



# A Cycloalkanol from Derivatization Studies on Vanillin: Evaluation of Antioxidant Activity of Obtained Derivatives'

Olawale Hakeem Oladimeji<sup>1,\*</sup>, Ekemini Samuel Idiong<sup>1</sup>, Udem Akpan Joseph<sup>1</sup>, Victor Udo Anah<sup>1</sup>, Moji Taibat Bakare-Odunola<sup>2</sup>, Stanislaus Ngaitad Njinga<sup>2</sup>, Saad Toyin Abdullahi<sup>2</sup>

<sup>1</sup>Department of Pharmaceutical & Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria

<sup>2</sup>Department of Pharmaceutical & Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Nigeria

## Email address:

wale430@yahoo.co.uk (O. H. Oladimeji), olawaleoladmeji70@gmail.com (O. H. Oladimeji),

hakeemoladmeji@uniuyo.edu.ng (O. H. Oladimeji)

\*Corresponding author

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**Abstract:** Background of study: Vanillin is a white monoclinic crystalline compound whose chemical nomenclature is p-hydroxy-m-methoxy benzaldehyde. It is a phenolic aldehyde with a pleasant flavor and popularly found in vanilla beans and roasted coffee amongst many other sources. It possesses antitumor and particularly antioxidant activity which formed the essence of this study. Objectives: The insidious presence of free radicals in the human body has become a worrisome concern. These chemical species continue to plague the human cells, tissues and organs resulting in different patho-physiological conditions such as cancers and neurodegenerative disorders like Alzheimer's disease and Parkinson's disease amongst many other ailments. The search for novel pharmacological compounds with the aim of checking these radicals led the choice of vanillin in this present study. Methodology: Vanillin was separately subjected to some derivatization reactions namely; acetylation, O-demethylation, reduction and oxidation. The melting points, refractive indices and optical rotations of the lead compound and derivatives were obtained. The antioxidant activities of the five compounds were determined using the DPPH (2, 2-diphenyl-1-picrylhydrazyl hydrate) test. Comparison of the obtained antioxidant activities was done to determine if any improvements could be seen in the derivatives. Results: The identities of the derivatives have been revealed to be vanillyl acetate (E-1), 3, 4-dihydroxy benzaldehyde or protocatechui aldehyde (E-2), o-methoxy-p-methyl cyclohexan-1-ol (J-1) and vanillic acid (J-2) respectively using the IR spectral technique. Vanillin, E-2 and J-2 gave marginal antioxidant activity of IC<sub>50</sub> of 0.81, 0.84 and 0.0.85 µg/mL respectively while J-1 and E-1 demonstrated moderately significant IC<sub>50</sub> of 0.59 and 0.63 µg/mL which compare favorably with 0.44 µg/mL elicited by Vitamin C (a standard antioxidant drug). It is pertinent to point out that the obtained reduced derivative is a substituted cycloalkanol (a saturated cyclic compound) instead of a substituted phenolic compound as was expected. Conclusion: The results from this study indicate that reduction and acetylation separately enhances the antioxidant activity of vanillin.

**Keywords:** Vanillyl Acetate, Protocatechui Aldehyde, o-methoxy-p-methyl Cyclohexan-1-ol, Antioxidant Activity

## 1. Introduction

Oxidative stress is linked to the imbalance between reactive oxygen species and reactive nitrogen species (ROS and RNS) production and antioxidant defense, leading to oxidative damage to lipids, proteins and genetic material that contributes to the development of a range of different patho-

physiological conditions [1]. Thus, when the body is unable to process and remove free radicals efficiently, oxidative stress (OS) occurs. This condition has been implicated in a number of disease conditions such as arthritis, atherosclerosis, vision loss, emphysema, respiratory ailments, auto-immune disorders, mental stress, Alzheimer's disease and Parkinson's disease amongst so many others. Some free radicals are

needed for the body to function effectively but their overload may be fatal. The body needs to maintain an equilibrium between free radicals and antioxidants so as to prevent oxidative stress. Hence, the need for efficient antioxidants can not be over emphasized. In the search for novel pharmacologically active compounds, vanillin was considered. It is a pleasant smelling aromatic compound occurring naturally in vanilla beans, roasted coffee, Chinese red pine and leptotes amongst many other sources. It is used as a flavoring agent in candies, ice cream, yoghurt, beverages, soda drinks, confectioneries and alcoholic liquors. In addition, it has tremendous applications in the fragrance and perfumery industries where it is used as an additive in making candles, incense, air-fresheners, creams, soaps, shampoos and ointments. It possesses antioxidant and antitumor properties and is used as a chemical intermediate in the production of pharmaceuticals, cosmetics and herbicides. In this present study, vanillin was subjected to acetylation, O-demethylation, reduction and oxidation reactions. The antioxidant activities ( $IC_{50}$ ) of the vanillin and the respective derivatives were obtained using the rapid bench-top DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) test [2-4]. Comparison of the antioxidant activities of the vanillin and derivatives was done with the aim of determining if any improvements in the obtained activities of the derivatives.

## 2. Materials and Methods

### 2.1. Reagents/Chemicals

DPPH (2,2-diphenyl-1-picryl hydrazyl hydrate), vanillin and vitamin C tablets were purchased from Tianjin Kernel Chemical Reagent Company, China, Sigma Aldrich Chemicals, Germany and Emzor Pharmaceuticals, Lagos, Nigeria respectively. Solvents and reagents namely, acetic acid, acetic anhydride, chloroform, di-ethyl ether, dichloromethane, ethanol, ethyl acetate, hydrochloric acid, iodine, magnesium sulphate, methanol, n-butanol, n-hexane, petroleum-ether, potassium permanganate, pyridine HCl, sodium borohydride, disodium hydrogen phosphate, sodium hydroxide, sulphuric acid and tetrahydrofuran were obtained as AnaLAR Grade Chemicals from British Drug House (BDH) Chemicals Limited, Poole, England.

### 2.2. Solubility Tests for Vanillin

Vanillin (0.01 g) was added to 2 mL of each of the following solvents namely, distilled water, methanol, ethyl acetate, ethanol, petroleum ether, n-hexane, n-butanol, dilute HCl, dilute  $H_2SO_4$  separately and observation was made for complete dissolution (solubility) or otherwise.

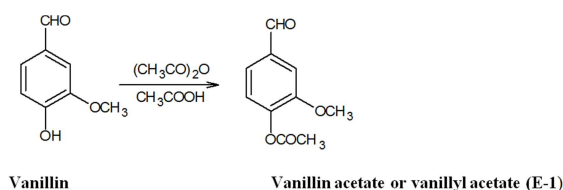
### 2.3. Determination of Melting Point

Vanillin (0.05 g) was filled to a quarter of the length of a micro-capillary tube and the melting point determined [5] using an Electro-thermal Melting Point apparatus (Electro-thermal Engineering Limited, England).

## 2.4. Derivatization Reaction

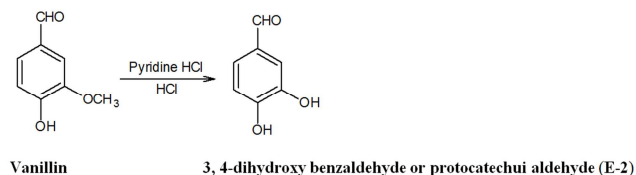
### 2.4.1. Acetylation

Vanillin (0.4 g) was dissolved in a mixture of acetic anhydride (10 mL) and acetic acid (10 mL) in a beaker. The solution was heated for 20 minutes and allowed to cool. Concentrated sulphuric acid (5 mL) was added as catalyst. Further heating was done for a few minutes. The mixture was covered with aluminum foil and kept in the refrigerator. After two weeks, an amorphous compound was formed in the beaker. 5 mL of warmed di-ethyl was added and the mixture warmed again for some minutes. The compound dissolved on warming but formed back after four hours, filtered, dried and then stored in the refrigerator for further studies [6, 7].



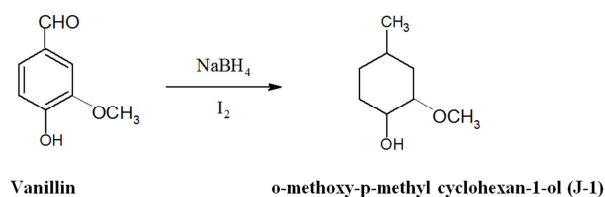
### 2.4.2. O-demethylation

Vanillin (0.4 g) was added to a solution of pyridine HCl (50 mL) and dilute hydrochloric acid (10 mL) and the mixture stirred until complete dissolution. The mixture was heated at 27°C for thirty minutes and allowed to cool. The resulting product was filtered, dried at water pump and then stored in a refrigerator [8].



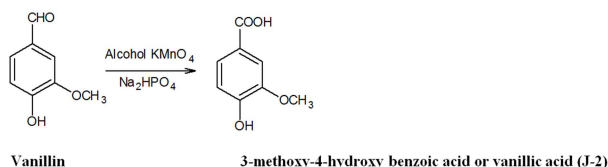
### 2.4.3. Reduction

A solution of vanillin (1.5 g) in tetrahydrofuran (20 mL) was slowly added to a suspension of sodium borohydride (0.45 g) in tetrahydrofuran (200 mL) at room temperature and left for ten minutes. The mixture was then stirred until evolution of gas ceased. Iodine (0.63 g) and tetrahydrofuran (20 mL) were added slowly to the mixture at a temperature of 0°C (in ice) with further evolution of hydrogen gas. The contents were further stirred for 1 hour. Dilute hydrochloric acid (5 mL) was added carefully and the mixture extracted with diethyl ether. The combined ethereal extract was washed with 3 M sodium hydroxide (30 mL), brine and dried over magnesium sulphate. Evaporation of the organic layer gave the reduced product [9].



#### 2.4.4. Oxidation

Vanillin (0.4 g) was weighed and added to a butanolic solution of potassium permanganate (50 mL) and the mixture stirred for ten minutes. Disodium hydrogen phosphate (5 mL) was added as a buffer to the mixture which was stirred for thirty (30) minutes and then stored in the refrigerator for a week for complete reaction [8].



#### 2.5. Determination of Specific Optical Rotation and Refractive Index

Each sample (0.05 g) was dissolved in methanol (10 mL). The tube of the Polarimeter (ADP-220, Bellingham Stanley, England) was filled with distilled water and the machine subsequently zeroed. The tube was refilled with 5 mL of sample and the optical rotation was measured at the wavelength ( $\lambda$ ) of sodium D line (589.3 nm) at 20.5°C. Similarly, the refractive index of sample was obtained on a refractometer (WAY-15, Abbe, England) at the wavelength ( $\lambda$ ) of sodium D line (589.3 nm) at 20.5°C [10, 11]. In a situation where the derivative was a liquid, 5 mL of sample was used for the determinations.

#### 2.6. Antioxidant Activity

Substances which are capable of donating electrons or hydrogen atoms can convert the purple-colored DPPH radical (2, 2-diphenyl-1-picrylhydrazyl hydrate) to its yellow-colored non-radical form; 1, 1-diphenyl-2-picryl hydrazine [12, 13]. This reaction can be monitored by spectrophotometry.

##### 2.6.1. Preparation of Calibration Curve for DPPH Reagent

DPPH (4 mg) was weighed and dissolved in methanol (100 mL) to produce the stock solution (0.004% w/v). Serial dilutions of the stock solution were then carried out to obtain the following concentrations; 0.0004, 0.0008, 0.0012, 0.0016, 0.0020, 0.0024, 0.0028, 0.0032 and 0.0036% w/v. The absorbance of each of the sample was taken at  $\lambda_m$  512 nm using the Ultra-Violet Spectrophotometer (Jenway 6405, USA). This machine was zeroed after an absorbance had been taken with a solution of methanol without DPPH which served as the blank.

##### 2.6.2. Determination of the Antioxidant Activity of Vanillin, Derivatives and Vitamin C

2 mg each of sample was dissolved in 50 mL of methanol. Serial dilutions were carried out to obtain the following concentrations; 0.0004 mg mL<sup>-1</sup>, 0.0008 mg mL<sup>-1</sup>, 0.0012 mg mL<sup>-1</sup>, 0.0016 mg mL<sup>-1</sup> and 0.0020 mg mL<sup>-1</sup> using methanol. 5 mL of each concentration was incubated with 5 mL of 0.004%

w/v methanolic DPPH solution for optimal analytical accuracy. After an incubation period of 30 minutes in the dark at room temperature (25 ± 2°C), observation was made for a change in the color of the mixture from purple to yellow. The absorbance of each of the samples was then taken at  $\lambda_m$  512 nm. The Radical Scavenging Activity (RSA%) or Percentage Inhibition (PI%) of free radical DPPH was thus calculated:  $RSA\% (PI\%) = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$ .  $A_{blank}$  is the absorbance of the control reaction (DPPH solution without the test sample) and  $A_{sample}$  is the absorbance of DPPH incubated with the sample. Vanillin / derivative / Vitamin C concentration providing 50% inhibition ( $IC_{50}$ ) was calculated from a graph of inhibition percentage against the concentration of the vanillin / derivative / vitamin C [14-16]. Vitamin C was used as a standard antioxidant drug.

#### 2.7. Thin-layer Chromatography of Samples

A portion of each solid sample (0.05 g) dissolved in methanol (2 mL) or 2 mL of liquid sample was applied on a 20 cm x 10 cm silica gel analytical plate (Merck, Germany) and then developed in a toluene: acetone: water (10:20:1) mixture in a chromatographic tank until optimal separation was observed. The retardation factor ( $R_F$ ) was then computed thus:

$$R_F = \text{distance moved by spot} / \text{distance moved by solvent front}$$

#### 2.8. Infra-red Spectroscopy of Samples

Each sample (0.2 g) or 2 mL (liquid) was analyzed for IR characteristics using the FTIR 84005 Spectrophotometer (Shimadzu, Japan).

#### 2.9. Ultra-Violet/Visible Spectroscopy of Samples

A portion of each sample (0.2 g) or 2 mL (liquid) was analyzed for UV/VS absorption characteristics using the Jenway 6405 UV/VS Spectrophotometer.

### 3. Results

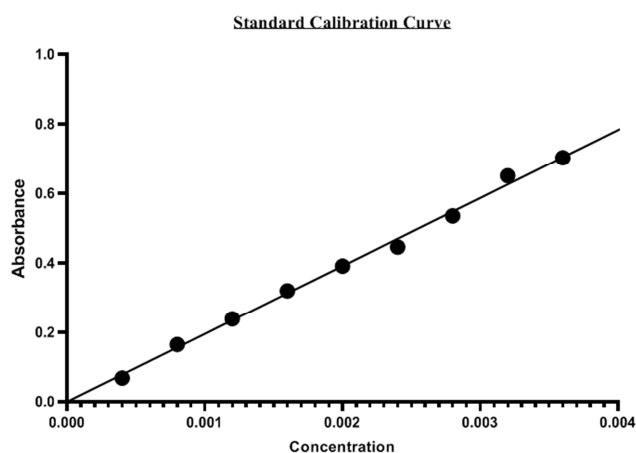


Figure 1. Graph of absorbance against concentration of methanolic solution of DPPH reagent.

**Table 1.** Preparation of calibration curve for DPPH reagent at  $\lambda_{\max}$  512 nm.

Concentration	Average absorbance ( $\pm 0.006$ )
0.0004	0.068
0.0008	0.165
0.0012	0.239
0.0016	0.320
0.0020	0.391
0.0024	0.446
0.0028	0.535
0.0032	0.651
0.0036	0.703

**Table 2.** Absorbance of samples incubated with DPPH at different concentrations at  $\lambda_{\max}$  512 nm (Blank absorbance of 0.004% methanolic DPPH reagent: 0.748).

Sample	0.0008 mg/mL <sup>-1</sup>	0.0016 mg/mL <sup>-1</sup>	0.0024 mg/mL <sup>-1</sup>
Vitamin C	0.061	0.053	0.052
Vanillin	0.325	0.320	0.275
E-1	0.307	0.223	0.164
E-2	0.395	0.351	0.304
J-1	0.259	0.189	0.146
J-2	0.391	0.349	0.301

Key: E-1=Vanillin acetate or vanillyl acetate or 4-O-acetyl vanillin)

E-2 =3, 4-dihydroxy benzaldehyde or Protocatechui aldehyde

J-1 =o-methoxy-p-methyl cyclohexan-1-ol

J-2 =3-methoxy-4-hydroxy benzoic acid (vanillic acid)

DPPH = 2, 2-Diphenyl-1-picrylhydrazyl hydrate.

**Table 3.** Radical scavenging activity (percentage inhibition%) of samples at different concentrations and IC<sub>50</sub> of samples.

Sample	0.0008 mg/mL <sup>-1</sup>	0.0016 mg/mL <sup>-1</sup>	0.0024 mg/mL <sup>-1</sup>	IC <sub>50</sub> (μg/mL <sup>-1</sup> )
Vitamin C	91.84	92.91	93.05	0.44
Vanillin	52.69	53.27	59.83	0.81
E-1	58.96	70.19	78.07	0.68
E-2	47.19	53.41	59.41	0.84
J-1	65.37	74.73	80.48	0.59
J-2	52.76	55.43	57.87	0.85

Key: Refer to Table 2 and RSA% (PI%) = Radical Scavenging Activity (Percentage Inhibition%).

IC<sub>50</sub> =Concentration at which 50% of DPPH is scavenged or inhibited

Vanillin: C<sub>8</sub>H<sub>8</sub>O<sub>3</sub>; mol. wt. (152.15 g/mol); white crystalline solid; m.pt. (80-82°C);  $[n]_D^{20}$  (1.5770);  $[\alpha]_D^{20}$  (0°);  $\lambda_{\max}$  (246 nm); R<sub>F</sub> (0.66); FTIR (cm<sup>-1</sup>): 1146 (C-O-C), 1575 (Ar-C=C), 1634 (C=O), 2847 (-CH stretching) and 3179 (Ar-OH).

Vanillin acetate (vanillyl acetate) (E-1): C<sub>10</sub>H<sub>10</sub>O<sub>4</sub>; mol. wt. (194.19 g/mol); amorphous dark yellow solid; m.pt. (79-81°C);  $[n]_D^{20}$  (1.5791);  $[\alpha]_D^{20}$  (0°),  $\lambda_{\max}$  (400 nm); R<sub>F</sub> (0.70); FTIR (cm<sup>-1</sup>): 1157 (C-O-C), 1597 (Ar-C=C), 1684 (C=O in -COCH<sub>3</sub>), 1745 (C=O), 2942 (-CH) and 2965 (-CH).

3,4-dihydroxy benzaldehyde (protocatechui aldehyde)

(E-2): C<sub>7</sub>H<sub>6</sub>O<sub>3</sub>; mol. wt. (138.12 g/mol); pale yellow resinoid compound; m.pt. (152-154°C),  $[n]_D^{20}$  (1.4874);  $[\alpha]_D^{20}$  (0°);  $\lambda_{\max}$  (246 nm); R<sub>F</sub> (0.23); FTIR (cm<sup>-1</sup>): 1600 (Ar-C=C), 1756 (C=O), 3245 (-OH) and 3487 (-OH).

o-methoxy-p-methyl cyclohexan-1-ol (J-1): C<sub>8</sub>H<sub>16</sub>O<sub>2</sub>; mol. wt. (144.14 g/mol); colorless liquid;  $[n]_D^{20}$  (1.5773);  $[\alpha]_D^{20}$  (0°);  $\lambda_{\max}$  (370 nm); R<sub>F</sub> (0.89); FTIR (cm<sup>-1</sup>): 1161 (C-O-C) and 3460 (-OH).

3-methoxy-4-hydroxy benzoic acid (vanillic acid) (J-2): C<sub>8</sub>H<sub>8</sub>O<sub>4</sub>; mol. wt. (168.14 g/mol); light yellow solid; m.pt.

(209-211°C),  $[n]_D^{20}$  (1.5773);  $[\alpha]_D^{20}$  (0°);  $\lambda_{\max}$  (248 nm); R<sub>F</sub> (0.53); FTIR (cm<sup>-1</sup>): 1610 (Ar-C=C), 1716 (C=O), 2991 (-CH) and 3490 (-OH).

## 4. Discussion

Vanillin is a white monoclinic crystalline compound with sweet smelling, pleasant and balsamic fragrance. It was put through some monographic determinations in this study where its identity, purity, integrity and suitability were established. The compound was observed to be soluble in ethanol, ethyl acetate, n-butanol, n-hexane, methanol and water when heated. However, it was insoluble in petroleum ether, dilute hydrochloric acid and dilute sulphuric acid. Its determined melting point and refractive index fall within standard literature values. The UV absorption at  $\lambda_{\max}$  (246 nm) is indicative of the presence of electron densities in Ar-C=C, -OH, -OCH<sub>3</sub> and HC=O chromophores while the retardation factor R<sub>F</sub> (0.66) shows that vanillin is moderately polar and hence likewise retarded on the silica gel used in the thin-layer chromatographic (TLC) analyses. The IR spectral matrix of

vanillin shows stretchings at 1146, 1575, 1634, 2847 and 3179  $\text{cm}^{-1}$  which are diagnostically characteristic of -C-O-C, Ar-C=C, -C=O, -CH and Ar-OH groups respectively. Vanillin acetate or vanillyl acetate or 4-O acetyl vanillin (E-1) which belongs to a class of aromatic phenol esters was synthesized as dark yellow compound with a flavor and fragrance similar to the odour of vanilla. The UV absorption at  $\lambda_{\text{max}}$  (400 nm) which is comparatively higher than that of the parent analogue (vanillin) indicates the presence of electron clouds over Ar-C=C, -OCH<sub>3</sub>, -OCOCH<sub>3</sub> and HC=O chemical species while retardation factor  $R_F$  (0.70) shows that acetyl product is comparably non-polar and hence weakly retarded on the silica gel plate. The IR spectrum of E-1 shows stretchings at 1157, 1597, 1684, 1745, 2942 and 2965  $\text{cm}^{-1}$  which are characteristic of -C-O-C, Ar-C=C, -CO in OCOCH<sub>3</sub> substitution of the derivative, -C=O and -CH respectively. It is instructive to note that the peaks in the acetyl product absorb at slightly higher than that those recorded in the IR spectrum of vanillin. Furthermore, the peak representing -C-O-C is particularly and diagnostically elucidative indicating that the hydrogen atom in the -OH had been replaced with-COCH<sub>3</sub> showing the acetylation of the vanillin had truly taken place. The O-demethylated product (3,4-dihydroxy benzaldehyde) (E-2) was synthesized as a pale yellow resin. In addition, the UV absorption at  $\lambda_{\text{max}}$  (246 nm) is the same as found with vanillin. However, the electrons are found delocalized over only Ar-C=C, -OH and HC=O chromophores without -OCH<sub>3</sub> while retardation factor  $R_F$  (0.23) indicates that E-2 is too polar because of the presence of 2-OH groups in the derivative. Hence, the product is strongly attracted to the OH groups of the silica gel and thereby making E-2 greatly retarded on the chromatographic plate. The IR spectrum of E-2 shows peaks at 1600, 1756, 3245 and 3487  $\text{cm}^{-1}$  which are indicative of Ar-C=C, -C=O and -OH chemical species. This derivative also known as protocatechui aldehyde is employed as a perfumery and flavoring agent. In addition, this aldehyde also serves as a precursor in the synthesis of vanillin by bio-transformation of cell cultures of *Capsicum frutescence* [17]. It is also used as an apoptosis inducer in human leukemia cells, possess anti-proliferative property against breast and colorectal cancers and cardio-protective activity [18]. The reduced product (o-methoxy-p-methyl cyclohexan-1-ol) (J-1) was synthesized as a colorless liquid. The UV absorption at  $\lambda_{\text{max}}$  (370 nm) is indicative of presence of electrons in -OH and -OCH<sub>3</sub> species while  $R_F$  (0.89) shows that the product is non-polar because of the presence of methyl cyclohexyl group thereby making J-1 largely un-retarded or un-hindered on the silica-coated plate. The IR spectrum of the derivative shows a matrix characterized by stretchings at 1161 and 3460  $\text{cm}^{-1}$  which are indicative of -C-O-C and -OH species. It needs to be stated that the reduction reagent ( $\text{NaBH}_4/\text{I}_2$ ) is supposed to selectively reduce the -C=O to -CH<sub>2</sub> [9]. Surprisingly, in addition to this supposition, the three (3) endocyclic Ar-C=C bonds were also reduced. Hence, the IR peaks at 1575 and 1634  $\text{cm}^{-1}$  in the vanillin (parent compound) representing the aromatic double bonds and

carbonyl species disappeared. This translates to losses of the aromatic character and carbonyl group in the derivative. This same scenario was observed in a previous study [3] where gallic acid was reduced to a new compound (3,4,5-trihydroxy cyclohexyl methanol) using  $\text{NaBH}_4/\text{I}_2$ . It is interesting to note that if the -C=O is attached to the aromatic ring, this reduction procedure will result in a product with both C=O and Ar-C=C double bonds being reduced simultaneously as has been observed in this present study and [3]. It will be of curious interest to know the reaction mechanism(s) by which this particular reduction is effectuated. Matter-of-factly, this assignment is currently being worked upon by our research team. However, if the C=O is far from the aromatic ring (when not found attached to the aromatic ring), the reduction involves only the C=O and not the Ar-C=C bonds as observed in [2, 9]. The oxidation of vanillin led to a compound which has been identified to be vanillic acid (J-2). It is a mono substituted hydroxy benzoic acid which is a plant metabolite and also a conjugate acid of a vanillate. The UV absorption at  $\lambda_{\text{max}}$  (248 nm) highlights the presence of electron clouds over Ar-C=C, -OCH<sub>3</sub>, -COOH and -OH chemical species while the retardation factor  $R_F$  (0.53) indicates that the oxidized product is moderately polar and hence likewise retarded on the silica gel plate. The IR spectrum of this derivative shows peaks at 1610, 1716, 2991 and 3490  $\text{cm}^{-1}$  reflecting Ar-C=C, -C=O, -CH and -OH respectively. J-2 has been reported to inhibit inflammatory pains, oxidative stress and cytokine production in mice [19] and also serves as an intermediate product in the bioconversion of ferulic acid to vanillin [20]. Generally the parent compound, vanillin and all other solid derivatives gave melting points and refractive indices which are consistent with literature values. Furthermore, it was observed that vanillin and all the derivatives showed optical rotation  $[\alpha]_D^{20}$  of 0° indicating that the compounds are optically inactive. Hence, none of these compounds will demonstrate laevorotation (-) (ability of a compound to rotate plane of light in anticlockwise direction) or dextro-rotation (+) (ability of a compound to rotate plane of light in a clockwise direction) [10, 11]. The preparation of a calibration curve was done for DPPH (2, 2-diphenyl-1-picryl hydrazyl hydrate) reagent with the aim of ascertaining its purity and suitability for use in the antioxidant determinations. The Beer-Lambert's Law is the basis of all absorption spectrophotometry [12, 21]. The calibration curve obtained indicates that the underlying principles behind the aforementioned Law were fulfilled as the curve (Figure 1) shows a straight line which passes through the origin. The reduction of the DPPH radical was determined by taking its absorption at a wavelength of  $\lambda_m$  512 nm. It was observed that the absorbance of DPPH decreased as the concentration of added free radical scavenger (vanillin /derivative/vitamin C) increased which suggested that the DPPH reagent was being reduced (Table 2). Furthermore, Table 3 shows radical scavenging activity (RSA%) or percentage inhibition (PI%) and the computed IC<sub>50</sub> values of vanillin /derivative/ vitamin C. The RSA% is an indicator of the antioxidant activity of

vanillin / derivative/ vitamin C. Interestingly, vanillin, O-demethylated product (E-2) and oxidized derivative (J-2) all demonstrated marginal antioxidant activity ( $IC_{50}$ ) of 0.81, 0.84 and  $0.85 \mu\text{g mL}^{-1}$  respectively. This is not surprising because the chemical structures of these three compound products contain(s) chemical species such as -OH,  $\text{HC}=\text{O}$  and / or -COOH which confer some hydrophilic character on the compounds. Hence, these compounds will experience some hindrance in reaching the active or allosteric sites where the pharmacological action of anti-oxidation is to be effectuated. However, the reduced product (J-1) and acetyl product (E-1) gave moderate antioxidant activity ( $IC_{50}$ ) of 0.59 and  $0.68 \mu\text{g mL}^{-1}$  respectively. The values particularly compare significantly with the antioxidant activity given by vitamin C (a standard antioxidant drug) at  $0.44 \mu\text{g mL}^{-1}$ . However, it should be highlighted that the reduced product (J-1) was slightly more active than E-1. From the foregoing, it can be inferred that both reduction and acetylation separately enhances the antioxidant activity of vanillin. Furthermore, J-1 contains the methyl cyclohexyl moiety which confers much more lyphophilic character on the molecule than -CHO, -OCH<sub>3</sub> and -OCOCH<sub>3</sub> in E-1. This physico-chemical feature in J-1 could have contributed to the observed higher antioxidant activity. Other equally efficient and reliable methods apart from DPPH test for determining the antioxidant activity of compounds include the hydrogen peroxide, nitric oxide, conjugated diene, superoxide, phosphomolybdenum, peroxyxynitrile and xanthine oxidase assay methods amongst many others [22-25].

## 5. Conclusion

This present study shows that the reduced derivative (o-methoxy-p-methyl cyclohexan-1-ol) and acetyl derivative (vanillin acetate) elicited comparably significant antioxidant activities as vitamin C (a standard antioxidant drug). However, the activity demonstrated by reduced product was slightly better than that of the acetyl product. Hence, reduction and acetylation both separately enhances antioxidant activity of vanillin.

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