ISSN: 1410-8917
Jurnal Kimia Sains & Aplikasi
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 27 (2) (2024): 72-82

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa

# Formulation and Synthesis of Vanillin from Clove Oil as a Chemosensor for Urea Detection in Urine

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https://doi.org/10.14710/jksa.27.2.72-82

Article Info

Article history:

Received: 17th November 2023
Revised: 05th March 2024
Accepted: 06th March 2024
Online: 20th March 2024

Keywords:
colorimetry; clove oil; urea; urine; vanillin

Abstract

Urea levels in urine play an important parameter in diagnosing bodily conditions through liver and kidney examinations. The typical reagent for detecting urea content in urine is para-dimethylaminobenzaldehyde (pDMAB). However, it has a drawback related to the instability of color in the resulting Schiff base compound (pDMAB-urea). In this study, the synthesized vanillin compound derived from clove oil serves as the foundational material for a urea chemosensor based on the colorimetric concept. The synthesized vanillin was characterized using FTIR, GC-MS, and $^1$H-NMR. The formulation of the vanillin compound as a sensor was conducted by assessing the suitable solvent, determining the optimal mass of vanillin, and evaluating the acid-base conditions of the sensor formulation system both qualitatively and quantitatively. Subsequently, the most effective formulation was selected for detecting urea in urine samples. The synthesis of vanillin yielded a purity level of 95%. The optimal formulation was obtained at an optimum mass of vanilla of 0.75 g in 50 mL of 96% ethanol and 10 mL of 10% NaOH. The color change in the sample was from colorless to greenish yellow (436 nm). The vanillin obtained was applied to urine samples with the best results at a sample dilution level of 10,000×.

## 1. Introduction

Urea is the primary product of the protein metabolism process, excreted by both the kidneys and liver. It is distributed throughout various bodily fluids, such as blood and urine. In human urine, urea concentration typically falls between 275 to 409 mmol/L [1, 2]. Therefore, the diagnosis of human physical conditions generally involves analyzing the urea levels in urine. The characteristics of urine can be determined through its color, transparency, and odor. Since urine exhibits different levels of color, detecting urea often employs colorimetric techniques for medical diagnosis [3, 4, 5]. Colorimetry is an analytical method based on color changes to ascertain the concentration of substances in a sample [6, 7].

Various methods have been employed to examine the urea levels in urine samples, including chromatography and spectroscopy. However, these methods are costly and involve complicated procedures [8]. Recent technological progress has simplified urea analysis in urine, with one approach utilizing natural material sensors [9]. Colorimetric chemosensors can be created by incorporating urea-specific chemical reagents. Using colorimetric sensors for urine analysis is highly effective in measuring urea concentration [10, 11].

Conventional analysis of urea content in urine generally employs para-dimethylaminobenzaldehyde (pDMAB) reagent. However, the color formed from the urea-pDMAB compound is less stable when exposed to direct sunlight [12]. Apart from pDMAB, diacetyl monoxime has also been investigated as a potential urea sensor reagent, albeit producing a less intense color [10, 13, 14]. Thus, there is a need for alternative reagents that can serve as the basis for urea sensors and generate more vivid colors. This study investigates the potential of vanillin compound, derived from clove oil synthesis, as a basic material for urea detection chemosensors. Vanillin

contains multiple chromophore groups capable of absorbing photons in the visible light spectrum [15].

Clove oil, extracted through the distillation of clove fruit—a prominent plantation commodity in Indonesia [16]—holds considerable commercial value due to its rich content of beneficial active compounds [17, 18, 19]. Among these, eugenol constitutes a significant portion, typically 80% to 90%, as a primary ingredient in vanillin synthesis [20, 21]. Despite the possibility of directly sourcing vanillin from the *Vanilla planifolia* plant, its limited availability in Indonesia leads to excessively high costs [22, 23]. In contrast, eugenol is the most cost- effective and environmentally friendly precursor for vanillin synthesis compared to alternatives such as ferulic acid [24] and guaiacol [25].

In the realm of urea detection, limited research has explored natural ingredients, with most studies focusing on biosensors [26, 27, 28]. Although biosensors are commonly employed for analyzing urea levels in food products, their application in urine urea detection remains underexplored. Biosensor production for urea analysis involves intricate procedures, prolonged material synthesis durations, and high-temperature requirements and is relatively less environmentally friendly. Conversely, employing vanillin as a base material offers the potential for detecting urea in urine through traditional synthesis methods known for their efficiency and cost-effectiveness.

## 2. Experimental

### 2.1. Equipment and Materials

The equipment used in this research included glassware, pH meter, analytical balance, electric heater (Thermo Scientific), magnetic stirrer, centrifuge, sonicator (Shimadzu Sonicator 5.7L), Fourier transform infrared spectrometer (Shimadzu FT-IR 8201 PC), UV-Vis spectrophotometer (Orion Aquamate 8100), gas chromatography-mass spectrometry (GCMS-TQ8040 NX) with specific analysis conditions (column oven temperature: 70°C; pressure: 30.0 kPa; column flow rate: 0.65 mL/minute), rotary evaporator (Shimadzu Rotation Evaporator QR 2005-S), and $^1$H-NMR spectroscopy with a frequency of 500 Mhz (JNM ECA 500).

The materials used in this research were clove oil obtained from Kranggan Market, double-distilled water, potassium hydroxide (KOH) (Sigma-Aldrich), 1 M sodium hydroxide (NaOH) solution (Sigma-Aldrich) and 10%, ethanol ($C_2H_5OH$) 96% (Merck), n-hexane ($C_6H_{14}$) 99% (Merck), hydrochloric acid solution (HCl) 25% and 1 M, diethyl ether ($(C_2H_5)_2O$) (Merck), sulfuric acid ($H_2SO_4$) 96-98% (Sigma-Aldrich), dichloromethane ($CH_2Cl_2$) (Merck), petroleum ether ($C_6H_{14}$) (Sigma-Aldrich), ethyl acetate solution ($C_4H_8O_2$) (Merck), cetrimonium bromide solid (CTAB) (Merck), potassium permanganate solid ($KMnO_4$) (Merck), urea solid ($CO(NH_2)_2$) (Merck), para-dimethylaminobenzaldehyde solid (pDMAB) (HIMEDIA) and anhydrous sodium sulfate solid ($Na_2SO_4.5H_2O$) (Merck).

### 2.2. Isolation of Eugenol from Clove Oil

Referring to research conducted by Widayat *et al.* [29], 8 g of NaOH was dissolved in 60 mL of distilled water. The solution was added with 20 g of clove oil. The mixture was then stirred until homogeneous and extracted. The upper layer was added with 1.6 g of NaOH dissolved in 16 mL of distilled water. The formed water layer was extracted with 10 mL of petroleum ether twice. The water phase was then acidified with 25% HCl solution to pH 3. Extraction with 10 mL of petroleum was done twice, and then the organic phase was washed twice with 10 mL of distilled water. The organic phase was dried with anhydrous $Na_2SO_4$ crystals, and the remaining solvent was evaporated. The isolation results were then analyzed using FTIR and GC-MS.

### 2.3. Eugenol Isomerization

According to the research conducted by Utomo and Setiati [30], 2 g of KOH was dissolved in 25 mL ethanol and thoroughly mixed in a three-neck flask. Subsequently, 5 mL of eugenol, with a eugenol-to-solvent ratio of 1:5, was added to the solution. The mixture was refluxed at 50-60°C for 4 hours. After cooling, the reflux results were neutralized by adding 1 M HCl. Then, the mixture was added with 50 mL of double-distilled water and extracted with diethyl ether. The isomerization results were then analyzed using FTIR.

### 2.4. Synthesis of Vanillin from Isoeugenol

The mixture containing 2.7 mL isoeugenol, 100 mL double-distilled water, 15 mL sulfuric acid (50%), 0.5 g CTAB, and 100 mL dichloromethane was stirred at room temperature [30]. Then, the mixture was oxidized using 9.8 g of $KMnO_4$ while maintaining the temperature below 30°C. The mixture was heated over a water bath until the color changed from blackish purple to colorless. After the mixture was allowed to cool, the precipitated $MnO_2$ was filtered. Residual solvent-containing products underwent extraction with dichloromethane. All organic layers that had been separated were then combined and washed with distilled water. Any remaining solvent was removed by drying with $Na_2SO_4$. The results were analyzed using FTIR, GC-MS, and $^1$H-NMR.

### 2.5. Study of the Potential of Vanillin Compounds as Urea Sensors in Urine

#### 2.5.1. Determination of Compatible Solvents

A 0.5 g of the synthesized vanillin was dissolved in different solvents, including distilled water, 96% ethanol, and 99% n-hexane. Subsequently, the solutions were centrifuged for 10 minutes. The filtrate was then observed for color changes, and the absorbance was measured using a UV-Vis spectrophotometer.

#### 2.5.2. Determination of the Optimum Mass of Vanillin

The synthesized vanillin was prepared by varying mass variations (0.15, 0.25, 0.50, 0.75, and 1 g) and then dissolved in 50 mL of 96% ethanol. Each mass variation was replicated twice and mixed with 10 mL of 10% NaOH or 1 M. Subsequently, 20 mL of each formulation was mixed with 10 mL of 0.1 M standard urea solution and

centrifuged for 10 minutes. The absorbance of the filtrate was then measured using a UV-Vis spectrophotometer.

#### 2.5.3. Determination of the Optimum Mass of pDMAB as a Comparator

Para-dimethylaminobenzaldehyde (pDMAB) solid was prepared by varying mass variations (0.15, 0.25, 0.50, 0.75, and 1 g) and dissolved in 50 mL of 96% ethanol. Then, 10 mL of 1 M HCl was added to each variation. Subsequently, 20 mL of the formulation was mixed with 10 mL of 0.1 M standard urea solution and centrifuged for 10 minutes. The absorbance of the filtrate was then measured using a UV-Vis spectrophotometer.

#### 2.5.4. Acidic and Alkaline Conditions

The optimum mass of vanillin was dissolved in 50 mL of ethanol, followed by the addition of the optimum concentration of NaOH. Subsequently, 20 mL of the mixture was mixed with 10 mL of 0.1 M standard urea solution. This procedure was made into three variations: an acidic condition with the addition of 1 M HCl, a neutral condition with distilled water, and a basic condition with the addition of 1 M NaOH. Afterward, the absorbance was measured using a UV spectrophotometer, and visual observation was conducted to note any color changes in the samples.

### 2.6. Application of Vanillin Compound as a Urea Sensor in Urine

The 20 mL of the optimum formulation of the vanillin sensor was mixed with 10 mL of urine sample, which was diluted at varying levels of 100, 1000, and 10,000×. Each mixture was centrifuged for 10 minutes. The absorbance of the filtrate was then measured using a UV-Vis spectrophotometer, and any alterations in color within the urine sample were observed.

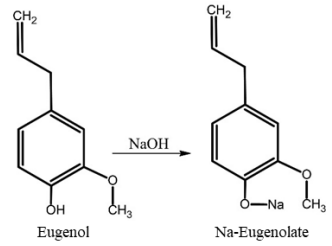


**Figure 1**. Saponification reaction mechanism of NaOH and eugenol in clove oil

## 3. Results and Discussion

### 3.1. Study of the Synthesis of Vanillin from Clove Oil

The process of synthesizing vanillin from clove oil involved three primary stages: the extraction of eugenol from clove oil, the isomerization of eugenol into isoeugenol, and the oxidation of isoeugenol into vanillin. The extraction of eugenol from clove oil occurred through a saponification reaction with NaOH. This reaction involved the combination of eugenol in clove oil with NaOH in an aqueous solvent, forming a water layer and Na-eugenolate, as illustrated in Figure 1. The yield of isolated eugenol from clove oil using this method was 65%.

The extracted eugenol from clove oil is the main precursor in the synthesis of vanillin. The synthesis process begins with an isomerization reaction, wherein eugenol transforms into isoeugenol via a prototropic rearrangement mechanism facilitated by forming free radicals. The sigmatropic rearrangement involves the shift or transfer of hydrogen [1, 8] within the molecule facilitated by interfacial processes. In contrast to light irradiation (photochemical), an electron in the HOMO (Highest Occupied Molecule Orbital) position does not experience excitation due to an increase in temperature (thermally induced).

The free radical reaction can be divided into two stages (Figure 2): withdrawal (abstraction) and re- insertion of protons. Initially, proton withdrawal occurs at the terminal alkene carbon atom (in this instance, benzyl) facilitated by KOH, forming a carbanion and releasing HB (proton complex with base). This stage is followed by the formation of a stable carbanion resonance structure. The second stage is the reintroduction of protons ($H^+$) into stable carbanions by HB. Meanwhile, this stage is followed by the formation of alkenes that are not in the terminal position [31].

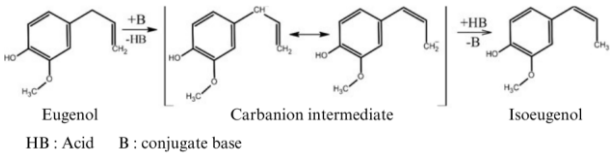


**Figure 2**. Free radical reaction mechanism of sigmatropic rearrangement [1, 3]

**Table 1**. Composition of compounds in isolated eugenol

| Compound name | Retention time (minute) | Height | Area% |
|---|---|---|---|
| Alpha-pinene | 6.597 | 422469 | 11.99 |
| Ocimene | 9.011 | 381219 | 0.30 |
| Eugenol | 20.609 | 22637424 | 81.69 |
| Copaene | 21.033 | 149842 | 0.15 |

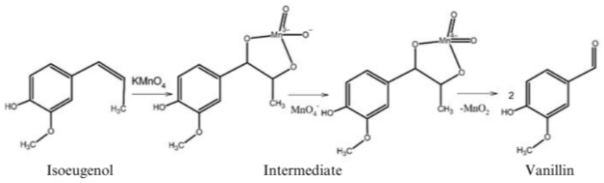


**Figure 3**. Mechanism of eugenol oxidation to vanillin in an acidic condition facilitated by the $MnO_4^-$ oxidizer

The synthesis of vanillin from the oxidation of isoeugenol, facilitated by a CTAB base transfer catalyst, occurs under acidic conditions. In this process, the oxidation reaction takes place with the $MnO_4^-$ anion, wherein a pair of $K^+$ ions are captured by the organic phase while $K^+$ is taken up by the base transfer catalyst. This mechanism leads to the oxidation of isoeugenol by $MnO_4^-$. Any olefin undergoing oxidation transforms into an aldehyde group, which can subsequently undergo further oxidation to yield a carboxylic acid [31]. The oxidation reaction equation is shown in Figure 3. Based on the synthesis, a vanillin yield of 60% was achieved.

### 3.2. Analysis of Compound Content in Isolation and Synthesis Results

The purity of both the isolated and synthesized products is evident through GC-MS analysis, as indicated by the percentage area of the chromatogram. Additionally, distinct retention times and molecular masses are observed in each chromatogram, facilitating the identification of the compounds in the products. Specifically, Table 1 shows the composition of compounds isolated from eugenol. Four prominent peaks are discernible in the chromatogram of isolated eugenol from clove oil, as depicted in Figure 4. Notably, the eugenol compound exhibits the highest peak, with a retention time of 20.61 minutes and a molecular weight of 164 g/mol, indicating its dominance among other compound components in the sample.

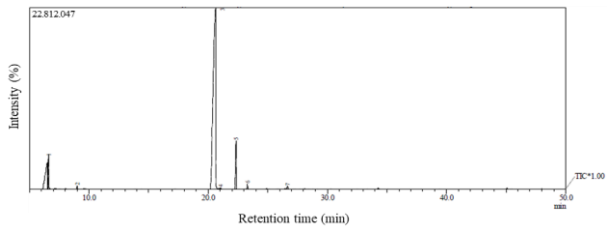


**Figure 4**. Chromatogram of compounds isolated from eugenol

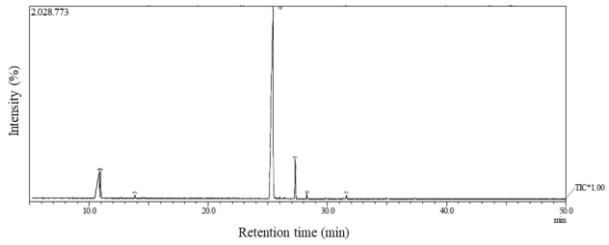


**Figure 5**. Chromatogram of clove oil

The extraction of eugenol from clove oil aligns with previous studies, indicating that clove oil typically comprises 80-85% eugenol [20, 21]. These findings corroborate with the chromatographic analysis of clove oil. Notably, the peak exhibiting the highest percentage area or purity is 78% (Figure 5). In the chromatogram of the synthesis of vanillin from isoeugenol, a prominent peak is observed, exhibiting a molecular mass of 151 g/mol, indicative of a high-purity vanillin compound. This is evident from the area percent value, which notably reaches 95%. Furthermore, the chromatogram depicted in Figure 6 reveals the presence of eugenol compounds, albeit in reduced quantities (2%) compared to the previous process, primarily due to its conversion to vanillin.

### 3.3. Functional Group Analysis of Isolation, Isomerization and Synthesis Results

Functional group analysis was conducted to verify whether the structure of the compound obtained from isolation, isomerization, and synthesis aligns with the predominant compound identified through GC-MS analysis and reaction mechanisms. Validation using FTIR spectroscopy was employed to ascertain the compound's structure by detecting characteristic functional groups present in eugenol, isoeugenol, and vanillin.

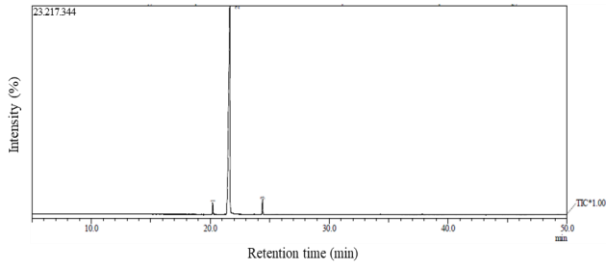


**Figure 6**. Chromatogram of the sample resulting from the synthesis of vanillin from isoeugenol

**Table 2**. Chemical shift and multiplicity of vanillin samples

| Position | Chemical shift (ppm) [multiplicity] | Chemical shift (ppm) [multiplicity] [32] |
|---|---|---|
| 1'-CHO | 9.545 [singlet] | 9.80 [singlet] |
| 3'-$OCH_3$ | 3.792 [singlet] | 3.95 [singlet] |
| 5 | 6.907 [duplet] | 7.02 [duplet] |
| 6 | 7.378 [duplet] | 7.40 [duplet] |

**Table 3**. Structural energy resulting from geometric optimization of vanillin, pDMAB, and urea compounds

| Compound name | Structural energy (kJ.mol$^{-1}$) | ΔE (kJ.mol$^{-1}$) |
|---|---|---|
| Vanillin | 154.498 | 46.119 |
| Vanillin-Urea (VU) | 108.379 | |
| pDMAB | 160.982 | 6.384 |
| pDMAB-Urea (pU) | 154.598 | |

Figure 7 shows the characteristic absorptions of eugenol, isoeugenol, and vanillin. Specifically, absorptions at 1265, 1512, and 1651 cm$^{-1}$ are observed in the vanillin spectrum, corresponding to trisubstituted benzene compounds in all three samples. Furthermore, in the IR spectrum of eugenol, an absorption at 3510 cm$^{-1}$ signifies the presence of the Ar-OH group, while an absorption at 1650 cm$^{-1}$ indicates the stretching of the alkene C=C group [30]. Meanwhile, in the IR spectrum of isoeugenol, the alkene absorption shifts to 1034 cm$^{-1}$, indicating a positional shift of the alkene double bond from the CH=CH$_2$ to the CH=CH position.

Additionally, there is a decrease in absorbance in the region around 1400 cm$^{-1}$, characteristic of methylene groups, and at 910 cm$^{-1}$, indicative of terminal C=C bonds. Moreover, the spectra of eugenol and isoeugenol in the range of 1500-1700 cm$^{-1}$ exhibit differences in shape. Specifically, the peaks in the eugenol spectrum appear widely spaced, whereas those in the isoeugenol spectrum appear denser. This alteration is attributed to the transition of the terminal alkene group in eugenol to a substituted form in isoeugenol, indicating successful isomerization [33]. In the IR spectrum of vanillin, an absorption peak at 1651 cm$^{-1}$ is observed, along with peaks at 2747 and 2862 cm$^{-1}$, indicating the presence of carbonyl groups (C=O) in the form of an aldehyde in the structure. These findings confirmed the successful synthesis of vanillin through the oxidation reaction of isoeugenol [30].

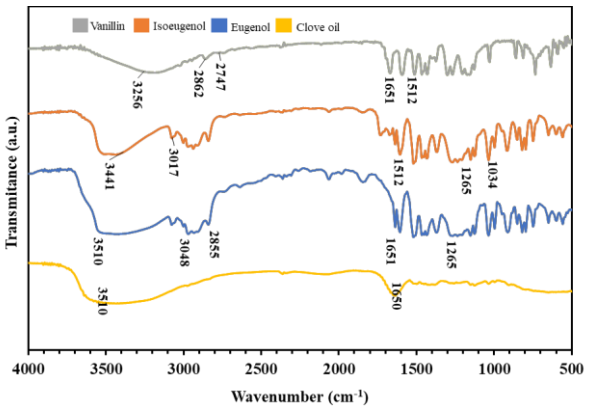


**Figure 7**. FTIR spectra of eugenol, isoeugenol, and vanillin

**Table 4**. Concentration of vanillin in various types of solvents

| Solvent | Maximum wavelength (nm) | Concentration (ppm) |
|---|---|---|
| Double-distilled water | 348 | 107 |
| Ethanol | 348 | 112 |
| n-Hexane | 310 | 47 |

### 3.4. Analysis of the Structure of Synthesized Vanillin

The synthesized vanillin was analyzed using $^1$H-NMR to determine the structure by identifying the position of the proton. Based on the NMR spectrum with heavy water ($D_2O$) solvent in Figure 8, a chemical shift of 9.545 ppm with singlet multiplicity appears. Table 2 reveals the chemical shifts of the protons in the aldehyde group attached to the benzene ring. The shift of 3.792 ppm with singlet multiplicity shows the presence of protons in the methoxy group of the methyl part (-OCH$_3$). Additionally, two other chemical shift values (6.907 and 7.378 ppm) are observed with duplet multiplicity, indicating the presence of protons in aromatic compounds (benzene). These findings were subsequently compared with NMR spectra analyses obtained from previous research [32]. Through the deduction method and comparison with existing literature, it was concluded that the structure of the compound obtained corresponds to vanillin.

### 3.5. Geometry Optimization of Vanillin-Urea Compound Structure

Molecular mechanics techniques were employed to optimize the geometries of vanillin, pDMAB, vanillin- urea, and pDMAB-urea structures. Geometric optimization is conducted to evaluate the energy of each molecule's structure. This structural energy indicates the stability of the compound formed. Lower energy levels signify greater stability, ensuring sustained color changes throughout the analysis. Structural energy computations utilized Avogadro software, employing MMF94 and UFF force fields tailored to organic molecules like vanillin, pDMAB, and urea. The energy calculations for each structure are detailed in Table 3, while molecular visualization outcomes for several of these compounds are depicted in Figure 9.

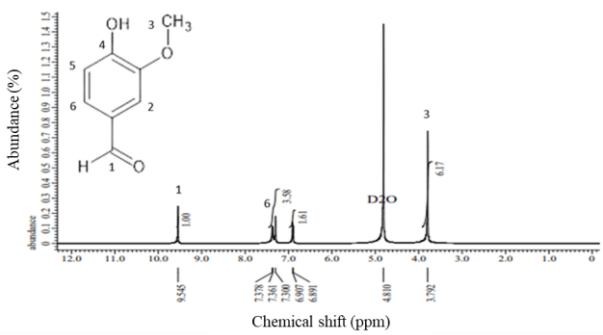


**Figure 8**. NMR spectrum of vanillin synthesized from isoeugenol

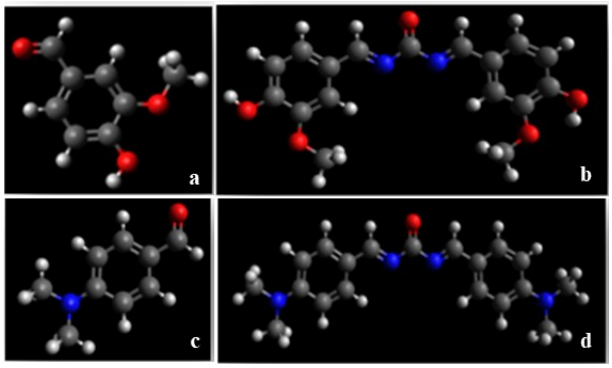


**Figure 9**. Optimized geometries of (a) vanillin, (b) vanillin-urea, (c) pDMAB, and (d) pDMAB-urea

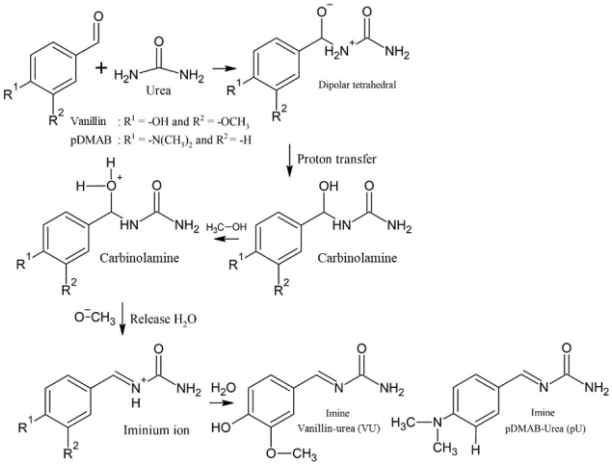


**Figure 10**. Formation reaction of vanillin-urea and pDMAB-urea compounds

The structural energy of the pDMAB molecule is greater than that of the vanillin molecule. This difference in energy value shows a substantial disparity in the charge distribution between the two molecules. Notably, the tertiary amine group ($R_3N$) in pDMAB tends to have a positive charge because it is an electron donor to the benzene ring, resulting in a fairly large charge difference in its structure.

Meanwhile, the structural energy of the VU compound is lower than that of pU when reacting with urea, indicating a higher degree of stability in the VU structure than pU. This statement is also supported by the ΔE data, where the energy difference for the VU compound is greater than that of pU. These findings indicate that the energy released in forming VU surpasses that of the formation of the pU compound. The process of formation of vanillin-urea and pDMAB-urea compounds is shown in Figure 10. The enhanced stability of VU compounds can be attributed to the presence of more pronounced resonance effects, particularly under alkaline conditions, compared to pU compounds. The resonance characteristics of the VU compound are depicted in Figure 11.

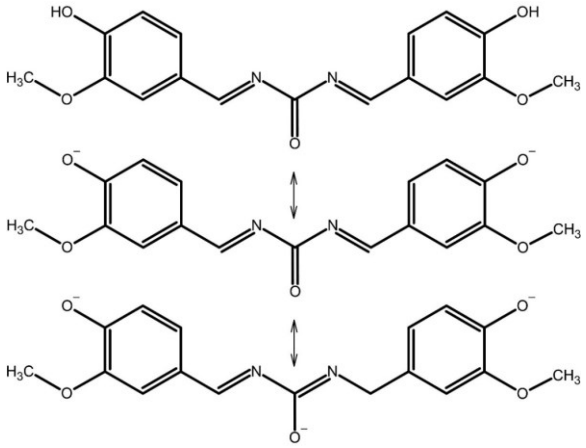

**Figure 11**. Resonance of vanillin-urea compounds

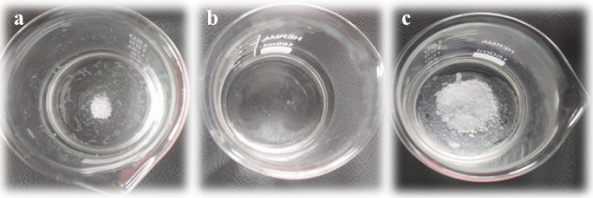


**Figure 12**. Vanillin in (a) double-distilled water, (b) ethanol, and (c) n-hexane

The conjugated system possesses the capability to absorb the visible light spectrum, thereby giving rise to colors perceivable by the human eye. Enhanced stability within the conjugated system facilitates a more efficient light spectrum absorption, leading to more vibrant colors. This correlation occurs due to the presence of a larger electron delocalization cloud, enabling stronger interactions with photons [34]. Conversely, diminished stability within the compound results in a reduction of electron delocalization clouds, thereby weakening the interaction with photons and consequently yielding less intense colors.

### 3.6. Vanillin-based Urea Sensor Formulation

#### 3.6.1. Determination of Compatible Solvents

Solvents are substances capable of dispersing vanillin solids when employed in a urea sensor application. The findings revealed that vanillin exhibited high solubility in 96% ethanol compared to 99% distilled water or n-hexane. This is because vanillin is a semipolar compound that is compatible with ethanol. The presence of hydroxyl, ether, and aldehyde groups renders vanillin polar. However, benzene as the main structure prevents its solubility in polar solvents like water.

Analysis was conducted to ascertain the concentration of vanillin dissolved in the three solvents using a UV-Vis spectrophotometer. Absorbance data was converted into concentrations using calibration curves specifically developed for each solvent type. The results of concentration calculations are shown in Table 4, while the qualitative analysis results are illustrated in Figure 12.

**Table 5**. One-way ANOVA statistical test results

| Group | Amount of data | Sum | Average | Variance | Variation | SS | df | M.S |
|---|---|---|---|---|---|---|---|---|
| pDMAB | 5 | 3.972 | 0.794 | 0.00364 | Mark between groups | 0.0645 | 2 | 0.03225 |
| Vanillin (NaOH 10%) | 5 | 4.775 | 0.955 | 0.00034 | Mark in the group | 0.0428 | 12 | 0.00356 |
| Vanillin (NaOH 1 M) | 5 | 4.360 | 0.872 | 0.00671 | Total | 0.1073 | 14 | - |

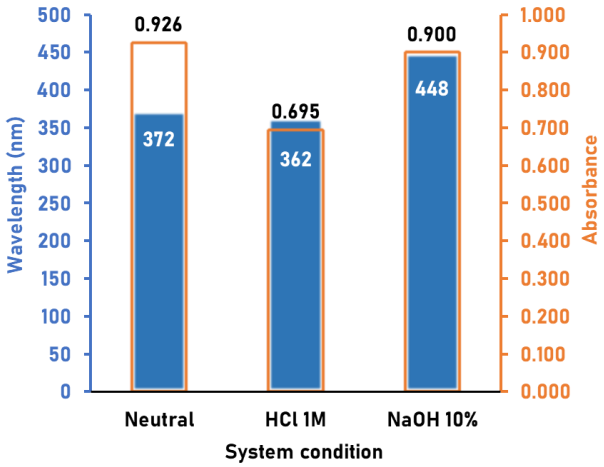


**Figure 13**. Acid-base conditions of urea sensor formulation based on vanillin compound

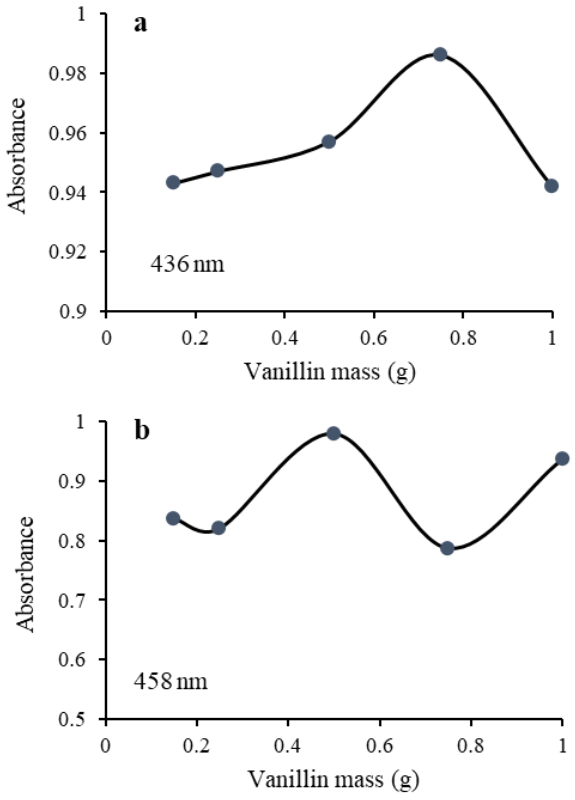


**Figure 14**. Optimum mass of vanillin with NaOH concentration of (a) 10% and (b) 1 M

### 3.6.2. Determination of Optimum Conditions in Acids and Bases

The formulation of the vanillin compound-based urea sensor is additionally affected by the pH levels of the solution. The study findings concerning the effects of acidic, neutral, and alkaline conditions on urea sensor formulations are depicted in the diagram presented in Figure 13. Alkaline conditions exhibit higher absorbance levels at a wavelength of 448 nm. This specific wavelength (λ) falls within the visible light spectrum, thereby inducing pronounced color changes.

Under acidic and neutral conditions, the highest absorbance is observed at wavelengths of 372 and 362 nm, respectively. These wavelengths fall outside the visible light spectrum (400-700 nm), resulting in no observable color change in the solution. Consequently, it can be deduced that an alkaline environment represents the optimal condition for formulating urea sensors. It facilitates maximum absorption at visible light wavelengths, particularly within the yellow complementary color range (380-450 nm). This phenomenon is attributed to a deprotonation reaction within the VU structure, leading to a resonance effect that stabilizes the structural state by intensifying electron cloud delocalization during photon interactions. Conversely, under acidic and neutral conditions, the delocalization system is hindered due to the absence of deprotonation reactions within the VU structure.

### 3.6.3. Determination of the Optimum Mass of Vanillin

The optimum mass of vanillin must be ascertained for formulating a urea sensor. The observed color transition is from colorless to yellow. The brightness level is discerned through the absorbance value and wavelength shift (λ), measured using a UV-Vis spectrophotometer. The base concentration utilized in the formulation also influences this process. The graph depicting the correlation between the mass of vanillin (in grams) and the corresponding absorbance is presented in Figure 14. The shift to yellow is induced by the VU compound absorbing its complementary color, purple, within the wavelength range of approximately 380-450 nm.

In Figure 14(a), employing a vanillin mass of 0.75 g yields the highest absorbance recorded at 0.986, observed at a wavelength of 436 nm. Conversely, in Figure 14(b), the utilization of 1 g of vanillin results in the highest absorbance, measured at 0.937, occurring at a wavelength

of 458 nm.Based on these data, it can be concluded that 10% NaOH is the best base concentration because it can reduce the amount of vanillin mass used and provide optimum absorbance in the purple wavelength range. Excessively high base solution concentrations may distort the VU structure, leading to instability in its interaction with photons, thereby diminishing the intensity of the reflected color.

### 3.6.4. Determination of the Optimum Mass of pDMAB as a Comparator

The pDMAB compound serves as a commonly utilized reagent for urine urea analysis. Hence, it is a suitable alternative compared to the vanillin-based urea sensor currently under development. The results depicting the determination of the optimal mass of pDMAB for detecting urea presence in urine are presented in Figure 15. The graph demonstrates that the optimum mass of pDMAB is 1 g, exhibiting an absorbance of 0.896 at a wavelength (λ) of 410 nm. Notably, when using 1 g of pDMAB, the resultant color change displayed the highest intensity compared to other masses tested.

The pDMAB and vanillin compounds share a common mechanism for urine detection. This mechanism relies on a condensation reaction to generate Schiff base compounds. The resulting reaction product induces a color change due to the presence of a chromophore group, specifically in the form of a C=N bond within its structure. The process of forming Schiff base compounds through the reaction between vanillin or pDMAB and urea is illustrated in Figure 16.

When comparing the two compounds, it is observed that pDMAB requires a greater mass to induce a color change within the visible light range compared to vanillin. Specifically, a mass of 1 g of pDMAB yields an optimal absorbance of 0.896 at a wavelength of 410 nm. In contrast, considering the variations in vanillin mass as depicted in the previous section (Figure 14), it is noted that 0.75 g of vanillin generates an optimal absorbance of 0.986 at a wavelength of 436 nm. This indicates that vanillin is more efficient in detecting urea in urine than pDMAB.

### 3.7. Statistical Test of the Effect of Sensor Reagent Type on Color Absorbance

Statistical analysis of the sensor reagent type was conducted to evaluate the impact on the color absorbance variations observed in urine samples. The analysis protocol entails identifying control, independent, and dependent variables. In this study, the control variable is the maximum wavelength (nm), while the independent variables encompass the type of sensor reagent and system conditions. The dependent variable is the maximum absorbance (au). The statistical analysis method was one-way ANOVA, supplemented by a Bonferroni-corrected α post-hoc test. The results of the one-way ANOVA statistical test for the pDMAB, vanillin (10% NaOH), and vanillin (1 M NaOH) sensor formulations are presented in Table 5.

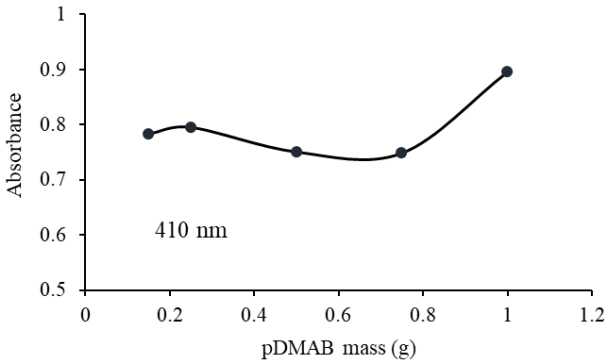


**Figure 15**. Optimum mass of pDMAB in detecting urea in urine

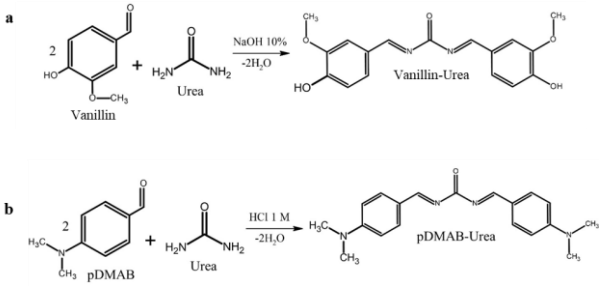


**Figure 16**. The condensation reaction between (a) vanillin and urea and (b) pDMAB with urea

**Table 6**. Bonferroni-correction post-hoc test results

| Group | P-value (T- test) | Alpha value (α) | Significant |
|---|---|---|---|
| pDMAB vs. Vanillin (NaOH 10%) | 0.00045835 | | Yes |
| Vanillin (NaOH 10%) vs. Vanillin (NaOH 1 M) | 0.05793943 | 0.016667 | No |
| Vanillin (NaOH 1 M) Vs. pDMAB | 0.12649899 | | No |

**Table 7**. Average absorbance value for each urea sensor reagent

| Group | Mean ± SD |
|---|---|
| pDMAB (410 nm) | 0.794 ± 0.06 |
| Vanillin + NaOH 10% (436 nm) | 0.955 ± 0.02 |
| Vanillin + NaOH 1 M (458 nm) | 0.872 ± 0.08 |

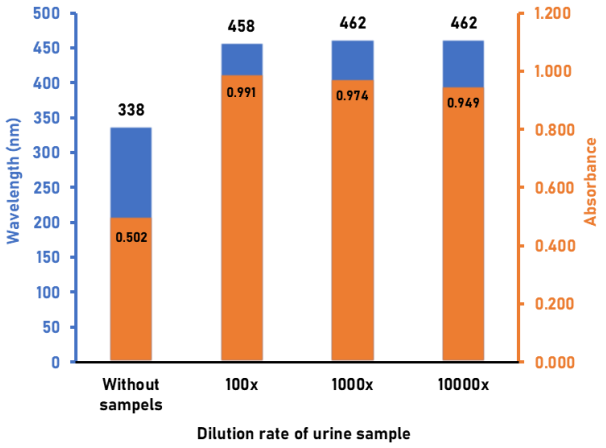


**Figure 17**. Variations in the dilution rate of urine samples

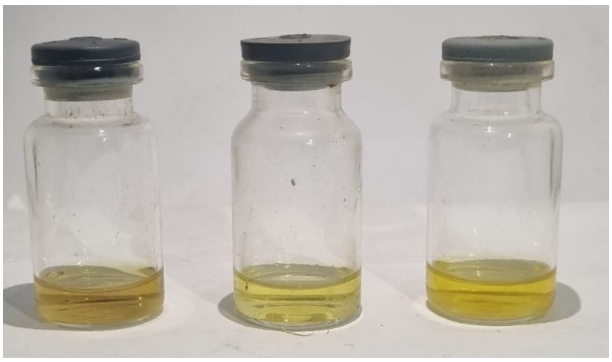

**Figure 18**. Visualization of color changes in the vanillin sensor during triplicate urea detection in urine samples

Based on the data in Table 5, the P-value obtained is 0.00401, which is smaller than the significance value (α) of 0.05 for the one-way ANOVA test. This indicates that the sensor reagent parameter type significantly influences the urine sample's color absorbance. Further investigation into the differences between groups can be conducted using the Bonferroni-correction post-hoc test method. The outcomes of the post-hoc statistical tests are presented in Table 6. Additionally, the comparison of sensor quality in detecting urea can be assessed through descriptive statistics, specifically by calculating the mean value ± standard deviation (SD). The calculation results of the average absorbance for each group are displayed in Table 7.

Based on the data in Table 6, a statistically significant difference is observed between the absorbance produced by pDMAB and vanillin (NaOH 10%). This difference in absorbance is evident from the considerably disparate average absorbance values of the two groups. Furthermore, according to the data in Table 7, the average absorbance value in the pDMAB system is lower than the vanillin + 10% NaOH system. This indicates that the vanillin + 10% NaOH system is capable of generating a more intense color than the pDMAB system.

### 3.8. Application of Vanillin Sensor Formulation to Urine Samples

Applying vanillin formulations to urine samples involved varying the level of sample dilution. This was done to determine the detection limit of urea concentration achievable by the vanillin sensor formulation. According to the data in Figure 17, it was observed that the 10,000× dilution level exhibited brighter colors than other variations. This variation demonstrated an optimal absorbance of 0.974 ± 0.02 (triplicate) at a wavelength of 462 nm. These findings suggest that the detection limit for urea in urine using the new vanillin formulation can be achieved at a sample dilution level of 10,000×. The color change induced by the vanillin sensor for detecting urea in the urine sample is illustrated in Figure 18. Additionally, observations of this color change have been conducted for 2 weeks following the addition of the vanillin sensor to the urine sample.

## 4. Conclusion

The vanillin compound was successfully synthesized from clove oil precursor with a purity of 95%. This synthesized vanillin was capable of reacting with urea to form a Schiff base compound, specifically vanillin-urea, through a condensation reaction. The resulting compound exhibited a greenish-yellow color at a wavelength of 436 nm. It has been demonstrated that this structure was notably more stable than pDMAB-urea, as evidenced by its structural energy value of 108.379 kJ/mol. Moreover, the optimal vanillin sensor formulation was determined to be 0.75 g of vanillin dissolved in 50 mL of 96% ethanol, added with 10 mL of 10% NaOH. During the application stage, it was found that the urine sample needed to be prepared with a dilution level of 10,000× to induce a significant color change. This change was more visibly pronounced when utilizing the vanillin sensor formulation.

## Acknowledgment

We extend our sincere gratitude to the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia, Gadjah Mada University, PKM Center UGM, Department of Chemistry, Gadjah Mada University, as well as the organic chemistry laboratory and analytical chemistry laboratory, for their invaluable guidance, financial support, and provision of facilities throughout our research.

## References


[1] Takao Sakurai, Toshiaki Irii, Kimiharu Iwadate, Simultaneous quantification of urea, uric acid, and creatinine in human urine by liquid chromatography/mass spectrometry, *Legal Medicine*, 55, (2022), 102011 https://doi.org/10.1016/j.legalmed.2021.102011

[2] Yuyang Bei, Kamile Arkin, Yuxin Zheng, Xuesong Ma, Jie Zhao, Huimin Jin, Qingkun Shang, Construction of a ratiometric fluorescent probe for visual detection of urea in human urine based on carbon dots prepared from Toona sinensis leaves and 5-carboxyfluorescein, *Analytica Chimica Acta*, 1240, (2023), 340733 https://doi.org/10.1016/j.aca.2022.340733

[3] Nura Ma'shumah, Sufiati Bintanah, Erma Handarsari, Hubungan Asupan Protein Dengan Kadar Ureum, Kreatinin, dan Kadar Hemoglobin Darah pada Penderita Gagal Ginjal Kronik Hemodialisa Rawat Jalan Di RS Tugurejo Semarang, *Jurnal Gizi*, 3, 1, (2014),

[4] Ardhiles Wahyu Kurniawan, Juliati Koesrini, Hubungan Kadar Ureum, Hemoglobin dan Lama Hemodialisa dengan Kualitas Hidup Penderita PGK, *Jurnal Ners dan kebidanan*, 6, 3, (2019), 292-299 https://doi.org/10.26699/jnk.v6i3.ART.p292-299

[5] Rahmi Nur Fahisyah, Nurlia Naim, Zulfian Armah, Pengaruh Variasi Lama Penyimpanan Reagen Enzim 1a Terhadap Hasil Pemeriksaan Ureum Darah Metode Berthelot, *Jurnal Media Analis Kesehatan*, 10, 1, (2019), 21-27 https://doi.org/10.32382/mak.v10i1.980

[6] Hanim Istatik Badi'ah, Pengaruh variasi Reagen asam dalam pembuatan sensor urea menggunakan Reagen Diacetil Monoxime-Thiosemicarbazide secara Adsorpsi pada Plat Silika Gel, Chemistry, Universitas Islam Negeri Maulana Malik Ibrahim, Malang, 2015

[7] Dang Soni, Riska Prasetiawati, Dian Novita Sari, Pengaruh lokasi terhadap kadar ion fluorida pada air sumur dan air PAM dengan metode kolorimetri, *Jurnal Ilmiah Farmako Bahari* 10, 1, (2019), 76-90 http://dx.doi.org/10.52434/jfb.v10i1.650

[8] Wei-Yi Zhang, Chun-Yan Zhang, Hang-Yu Zhou, Tao Tian, Hua Chen, Hao Zhang, Feng-Qing Yang, Paper-based sensor depending on the Prussian blue pH sensitivity: Smartphone-assisted detection of urea, *Microchemical Journal*, 181, (2022), 107783 https://doi.org/10.1016/j.microc.2022.107783

[9] Nadtinan Promphet, Wisarttra Phamonpon, Wimala Karintrithip, Pranee Rattanawaleedirojn, Kanokwan Saengkiettiyut, Yuttanant Boonyongmaneerat, Nadnudda Rodthongkum, Carbonization of self-reduced AuNPs on silk as wearable skin patches for non-invasive sweat urea detection, *International Journal of Biological Macromolecules*, 242, (2023), 124757 https://doi.org/10.1016/j.ijbiomac.2023.124757

[10] Amir Rostami, Hassan Soleimani, Zahra Samavati, Nasrin Khodapanah, Hojjatollah Soleimani, Surajudeen Sikiru, Mohammed Falalu Hamza, Vahid Khosravi, Real-time monitoring of urea slow release through fiber Bragg grating sensors, *Measurement*, 221, (2023), 113464 https://doi.org/10.1016/j.measurement.2023.113464

[11] Hermin Sulistyarti, Akhmad Sabarudin, Yudha Ikoma Istanti, Eka Ratri Noor Wulandari, Penentuan Kreatinin dalam Urin secara Kolorimetri dengan Sequential Injection-Flow Reversal Mixing (SI-FRM), *Jurnal Berkala Ilmiah Sains dan Terapan Kimia*, 5, 2, (2011), 158-164

[12] Irina Timofeeva, Polina Davletbaeva, Alexey Moskvin, Andrey Bulatov, p-Dimethylaminobenzaldehyde-based chemosensor for on-site sensing of ammonia precursor in concrete, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 253, (2021), 119556 https://doi.org/10.1016/j.saa.2021.119556

[13] Noah James Langenfeld, Lauren Elizabeth Payne, Bruce Bugbee, Colorimetric determination of urea using diacetyl monoxime with strong acids, *PLoS ONE*, 16, 11, (2021), e0259760 https://doi.org/10.1371/journal.pone.0259760

[14] Taher Alizadeh, Mohammad Reza Ganjali, Faride Rafiei, Trace level and highly selective determination of urea in various real samples based upon voltammetric analysis of diacetylmonoxime-urea reaction product on the carbon nanotube/carbon paste electrode, *Analytica Chimica Acta*, 974, (2017), 54-62 https://doi.org/10.1016/j.aca.2017.04.039

[15] Sekar Ayu Maharani, Adella Aisiyah, Diva Rizqi Salsabilla, Regita Nailuvar, Isolasi Senyawa Aktif Lignan dari Beberapa Tanaman, *Equivalent: Jurnal Ilmiah Sosial Teknik*, 4, 1, (2022), 73-81 https://doi.org/10.59261/jequi.v4i1.65

[16] Sayyid Syauqas Amarullah, Aldika Saputra, Devi Sartika Marni, Putri Athirah, Nur'Alian Nur'Alian, Izwar Izwar, Pemanfaatan Produk Lokal Berbasis Minyak Serai Wangi (*Cymbopogon nardus* (l) rendle) Dalam Rangka Peningkatan Kapasitas Ekonomi Masyarakat Desa Pantan Reduk, *Jurnal Pengabdian Masyarakat Bangsa*, 1, 7, (2023), 956 - 963 https://doi.org/10.59837/jpmba.v1i7.290

[17] Franky Reintje Tulungen, Cengkeh dan Manfaatnya Bagi Kesehatan Manusia Melalui Pendekatan Competitive Intelligence, *Biofarmasetikal Tropis (The Tropical Journal of Biopharmaceutical)*, 2, 2, (2019), 158-169 https://doi.org/10.55724/jbiofartrop.v2i2.128

[18] Tresno Saras, *Cengkeh : Keajaiban Herbal dalam Pengobatan dan Kesehatan*, Tiram Media, 2023,

[19] Sayu Kade Sintia Dewi, Made Antara, Gede Mekse Korri Arisena, Pemasaran Cengkeh di Desa Penyaringan Kecamatan Mendoyo, Kabupaten Jembrana, Bali, Indonesia, *Agro Bali: Agricultural Journal*, 4, 2, (2021), 246-259 https://doi.org/10.37637/ab.v4i2.719

[20] İsmail Tarhan, A robust method for simultaneous quantification of eugenol, eugenyl acetate, and β-caryophyllene in clove essential oil by vibrational spectroscopy, *Phytochemistry*, 191, (2021), 112928 https://doi.org/10.1016/j.phytochem.2021.112928

[21] Saiful Hadi, Pengambilan Minyak Atsiri Bunga Cengkeh (*Clove Oil*) Menggunakan Pelarut N-Heksana dan Benzena, *Jurnal Bahan Alam Terbarukan*, 1, 2, (2012), 25-30

[22] Alberto Peña-Barrientos, María de J. Perea-Flores, Hugo Martínez-Gutiérrez, Olga A. Patrón-Soberano, Francisco E. González-Jiménez, Miguel Á Vega-

Cuellar, Gloria Dávila-Ortiz, Physicochemical, microbiological, and structural relationship of vanilla beans (*Vanilla planifolia*, Andrews) during traditional curing process and use of its waste, *Journal of Applied Research on Medicinal and Aromatic Plants*, 32, (2023), 100445 https://doi.org/10.1016/j.jarmap.2022.100445

[23] Fadhlan Zuhdi, Khoiru Rizqy Rambe, Daya saing ekspor cengkeh Indonesia di pasar global, *Jurnal Sosial Ekonomi Pertanian dan Agribisnis*, 17, 2, (2021), 165-173 https://doi.org/10.20961/sepa.v17i2.43784

[24] Rebeca Yépez, Juan F. Illescas, Paulina Gijón, Manuel Sánchez-Sánchez, Eduardo González-Zamora, Rosa Santillan, J. Raziel Álvarez, Ilich A. Ibarra, Julia Aguilar-Pliego, HKUST-1 as a Heterogeneous Catalyst for the Synthesis of Vanillin, *JoVE (Journal of Visualized Experiments)*, 113, (2016), e54054 https://dx.doi.org/10.3791/54054

[25] Rosaria Ciriminna, Alexandra Fidalgo, Francesco Meneguzzo, Francesco Parrino, Laura M. Ilharco, Mario Pagliaro, Vanillin: The Case for Greener Production Driven by Sustainability Megatrend, *ChemistryOpen*, 8, 6, (2019), 660-667 https://doi.org/10.1002/open.201900083

[26] O. Y. Saiapina, V. M. Pyeshkova, O. O. Soldatkin, V. G. Melnik, B. Akata Kurç, A. Walcarius, S. V. Dzyadevych, N. Jaffrezic-Renault, Conductometric enzyme biosensors based on natural zeolite clinoptilolite for urea determination, *Materials Science and Engineering: C*, 31, 7, (2011), 1490-1497 https://doi.org/10.1016/j.msec.2011.06.003

[27] Farzaneh Shalileh, Hossein Sabahi, Mehdi Dadmehr, Morteza Hosseini, Sensing approaches toward detection of urea adulteration in milk, *Microchemical Journal*, 193, (2023), 108990 https://doi.org/10.1016/j.microc.2023.108990

[28] Fernanda L. Migliorini, Rafaela C. Sanfelice, Luiza A. Mercante, Rafaela S. Andre, Luiz H. C. Mattoso, Daniel S. Correa, Urea impedimetric biosensing using electrospun nanofibers modified with zinc oxide nanoparticles, *Applied Surface Science*, 443, (2018), 18-23 https://doi.org/10.1016/j.apsusc.2018.02.168

[29] Widayat Widayat, Hadiyanto Hadiyanto, Bambang Cahyono, Ngadiwiyana Ngadiwiyana, Optimization of eugenol extraction from clove oil using response surface methodology, *Modern Applied Science*, 9, 11, (2015), 68-76 https://doi.org/10.5539/mas.v9n11p68

[30] Suryadi Budi Utomo, Tresni Setiati, Application of Calixarene as A Phase Transfer Catalyst in Vanilin Synthesis from Eugenol, *2019*, 4, 3, (2019), 179-188 https://doi.org/10.20961/jkpk.v4i3.34993

[31] Wahyudi Priyono Suwarso, Tony Sukri, Hendra Wijaya, Reaksi Penataan Ulang Sigmatropik Hidrogen [1, 3] Secara Termal dan Reaksi Penataan Ulang Prototropik [1, 3] yang Dikatalisis oleh Katalis Transfer Fase (PTC),[18]-Crown Ether-6: Semi-Sintesis Vanili dari Eugenol, *Makara Journal of Science*, 6, 1, (2002), 14

[32] Bolaji B. Oluremi, Paul M. Osamudiamen, Johnson A. Adeniji, Olapeju O. Aiyelaagbe, Anti-measles virus activity of 4-hydroxy-3-methoxy benzaldehyde (Vanillin) isolated from *Xylopia aethiopica* (Dunal) A. rich, *Scientific African*, 19, (2023), e01506 https://doi.org/10.1016/j.sciaf.2022.e01506

[33] Babur Z. Chowdhry, John P. Ryall, Trevor J. Dines, Andrew P. Mendham, Infrared and Raman Spectroscopy of Eugenol, Isoeugenol, and Methyl Eugenol: Conformational Analysis and Vibrational Assignments from Density Functional Theory Calculations of the Anharmonic Fundamentals, *The Journal of Physical Chemistry A*, 119, 46, (2015), 11280-11292 https://doi.org/10.1021/acs.jpca.5b07607

[34] Zheng Zhao, Haoke Zhang, Jacky W. Y. Lam, Ben Zhong Tang, Aggregation-Induced Emission: New Vistas at the Aggregate Level, *Angewandte Chemie International Edition*, 59, 25, (2020), 9888-9907 https://doi.org/10.1002/anie.201916729