Antibacterial Activity of Constituents from *Piper retrofractum* Vahl. and *Piper arborescens* Roxb.

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Summary

The present study was carried out to determine the antibacterial activities from the isolated phytochemicals of *Piper retrofractum* and *Piper arborescens*. Fractionation and purification of the extract of *P. retrofractum* yielded piperine (1), methyl piperate (2) and *N*-isobutyl-2*E*,4*E*,14*Z*-eicosatrienamide (3), while extracts of *P. arborescens* yielded sesartemin (4), diayangambin (5), and 3-(3,4-dimethoxybenzyl)-4-(3',4',5'-trimethoxybenzyl)-tetrahydrofuran-2-ol (6). The structures of the isolated phytochemicals were established by analysis of their spectroscopic data, as well as the comparison with that of reported data. The antibacterial activity of the isolated phytochemicals was also evaluated by using the micro-dilution method. Compound (1) was found to show the significant activity against *Staphylococcus aureus* and *Bacillus subtilis* with MIC values of 225 µg/mL. This study reports amides and lignans as compounds that could be involved in antibacterial activity of *Piper retrofractum* and *Piper arborescens*. However, more detailed experiments are needed to explore the underlying antibacterial mechanism further.

Key words

phytochemical, bioactivity, extract, amide, lignan

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Introduction

In recent years, numerous studies have published that the main antibacterial ingredients of plant extracts are usually terpenes, plant essential oils, and especially, polyphenolic compounds (Altemimi et al., 2017). Liu et al. (2017) demonstrated that many of the extracts (especially medicinal herbs) contained high levels of polyphenols and possessed strong antibacterial ability against *Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli,* and *Salmonella anatum*. Catechins, a kind of polyphenols, were found in high amounts in green tea and had strong antibacterial activity against various microorganisms (Nakayama et al., 2015). Ellagitannins, the main polyphenolic compounds of *Rubus* and *Fragaria* genera showed different extents of effectiveness in inhibiting the growth of selected Gram-negative intestinal bacteria (strains of *Salmonella, Staphylococcus, Helicobacter, Escherichia, Clostridium, Campylobacter,* and *Bacillus* (Klewicka et al., 2016). Ferulic and gallic acids had antimicrobial activity against *E. coli, S. aureus,* and *L. monocytogenes* (Dasagrandhi et al., 2018). Therefore, efforts are being made to develop and utilize these antibacterial phenolic ingredients of plant extracts to replace chemical synthetic preservatives, which will benefit in reaching people's requirements of safe and green food.

The genus *Piper* (Piperaceae) comprises more than 700 species of worldwide distribution. Species in this genus have high commercial and medicinal importance. Extensive phytochemical investigations of *Piper* species from all parts of the world have led to the isolation of a number of physiologically active compounds, inclusive of alkaloids, flavones, dihydrochalcones, kawapyrones, lignans, neolignans, propenylphenols, and terpenoids (Salleh et al., 2014, 2015a, 2019a, 2019b).

Piper retrofractum Vahl, known as *cabe jawa* in Indonesia, is native to Southeast Asia and mostly cultivated in Indonesia and Thailand (Lim, 2012). The roots and fruits of *P. retrofractum* can act as stimulants and are used to treat asthma, bronchitis, hemorrhoids, fever, liver diseases, jaundice, edema, and abdominal pain (Kubo et al., 2013). Both the unripe and ripe fruits of *P. retrofractum* have been used as a spice in curries, preserves, and pickles. Phytochemical studies have characterized neurotrophic compounds, as well as anti-obesity effects of alkaloids and the anti-leishmanial activity of amides and lignans found in *P. retrofractum* (Kim et al., 2011; Bodiwala et al., 2007).

Piper arborescens Roxb. is a creeping shrub distributed in Lanyu Island of Taiwan, Philippines, and the Malay Peninsula and Archipelago. The leaves have been used for treating rheumatism (Lin and Lu, 1996). Phytochemical studies on this plant have led to the isolation of pyridone alkaloids, cyclobutanoid alkaloids, and lignans (Du et al., 1990; Lee et al., 2004; Tsai et al., 2005). The methanolic stem extract has shown significant antiplatelet aggregation activity and cytotoxicity activities, while chloroform stems extract was found to display significant activity against proliferation of KB and P-388 lymphocytic leukemia cell lines (Tsai et al., 2005; Geran et al., 1972). The present study was conducted to report a detailed phytochemical study of *P. retrofractum* and *P. arborescens* and their antibacterial activity of extracts and their constituents.

Material and Methods

Plant materials

The fruits of *P. retrofractum* (PR01) were bought from a spice shop at Pandan, Johor Bahru, Malaysia in June 2010. The plant was identified by Shamsul Khamis and deposited at Universiti Teknologi Malaysia. The stem of *P. arborescens* (UiTMKS3001) was collected from Borneo in July 2010, and identified by Khong Heng Yen. The plant was deposited at Natural Products Research & Development Centre (NPRDC), UiTM Sarawak. All samples were ground and kept at room temperature.

General experimental procedures

Vacuum liquid chromatography (VLC) and column chromatography (CC) was performed using Merck silica gel 60 (230-400 mesh) and Merck silica gel 60 (70-230 mesh), respectively. Centrifugal preparative thin layer chromatography (Chromatotron, model 7924T) with 1, 2, and 4 mm precoated SiO_2 plate thickness, prepared by Merck silica gel 60 PF_{254} was used for purification of compounds. Thin-layer chromatography (TLC) was performed on 0.20 mm precoated silica gel aluminum sheets (Merck Kieselgel 60 F_{254}). Spots were visualized by UV light (254 and 365 nm) with vanillin-sulphuric acid spraying agent. Leica Gallen III Melting Point Apparatus equipped with the microscope was used to determine the melting point. The ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance 400 MHz spectrophotometer. Solvents such as $CDCl_3$, acetone-d_6, and DMSO-d_6 were needed to dissolve the samples. The residual solvent was used as an internal standard. The infrared (IR) spectra were recorded on Nicolet Avatar 370 DTGS spectrophotometer as a thin film (NaCl) for liquid samples or KBr pellets for solid samples. Mass spectra were obtained from the University College of London, United Kingdom. Solvents such as *n*-hexane, diethyl ether (Et_2O), chloroform ($CHCl_3$), ethyl acetate (EtOAc), and methanol (MeOH) were of analytical grade.

Extraction and isolation

The powdered dried fruits of *P. retrofractum* (2 kg) were extracted for 16 hours with soxhlet extractor and repeated until three times with methanol to yield the methanol extract (22.5 g) as a semi-solid brown gum. The methanol extract (15 g) was subjected to VLC with SiO_2 using gradient combination of *n*-hexane, $CHCl_3$, EtOAc, and MeOH to give 14 fractions. The fractions which showed similar TLC profiles were combined to give six new combined fractions named as PRM 1-6. PRM 3-5 were purified by CC to afford piperine (1) (*n*-hexane:$CHCl_3$, 70:30), methyl piperate (2) (*n*-hexane:$CHCl_3$, 50:50) and *N*-isobutyl-2*E*,4*E*-hexadecadienamide (3) (*n*-hexane:$CHCl_3$, 40:60).

The MeOH extract (17.0 g) of the stems of *P. arborescens* was fractionated by VLC using *n*-hexane, $CHCl_3$, EtOAc, acetone, and MeOH as solvents to yield 14 fractions. Fractions with the same TLC profile were combined to afford five new fractions labeled as PASM 1-5. PASM 3-5 were purified by CC to afford sesartemin (4) (*n*-hexane: EtOAc, 70:30), diayangambin (5) (*n*-hexane: EtOAc, 65:35), and 3-(3,4-dimethoxybenzyl)-4-(3',4',5'-trimethoxybenzyl)-tetrahydrofuran-2-ol (6) (*n*-hexane: EtOAc, 50:50).

Antibacterial activity

The test microorganisms, *Staphylococcus aureus* (ATCC29737), *Bacillus subtilis* (ATCC6633), *Pseudomonas aeruginosa* (ATCC9027), and *Escherichia coli* (ATCC10536) were used. The bacteria were cultured in an appropriate nutrient broth (NB) at 37°C overnight. The concentrations of the cultures were adjusted to obtain turbidity comparable to that of 0.5 McFarland standard. Minimum inhibitory concentration (MIC) was determined by the broth microdilution method using 96-well microplates (Salleh et al., 2015b). Each sample was dissolved in DMSO to get 1800 µg/mL stock solution. A number of wells were reserved in each plate for positive and negative controls. Sterile broth (100 µL) was added to well from row B to H. The stock solutions of samples (100 µL) were added to wells at row A and B. Then, the mixture of samples and sterile broth (100 µL) at row B was transferred to each well in order to obtain a twofold serial dilution of the stock samples (concentration of 1800, 900, 450, 225, 112.5, 56.7, 28.3 and 14.1 µg/mL). The inoculate (100 µL) was added to each well. Plates were incubated at 37°C for 16-20 hours. Microbial growth was indicated by the presence of turbidity and a pellet at the bottom of the well. Samples from the MIC study which did not show any growth of bacteria were removed from each well (10 µL) and then subcultured on the surface of the freshly prepared nutrient agar in 50 mm×15 mm disposable Petri dishes. Then, the petri dishes were inverted and incubated for 16-20 h at 37°C. After 16-20 h, the number of surviving organisms was determined. Minimum bactericidal concentration (MBC) was defined as the lowest extract concentration at which 99.9% of the bacteria were killed. Streptomycin sulfate was used as a positive control.

Results and Discussion

Six compounds have been isolated from the fruits of *P. retrofractum* and stem of *P. arborescens*. They are identified as piperine (1), methyl piperate (2), *N*-isobutyl-2*E*,4*E*,14*Z*-eicosatrienamide (3) from the fruits; while sesartemin (4), diayangambin (5), and 3-(3,4-dimethoxybenzyl)-4-(3',4',5'-trimethoxybenzyl)-tetrahydrofuran-2-ol (6) from the stem part. The structural elucidation of these compounds was based on their spectroscopic data and by comparison of these data with the literature. The chemical structures (1-6) are shown in Fig. 1. Most of these compounds have been isolated previously from *Piper* genus. Compound (1) has been isolated from *P. attenuatum, P. betle, P. chaba, P. cubeba,* and *P. longum* (Parmar et al., 1997); compound (2) has been isolated from *P. schmidtii* (Tyagi et al., 1995), *P. longum* (Lee et al., 2008), and *P. sarmentosum* (Rukachaisirikul et al., 2004); compound (3) from *P. caninum* and from the same species collected from Indonesia (Salleh et al., 2015; Kikuzaki et al., 1993); compounds (4) and (5) have been isolated from *P. fimbriulatum* (Solis et al., 2005); while compound (6) was isolated for the first time from *Piper* species. Previously, phytochemicals from *P. retrofractum* resulted in the isolation and characterization of several amides (Chatterjee, 1991; Banerji et al., 1985; Krishnamurthi, 1969). Amides are the major constituents of several species of *Piper*. A review of this genus was published dealing with chemical distribution and biological activities of amides (Parmar et al., 1997). Fifteen years later, an update with new chemical constituents of this genus was described together with a compilation of ^{13}C NMR data, and biological activities of these compounds were reported (Nascimento et al., 2012). A comparison of the compounds isolated in the last decade with those previously described indicates increased structural diversity of piperamides, probably due to advances in instrumental techniques of isolation and chemical identification. Piperine (1) has been the major component in the fruits of *P. retrofractum*. It was the first amide isolated from *Piper* species and was reported to act as central nervous system depressant, antifeedant, analgesic, antipyretic, anti-inflammatory agent, and antioxidant (Mittal et al., 2000).

In this study, for antibacterial activity we used Gram-positive and Gram-negative bacteria that are capable of causing infections in humans and/or are able to develop resistance to antibiotics, such as *Bacillus subtilis*, a saprophyte. However, some species accidentally cause infections: *Escherichia coli* a versatile gastrointestinal pathogen that is involved in epidemics of infantile diarrhea; *Pseudomonas aeruginosa* an opportunistic pathogen that initiates infection in people with low resistance, being resistant to many antibiotics and commonly found infecting hospitalized patients, and *Staphylococcus aureus* that is the major cause of hospital-acquired infections in nurseries, surgeries and invasive medical procedures. Some strains have been very resistant to several antibiotics (Pinto et al., 2012). The antibacterial activities of the extracts/compounds are shown in Table 1. The *P. retrofractum* and *P. arborescens* extract were found to have moderate to weak activity against all the tested bacteria. Antibacterial screening of the pure compounds showed that compound (1) was active against Gram-positive bacteria: *Bacillus subtilis* and *Staphylococcus aureus* with MIC values of 225 µg/mL. Gram-positive bacteria are usually more sensitive to antibiotics than the Gram-negative ones (Madigan and Martinko, 2004). This is expected because the outer membrane of Gram-negative bacteria is known to present a

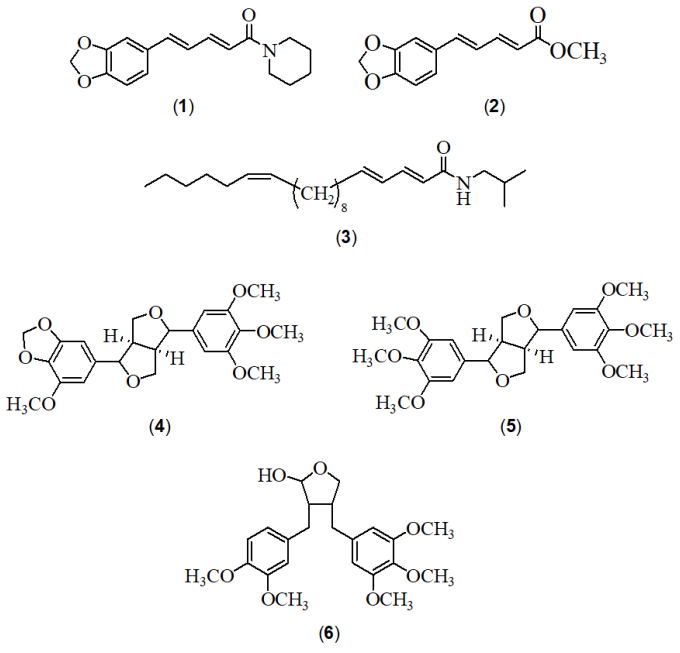

Figure 1. Chemical structures of compounds: (1) piperine, (2) methyl piperate, (3) piperine *N*-isobutyl-2*E*,4*E*,14*Z*-eicosatrienamide, (4) sesartemin, (5) diayangambin, and (6) 3-(3,4-dimethoxybenzyl)-4-(3',4',5'-trimethoxybenzyl)-tetrahydrofuran-2-ol

Table 1. Antibacterial activity of extracts and compounds from *Piper retrofractum* and *Piper arborescens*

Samples/ Microorganism	Gram-positive bacteria				Gram-negative bacteria			
	Bacillus subtilis		*Staphylococcus aureus*		*Pseudomonas aeruginosa*		*Escherichia coli*	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
PRFM	450	900	450	900	900	1800	900	1800
PASM	450	900	450	900	900	1800	900	1800
(1)	**225**	**225**	**225**	**225**	900	1800	900	1800
(2)	450	900	450	900	900	1800	900	1800
(3)	450	900	450	900	900	1800	900	1800
(4)	>1800	>1800	>1800	>1800	>1800	>1800	>1800	>1800
(5)	>1800	>1800	>1800	>1800	>1800	>1800	>1800	>1800
SS	14.1	14.1	14.1	14.1	14.1	14.1	14.1	14.1

Data represent mean ±SD; MIC - minimum inhibitory concentration (μg/mL); MBC - minimum bactericidal concentration (μg/mL); PRFM - *P. retrofractum* methanol fruit extract; PASM - *P. arborescens* methanol stem extract; SS - streptomycin sulfate

barrier to penetration of numerous antibiotic molecules, and the periplasmic space contains enzymes that are able to break down foreign molecules introduced from outside. Besides, the antibiotic that acts upon both Gram-positive and Gram-negative bacteria, a broad-spectrum antibiotic, has a wider medical usage and those that act on only a single group of organisms, narrow-spectrum antibiotic, may be quite valuable for the control of microorganisms that fail to respond to other antibiotics (Holetz et al., 2002). Piperine (1) has been previously reported to give significant antibacterial activity against some bacterial strains (Chavarria et al., 2016). It was found to be active against *P. aeruginosa* (MIC of 250 μg/mL) and *S. aureus* (MIC of 250 μg/mL), and reduced the MIC of mupirocin fourfold and of ciprofloxacin eightfold; and was found to inhibit efflux pumps in *S. aureus* (Mirza et al., 2011; Starvi et al., 2006). In addition, *N*-isobutyl-(2*E*,4*E*,14*Z*)-eicosatrienamide isolated from *P. caninum* showed good activity towards *B. subtilis* (MIC of 125 μg/mL), meanwhile 2*E*,4*E*,8*Z*-*N*-isobutyleicosatrienamide isolated from *P. nigrum* was active against *S. aureus, B. subtilis* (MIC of 34 mM) and *B. sphaericus* (MIC of 17 mM).

Piperine (1) - Yellowish needles (1.30 g, 1.43%); m.p 130-131°C; IR υ_{max}(KBr) cm^{-1}: 2924 (C-H), 1634 (C=O), 1612, 1584 (C=C), 1032 (C-O); ^{1}H-NMR ($CDCl_3$, 400 MHz) (Fig. 2): δ 7.37 (1H, dd, *J* = 14.8, 10.4 Hz, H-3), 6.96 (1H, d, *J* = 1.6 Hz, H-2'), 6.87 (1H, dd, *J* = 8.0, 1.6 Hz, H-6'), 6.74 (3H, m, H-4, H-5, H-5'), 6.42 (1H, d, *J* = 14.8 Hz, H-2), 5.95 (2H, s, OCH_2O), 3.61 (4H, m, H-6'', H-2''), 1.60 (6H, m, H-4'', H-3'', H-5''); ^{13}C-NMR ($CDCl_3$, 100 MHz) (Fig. 3): δ 165.4 (C-1), 148.2 (C-4'), 148.1 (C-3'), 142.5 (C-3), 138.2 (C-5), 131.0 (C-1'), 125.4 (C-4), 122.5 (C-6'), 120.1 (C-2), 108.5 (C-5'), 105.7 (C-2'), 101.3 (OCH_2O), 46.9 (C-6''), 43.2 (C-2''), 26.7 (C-3''), 25.6 (C-5''), 24.7 (C-4''); EIMS: *m/z* 285 [M^+, $C_{17}H_{19}O_3N$].

Methyl piperate (2) - Yellowish needles (0.26 g, 0.28%); m.p 138-139°C; IR υ_{max} (KBr) cm^{-1}: 2925 (C-H), 1707 (C=O), 1617, 1607 (C=C), 1039 (C-O); ^{1}H-NMR ($CDCl_3$; 400 MHz) (Fig. 4): δ 7.40 (1H, dd, *J* = 15.2 and 10.8 Hz, H-3), 6.98 (1H, d, *J* = 8.0 Hz, H-6'), 6.79 (1H, d, *J* = 15.6 Hz, H-5), 6.77 (1H, d, *J* = 8.0 Hz, H-5'), 6.70 (1H, dd, *J* = 15.6 and 10.8 Hz, H-4), 5.97 (2H, s, OCH_2O), 5.93 (1H, d, *J* = 15.2 Hz, H-2), 3.74 (3H, s, OCH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz) (Fig. 5): δ 167.6 (C-1), 148.6 (C-3'), 148.3 (C-4'), 144.9 (C-3), 140.3 (C-5), 130.5 (C-1'), 124.5 (C-4), 122.9 (C-2), 119.9 (C-6'), 108.6 (C-5'), 105.8 (C-2'), 101.3 (-OCH_2-), 51.5 (-OCH_3); EIMS: *m/z* 232 [M^+, $C_{13}H_{12}O_4$].

***N*-Isobutyl-2*E*,4*E*,14*Z*-eicosatrienamide (3)** - White solid (157 mg, 0.17%); m.p 65-67°C; IR υ_{max} (KBr) cm^{-1}: 3301 (C-H), 2917 (C-H), 1654 (C=O), 1609 (C=C); ^{1}H-NMR ($CDCl_3$, 400 MHz) (Fig. 6): δ 7.17 (1H, dd, *J* = 15.2 and 10.0 Hz, H-3), 6.20-5.90 (3H, m, H-5, H-4, -NH), 5.82 (1H, d, *J* = 15.2 Hz, H-2), 5.32 (2H, br s, H-14, H-15), 3.12 (2H, t, *J* = 6.0 Hz, H-1'), 2.13 (2H, m, H-6), 2.00 (4H, m, H-13, H-16), 1.77 (1H, m, H-2'), 1.31 (2H, d, *J* = 6.8 Hz, H-7) 1.30-1.24 (16 H, br. s, 8×CH2), 0.91 (6H, d, *J* = 6.8 Hz, H-3', H-4'), 0.86 (3H, t, *J* = 7.3 Hz, H-20); ^{13}C-NMR ($CDCl_3$, 100 MHz) (Fig. 7): δ 166.6 (C-1), 142.9 (C-3), 141.1 (C-5), 129.9 (C-14, C-15), 128.3 (C-4), 121.9 (C-2), 46.9 (C-1'), 32.8 (C-6), 31.9 (C-18), 29.8-28.9 (C-7-12, C-17), 27.2 (C-13, C-16), 23.5 (C-19), 20.2 (C-3', C-4'), 14.7 (C-20); CIMS: *m/z* 361 [M^+, $C_{24}H_{43}NO$].

Sesartemin (4) - White solid (254 mg, 1.49%); m.p 148-150°C; IR υ_{max} (KBr) cm^{-1}: 3444 (C-H), 2927 (C-H), 1591 (C=C), 1129 (C-O); ^{1}H-NMR ($CDCl_3$, 400 MHz) (Fig. 8): δ 6.55 (3H, br. s, H-2', H-2'', H-6''), 6.47 (1H, s, H-6'), 5.93 (2H, s, OCH_2O), 4.79 (1H, d, *J* = 6.8 Hz, H-4), 4.37 (1H, d, *J* = 6.8 Hz, H-1), 4.09 (1H, m, H-3a), 3.88 (2H, s, H-6), 3.83 (9H, s, OCH_3-3', OCH_3-3'', OCH_3-5''), 3.79 (3H, s, OCH_3-4''), 3.29 (2H, d, *J* = 4 Hz, H-3), 2.86 (1H, d, *J* = 6.8 Hz, H-6a); ^{13}C-NMR ($CDCl_3$, 100 MHz) (Fig. 9): δ 153.4 (C-3'', C-5''), 148.8 (C-5'), 143.5 (C-4'), 137.6 (C-1''), 136.7 (C-1'),

134.1 (C-4"), 132.9 (C-3'), 105.0 (C-2'), 102.9 (C-2", C-6"), 101.4 (OCH_2O), 99.8 (C-6'), 87.7 (C-1), 82.0 (C-4), 70.9(C-3), 69.7(C-6), 60.8 (OCH_3-3'), 56.6 (-OCH_3-4"), 56.1 (OCH_3-3", OCH_3-5"), 54.5 (C-6a), 50.0 (C-3a); EIMS: *m/z* 430 [M^+, $C_{23}H_{26}O_8$].

Diayangambin (5) - White solid (198.3 mg, 1.98%); m.p 150-152°C; IR υ_{max} (KBr) cm^{-1}: 2927 (C-H), 1592 (C=C), 1130 (C-O); ^{1}H-NMR ($CDCl_3$, 400 MHz) (Fig. 10): δ 6.57 (4H, s, H-2', H-6', H-2", H-6"), 4.87 (2H, d, *J* = 4.8 Hz, H-1, H-4), 3.84 (12H, s, OCH_3-3', OCH_3-5', OCH_3-3", OCH_3-5"), 3.82 (6H, s, OCH_3-4', OCH_3-4"), 3.70 (2H, dd, *J* = 9.6 and 1.6 Hz, H-3), 3.55 (2H, dd, *J* = 9.6 and 4.8 Hz, H-6), 3.17 (2H, m, H-3a, H-6a); ^{13}C-NMR ($CDCl_3$, 100 MHz) (Fig. 11): δ 153.2 (C-3', C-5', C-1", C-3", C-5"), 137.1 (C-1'), 134.6 (C-4', C-4"), 103.3 (C-2', C-6', C-2", C-6"), 84.1 (C-1, C-4), 68.9 (C-3, C-6), 60.9 (OCH_3-4', OCH_3-4"), 56.1 (OCH_3-3', OCH_3-5', OCH_3-3", OCH_3-5"), 49.4 (C-3a, C-6a); EIMS: *m/z* 447 [M^{+1}, $C_{24}H_{30}O_8$].

3-(3,4-Dimethoxybenzyl)-4-(3',4',5'-trimethoxybenzyl)-tetrahydrofuran-2-ol (6) – Brown amorphous solid (135 mg, 0.79%); IR υ_{max} (KBr) cm^{-1}: 3200 (OH), 2995 (C-H), 1590 (C=C), 1360 (C-O); ^{1}H-NMR ($CDCl_3$, 400 MHz) (Fig. 12): δ 6.81 (1H, d, *J* = 8.8 Hz, H-5), 6.77 (1H, d, *J* = 2 Hz, H-2), 6.76 (1H, d, *J* = 8.8 Hz, H-6), 6.75 (2H, s, H-6', H-2'), 4.22 (1H, d, *J* = 6.8 Hz, H-9), 3.87 (15H, s, 3,4,3',4',5'-OCH_3), 3.72 (2H, m, H-9'), 2.92 (2H, m, H-7'), 2.67 (2H, m, H-7), 2.35 (2H, m, H-8, H-8'); ^{13}C-NMR ($CDCl_3$, 100 MHz) (Fig. 13): δ 155.5 (C-5), 153.9 (C-3'), 151.2 (C-3), 148.9 (C-4), 147.6 (C-4'), 132.8 (C-1, C-1'), 120.1 (C-6), 111.7 (C-2', C-6'), 111.4 (C-5, C-2), 99.9 (C-9), 70.8 (C-9'), 60.0 (4-OCH_3), 55.8 (3,3',4',5'-OCH_3), 35.5 (C-7'), 30.3 (C-7), 26.9 (C-8, C-8'); EIMS: *m/z* 418 [M^+, $C_{23}H_{30}O_7$].

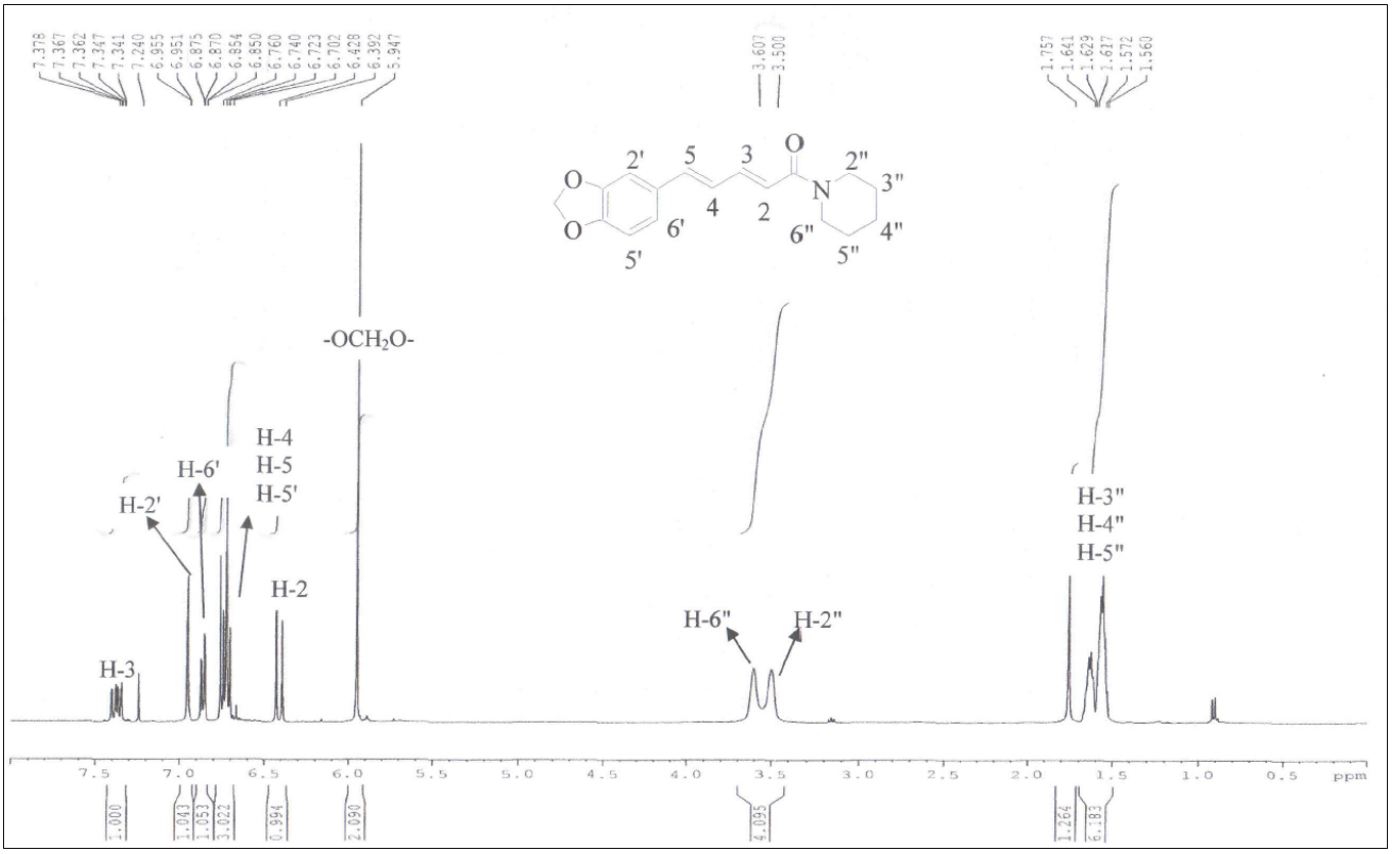

Figure 2. ^{1}H NMR spectrum of piperine

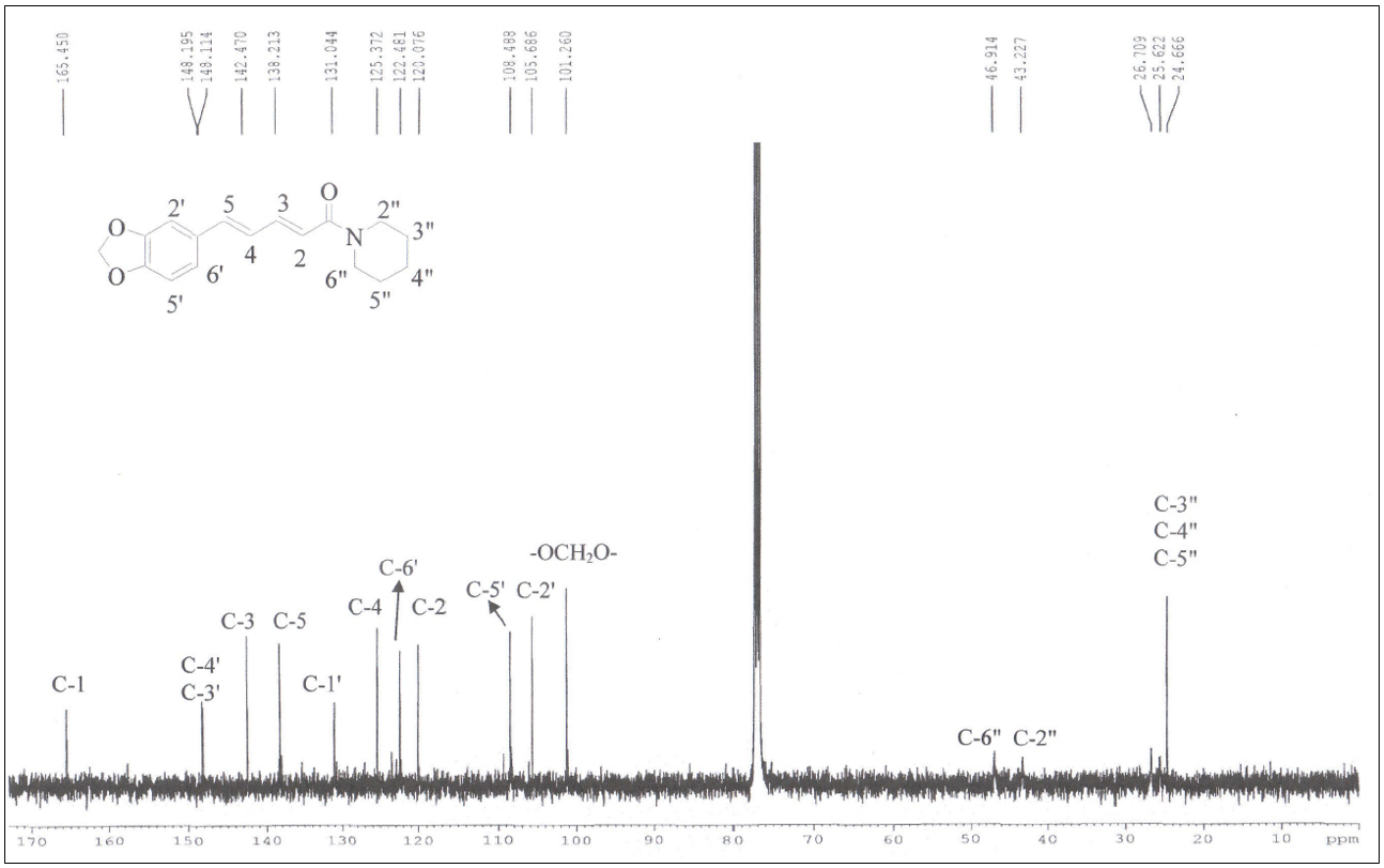

Figure 3. $_{13}$C NMR spectrum of piperine

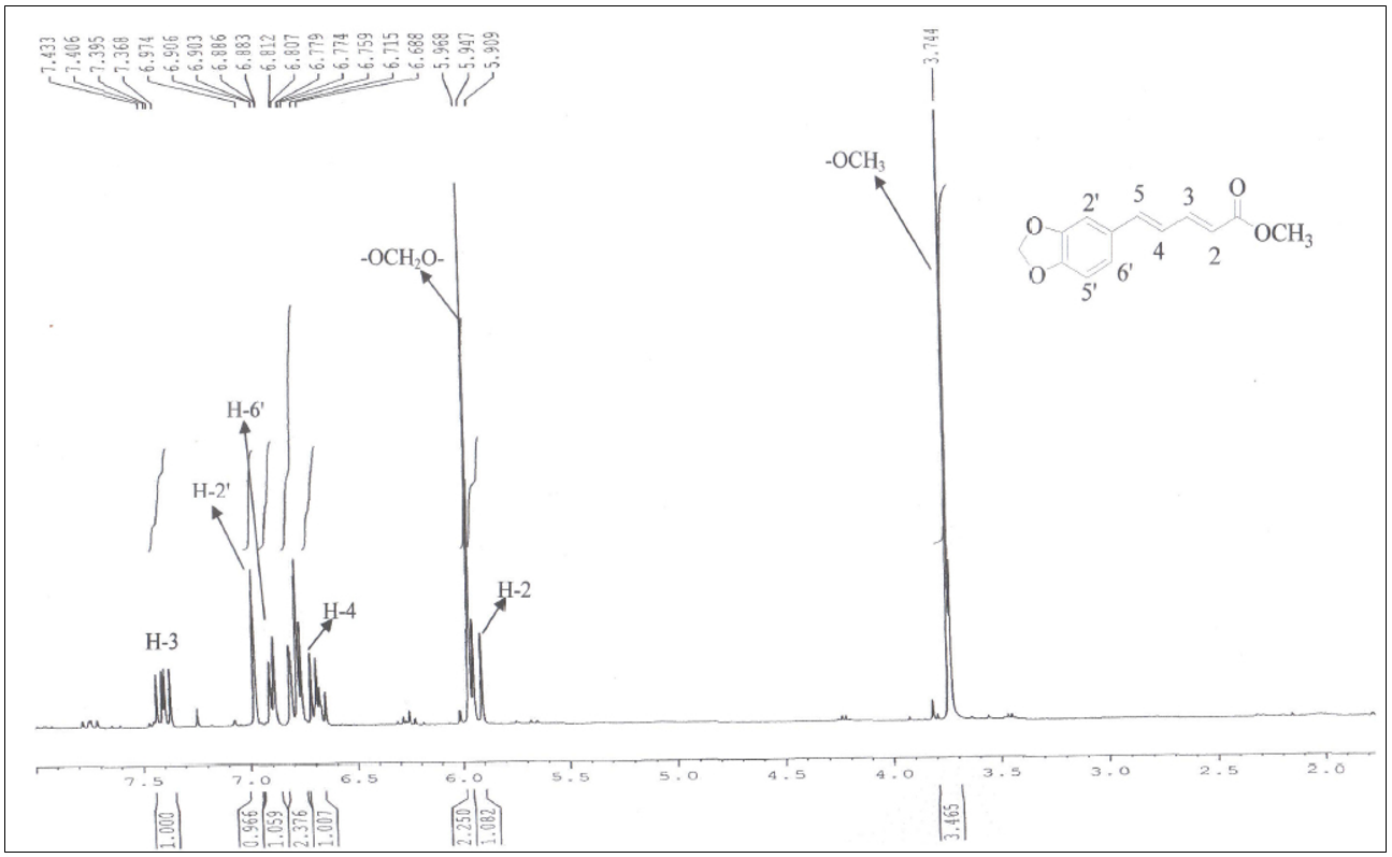

Figure 4. ^{1}H NMR spectrum of methyl piperate

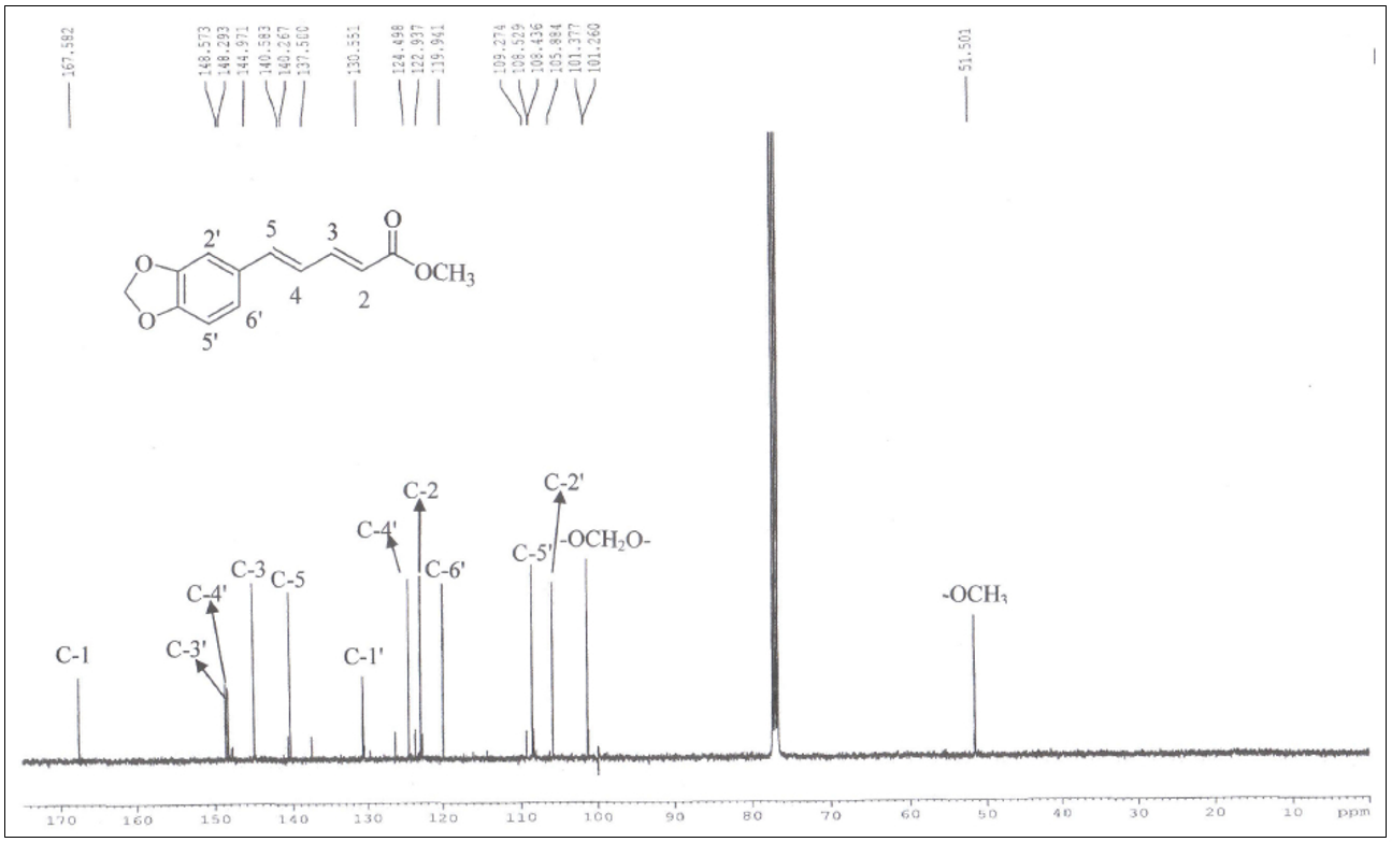

Figure 5. $_{13}$C NMR spectrum of methyl piperate

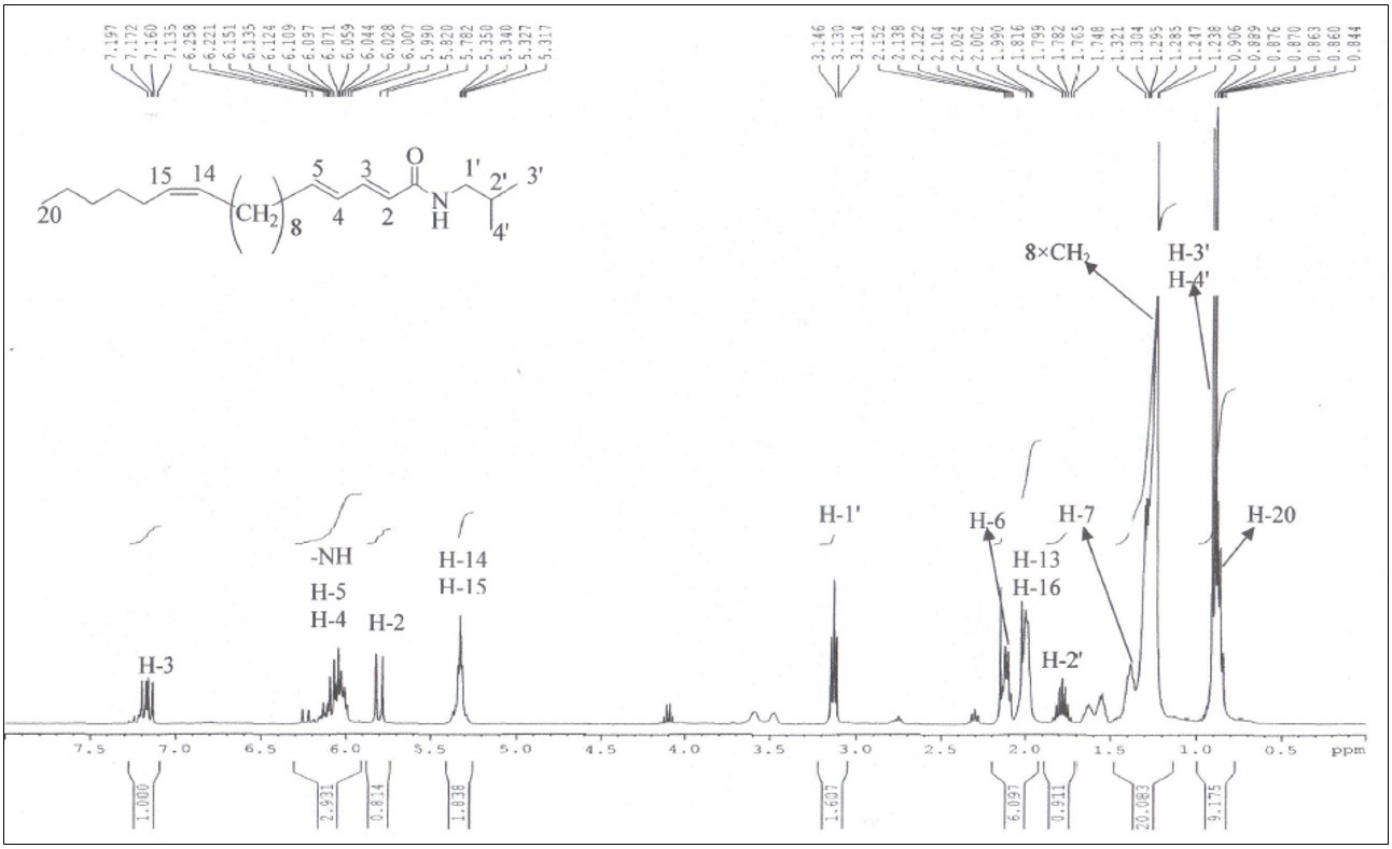

Figure 6. ^{1}H NMR spectrum of *N*-isobutyl-2*E*,4*E*,14*Z*-eicosatrienamide

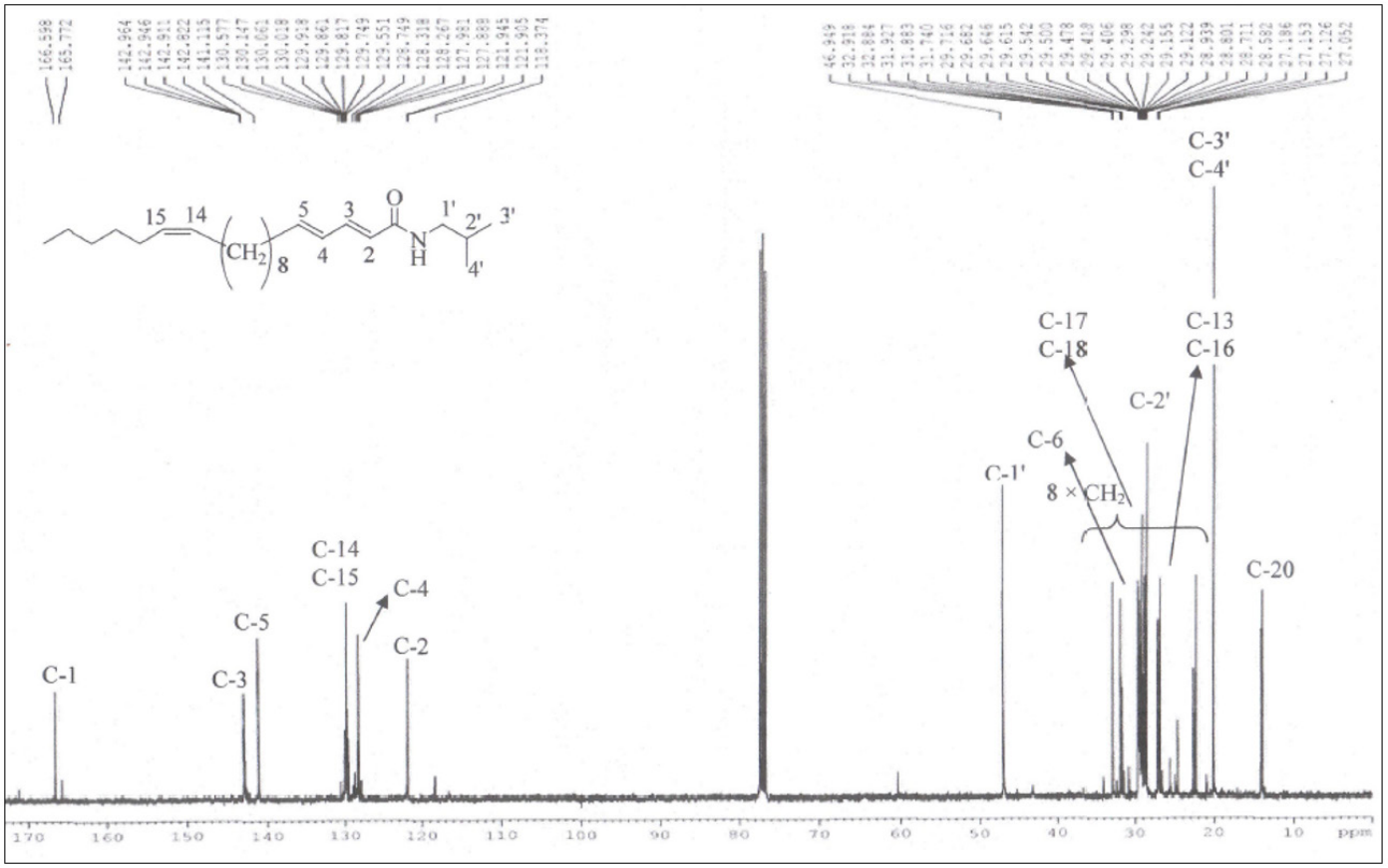

Figure 7. ^{13}C NMR spectrum of *N*-isobutyl-2*E*,4*E*,14*Z*-eicosatrienamide

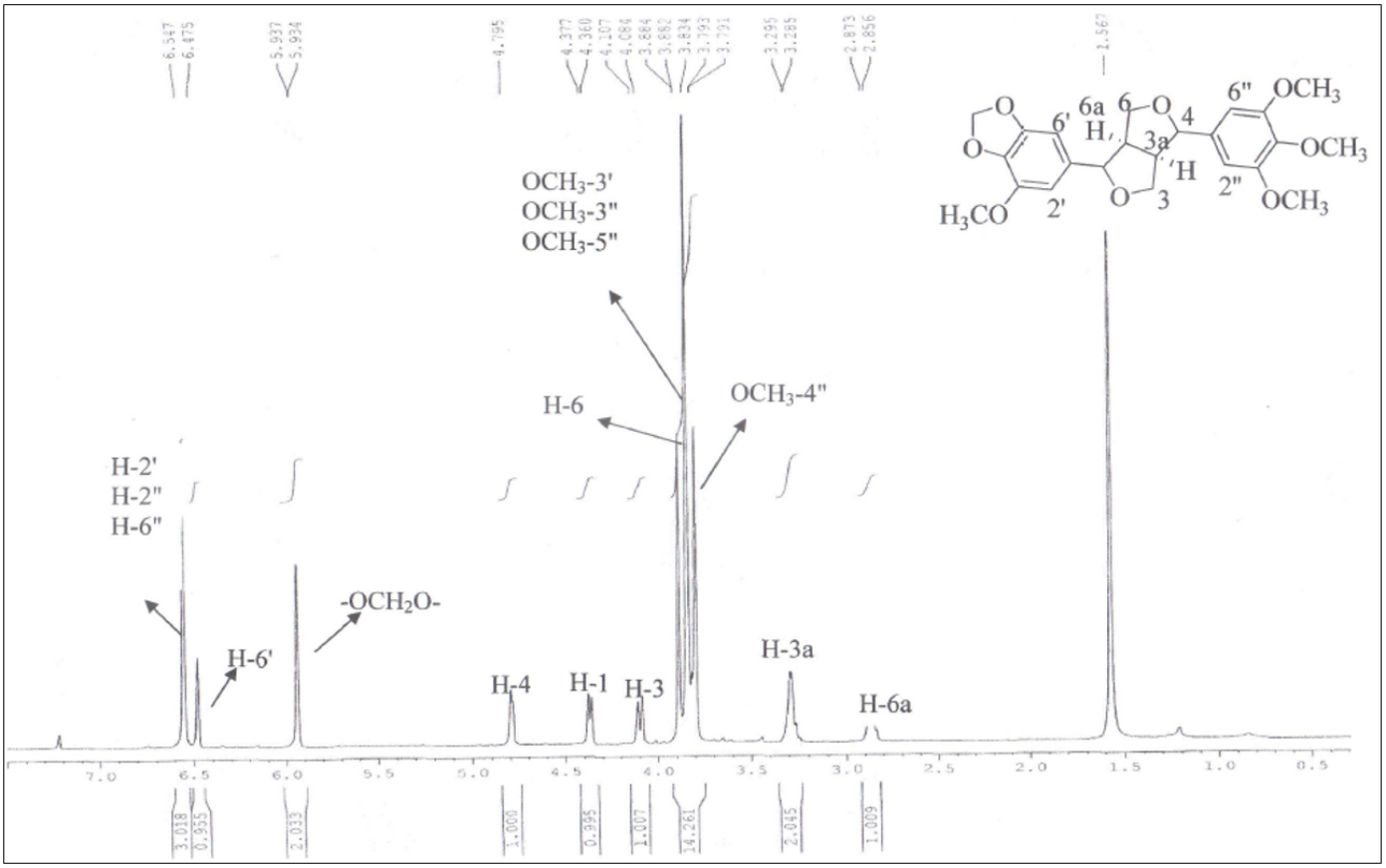

Figure 8. ^{1}H NMR spectrum of sesartemin

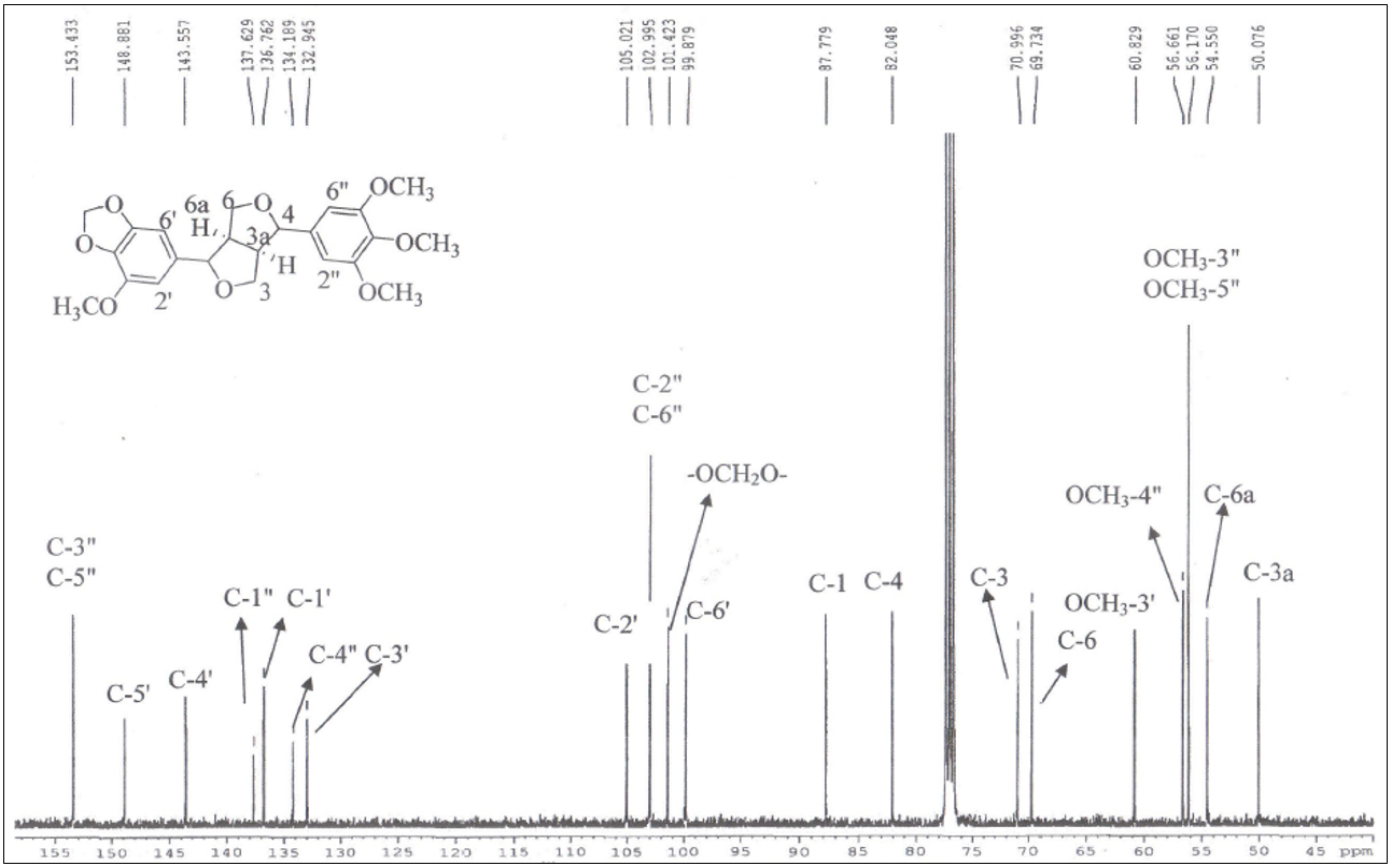

Figure 9. ^{13}C NMR spectrum of sesartemin

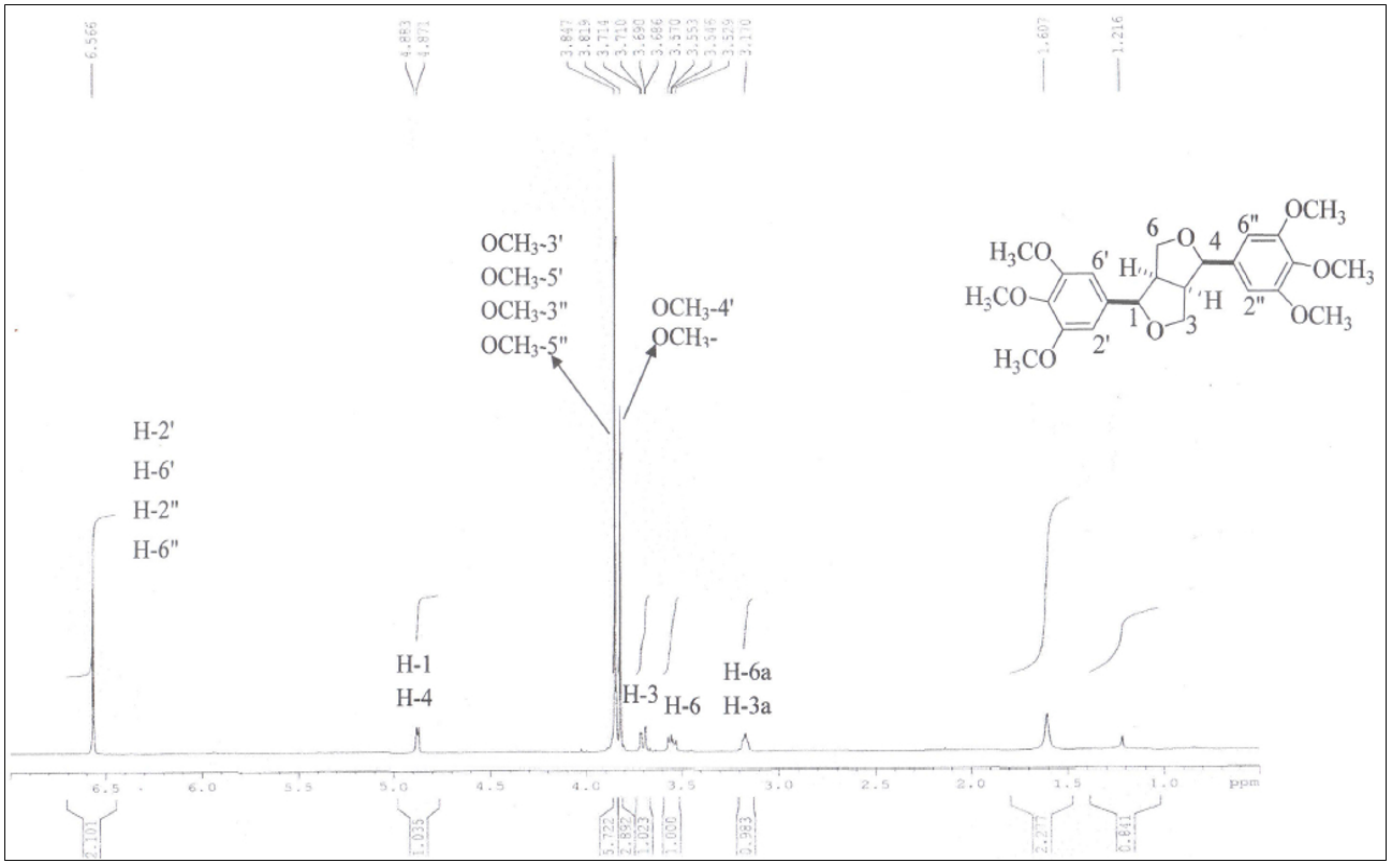

Figure 10. ^{1}H NMR spectrum of diayangambin

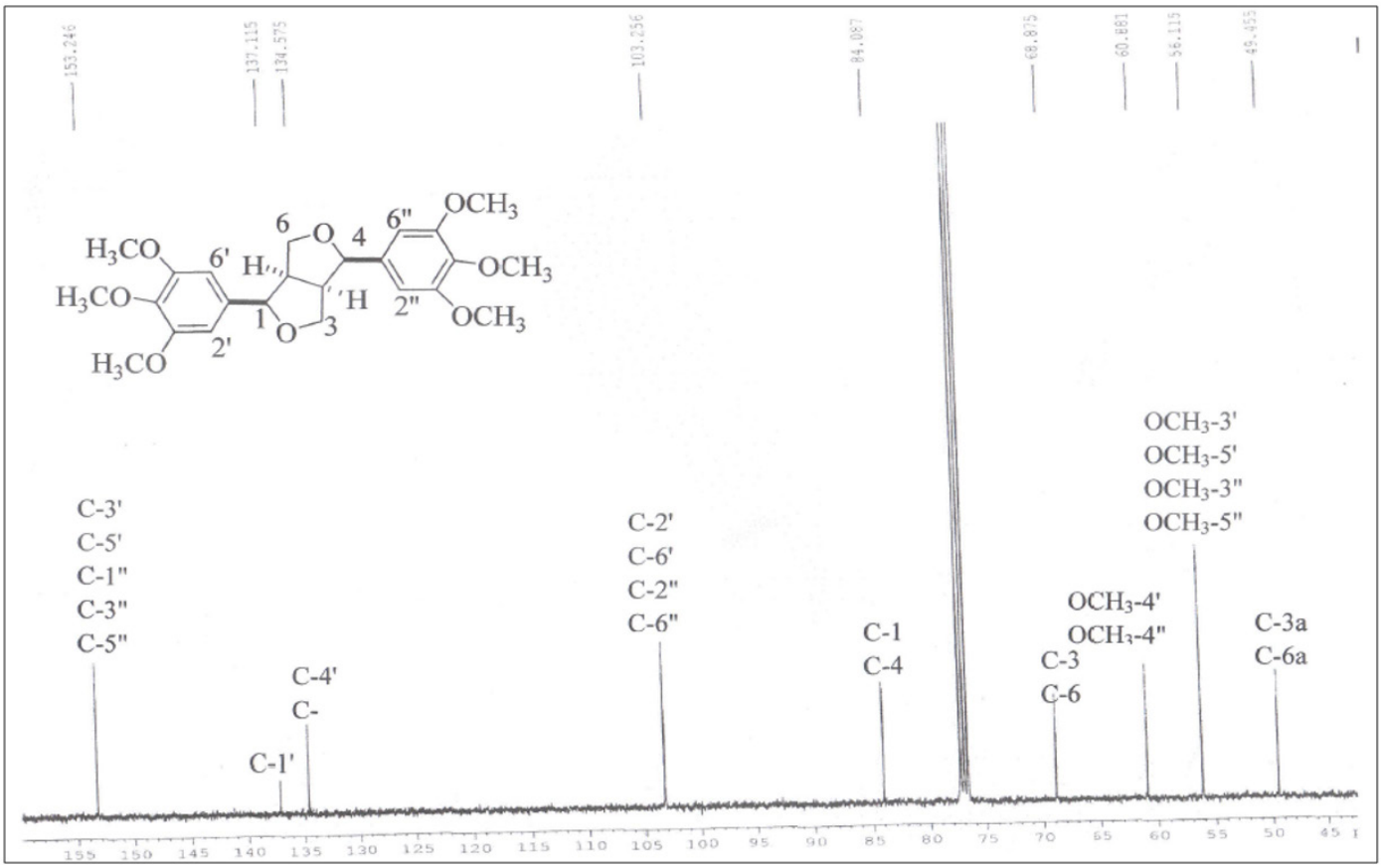

Figure 11. ^{13}C NMR spectrum of diayangambin

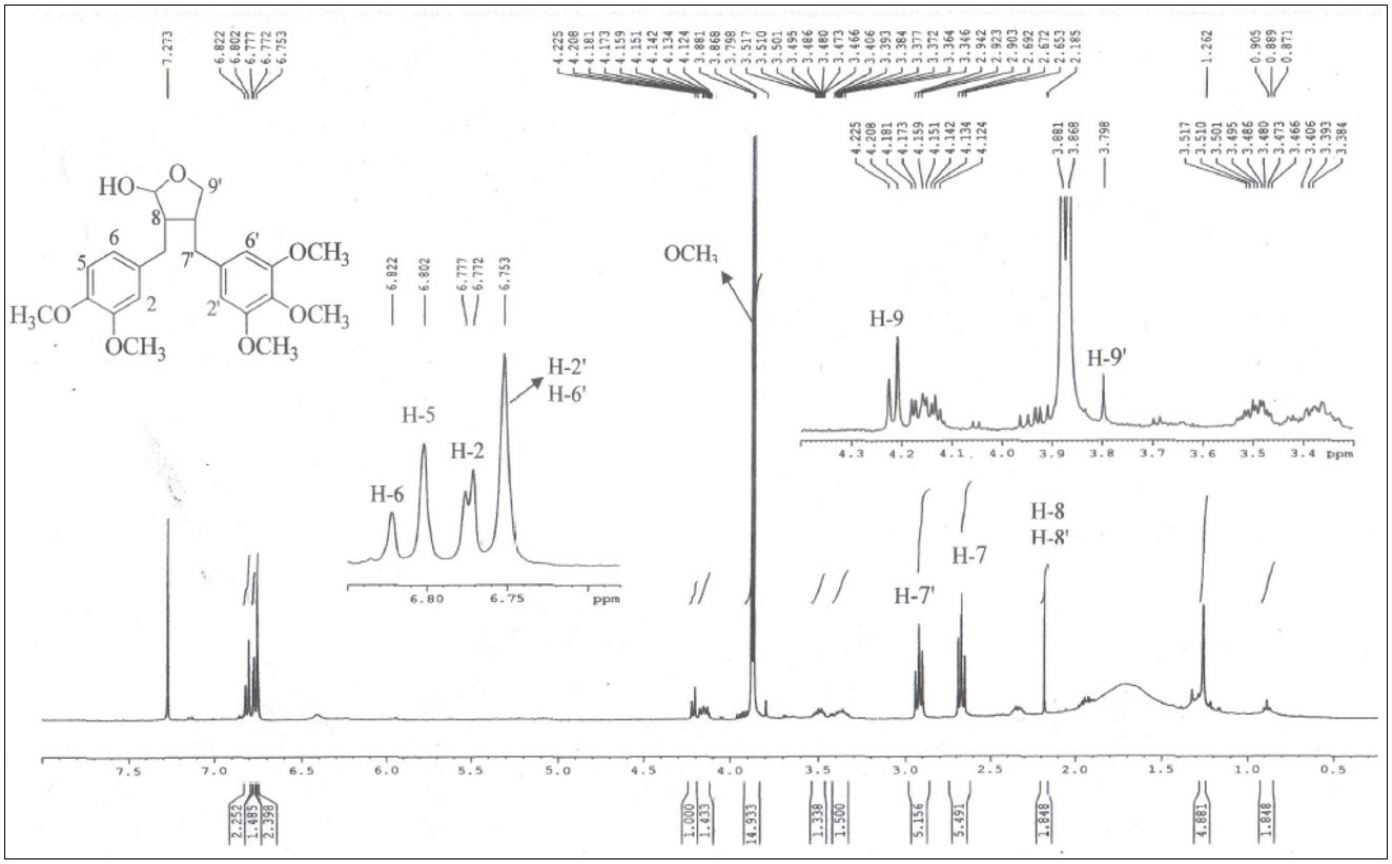

Figure 12. ^{1}H NMR spectrum of 3-(3,4-dimethoxybenzyl)-4-(3',4',5'-trimethoxybenzyl)-tetrahydrofuran-2-ol

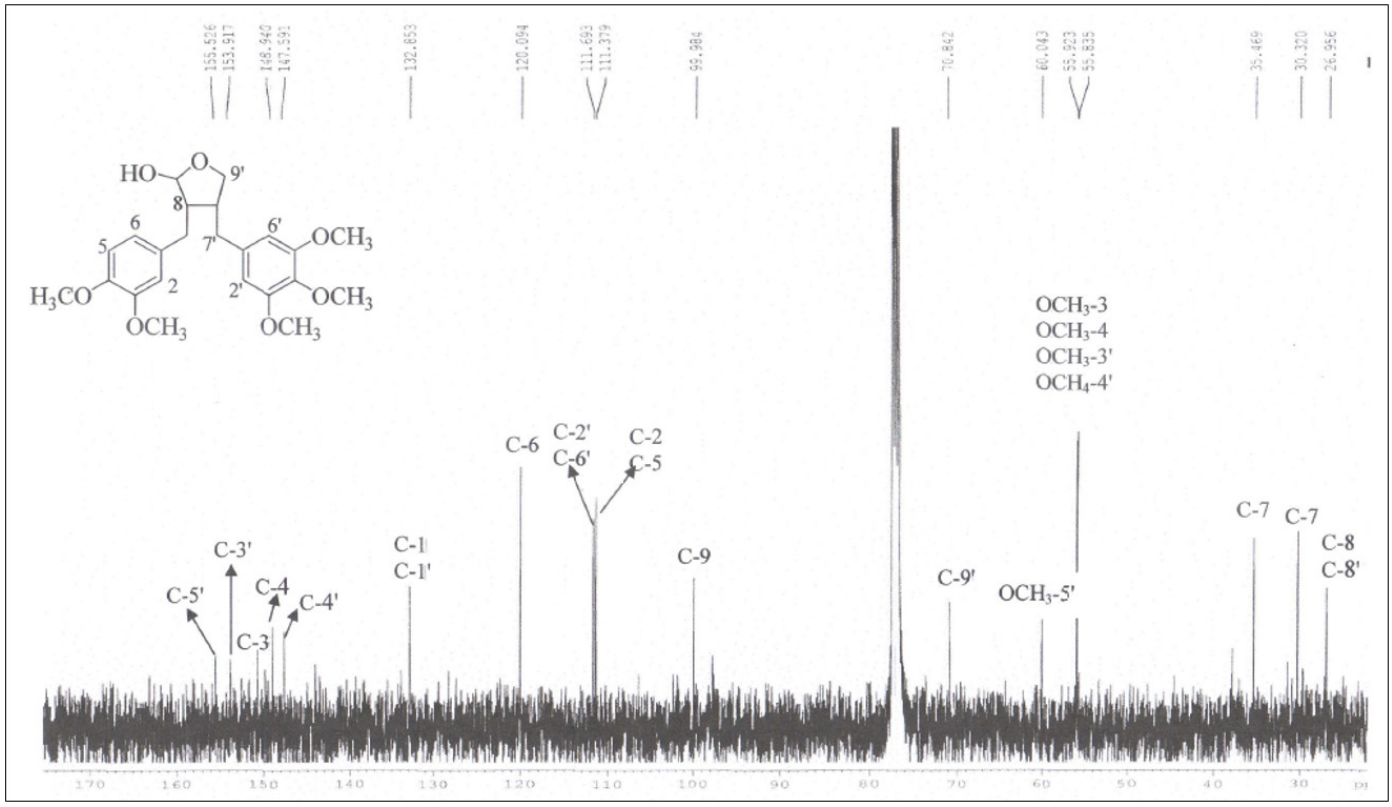

Figure 13. ^{13}C NMR spectrum of 3-(3,4-dimethoxybenzyl)-4-(3',4',5'-trimethoxybenzyl)-tetrahydrofuran-2-ol

Conclusion

In conclusion, this study reports amides and lignans as compounds from *Piper retrofractum* and *Piper arborescens*. These results also suggest that the extracts could serve as a potential source of bioactive compounds. Additionally, the antibacterial activity against the pathogenic bacteria may also need to be evaluated for the treatment of infections. Besides, further research is needed in which the extract could possibly be exploited for pharmaceutical use.

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References

Altemimi A., Lakhssassi N., Baharlouei A., Watson D.G., Lightfoot D.A. (2017). Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. Plants 6: 42-65.

Banerji A., Bandyopadhyay D., Sarkar M., Siddhanta A.K., Pal S.C., Ghosh S., Abraham K., Shoolery J.N. (1985). Structural and synthetic studies on the retrofractamides-new amide constituents of Piper retrofractum. Phytochemistry 24(2): 279-284.

Bodiwala H.S., Singh G., Singh R., Dey C.S., Sharma S.S., Bhutani K.K., Singh I.P. (2007) Antileishmanial amides and lignans from *Piper cubeba* and *Piper retrofractum*. J Nat Med 61: 418-421.

Chatterjee A. (1991). The Treatise on Indian Medicinal Plants, Vol. 1. Council of Scientific and Industrial Research (India), New Delhi.

Chavarria D., Silva T., Magalhaes Silva D., Remiao F., Borges F. (2016). Lessons from black pepper: Piperine and derivatives thereof. Expert Opin Ther Pat 26: 245-264.

Dasagrandhi C., Park S., Jung W.K., Kim Y.M. (2018). Antibacterial and biofilm modulating potential of Ferulic Acid-Grafted chitosan against human pathogenic bacteria. Int J Mol Sci 19: E2157.

Duh C.Y., Wu Y.C., Wang S.K. (1990). Cytotoxic pyridone alkaloids from *Piper aborescens*. Phytochemistry 29: 2689-2691.

Geran R.I., Greenberg N.H., Macdonald M.M., Schumacher A.M., Abbott B.J. (1972). Protocols for screening chemical agents and natural products against animal tumors and other biological systems. Cancer Chemother Rep 7: 7-9.

Holetz F.B., Pessini G.L., Sanches N.R., Cortez D.A.G., Nakamura C.V., Dias Filho B.P. (2002). Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem Inst Oswaldo Cruz 97: 1027-1031.

Kikuzaki H., Kawabata M. (1993). LC-MS Analysis and structural determination of new amides from Javanese long pepper (*Piper retrofractum*). Biosci Biotech Biochem 57: 1329-1333.

Kim K.J., Lee M.S., Jo K., Hwang J.K. (2011). Piperidine alkaloids from *Piper retrofractum* Vahl. protect against high-fat diet-induced obesity by regulating lipid metabolism and activating AMP-activated protein kinase. Biochem Biophys Res Commun 411: 219-225.

Klewicka E., Sojka M., Klewicki R., Kolodziejczyk K., Lipinska L., Nowak A. (2016). Ellagitannins from raspberry (*Rubus idaeus* L.) fruit as natural inhibitors of *Geotrichum candidum*. Molecules 21: 908-922.

Krishnamurthi A. (1969). Wealth of India. Council of Scientific and Industrial Research (India), New Delhi.

Kubo M., Ishii R., Ishino Y., Harada K., Matsui N., Akagi M., Kato E., Hosoda S., Fukuyama Y. (2013). Evaluation of constituents of *Piper retrofractum* fruits on neurotrophic activity. J Nat Prod 76: 769-773.

Lee F.P., Chen Y.C., Chen J.J., Tsai I.L., Chen I.S. (2004). Cyclobutanoid amides from *Piper arborescens*. Helvet Chim Acta 87: 463-468.

Lee S.A., Hwang J.S., Han X.H., Lee C., Lee M.H., Choe S.G., Hong S.S., Lee D., Lee M.K., Hwang B.Y. (2008). Methyl piperate derivatives from *Piper longum* and their inhibition of monoamine oxidase. Arch Pharm Res 31(6): 679-83.

Lim T.K. (2012). In Edible Medicinal and Non-medicinal Plants In; Springer Science & Business: Netherlands.

Lin T.T., Lu S.Y. (1996). Piperaceae, in – Flora of Taiwan, 2nd Ed., Editorial Committee of the Flora of Taiwan, Taipei, Taiwan.

Liu Q., Meng X., Li Y., Zhao C.N., Tang G.Y., Li H.B. (2017). Antibacterial and antifungal activities of spices. Int J Mol Sci 18(6): 1283-1345.

Madigan M., Martinko J. (2004). The Bacteria. In: Brock – Biology of Microorganisms. New Jersey: Prantice Hall.

Mirza Z.M., Kumar A., Kalia N.P., Zargar A., Khan I.A. (2011). Piperine as an inhibitor of the MdeA efflux pump of *Staphylococcus aureus*. J Med Microbiol 60: 1472-1478.

Mittal R., Gupta R.L. (2000). *In vitro* antioxidant activity of piperine. Methods Find Exp Clin Pharmacol 22: 271-274.

Nakayama M., Shimatani K., Ozawa T., Shigemune N., Tomiyama D., Yui K., Katsuki M., Ikeda K., Nonaka A., Miyamoto T. (2015). Mechanism for the antibacterial action of epigallocatechin gallate (EGCg) on *Bacillus subtilis*. Biosci Biotechnol Biochem 79(5): 845-854.

Nascimento J.C., Vanderlúcia F.P., Jorge M.D., Juceni P.D. (2012). Occurrence, biological activities and ^{13}C NMR data of amides from *Piper* (Piperaceae). Quím Nova 35(11): 2288-2311.

Pinto D.S., Duarte F.M., Costa J.I.V., de Almeida Filho G.G., Alves H.S., de Chaves M.C., Pessôa H.L.F. (2012). Antibacterial and hemolytic activities from *Piper montealegreanum* Yuncker (Piperaceae). Anti-Infective Agents 10: 1-5.

Rukachaisirikul T., Siriwattanakit P., Sukcharoenphol K., Wongvein C., Ruttanaweang P., Wongwattanavuch P., Suksamrarn A. (2004). Chemical constituents and bioactivity of *Piper sarmentosum*. J Ethnopharmacol 93: 173-176.

Salleh W.M.N.H.W., Ahmad F., Khong H.Y. (2014). Antioxidant and anti-tyrosinase activities from *Piper officinarum* C.DC (Piperaceae). J Appl Pharm Sci 4(5): 87-91.

Salleh W.M.N.H.W., Ahmad F., Khong H.Y. (2015a). Chemical constituents from *Piper caninum* and antibacterial activity. J Appl Pharm Sci 5(6): 20-25.

Salleh W.M.N.H.W., Ahmad F., Khong H.Y., Zulkifli R.M. (2015b). Chemical compositions and biological activities of essential oils of *Beilschmiedia glabra*. Nat Prod Commun 10(7): 1297-1300.

Hashim N.A., Ahmad F., Salleh W.M.N.H.W., Khamis S. (2019). A new amide from *Piper maingayi* Hk.F. (Piperaceae). Nat Prod Commun 1:1-5.

Salleh W.M.N.H.W., Hashim N.A., Abdullah N., Khong H.Y. (2019). Aporphine alkaloids from *Piper erecticaule* and acetylcholinestearse inhibitory activity. B Latinoam Caribe Pl 18(5): 527-532.

Solis P.N., Olmedo D., Nakamura N., Calderon A.I., Hattori M., Gupta M.P. (2005). A new larvicidal lignan from *Piper fimbriulatum*. Pharm Biol 43: 378-381.

Stavri M., Piddock L.J., Gibbons S. (2006). Bacterial efflux pump inhibitors from natural sources. J Antimicrob Chemother 59: 1247-1260.

Tsai I.L., Lee F.P., Wu C.C., Duh C.Y., Ishikawa T., Chen J.J., Chen Y.C., Seki H., Chen I.S. (2005). New cytotoxic cyclobutanoid amides, a new furanoid lignin and anti-platelet aggregation constituents from *Piper aborescens*. Planta Med 71: 535-542.

Tyagi O.M., Prasad A.K., Wengel J., Boll P.M., Olsen C.E., Parmar V.S., Sharma N.K., Jha A., Bisht K.S. (1995). Neolignans from *Piper schmidtii* and reassignment of the structure of schmiditin. Acta Chem Scand 49: 142-148.

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