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Syntheses, Characterization, Antioxidant Activity, and Toxicity Evaluation of Schiff Base Derivates from O-Vanillin

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ABSTRACT

Three Schiff bases (1a, 1b, and 1c) have been prepared from the reaction of *o*-vanillin with primary amine (aniline, *p*-toluidine, and *p*-anisidine). Schiff base derivates from *o*-vanillin were synthesized using the grinding method for 20 minutes. Physical properties were observed based on color, melting point, and solubility. Synthesis products were also characterized using FTIR, GCMS, ^{1}H-NMR, and ^{13}C-NMR. The antioxidant activity of the Schiff base was tested using DPPH. While the toxicity test uses the BSLT method. The result of this synthesis and characterization Schiff base (1a, 1b, and 1c) showed that the Schiff base compound was formed into 2-methoxy-6 (phenyliminomethyl) phenol; 2-methoxy-6- (((4-methylphenyl) imino) methyl) phenol; and 2-methoxy-6-(((4-methoxyphenyl) imino) methyl) phenol. The result of NMR analysis, on ^{1}H-NMR spectrum showed the shift chemical at 8,5-8,6 ppm which indicates the typical peak of proton (-HC=N-). Meanwhile, the ^{13}C-NMR spectrum shown the shift chemical at 160-162 ppm which indicates the typical peak of carbon (-C=N-). The result of antioxidant activity showed that all Schiff base was antioxidant quite low ability with value of EC_{50} is 106.2-196.4 ppm. Meanwhile, the result of toxicity test showed that all Schiff base was anticancer with an LC_{50} value of 9.99-22.29 ppm.

Key word: Schiff Base, synthesis, characterization, antioxidant, toxicity

INTRODUCTION

Schiff base is a synthetic organic compound that has a lot of potential as an antioxidant, anti-bacterial [1], anticancer [2,3], antiviral [4] and anti-malarial [5]. The antioxidant potential of Schiff base compounds is supported by the presence of a lone pair in the sp2 hybridized orbital of the azomethine group (-C=N-). The presence of non bonding electrone in this group has an important role chemically and biologically. Schiff base compounds can be applied in the pharmacy chemistry and medicine. One of the benefits of Schiff's base that can be applied in the health sector is as antioxidant. Antioxidants are generally hydrogen donors or electron donors to the reactive sites of radical molecules [6–8]. Besides having potential as an antioxidant, Schiff base also has biological activities such as anti-cancer. Screening for the bioactivity of Schiff base compounds can be carried out using a toxicity test [9,10]. Based on Shoaib et al. [10], that the toxicity test of Schiff's basic compound of 4-aminoantipyrine and vanillin, showed an LC_{50}=5 ppm. While the results of the Schiff base toxicity test of 4-aminoantipyrine and 2-hydroxy benzaldehyde showed an LC50=1 ppm. Meanwhile, according to Mc Laughlin et al. [11] the LC_{50} value of compounds in <30 ppm

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show that compound can classified as a compound that has cytotoxicity and potentially as an anti-cancer.

Schiff base usually formed by condensation of carbonyl compounds and primary amines. This compounds can be synthesis using conventional method and green synthesis. Synthesis using conventional methods requires organic solvents and a relatively longer time [12–14]. While the green synthesis method is more environmentally friendly and effective [15]. So, in this study using the grinding method for synthesis of Schiff base derived from o-vanillin. The synthesized products were characterized using spectroscopy methodology. Antioxidative activity is evaluated by capability to scavenging the DPPH radical [16,17], whereas the toxicity is evaluated following brine shrimp lethality test [18].

EXPERIMENT

Chemicals and instrumentation

The chemical used include o-Vanillin (Merck), aniline (Merck), p-toluidine (Merck), p-anisidine (Merck), ethanol (Merck), DPPH (*Smartlab*), ascorbic acid, BHT and baker's yeast.

Melting point test using melting point apparatus STUART type SMP11. The elucidation of compounds using FTIR VARIAN type FT 1000, GCMS SM QP2010S SHIMADZU, and Agilent DD2 NMR (chloroform-D used as solvent, 500 MHz (^{1}H) and 125 MHz (^{13}C). Analysis of DPPH level using a spectrophotometer UV-Vis Shimadzu.

Reaction procedure

The synthesis of Shiff base derived from o-vanillin with a variety of primary amine compounds (aniline, p-toluidine, and p-anisidine) used a ratio 1:1. The mixture of the two reactants was grinded for 20 minutes at room temperature. The synthesized product was kept in a desiccator to constant weight. Synthesis results were characterized using melting point test, FTIR, GCMS, ^{1}H-NMR, and ^{13}C-NMR.

Antioxidative test

Antioxidant activity test [10,16] using DPPH (0.2 mM) with variations in Schiff base concentrations of 5, 10, 15, 20, and 30 ppm. The absorbance data obtained was processed using the GraphPad Prism 7 application and analyzed to obtain the EC_{50} value.

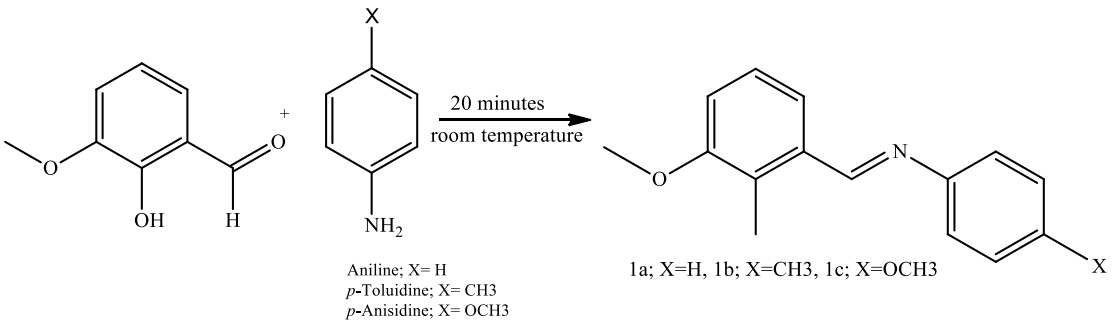
Toxicity evaluation

Toxicity test using the BSLT (Brine Shrimp Lethality Test) method [19,20]. The test animal used was Artemia salina Leach shrimp larvae. Variations in the concentration of the samples used were 10, 15, 20, 25, 30 and 35 ppm. Each sample concentration was repeated 5 times. The % mortality data obtained was processed to obtain an LC_{50} value (using probit analysis on the MINITAB 16 program with a 95% confidence level).

RESULT AND DISCUSSION

Synthesis and characterization of Schiff base derivates *o*-Vanillin

The reaction in synthesis of Schiff base compounds is addition elimination. In this reaction, the carbonyl compound acts as an electrophile, while the primary amine compound acts as a nucleophile. The reaction for the formation of Schiff base compounds is presented in Scheme 1. While the success of the formation of Schiff base compounds can be seen based on the observation Table 1 and Figure 1.



Scheme 1. Reaction of carbonyl compounds (o-vanillin) with primary amines (aniline, p-toluidine, p-anisidine)

Table 1. Physical data of synthesized compounds and reactants
.

Compound	Molecular Formula	Physical State	Melting Point (°C)	Yield (%)	Solubility
o-Vanillin	$C_8H_8O_3$	Yellow pale	40-42	-	Low solubility in aquades, Dissolves completely in 2 M NaOH
X=H	C_6H_7N	Brown Liquid	-6	-	-
1a	$C_{14}H_{13}NO_2$	Orange	75-77	94.80	Soluble in 2 M NaOH
X=CH_3	C_7H_9N	White Solid	44	-	-
1b	$C_{15}H_{15}NO_2$	Orange pale	95-97	98.33	-
X=OCH_3	C_7H_9NO	Black Solid	55-59	-	-
1c	$C_{15}H_{15}NO_3$	Brown Pale	83.67-85.67	99.04	Insoluble in aquades, completely soluble in 0.2 M NaOH

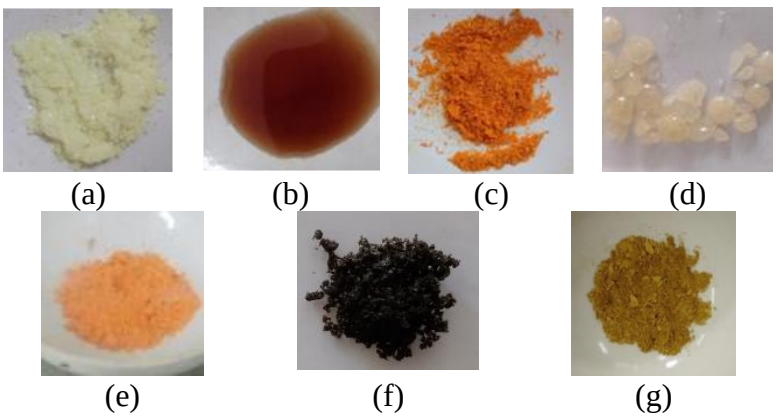


Figure 1. Organoleptic compounds (a) o-vanillin, (b) X=H, (c) 1a, (d) X=CH_3, (e) 1b, (f) X=OCH_3, (g) 1c

Based on the observations, the difference in color and melting point between the synthesized product and the reactants could be an indication of the formation a Schiff base compounds.

FTIR identification is used to determine functional groups. The results of the absorption band analysis on the spectra are presented in Table 2. The interpretation of the absorption band refers to the reference wavelength [21,22]. Comparison of the IR spectra of the reactants with the synthesized products aims to determine whether there are new absorptions in the product spectra which indicate the formation of imine groups. (C=N). In addition, it is also to determine the typical absorption of reactants that do not appear in the Schiff base compound spectra.

Table 2. Comparison of functional group wavelength for reactants and Schiff base products

Functional Group	**Reference**	**Wavelength (cm^{-1})**						
		Carbonyl	**Primary Amine**			**Schiff Base**		
		o-Vanilin	X=H	X=CH_3	X=OCH_3	1a	1b	1c
N-H *stretch* (primary amine)	3550-3330[b]	-	3432 & 3358	3417 & 3335	3422 & 3348	-	-	-
C=O *stretch*	1665-1625[b]	1642	-	-	-	-	-	-
-C=N-	1645-1605[b]	-	-	-	-	1614	1617	1616
-OH *stretch*	3550-3200[a]	3480	-	-	-	3464	3483	3469
C_{sp3}-H *strectch*	2975-2840[b]	2939	-	2920	2839	2917	2923	2940
C_{sp2}-H *strectch*	3100-3000[a]	3015	3035	3012	3073	3077	3040	3074
C-O Alkoxy	1275-1200[a]	1258	-	-	1236	1254	1259	1248
C=C aromatic	1600-1450[b]	1588	1498	1514	1507	1586	1508	1509
Overtune aromatic	2000-1600[b]	1908-1680	1929-1781	1977 - 1622	2000-1600	1984-1660	1950-1680	2000-1600
aromatic C_{sp2}-H *bend*	900-700[b]	762-717	754-690	812	764-719	782-691	778-733	778-734

[a]Ref [21], [b]Ref [22]

Characterization using GCMS aims to determine the molecular weight (m/z value) of the synthesized compound and its purity. The characterization results using GCMS for each compound (1a, 1b, and 1c) appear as one peak with an area of 100%. While the results of GCMS analysis in Table 3, show the m/z value of molecular ions according to the molecular weight (MW) of each Schiff base compound. Molecular ions with m/z values of 227, 241, and 257 indicated the MW of the basic Schiff of 2-methoxy-6(phenyliminomethyl)phenol; 2-methoxy-6-(((4-methylphenyl) imino) methyl) phenol; and 2-methoxy-6-(((4-methoxy phenyl) imino) methyl) phenol, respectively.

Table 3. Data analysis of GCMS characterization

Schiff Base Compound	Relative Mass (Mr)	Molecular Ions *m/z*	Base Peak (*m/z*)	Retention Time (minutes)
1a	227	227	77	38.67
1b	241	241	107	44.18
1c	257	257	257	26.65

The purity of the three Schiff bases is 100% without any residual reactants or by-products. The difference in retention time indicates the difference in the degree of polarity of each of the Schiff base compounds (1a, 1b, dan 1c).

Characterization using ^{1}H-NMR aims to determine the number of hydrogen environments and the number of hydrogen atoms in all synthesis products. The ^{1}H-NMR spectra of all the synthetic products are shown in Figure 2-4, and the interpretation results are shown in Table 4.

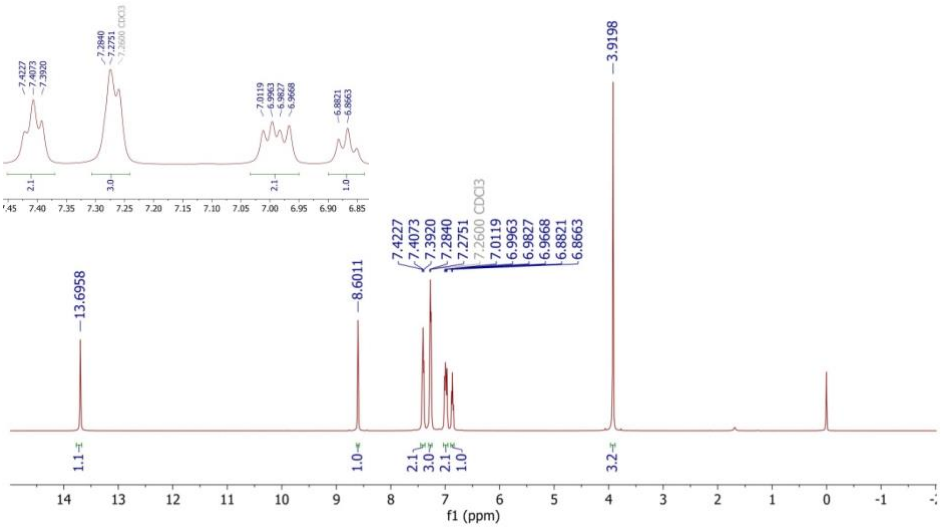


Figure 2. ^{1}H-NMR spectra of compound 1a

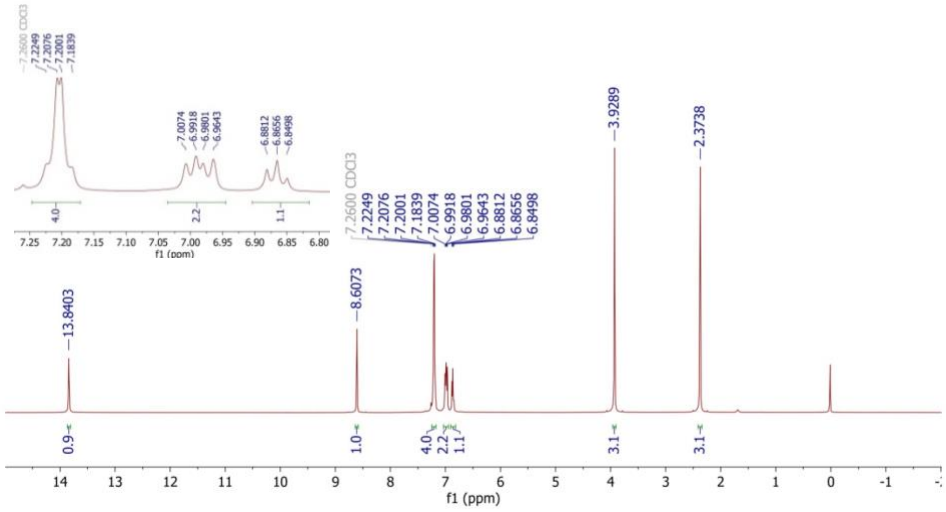


Figure 3. ^{1}H-NMR spectra of compound 1b

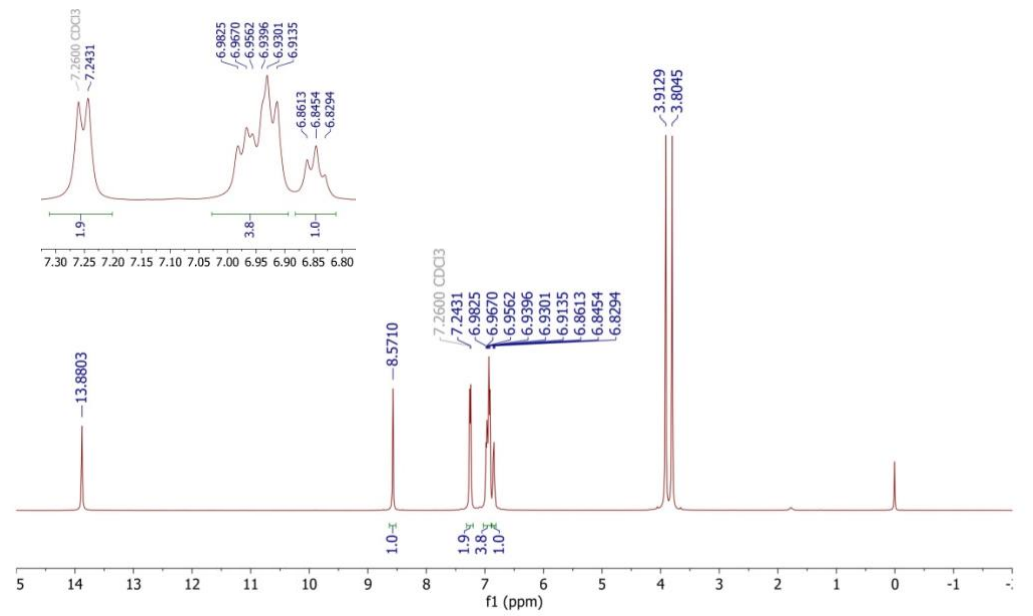


Figure 4. [1]H-NMR spectra of compound 1c

Table 4. [1]H-NMR data of Schiff base compounds 1a, 1b, and 1c

Schiff Base Compound	Molecular Formula	Chemical shift (ppm)					
		OH (s)	CH=N (s)	Ar-H (m)	OCH_3 (s)	CH_3 (s)	X' (s)
1a	$C_{14}H_{13}NO_2$	13.69 (1H)	8.60 (1H)	6.87-7.42 (8H)	3.92 (3H)	-	-
1b	$C_{15}H_{15}NO_2$	13.84 (1H)	8.61 (1H)	6.85-7.23 (7H)	3.93 (3H)	2.37 (3H)	-
1c	$C_{15}H_{15}NO_3$	13.88 (1H)	8.16 (1H)	6.83-7.24 (7H)	3.92 (3)	-	3.81 (3H)

[a] X' = OCH_3 for compound 1c
[b] Multiplicity is given as s= singlet, m=multiplet

The spectrometer [13]C-NMR is used to determine the number of carbon environments and the number of carbon atoms of all synthesis products. The result spectra of all synthesis products and their interpretation results are shown in Figures 5-7.

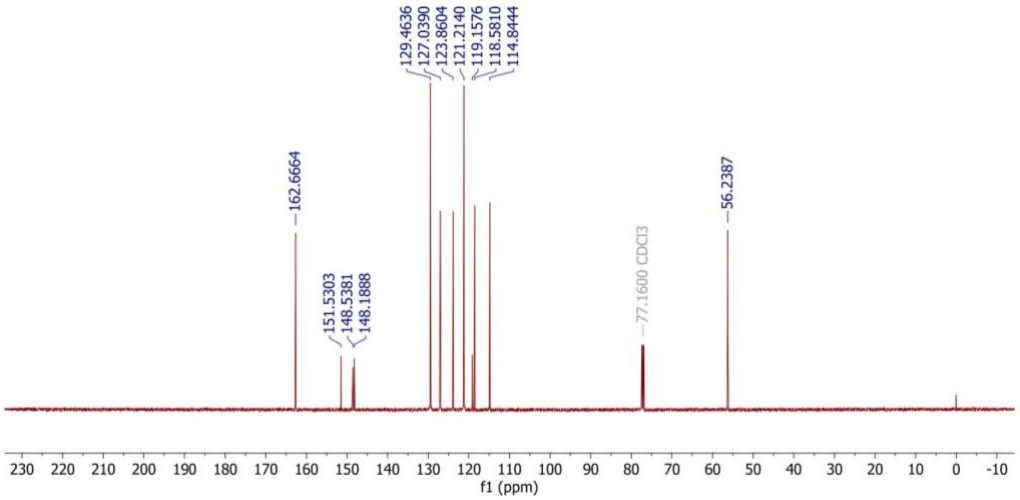


Figure 5. ^{13}C-NMR spectra of compound 1a

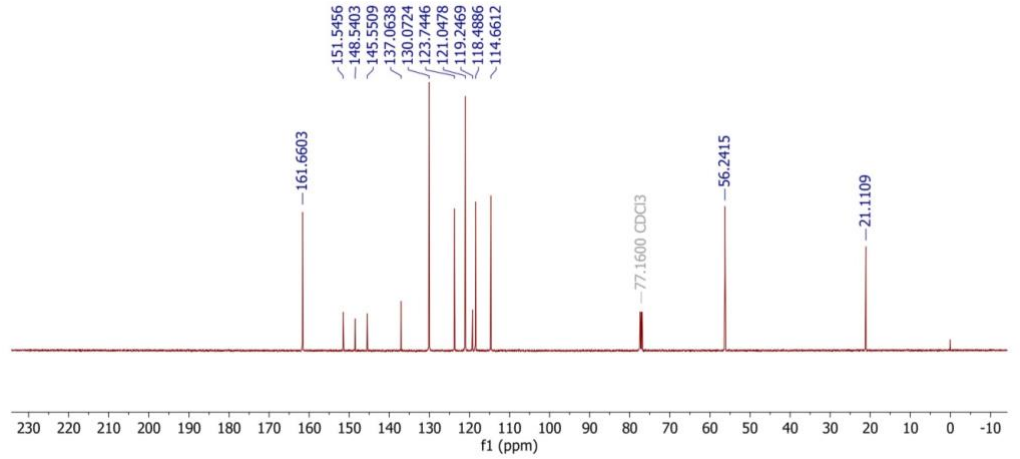


Figure 6. ^{13}C-NMR spectra of compound 1b

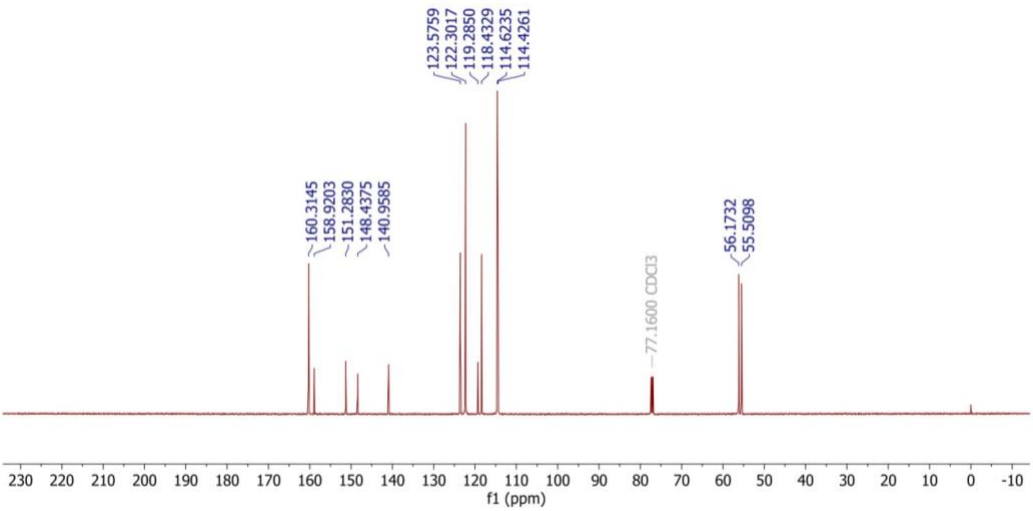


Figure 7. ^{13}C-NMR spectra of compound 1c

Table 5. ^{13}C-NMR data of Schiff base compounds 1a, 1b, and 1c

Schiff Base Compound	Molecular Formula	Chemical shift (ppm)					
		OCH_3	C-Ar	C-Ar	C imine	CH_3	X[a]
1a	$C_{14}H_{13}NO_2$	56.23	114.84	148.53	162.66	-	-
			121.21	148.18			
			123.86	118.58			
			121.21	151.53			
			129.46				
			127.03				
			129.46				
			121.21				
1b	$C_{15}H_{15}NO_2$	56.24	114.66	151.54	161.66	21.11	-
			119.24	145.55			
			123.74	118.48			
			121.04	148.54			
			130.07	137.06			
			130.07				
			121.04				
1c	$C_{15}H_{15}NO_3$	56.17	118.43	151.28	160.31	-	55.50
			123.57	148.43			
			123.57	119.28			
			122.30	140.95			
			114.62	158.92			
			114.62				
			122.30				

[a] X' = CH_3, methoxy for compound 1c

Antioxidant Activity

Determination of the antioxidant activity of the basic Schiff compound derived from o-vanillin using the DPPH method with λ max= 517.0 nm [7,16]. The presence of activity in the compound can be indicated by the change in color of the DPPH solution from purple to pale purple to yellow. The data obtained from this test is the absorbance of DPPH in samples and blanks, then processed to obtain % antioxidant activity. Data on antioxidant test results are presented in Table 6.

Table 6. Antioxidant activity of Schiff base derivatives o-vanillin and comparisons

Sample	Antioxidant Activity (%)					
	5 ppm	10 ppm	15 ppm	20 ppm	25 ppm	30 ppm
BHT	37.04	48.52	57.10	59.75	62.25	65.49
o-Vanillin	1.97	4.55	7.82	9.19	10.97	12.56
1a	4.83	6.42	10.00	11.61	14.30	16.24
1b	2.98	4.29	6.51	7.37	10.54	13.70
1c	22.07	26.77	29.90	33.28	34.72	37.69

Based on Table 6, it shows that the percentage of antioxidant activity is directly proportional to the increase of the sample concentration. Percentage of activity data is processed using the GraphPad Prism 7 application to produce EC_{50} values (Table 7).

Table 7. EC_{50} value of Schiff base compounds 1a, 1b, 1c, and comparisons

Sample	EC_{50} (ppm)
o-Vanillin	217.0
1a	196.4
1b	167.4
1c	106.2
BHT	10.88
Vitamin C*	3.585

The EC_{50} values for compounds 1a, 1b and 1c and o-Vanillin are high compare to the BHT and Vitamin C. The higher the EC_{50} value indicates the weaker the activity of the compound in reducing DPPH radicals. Although it has a weak antioxidant activity, the EC_{50} value of Schiff's base compound which ranges from 106.2 to 196.4 ppm. This value indicates the potential as an antioxidant to scavenge the DPPH radicals. This is evidenced by the percentage of antioxidant activity which increases with increasing concentration.

Toxicity Test

Toxicity test is a screening to determine the biological activity [20] of Schiff base compounds. Based on the test results in Table 8, compounds 1a, 1b, and 1c have LC_{50} values in the range of 9.99-22.29 ppm. The blank and reactant mortality is zero. Thus the LC_{50}-24 value does not determine.

Table 8. Mortality data of Schiff base derivatives o-vanillin and comparisons

Compounds	Mortality						LC_{50} (ppm)
	10 ppm	15 ppm	20 ppm	25 ppm	30 ppm	35 ppm	
Blanko	0	0	0	0	0	0	-
o-Vanillin	0	0	0	0	0	0	-
X=H	0	0	0	0	0	0	-
1a	25	40	45	50	50	50	9.9914
X=CH3	0	0	0	0	0	0	-
1b	15	20	25	30	40	45	17.3822
X=OCH3	0	0	0	0	0	0	-
1c	5	10	15	25	45	50	22.2899

Based on the test results, the Schiff base compounds 1a, 1b, and 1c have toxic properties from reactants that do not have toxic properties at various concentrations used. The compound is toxic with an LC_{50} value of < 1000 ppm. The level of toxicity is classified based on the LC_{50} value, LC_{50} < 30 ppm is very toxic, LC_{50} 30-1000 ppm is toxic, and LC_{50} > 1000 ppm is not toxic [19]. Schiff base compounds are toxic because of the extension of the conjugation system, the imine group, and the two synergistic aromatic rings.

CONCLUSION

The synthesis of the three Schiff base compounds (1a, 1b, and 1c) produced products with different characteristics. The products resulted with 100% purity, and the antioxidative activity show a weak activity for compounds 1a, 1b, and 1c. While the toxicity test indicate potential candidate as anticancer compound.

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