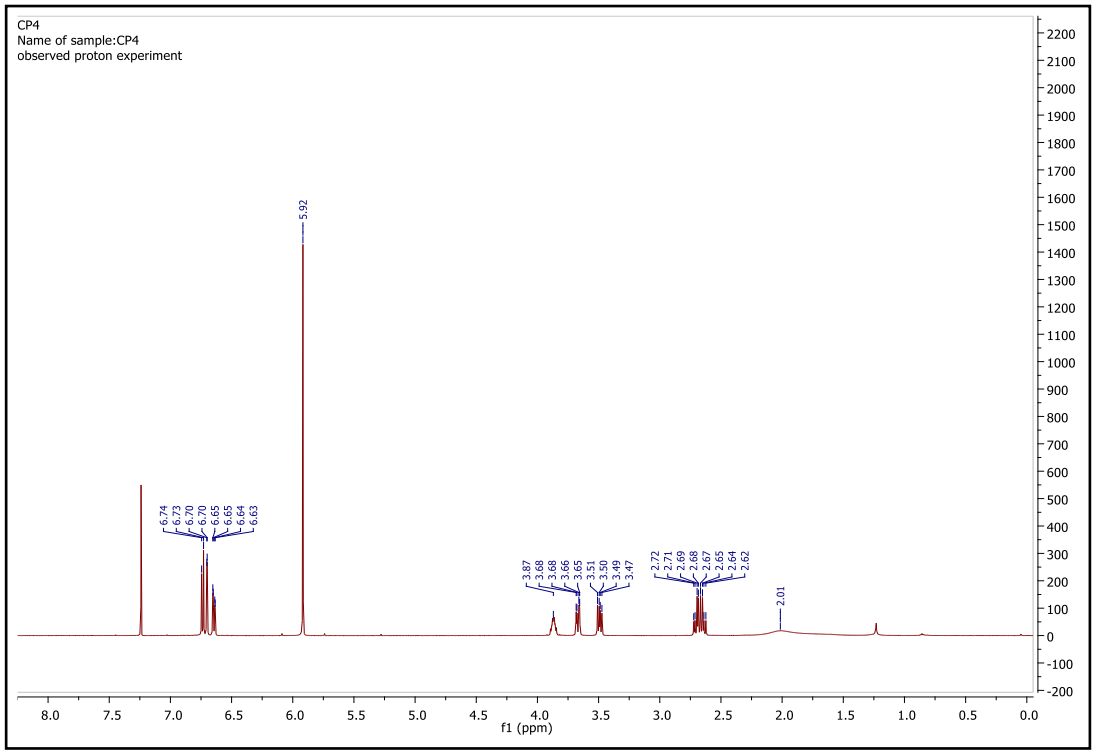


Figure 24. The 500 MHz ^{1}H NMR spectrum of compound **CP4** in $CDCl_3$

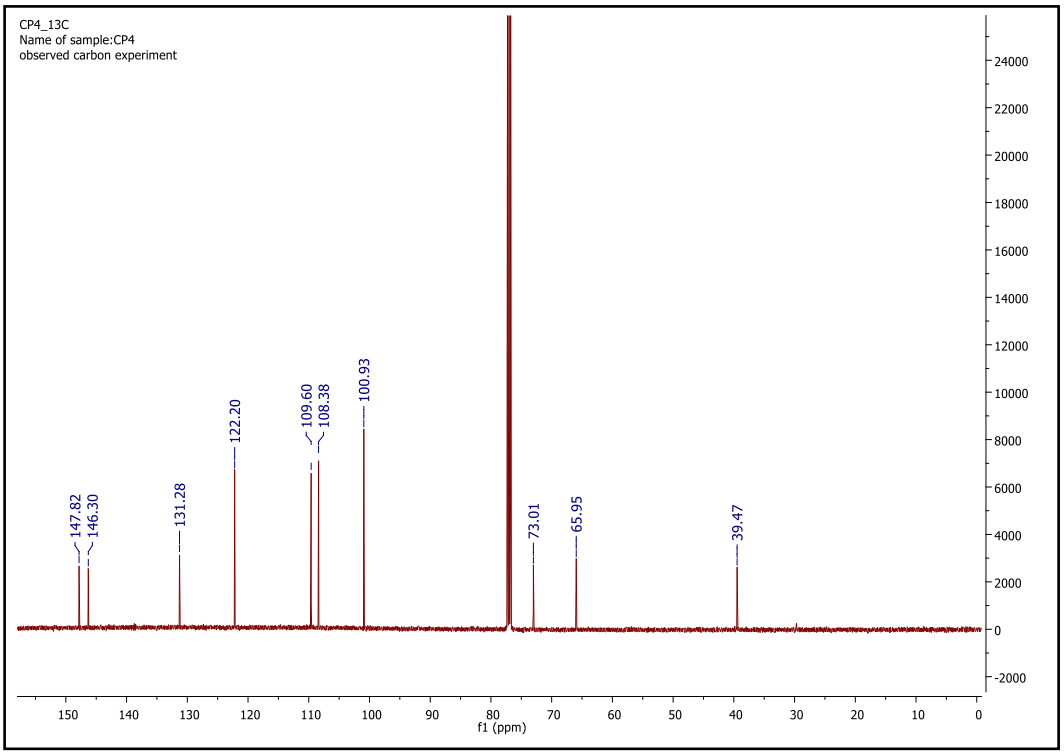


Figure 25. The 125 MHz ^{13}C NMR spectrum of compound **CP4** in $CDCl_3$

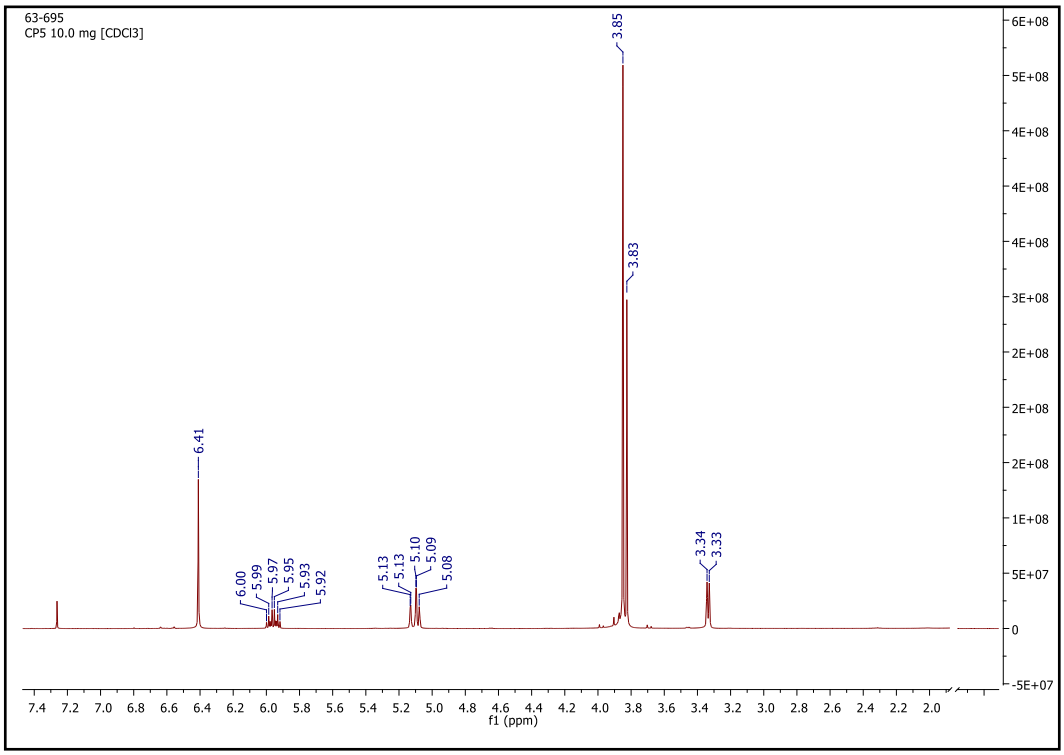


Figure 26. The 500 MHz ^{1}H NMR spectrum of compound **CP5** in $CDCl_3$

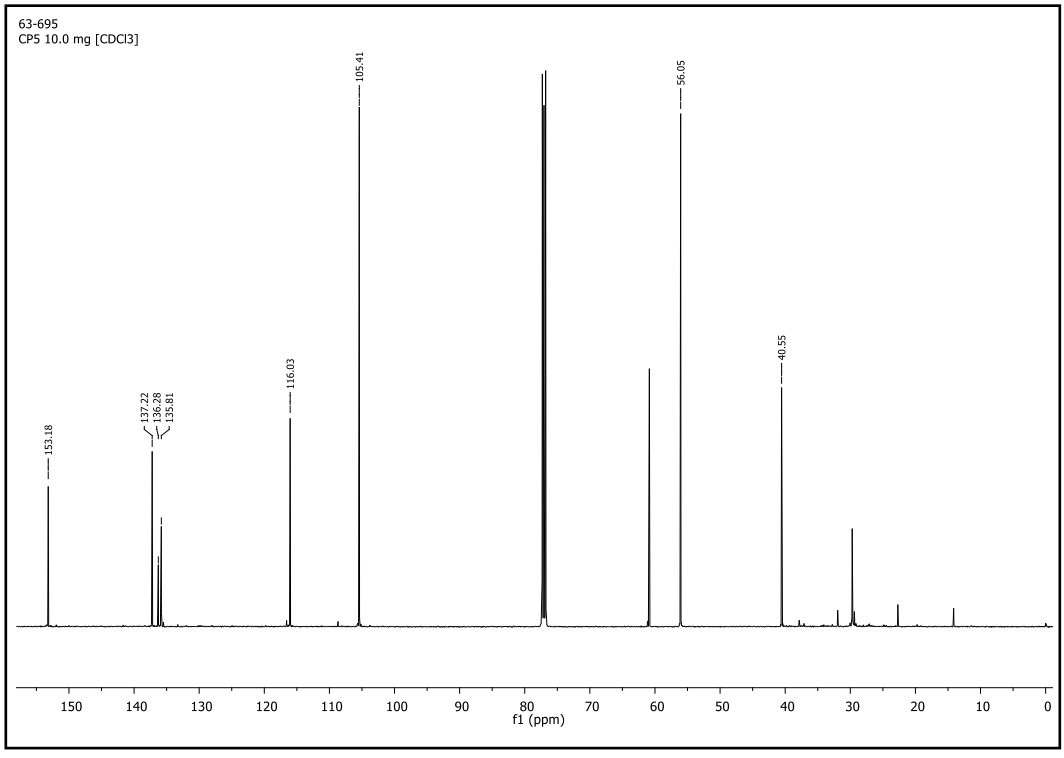


Figure 27. The 125 MHz ^{13}C NMR spectrum of compound **CP5** in $CDCl_3$

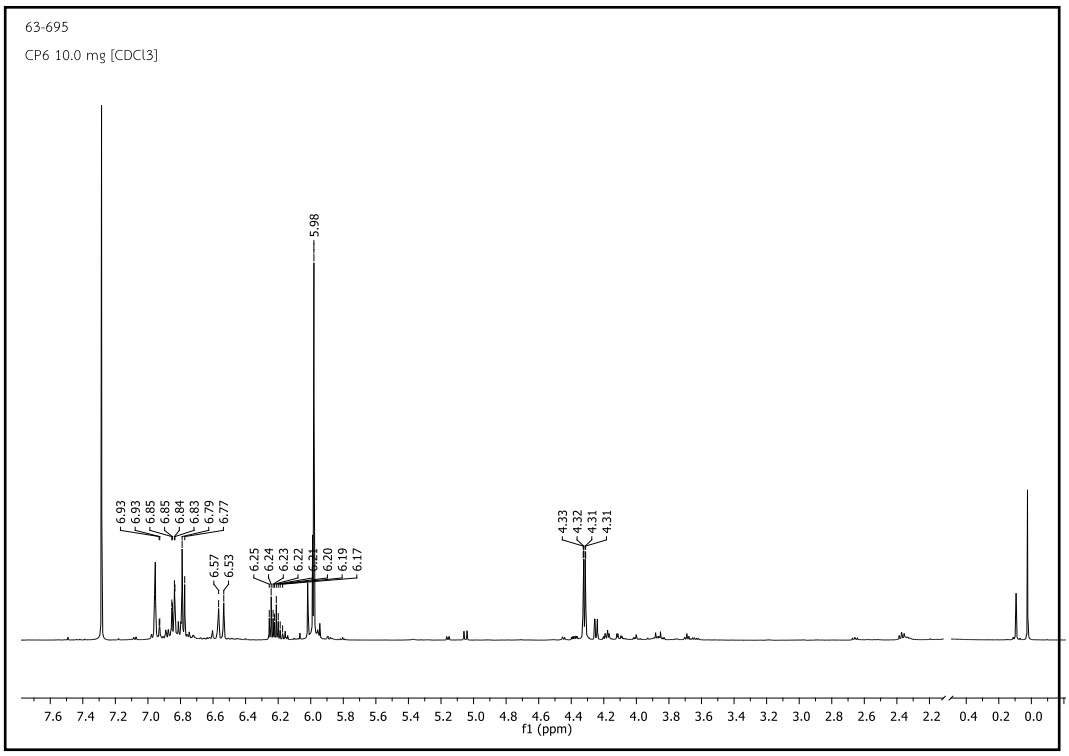


Figure 28. The 500 MHz ^{1}H NMR spectrum of compound **CP6** in $CDCl_3$

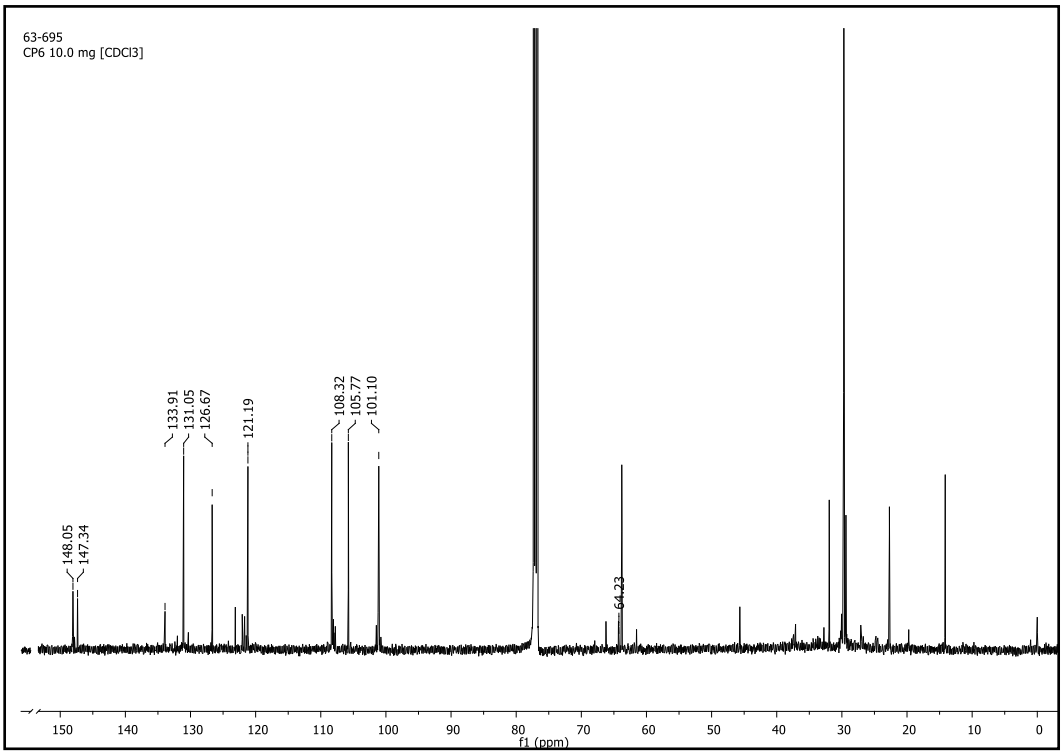


Figure 29. The 125 MHz ^{13}C NMR spectrum of compound **CP6** in $CDCl_3$

APPENDIX E

The regression line of ascorbic acid for antioxidant activities

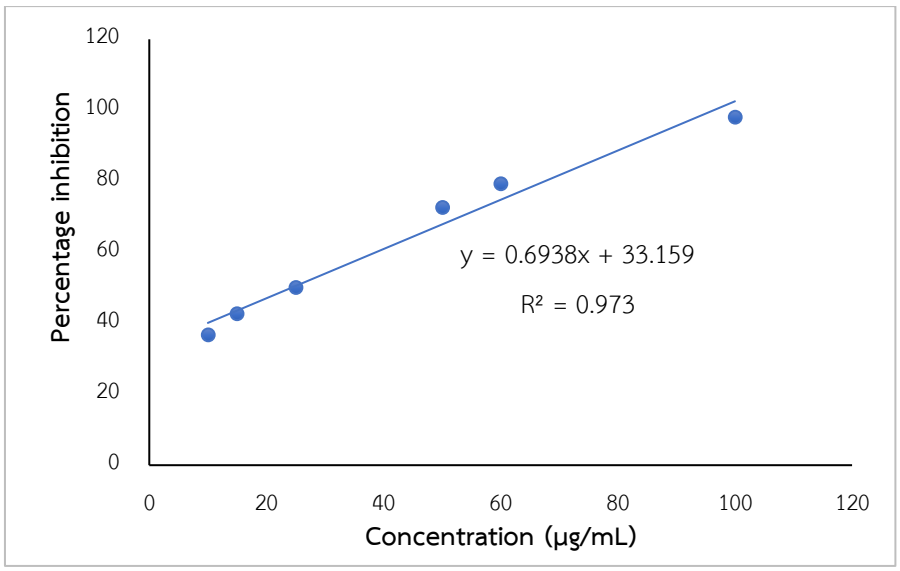


Figure 30. The regression line of ascorbic acid for antioxidant activities

VITAE

Name Pornpimoll Muanchaichum

Student ID 6240320403

Educational Attainment

Degree	Name of Institution	Year of Graduation
B.Sc. (Chemistry for industry)	Prince of Songkla University	2018

Scholarship Awards during Enrolment

1. The Scholarship of outstanding Academic Performance Funding Fiscal Year 2018

2. The Graduate School, Prince of Songkla University, Funding for Thesis Fiscal Year 2020

List of Publication and Proceeding

Muanchaichum, P., Pechwang, J., Klaiklay, S., & Chumkaew, P. (2020). ฤทธิ์ต้านแบคทีเรียของสารสกัดหยาบจากต้นเทพทาโร. *Thaksin Procedia*, *2020*(2), 43-50.

Muanchaichum, P., Pechwang, J., Klaiklay, S., & Chumkaew, P. (2020). Evaluation of the antibacterial activity of crude extracts from *Cinnamomum porrectum*. In Proceeding of 17th Kasetsart University National Conference 2020, Kasetsart University, Kamphaeng saen campus, Thailand, 2 December, 4944-4941.