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LARVICIDAL ACTIVITY OF THE SYNTHESISED LIGNANS, NEOLIGNANS, AND COUMARIN AGAINST *Crocidolimia binotalis* 2ND INSTAR LARVAE

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Abstract: Five compounds comprising of 8-O-4'-neolignan (7), two arylnaphthalene lignans (5, 8), aryldihydrobenzofuran neolignan (4), and lignan (6) were synthesised by enzymatic coupling reaction using horseradish peroxidase (HRP) between vanillin (1) with methyl ferulate (2) or methyl sinapate (3). All of these compounds, as well as previously synthesised palladium-catalysed coupling products of neolignan (9), 8-O-4'-neolignan (10), arylcoumarin (11), and lignan (12), were examined for larvicidal activity against *Crocidolomia binotalis* (*C. binotalis*) 2nd instar larvae. It was revealed that seven out of nine synthesised compounds had a mortality rate of more than 90% after 24 hours of exposure. Neolignan (10) and lignan (6) demonstrated strongest larvicidal activity with LD_{50} = 2.218 mg/L and LD_{50} = 1.678 mg/L, respectively compared to the standard azadirachtin (LD_{50} =2.818 mg/L). The results showed that the synthesised compounds have a high potential for use in the control of *C. binotalis* larvae and could be used in the development of new and more effective compounds as larvicides.

Keywords: *Lignan, neolignan, coumarin, horseradish peroxidase, larvicidal*

1. Introduction

Insects pose a significant threat to crops because they can consume plant leaves, roots, and stems hence rendering them to be unfit for consumption, other uses, and potentially damaging the plants. Insecticides are chemicals that are used in crop plantations to control insect and disease infestations. Insecticides are widely available on the market and can be classified in a variety of ways depending on the (i) chemical structure (natural, synthetic, organic, inorganic), (ii) toxicity (extreme, high, moderate, less), (iii) stages in the life cycle (larvicides, pupicides, ovicides, adulticides), (iv) mode of entry (systemic, contact, stomach, fumigant, repellent), and (iv) mode of action (AChE inhibitors, GABA chloride channel blockers, ecdysone receptor agonists, sodium channel modulators, juvenile hormone mimics, inhibitors of chitin biosynthesis, ryanodine receptor modulators, and others) (IRAC Website; CFR Website; Akashe et al., 2018; Yadav & Devi, 2017). Crop protection with insecticides has been extremely beneficial to agriculture, particularly in terms of increasing yield production. However, the development of resistance of plant pathogens to conventional insecticides, as well as toxic effects and environmental concerns, piqued researchers' interest in developing synthetic or natural in the origin of new insecticides.

Novel insecticides are abundant in higher plants and have been used worldwide (Isman, 2006). Many plant species, particularly those native to the tropics, have the potential to be used as bioinsecticides (Lewis et al., 2016). As they are frequently biodegradable and active against a limited range of species, these insecticides may lead to the development of new classes of safer insect control agents. They can also be suitable materials for use in integrated pest management (Hikal et al., 2017; Hubbard et al., 2014).

Lignans, neolignans, and coumarins are among the plant insecticides that have been widely reported. Lignans and phenolic compounds originating from C_6C_3 precursors function as insect-feeding regulators or insect growth and development regulators. These compounds interact with the insect's endocrine system, which is involved in insect development (Harmatha & Dinan, 2003). Examples of lignans activities include an insecticidal activity of podophyllotoxin against 5th instar larvae of *Pieris rapae* L. (Di et al., 2007) and the 3rd instar larvae of *Mythimna separata* (Xu & Xiao, 2009); antifeedant activity of gomisin J against *Tribolium castaneum* adults (Guo et al., 2020); repellent and feeding deterrent activities of (-)-sesamin against *T. castaneum* adults (Wang et al., 2020); insecticidal activity of haedoxan A

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against 3rd instar larvae of *M. separata (*Li et al., 2019), and insecticidal activity of phrymarolin B against 4th instar larvae of *M. separata* (Xiao et al., 2013). Examples for neolignans include the growth inhibition activity of licarin A against neonate larvae of *Spodoptera litura* (González-Coloma et al., 1994) and insecticidal activity of honokiol against the 3rd instar larvae of *M. separata* (Yang et al., 2015). Whereas, the example for coumarins activities include acaricidal and growth inhibition activities of osthole ester against *M. separata* and *Tetranychus cinnabarinus* (Shan et al., 2022), insecticidal activity of psoralen against neonate larvae of *Spodoptera frugiperda* (Ayil-Gutiérrez et al., 2015), and insecticidal activity of surangin B against the 3rd instar larvae of *Plutella xylostella* (Issakul et al., 2011).

This study investigated the insecticidal activity of new potential lignans, neolignans, and coumarins against *C. binotalis* larvae. *C. binotalis* was chosen as the target pest because it is a common pest in Malaysia for vegetable crops replacing *P. xylostella*, *S. litura*, and *Hellula undalis* (Lim et al., 1996; Ooi & Kelderman, 1979). Another reason for using *C. binotalis* in this study is because of their ease of rearing and availability. There is a lack of reports on the effect of synthetic compounds on this pest in Malaysia (Hashim & Ibrahim, 2003; Hashim et al., 2002; Ng et al., 2003), as well as in neighbouring countries such as Indonesia (Sastrosiswojo & Setiawati, 1992; Shepard & Schellhorn, 1994) and India (Kannan et al., 2011; Srinivasan & Veeresh, 1986).

Previously, we investigated the palladium-catalysed Heck coupling reaction between a series of methyl cinnamate derivatives and an activated C-I bond of iodovanillin or a non-activated C-H bond of vanillin (Juhan et al., 2018). Furthermore, the aim of this research is to employ an enzymatic method to perform the coupling reaction of vanillin (**1**) and methyl ferulate (**2**) or methyl sinapate (**3**) and to investigate the larvicidal activity of all coupling products obtained from both chemical and enzymatic coupling methods. In the modified enzymatic coupling reaction, HRP as a catalyst in the presence of hydrogen peroxide (H_2O_2) and phosphate buffer (pH7.2) was used to synthesise lignan and neolignan derivatives (Wakimoto et al., 2009).

It was discovered that the structures of the compound play an important role in enhancing insecticidal activities such as introducing methoxy group, which is essential for maintaining the activities, at a specific position (Di et al., 2007). The formation of ester derivatives (Xu & Xiao, 2009), the number of methylenedioxy, and the position of hydroxyl groups also demonstrated promising activities (Guo et al., 2020; Kirst, 2010). The presence of different functional groups in their structures such as hydroxyl, methoxy, and ester led to the selection of vanillin, iodovanillin, and all methyl cinnamate derivatives for our study. These three functional groups were found in the structures of commercially available insecticides such as azadirachtin (Lin et al., 2021), organophosphates (Rathnayake & Northrup, 2016; Roger et al., 1969), and carbamates (Metcalf, 1971) that contributed to their activities. Based on these considerations, it was hypothesised that the products of these coupling reactions would have comparable or better insecticidal activity.

2. Experimental

2.1 General

All commercially available solvents and chemicals were directly used without further purification. Precoated aluminium backed plates of thin layer chromatography (TLC) (Silica gel 60 F245, Merck KGaA) were used to monitor all reactions. TLC plates were visualised using UV Lamp UVGL-58 and stained with basic $KMnO_4$ solution. Silica gel 60 (100-150 mesh) was used for purification using column chromatography. The organic layers were dried over sodium sulphate and concentrated using a Buchi Switzerland rotavapor R-215. IR spectra were recorded using the Perkin-Elmer FT-IR Model Spectrum 100 series. The NMR spectra were recorded at 500 MHz on a JEOL JNM-ECX500 using $CDCl_3$ as the solvent. Chemical shifts were measured in ppm in relation to the TMS signal. Melting points were measured three times for each compound using a digital Electrothermal IA9000 Series. EIMS spectra were recorded using a Shimadzu QP5050A and HRMS spectra were obtained using an AGILENT 6550 iFunnel Q-TOF.

2.2 Coupling Reactions

2.2.1 General Procedure

Vanillin (**1**) (1.0 mmol) and methyl ferulate (**2**) or methyl sinapate (**3**) (1.0 mmol) were added into phosphate buffer (pH 7.2, 23 mL) and stirred for 10 minutes. The reaction mixture was further treated with HRP (0.4 mg). 1 M of H_2O_2 (1.8 mL) was added slowly into the mixture within 30 minutes and the mixture was continuously stirred for 3 days at room temperature. The reaction was terminated with ethyl acetate (AcOEt) (10 mL) and subsequently washed with 1 M of HCl (3×10 mL) and saturated brine solution (3×30 mL) before being dried, evaporated, and purified (Wakimoto et al., 2009).

Methyl 5-((*E*)-2-(methoxycarbonyl)vinyl)-2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-7-methoxybenzofuran-3-carboxylate (**4**): R_f 0.25 (hexane:AcOEt, 3:2); white solid; yield, 20%; mp 143-144°C (lit, 151-152°C) (Isman, 1993); ν_{max} (UATR, cm^{-1}) 3381, 2953, 1725, 1631, 1604, 1436, 1158, 1137; δ_H (500 MHz, $CDCl_3$, ppm) 7.63 (d, 1H, *J* 16.0 Hz, C*H*=C), 7.17 (s, 1H, *H*-Ar), 7.01 (s, 1H, *H*-Ar), 6.90 (d, *J* 8.0 Hz, 1H, *H*-

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Ar), 6.89 (s, 1H, *H*-Ar), 6.88 (d, *J* 8.0 Hz, 1H, *H*-Ar), 6.30 (d, *J* 16.0 Hz, 1H, C*H*=C), 6.09 (d, *J* 8.0 Hz, 1H, C*H*-C), 5.70 (br. s, 1H, O*H*), 4.33 (d, *J* 8.0 Hz, 1H, C*H*-*C*), 3.90 (s, 3H, CH_3), 3.86 (s, 3H, CH_3), 3.82 (s, 3H, CH_3), 3.79 (s, 3H, CH_3); δ_C (125 MHz, $CDCl_3$, ppm) 170.7, 167.6, 150.0, 146.7, 146.1, 145.5, 144.7, 131.4, 128.6, 125.7, 119.4, 117.9, 115.6, 114.5, 112.2, 108.7, 87.5, 56.1, 56.0, 55.5, 52.8, 51.6; *m/z* (HRMS) 414.1321 $[M]^+$ (Calcd for $C_{22}H_{22}O_8$: 414.1315), 437.1215 $[M+Na]^+$ (Calcd for $C_{22}H_{22}O_8Na$: 437.1212).

Dimethyl-1,2-dihydro-6-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-methoxynaphthalene-2,3-dicarboxylate (**5**): R_f 0.30 (hexane:AcOEt, 3:2); brown solid; yield, 3%; mp 136-138°C; ν_{max} (UATR, cm^{-1}) 3405, 2944, 1703, 1509, 1215; δ_H (500 MHz, $CDCl_3$, ppm) 7.64 (s, 1H, C*H*=C), 6.82 (s, 1H, H-Ar), 6.70 (d, *J* 8.0 Hz, 1H, *H*-Ar), 6.68 (s, 1H, *H*-Ar), 6.60 (d, *J* 2.3 Hz, 1H, *H*-Ar), 6.39 (dd, *J* 2.3, 8.0 Hz, 1H, *H*-Ar), 4.53 (d, *J* 2.3 Hz, 1H, C*H*-C), 3.96 (d, *J* 3.4 Hz, 1H, C*H*-C), 3.89 (s, 3H, CH_3), 3.77 (s, 3H, CH_3), 3.73 (s, 3H, CH_3), 3.61 (s, 3H, CH_3); δ_C (125 MHz, $CDCl_3$, ppm) 172.9, 167.1, 147.7, 146.4, 145.8, 144.4, 137.7, 134.3, 131.2, 123.9, 122.4, 120.4, 115.6, 114.2, 112.2, 110.1, 56.0, 55.8, 52.4, 51.9, 47.2, 45.6; *m/z* (HRMS) 414.1315 $[M]^+$ (Calcd for $C_{22}H_{22}O_8$: 414.1315), 437.1208 $[M+Na]^+$ (Calcd for $C_{22}H_{22}O_8Na$: 437.1212).

(2*E*,3*E*)-Dimethyl 2,3-bis(4-hydroxy-3,5-dimethoxybenzylidene)succinate (**6**): R_f 0.28 (petroleum ether:AcOEt, 2:3); yellow solid; yield, 14%; mp 129-130°C; ν_{max} (UATR, cm^{-1}) 3413, 2944, 1705, 1598, 1510, 1234; δ_H (500 MHz, $CDCl_3$, ppm) 7.81 (s, 2H, 2×C*H*=C), 6.80 (s, 4H, 4×*H*-Ar), 5.77 (br. s, 2H, 2×O*H*), 3.76 (s, 12H, 4×CH_3), 3.66 (s, 6H, 2×CH_3); δ_C (125 MHz, $CDCl_3$, ppm) 167.7, 146.9, 142.5, 136.7, 125.9, 124.7, 107.1, 56.1, 52.4; *m/z* (EIMS) 474 (M^+, $C_{24}H_{26}O_{10}$ requires 474).

(*Z*)-Methyl 2-(4-formyl-2-methoxyphenoxy)-3-(4-hydroxy-3,5-dimethoxyphenyl) acrylate (**7**): R_f 0.43 (hexane:AcOEt, 3:2); white solid; yield, 29%; mp 69-70°C; ν_{max} (UATR, cm^{-1}) 3404, 2928, 1702, 1512, 1260; δ_H (500 MHz, $CDCl_3$, ppm) 9.82 (s, 1H, C*H*O), 7.49 (s, 1H, *H*-Ar), 7.38 (s, 1H, C*H*=C), 7.32 (d, *J* 8.0 Hz, 1H, *H*-Ar), 6.97 (s, 2H, 2×*H*-Ar), 6.85 (d, *J* 8.0 Hz, 1H, *H*-Ar), 5.77 (br. s, 1H, O*H*), 3.98 (s, 3H, CH_3), 3.77 (s, 3H, CH_3), 3.76 (s, 6H, 2×CH_3); δ_C (125 MHz, $CDCl_3$, ppm) 190.7, 163.6, 150.9, 149.4, 146.9, 137.1, 136.9, 131.7, 128.9, 126.3, 123.1, 113.1, 110.2, 107.6, 56.1, 56.0, 52.6; *m/z* (HRMS) 388.1120 $[M]^+$ (Calcd for $C_{20}H_{20}O_8$: 388.1158), 411.0996 $[M+Na]^+$ (Calcd for $C_{20}H_{20}O_8Na$: 411.1056).

Dimethyl-1,2-dihydro-7-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-6,8-dimethoxynaphthalene-2,3-dicarboxylate (**8**): R_f 0.22 (hexane:AcOEt, 2:3); yellow solid; yield, 14%; mp 181-183°C; ν_{max} (UATR, cm^{-1}) 3437, 2949, 1701, 1600, 1447, 1189, 1096; δ_H (500 MHz, $CDCl_3$, ppm) 7.62 (s, 1H, C*H*=C), 6.68 (s, 1H, *H*-Ar), 6.25 (s, 2H, 2×*H*-Ar), 5.81 (br. s, 1H, O*H*), 5.36 (br. s, 1H, O*H*), 4.97 (s, 1H, C*H*-C), 3.99 (s, 1H, C*H*-C), 3.90 (s, 3H, CH_3), 3.73 (s, 9H, 3×CH_3), 3.62 (s, 6H, 2×CH_3); δ_C (125 MHz, $CDCl_3$, ppm) 172.4, 167.0, 146.8, 144.9, 140.9, 137.6, 133.5, 123.8, 123.4, 122.8, 107.3, 104.3, 60.6, 56.3, 52.5, 51.9, 46.5, 39.4; *m/z* (HRMS) 474.1548 $[M]^+$ (Calcd for $C_{24}H_{26}O_{10}$: 474.1526), 497.1442 $[M+Na]^+$ (Calcd for $C_{24}H_{26}O_{10}Na$: 497.1424).

2.3 Bioassay

C. binotalis larvae (cabbage head caterpillar) that are used in this research were collected from a laboratory colony reared on cabbage plants or using an artificial diet from the Genebank and Seed Centre, Malaysian Agricultural Research and Development Institute (MARDI), Serdang.

The larvicidal activity of the synthesised compounds was assessed with a slight modification using a conventional leaf disc method (Wu et al., 2016). Leaf discs (150 mm in diameter) were trimmed with a cork borer from clean organic cabbage leaves and kept in a moist chamber. The solution of the synthesised compounds and azadirachtin in acetone was prepared at 100 ppm for screening. The leaf discs were immersed in acetone alone or in an acetone solution containing the compounds for 5 seconds before being air dried at room temperature. Ten 2nd instar larvae of *C. binotalis* of the same size (2-3 hours pre-starved) and five treated leaf discs were positioned into the test container and covered with a moistened towel. One container was considered a single replicate and the experiment was conducted with three replicates of each compound (29-32 larvae). The untreated leaves were used as a negative control and the leave treated with azadirachtin was used as the positive control. The number of larvae that died at 24 hours was recorded and the values of corrected mortality (CM) were determined as follows: CM rate (%) = (T – C) × 100/(100% – C), where C is the mortality rate of the negative control and T is the mortality rate of the treated *C. binotalis* (Ren et al., 2020). When larvae did not react to the stimulation or did not move after being touched, they were considered dead. All the screened compounds with less than a 50% of mortality rate are considered not active and will not be further subjected to LD_{50} (lethal dose causes 50% mortality) determination.

The toxicity of the active compounds was tested in acetone at up to five different concentrations ranging from 0 to 15 ppm. The LD_{50}, 95% confidence limits (CLs), the slope of the concentration-mortality curve, and the standard error of the slope were calculated using Polo Plus software by probit analysis. The differences between LD_{50} values were considered significant if 95% of the CLs did not overlap.

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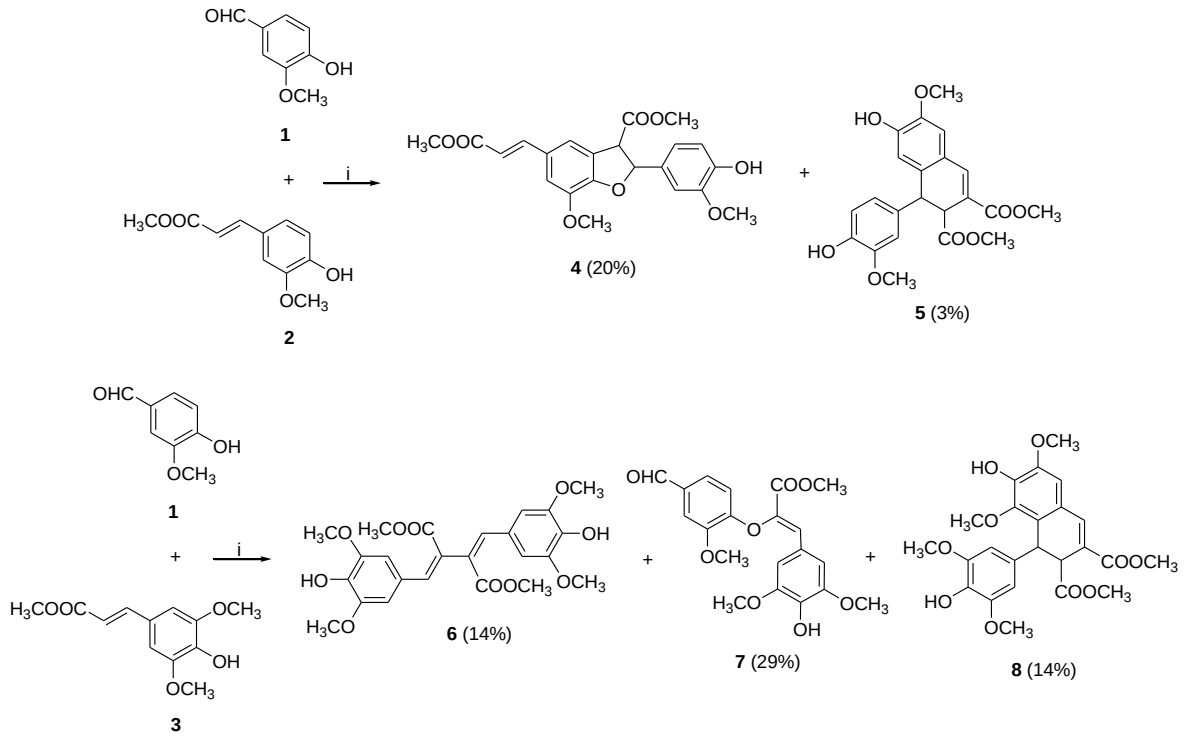
3. Results and Discussion

3.1 Enzymatic Coupling Reaction

The reaction applied for the vanillin (**1**) and methyl cinnamate derivatives (**2**) and (**3**) was adapted from Wakimoto et al. (2009). First, both compounds (**2**) and (**3**) were prepared from their respective acids via common Fischer esterification (Juhan et al., 2018). The reaction of vanillin (**1**) and methyl ferulate (**2**) with HRP yielded compounds (**4**) and (**5**), whereas the reaction with methyl sinapate (**3**) produced compounds (**6**-**8**). All compounds were obtained through the homocoupling of methyl ferulate (**2**) or methyl sinapate (**3**) except for compound (**7**) whereby this compound was obtained through heterocoupling of vanillin (**1**) and methyl sinapate (**3**) (Scheme 1). This finding demonstrated that the enzymatic reaction preferred the formation of dihydrobenzofuran neolignan (**4**) and arylnaphthalene type of lignans (**5**, **8**). However, these reactions contributed to a low yield (3-29%) with a high recovery of starting material even though the reaction time was increased up to three days. Spectroscopic data of lignan (**6**) and 8-O-4' neolignan (**7**) were reported in our previous study (Juhan et al., 2018) while the others were discussed in this paper and compared with data from the literature.



Scheme 1. The enzymatic coupling reaction between vanillin (**1**) with methyl ferulate (**2**) or methyl sinapate (**3**). Reagents and conditions: i) HRP, 1 M of H_2O_2, phosphate buffer pH7.2, RT, 3d. All of the synthesised compounds were separated using column chromatography. The same lignan (**6**) and neolignan (**7**) were also produced in the previous coupling reaction with a palladium catalyst (Figure 1).

All synthesised compounds in this work (**4**-**8**) and all compounds synthesised earlier (**9**-**12**) using palladium-catalysed coupling reaction (Juhan et al., 2018) (Figure 1) were further studied for their larvicidal activity. To the best of our knowledge, no research on the larvicidal activity of these synthesised compounds **(4**-**12)** against *C. binotalis* has been reported.

Neolignan (**4**) and lignan (**5**) were obtained both as white and brown solids with a melting point of 145-144°C and 136-138°C, respectively. The IR spectrum for both compounds showed the presence of hydroxyl groups with a broad band at 3381 cm^{-1} and 3405 cm^{-1}, whereas the band for carbonyl of esters appeared at 1725 cm^{-1} and 1703 cm^{-1}, respectively. Both neolignan (**4**) and lignan (**5**) shared the same molecular formula of $C_{22}H_{22}O_8$, which their HRMS analyses gave *m/z* 414.1324 $[M]^+$ and *m/z* 414.1315 $[M]^+$ (Calculated for $C_{22}H_{22}O_8$ requires 414.1315).

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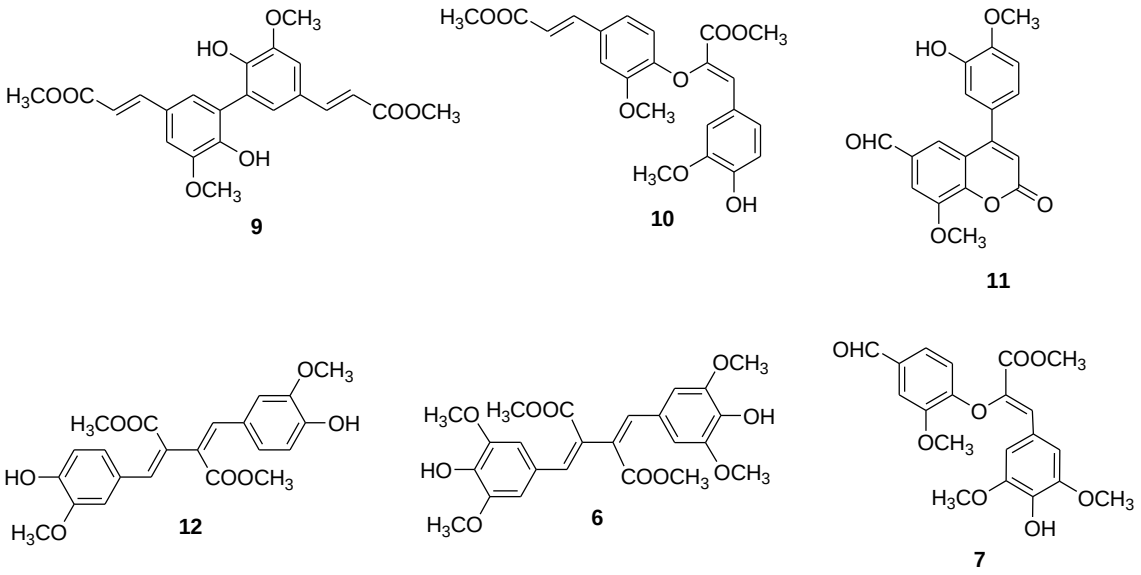

Figure 1. Neolignans (**7**, **9**-**10**), coumarin (**11**), and lignans (**6**, **12**) were synthesised via a palladium-catalysed coupling reaction of vanillin (**1**) with methyl ferulate (**2**) or methyl sinapate (**3**) (Juhan et al., 2018).

In both ^{1}H NMR spectra, two singlets of methoxy protons and two singlets of methyl protons of ester appeared at *δ* 3.90, 3.86, 3.82, and 3.79 ppm for compound (**4**), whereas at *δ* 3.89, 3.77, 3.73, and 3.61 ppm for compound (**5**). Two sets of a doublet in (**4**) appeared at *δ* 4.33 ppm and 6.09 ppm corresponding to H3 and H2, and another two sets (*J*=16 Hz) were presented at *δ* 6.30 ppm and 7.63 ppm referring to the two *trans* protons of H2'' and H1''. Two doublets of H1 and H2 in (**5**) were observed at *δ* 4.53 ppm and 3.96 ppm. It was confirmed that compound (**5**) was bonded and cyclised through the alkene functional group by the signal of only one proton observed at *δ* 7.64 ppm (s) representing H4. Besides that, compound (**5**) showed two broad peaks at *δ* 5.51 ppm and 5.84 ppm representing hydroxyl groups attached to a benzene ring at C7 and C4' while only one OH peak appeared at *δ* 5.70 ppm for compound (**4**).

The ^{13}C NMR supports the structure of the compounds by showing two carbons in the downfield region at *δ* 170.7 ppm and 167.6 ppm for neolignan (**4**) and *δ* 172.9 ppm and 167.1 ppm for lignan (**5**) referring to the two carbonyl esters in each compound. The formation of dyhdrobenzofuran ring was established by the presence of signals at *δ* 87.5 ppm and 56.0 ppm for C2 and C3, respectively. In contrast, the naphthalene type of lignan (**5**) was represented by the peaks observed at *δ* 137.7 ppm (C4), 47.2 ppm (C2), and 45.6 ppm (C1). All NMR data of compound (**4**) agree with the data from Moussouni et al. (2011).

The HMBC analysis of compound (**4**) also supports the proposed structure by showing correlations between H2 with C3, C1', C2', and C6'. On the other hand, there were also correlations between H3 with C2, C3a, and C1'. The HMBC analysis of compound **5** showed correlations between H1 with C2, 2-$COOCH_3$, C3, C4a, C8, C1', and C6'. Other correlations between H2 with C1, 2-*C*$OOCH_3$, C3, 3-$COOCH_3$, C4a, and C1' were also observed (Figure 2).

Lignan (**8**) with a melting point of 181-183°C was obtained as a yellow solid formed by the free radical coupling mechanism. This compound was isolated previously from the ethanol extract of rapeseed (*Brassica napus L.*) and is known as dimethyl (±)-thomasidioate by Fang et al. (2012). The IR analysis for compound (**8**) revealed a broad band at 3437 cm^{-1} referring to a hydroxyl group while the presence of a carbonyl aldehyde absorption was observed at 1701 cm^{-1}. Compound (**8**) has a molecular formula of $C_{24}H_{26}O_{10}$ as indicated by analysis of its HRMS with *m/z* 474.1548 $[M]^{+}$ (calculated for $C_{24}H_{26}O_{10}$ requires 474.1526). Therefore, compound (**8**) is thus confirmed to be a combination of two methyl sinapate (**3**) units.

The integration of the ^{1}HNMR spectrum presented one proton at *δ* 6.68 ppm belonging to H5 for the first unit of methyl sinapate (**3**). Two protons appeared as singlets (*δ* 6.25 ppm) attributed to the symmetrical H2' and H6' in the second unit of methyl sinapate (**3**) suggesting bonds between C8a and C1. An olefinic proton (H4) was observed at *δ* 7.62 ppm while another two protons assigned to H2 and H1 appeared at *δ* 3.99 ppm and 4.97 ppm, respectively. In support of the assignments, ^{13}C NMR analysis depicted two carbon signals at *δ* 39.4 ppm and 46.5 ppm corresponding to C1 and C2, respectively. Two carbonyl esters are seen at *δ* 172.4 ppm and 167.0 ppm. NMR data of this compound agreed with the previously reported data (Fang et al., 2012).

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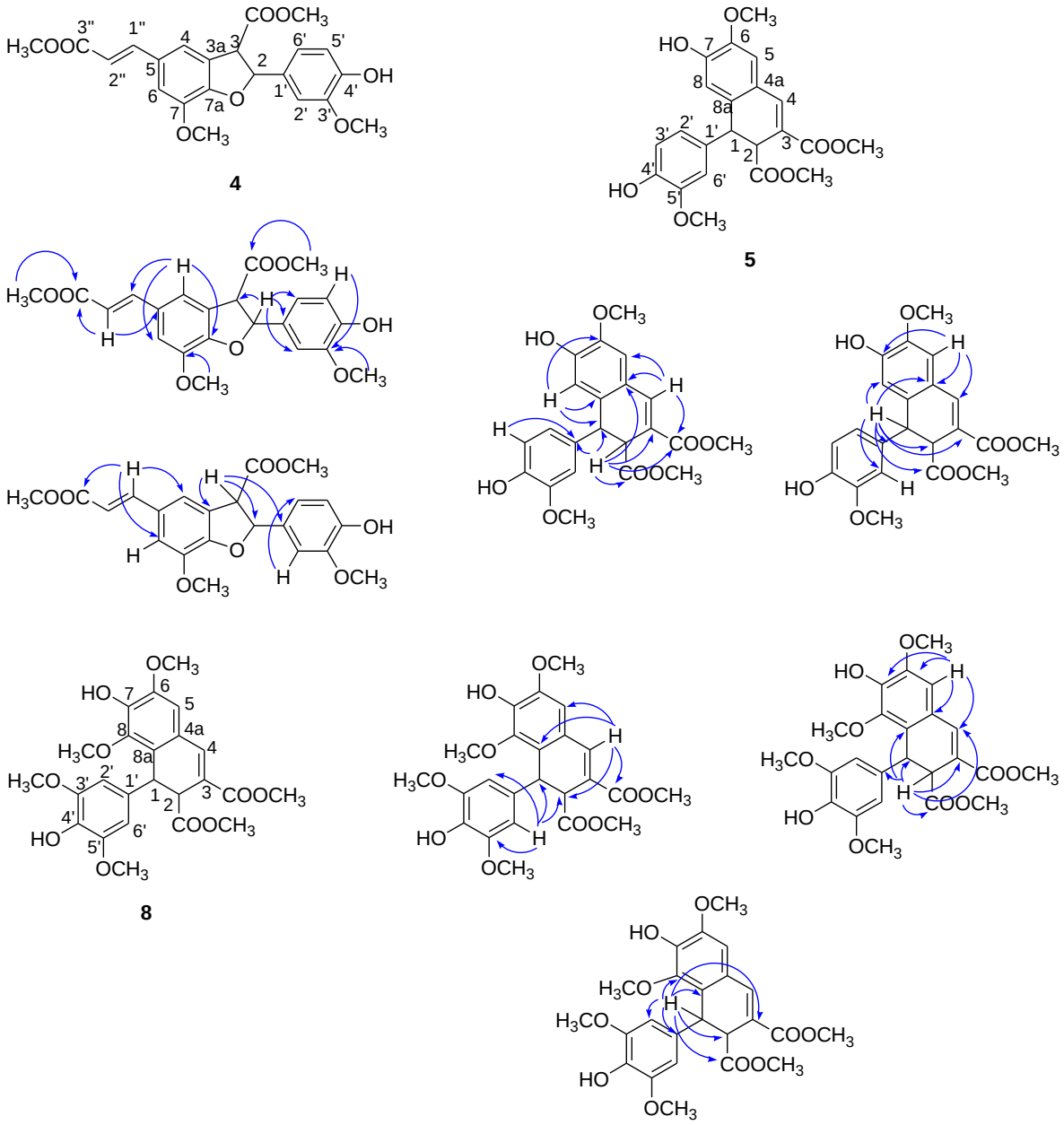

Figure 2. HMBC correlations of compounds **4-5** and **8**.

The HMBC measurement of (**8**) exhibited correlations between H1 with C2, 2-$COOCH_3$, C3, C8, C8a, C1', C2', and C6' thus rationalised the position of C1 to the ring. The correlations between H2 with C1, C1', 2-$COOCH_3$, C3, C4, and C8a, and H4 with C2, 3-$COOCH_3$, C5, and C8a supported the proposed structure. Besides that, the benzene protons of H2' and H6' showed a correlation with C3', C5', C1, and C2 meanwhile the benzene proton of H5 was correlated with C4, C4a, C6, and C7 (Figure 2). With all these findings, it can be deduced that the bond between the two units of methyl sinapate (**3**) forms a naphthalene type of lignan.

3.2 Larvicidal Activity

At a concentration of 100 ppm, 5,5-neolignan (**9**), 8-O-4'-neolignan (**10**), coumarin (**11**), lignans (**6**, **12**), and dihydrobenzofuran neolignan (**4**) demonstrated strong activity with a mortality rate of more than 90% (Table 1). It is worth noting that the presence of two ester groups in the structures is important for larvicidal activity. This hypothesis was supported by comparisons with commercial insecticides azadirachtin and toosendanin, both of which have three and two ester groups, respectively. Among the larva species

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tested with these two standards are *Actebia fennica*, *Mamestra configurata*, *Malanchra picta*, *S. litura* (Isman, 1993), and *S. frugiperda* (Céspedes et al., 2015). Other commercial insecticides with ester groups, such as organophosphates (malathion and diazinon) and carbamates (methomyl), are used to control the fall armyworm, *S. frugiperda* (Yu, 1992) and the addition of an ester group to podophyllotoxin against the oriental armyworm, *M. separata* (Xu & Xiao, 2009) also proved this finding.

Table 1. Larvicidal screening results of synthesised compounds (4-12) and against the 2nd instar larvae of *C. binotalis* at a concentration of 100 ppm for 24 hours of exposure.

Compound	Corrected Mortality Rate (%)[a]
Acetone[b]	0.00
Azadirachtin[c]	100.00 ± 0.00
9	96.66 ± 5.77
10	93.10 ± 0.00
11	100.00 ± 0.52
12	90.30 ± 5.53
4	93.33 ± 5.77
5	0.00
6	96.77 ± 5.25
7	50.06 ± 3.23
8	51.72 ± 5.77

[a] Data are expressed as means ± SE from experiments with three replicates. Corrected as Xu et al., 2020 (Ren et al., 2020). [b] Negative control. [c] Positive control.

Compound (**10**) was observed to have good activity compared to moderate activity seen in compound (**7**) with mortality rates of 93.10% and 50.06%, respectively. The structural differences between these two neolignans demonstrated that the presence of extra ester groups in compound (**10**) made it more active in terms of larvicidal activity. Other findings revealed that uncyclized lignans (**6**, **12**) were more active with mortality rates of 90.3% and 96.77%, respectively compared to the cyclised lignans (**5**, **8**) with mortality rates of 0.00% and 51.72% (Table 1). It was thought that the presence of more double bonds in the uncyclised lignans (**6**, **12**) resulted in increased larval mortality.

When similar structures of lignans as seen in compounds (**5**) and (**8**) were compared, it was discovered that compound (**8**) has higher larvicidal activity than compound **(5**) due to the extra methoxy groups (Table 1). The presence of a methoxy group has been reported to increase podophyllotoxin's insecticidal activity against *P. rapae* L. 5th instar larvae (Di et al., 2007). It was also discovered that the presence of two methoxy groups in methyleugenol increased its activity against *S. litura* (Bhardwaj et al., 2010). Coumarin (**11**), like azadirachtin, demonstrated the highest activity with 100% larvae mortality. This finding indicated that the presence of a hydroxyl group in the structure could increase bioactivity. Previous research found that coumarin derivatives with hydroxyl groups had the most potent insecticidal activity against *Musca domestica* adults and *Aedes albpictus* 4th instar larvae (Wang et al., 2011).

Table 2. Toxicity of compounds (**4**), (**6**), and (**9**-**12**) against the 2nd instar larvae of *C. binotalis*.

Compound	Total insect used	Slope ± SE	LD_{50} (mg/L) (95% CL)[a]	TR[b]
Azadirachtin	197	1.728 ±0.460	2.818 (1.213-4.086)	1.7
9	182	3.288 ± 0.584	3.537 (2.823-4.193)	2.1
10	181	2.080 ± 0.542	2.218 (1.088-2.988)	1.3
11	182	5.326 ± 0.906	4.269 (2.488-5.515)	2.5
12	182	3.097 ± 0.766	4.673 (3.786-6.166)	2.8
4	152	4.499 ± 1.559	9.849 (8.297-15.144)	5.9
6	150	2.107 ± 0.520	1.678 (0.968-2.335)	1

[a] Where 95% of CLs do not overlap, LC_{50} values differ significantly. [b] Toxicity ratio (TR) = LD_{50} of a compound / LD_{50} of compound (**6**).

All compounds with high mortality rates were subjected to LD_{50} determination (Table 2). The results showed that all compounds were toxic to *C. binotalis* larvae. The most active compounds were 8-O-4'-neolignan (**10**) and lignan (**6**) with LD_{50} values of 2.218 mg/L and 1.678 mg/L, respectively. These values are lower than the commercial standard azadirachtin (LD_{50} = 2.818 mg/L). Compound (**6**) outperformed azadirachtin in terms of larvicidal activity by 1.6 times. In comparison, compound (**10**) has one double bond and one oxygen atom, whereas compound (**6**) has two double bonds between two phenyl groups to form a continuous long-conjugated double bond. These distinct properties, combined with the presence of methoxy (Di et al., 2007), ester (Che et al., 2013), and hydroxyl (Gua et al., 2022) groups in the structures enhanced their larvicidal activity.

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When compared to the standard, compounds (**9**), (**11**), and (**12**) showed significant toxic effects with LD_{50} values of 3.537 mg/L, 4.269 mg/L and 4.673 mg/L, respectively (Table 2). Compound (**4**) was found to be the least toxic among the studied compounds due to the separated conjugated system by the formation of the dihydrofuran ring. The presence of a methyl cinnamate fragment attached to a dihydrofuran ring likely contributed to its low toxicity as reported for methyl 4-methoxycinnamate against *S. litura* (Bhardwaj et al., 2010).

4. Conclusion

Five coupling products, 8-O-4'-neolignan (**7**), two arylnaphthalene lignans (**5, 8**), aryldihydrobenzofuran neolignan (**4**), and lignan (**6**) were synthesised via enzymatic coupling reactions. In comparison, all synthesised compounds and four chemical-catalysed coupling products (**9-12**) were screened against *C. binotalis* larvae in which seven compounds were active and proceeded with LD_{50} identification. 8-O-4'-Neolignan (**10**) and lignan (**6**) showed the strongest larvicidal activities with LD_{50} lower than the standard azadirachtin. Both compounds may be effective to conventional synthetic insecticides in the pest control of *C. binotalis*.

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