SYNTHESIS AND ANTIOXIDANT ACTIVITY STUDY OF NEW MANNICH BASES DERIVED FROM VANILLIC ACID

H. Hayun*, I. Gavrilă, S. Silviana, A.E.K. Siahaan, R.F. Vonna
and M.I. Latifah

¹Faculty of Pharmacy, Universitas Indonesia, Depok, 16426, West Java, Indonesia.

*E-mail: hayun@farmasi.ui.ac.id

ABSTRACT

We synthesized five new Mannich bases compounds from vanillic acid and evaluated for their antioxidant activities using a free-radical DPPH and FRAP methods. The compounds' structures were confirmed based on infrared, proton NMR, carbon NMR, and mass spectra. In this study, the compound 5-(pyrrolidin-1-ylmethyl)vanillic acid (**2e**) showed the best free-radical scavenger activity, while compound 2-[(diethylamino)methyl]vanillic acid (**2c**) showed the best as a ferric reductant. Mannich bases enhanced the free-radical scavenger activity but decreasing the ferric reduction potential of the parent compounds. The compounds were found to be moderate antioxidant agents.

Keywords: Vanillic Acid, Mannich Bases, Synthesis, Antioxidant, DPPH, FRAP.

© RASĀYAN. All rights reserved

INTRODUCTION

The excess of free radicals that are not neutralized by the human body's antioxidants defenses is believed as an inducing agent of the oxidative stress. It induced the damages of various species of biological molecules, which leads to some degenerative diseases, such as Alzheimer's, Parkinson's, atherosclerosis, cancer, arthritis, immunological incompetence, neurodegenerative disorders, and even aging. The unhealthy lifestyle, cigarette smoking, pollution, drugs, illness, stress, etc. compounded the risk of diseases due to that condition. On the other hand, food consumption, which rich in antioxidants such as fruits, vegetables, nuts, and the use of exogenous antioxidants as diet, plays an essential role in enhancing endogenous antioxidants for neutralizing the excess of the free radicals.¹⁻⁴

Phenolic compounds are a major determinant of antioxidant potentials of foods.⁵ The compounds have one or more hydroxyl groups and range from simple to highly polymerized molecules.⁶ Vanillic acid is a natural phenolic, a class of para-hydroxybenzoic acid or paraben that has moderate antioxidant, anti-inflammatory, and anticancer activity.⁷⁻⁹

Many aminoalkyl derivatives have been prepared using the Mannich reaction to develop more potent bioactive compounds.¹⁰⁻¹¹ Some Mannich bases derived from phenol compounds, such as several chalcones, thymol, and flavanone, exhibited better antioxidant activity than the corresponding parent compounds.¹²⁻¹³ However, there was no report about the effect of Mannich bases substitutions on the biological activity of vanillic acid. To further understand that effect and to find new antioxidant agents, hence we synthesized the Mannich bases compounds from vanillic acid and tested their antioxidant activity.

EXPERIMENTAL

Material and General Procedures

We purchased solvents and chemicals from commercial sources (Sigma-Aldrich, USA; Merck, Germany; and Mallinckrodt, USA). Silica gel 60 (Merck, Germany) was used to column chromatography. The compounds' purity was tested using TLC-silica gel 60 GF₂₅₄ (Merck, Germany), while their melting points were measured using the StuartTM melting point apparatus (Stuart Scientific, UK) and were uncorrected.

FTIR-8400S Spectrophotometer (Shimadzu, Japan), NMR spectrometer (Agilent, USA) at 500 MHz and 125 MHz were used to record infrared, proton NMR and carbon NMR spectra respectively. CDCl_3 or CD_3OD was used as solvents. High-Resolution Mass Spectrometer (HR-MS) (LCT Premier XE, Waters, USA) in ESI mode was used to record mass spectra.

Synthesis of Vanillic Acid (VA) (1)

VA (1) was synthesized through vanillin oxidation using a mixture of sodium hydroxide and potassium hydroxide at high temperature as reported previously, with slight modification.¹⁴ Vanillin (0.1 mol) was added stepwise while stirring within 10-12 min into a hot mixture (160°C) of sodium hydroxide (0.43 mol), potassium hydroxide (0.27 mol) and 5 ml of water in a stainless steel beaker. Initially, the temperature will rise to 180 - 195°C but then dropped to 150 - 160°C after stirring continuously. Water (100 ml) was poured to dissolve the mixture, and then sulfur dioxide gas was flowed for 1 min and cooled to r.t. After that, hydrochloric acid 6N (112 ml) was added to pH 3.2 and cooled over an ice bath. The crystals formed were filtered and washed using cooled distilled water (150 ml). The crystal then was dried in an oven vacuum at 50°C for 7 h to get 1.

Synthesis of the Mannich Bases 2a-e

The compounds (2a-e) were synthesized by the amino-alkylation of VA (1) through a Mannich reaction refers to the preparation of Mannich bases derivatives of curcumin analog and dehydrozingerone reported previously.¹⁵⁻¹⁶ A mixture of formaldehyde solution (80 mmol) and corresponding secondary amines (80 mmol) was mixed with 18 mL solution of 1 (20 mmol) in ethanol, heated under reflux at $\pm 50^\circ\text{C}$ during 30 min. It stirred until a completed reaction at 25°C for 12-24 h (TLC monitoring). The mixture then was refrigerated overnight, and the resulting precipitate was filtered and washed using cooled acetone. Recrystallization or column chromatography of crude product afforded pure compound 2a-e.

In vitro Antioxidant Assay

The antioxidant activities of synthesized compounds (2a-e) and vanillic acid evaluated spectrophotometrically following the procedure of free-radical DPPH scavenger and Ferric ion Reducing Activity Potential (FRAP) as reported previously, with slight modification.^{5, 17-18} Quercetin was used as a standard.

DPPH Free Radical Scavenger

To a series of solutions contained six various concentrations of the test compounds or the standard in methanol, methanolic solution of 1,1-diphenyl-2-picrylhydrazine (DPPH, 100 $\mu\text{g}/\text{ml}$) was added. The mixtures were stirred and incubated at a darkened room temperature for 30 min, and their absorbance was measured at 517 nm against the blank. The scavenging activity (%) was calculated using a formula:

$$\% \text{ Scavenging} = \frac{(\text{Ac} - \text{At})}{\text{At}} \times 100$$

Ac = the absorbance of negative control

At = the absorbance with tested compound

The IC_{50} value of scavenging capacity was determined by plotting the scavenging activity (%) versus concentration.

Ferric Reduction Activity Potential (FRAP)

The fresh FRAP reagent was prepared as reported by Hossain et al.⁵ To a series solution of various concentrations of the test compounds or the standard in methanol, the FRAP reagents were added, stirred and incubated at 37°C for 6 min. The absorbance of the intense blue color of the ferrous tripyridyl-triazine complex (Fe^{2+} -TPTZ) formed from the reduction of ferric tripyridyl-triazine complex (Fe^{3+} -TPTZ) was measured at 595 nm against the blank. The percentage of a ferrous equivalent of the compound was calculated using a formula:

$$\% \text{ Ferrous equivalent} = \frac{(\text{Absorbance of test compound})}{(\text{Absorbance of } 1000 \mu\text{M FeSO}_4)} \times 100$$

The ferric reduction activity potential (FRAP) of the compounds expressed as the concentration needed to reduce Fe^{3+} to Fe^{2+} equivalent to 50% of 1000 μM FeSO_4 (EC_{50}) was calculated by plotting the % ferrous equivalent against concentration.

Physical and Spectral Data of The Synthesized Compounds

Vanillic Acid (1)

White-yellowish powder, 91.41% yield, mp 210-212°C, Rf: 0.53, λ (MeOH): 258.5 nm and 289 nm. IR (KBr, cm^{-1}): 3483 (O-H carboxylates), 3083–2750 (O-H phenol), 1681 (C=O carboxylates), 1599 and 1433 (C=C aromatic), 1456 and 1381 (CH_3), 1112 (C-O eter), 1028 (C-O carboxylates), 918 and 761 (1,2,4-trisubstitution). $^1\text{H-NMR}$ (CD_3OD , δ ppm): 7.56 (d, 1H of H-Ar), 7.54 (s, 1H of H-Ar), 6.83 (d, 1H of H-Ar), 3.87 (s, 3H of $-\text{OCH}_3$). $^{13}\text{C-NMR}$ (CD_3OD , δ ppm): 170.02 (1C, C=O), 152.55 (1C, COCH_3 aromatic), 148.54 (1C, C-OH aromatic), 125.24 (1C, aromatic), 122.94 (1C, aromatic), 115.77 (1C, aromatic), 113.69 (1C, aromatic), 56.32 (1C, $-\text{O}-\text{CH}_3$).

2-Methoxy-4,6-bis[(dimethylamino)methyl]phenol (2a)

Brown viscous liquid, 18.23% yield, λ_{max} (MeOH): 286 nm. IR (KBr, cm^{-1}): 1600 and 1458 (C=C aromatic), 1496 and 1363 (alkyl), 1153 (C-O), 1087 (C-N). $^1\text{H-NMR}$ (CDCl_3 , δ ppm): 6.71 (d, 1H of H-Ar), 6.46 (d, 1H of H-Ar), 3.81 (s, 3H of $-\text{OCH}_3$, C_8), 3.56 (s, 2H of $\text{Ar}-\text{CH}_2-\text{N}$), 3.24 (s, 2H of $\text{Ar}-\text{CH}_2-\text{N}$), 2.26 (s, 6H of dimethylamine), and 2.16 (s, 6H of dimethylamine). $^{13}\text{C-NMR}$ (CDCl_3 , δ ppm): 147.64 and 146.28 (2C, CAr-O); 128.9 and 121.6 (2C, CAr- CH_2-N); 121.0 and 111.9 (2C, aromatic); 64.28 and 62.59 (2C, Ar- CH_2-N); 55.90 (1C, CH_3-O); 45.21 and 44.48 (4C, CH_3-N of dimethylamine).¹⁹ HR-MS (m/z) observed 239.1760 ($\text{M}+\text{H}$)⁺, calcd. masses for $\text{C}_{13}\text{H}_{23}\text{N}_2\text{O}_2$: 239.1760 (error 0.0 ppm).

2-Methoxy-4,6-di [(morpholine-4-yl) methyl]phenol (2b)

Brownish white powder, 40.68% yield, mp 110-111°C, λ_{max} (MeOH): 283.5 nm. IR (KBr, cm^{-1}): 2949 and 2906 (C-H alkyl), 1597 and 1473 (C=C aromatic), 1458 and 1365 (alkyl), 1155 (C-N) 1114 (C-O). $^1\text{H-NMR}$ (CD_3OD , δ ppm): 6.68 (d, 1H of H-Ar), 6.89 (d, 1H, of H-Ar), 3.40 (s, 2H, CH_2-N of morpholine), 3.67 (t, 4H, O- CH_2 of morpholine), 3.66 (s, 2H, CH_2-N of morpholine), 3.69 (t, 4H, O- CH_2 of morpholine), 3.83 (s, 3H of $-\text{OCH}_3$), 2.43 (t, 4H, N- CH_2 of morpholine), and 2.52 (t, 4H, N- CH_2 of morpholine). $^{13}\text{C-NMR}$ (CD_3OD , δ ppm): 124.14, 113.69, 128.74 and 122.44 (4C, CAr), 146.68 and 148.86 (2C, CAr), 60.72 (1C, CH_2-N of morpholine), 54.06 (2C, N- CH_2 of morpholine), 67.63 (2C, O- CH_2 of morpholine), 64.19 (1C, CH_2-N of morpholine), 54.52 (2C, N- CH_2 of morpholine), 67.76 (2C, O- CH_2 of morpholine), 56.45 (1C, CH_3-O , C_{23}).¹⁹ HR-MS (m/z) observed 323.1978 ($\text{M}+\text{H}$)⁺, calcd masses for $\text{C}_{17}\text{H}_{27}\text{N}_2\text{O}_4$: 323.1892 (error 2.2 ppm).

3-[(Diethylamino)methyl]vanillic Acid (2c)

Dark brown viscous liquid, 12.26% yield, λ_{max} (MeOH): 253.5 nm and 289 nm. IR (KBr, cm^{-1}): 3583–3017 (O-H), 2974 (C-H), 1564 and 1390 (COO^- carboxylate), 1496 and 1284 (C=C aromatic), 1458 and 1246 (alkyl), 1084 (C-N). $^1\text{H-NMR}$ (CD_3OD , δ ppm): 7.61 (s, 1H of H-Ar), 7.53 (d, 1H of 4.19 (s, 2H of $\text{Ar}-\text{CH}_2-\text{N}$), 3.89 (s, 3H of $-\text{OCH}_3$), 3.06 (q, 4H, $-\text{CH}_2-\text{N}$ of diethylamine), and 1.30 (t, 6H, C- CH_3 of diethylamine). $^{13}\text{C-NMR}$ (CD_3OH , δ ppm): 174.26 (1C, C=O carboxylate), 150.60 and 148.51 (2C, CAr-O), 129.40, 125.83, 117.26, and 114.46 (4C, CAr), 56.45 (1C, O- CH_3), 53.51 (1C, $\text{Ar}-\text{CH}_2-\text{N}$), 48.04 (2C, $-\text{CH}_2-\text{N}$ of diethylamine), 9.72 (2C, $-\text{CH}_3$ of diethylamine).¹⁹ HR-MS (m/z) observed 254.1392 ($\text{M}+\text{H}$)⁺, calcd masses for $\text{C}_{13}\text{H}_{20}\text{NO}_4$: 254.1314 (error 0.03 ppm).

5-[(4-Methylpiperazin-1yl)methyl]vanillic Acid (2d)

Brownish white crystal needle, 36.58% yield, mp 127-129°C, λ_{max} (MeOH): 345.5 nm. IR (KBr, cm^{-1}): 2974 - 2825, (C-H alkyl), 1608 and 1444 (COO^- anionic), 1564 and 1462 (C=C aromatic), 1494, and 1388 (alkyl), 1111 (C-O), and 1084 (C-N). $^1\text{H-NMR}$ (CD_3OD , δ ppm): 7.51 (d, 1H of H-Ar), 7.45 (d, 1H of H-Ar), 3.88 (s, 3H of $-\text{OCH}_3$), 3.79 (s, 2H of $\text{Ar}-\text{CH}_2-\text{N}$), 2.71 (m, 8H, $-\text{CH}_2-\text{N}-\text{CH}_2$ of piperazine), and 2.42 (s, 3H, N- CH_3 of piperazine). $^{13}\text{C-NMR}$ (CD_3OD , δ ppm): 174.02 (1C, C=O carboxylate), 150.16 and 148.28 (2C, CAr-O), 128.22, 125.13, 121.51, and 113.48 (4C, CAr), 59.34 (1C, OCH_3), 56.43 (1C,

Ar-CH₂-N), 55.20 (2C, C-N of piperazine), 52.44 (2C, C-N of piperazine), 45.27 (1C, N-CH₃ of piperazine).¹⁹ HR-MS (m/z) observed 281.1497 (M+H)⁺, calcd masses for C₁₄H₂₁N₂O₄: 281.1423 (error - 1.4 ppm).

5-[(Pyrrolidin-1-yl)methyl]vanillic Acid (2e)

Brownish granule, 31.19% yield; mp 195-198°C, λ_{max} (MeOH): 243.5 nm. IR (KBr, cm⁻¹): 3620-2904 (OH), 1667 (C=O carboxylate), 1456 (alkyl), 1113 (C-O), 1088 (CN), 1028 (C-OH carboxylic). ¹H-NMR (CD₃OD, δ ppm): 2.05 (p, 4H, CH₂ of pyrrolidine), 3.2 (t, 4H, N-CH₂ of pyrrolidine), 3.89 (s, 3H of OCH₃), 4.23 (s, 2H of Ar-CH₂-N), 7.59 ppm (d, 2H of HAr). ¹³C-NMR (CD₃OH, δ ppm): 24.04 (2C, CH₂ of pyrrolidine), 54.46 (2C, CH₂ of pyrrolidine), 55.05 (1C, CH₂-Ar), 56.46 (1C, CH₃-O-Ar), 114.35, 118.66, 125.89 and 129.79 (4C, CAr), 148.41 and 149.96 (2C, CAr-O), 174.62 (1C, C=O carboxylate).¹⁹ HR-MS (m/z) observed 252.1238 (M+H)⁺, calcd masses for C₁₃H₁₈NO₄: 252.1157 (error 0.8 ppm).

RESULTS AND DISCUSSION

Chemistry

Five new Mannich bases compounds from vanillic acid (**2a-e**) were synthesized successfully. The synthesis methods were summarized in Scheme-1. The substitutions of dimethylamine and morpholine Mannich bases were obtained di-Mannich bases of 2-methoxyphenol (**2a-b**), while the substitution of diethylamine, N-methylpiperazine, and pyrrolidine Mannich bases was obtained Mannich bases derivatives of vanillic acid (**2c-e**).

Scheme-1: The Synthesis of Title Compounds

The disappearance of a sharp peak of OH phenolic in the infrared spectra of prepared compounds at 3483 cm⁻¹ indicating hydrogen bonding formation between OH and N of the Mannich base.²⁰ The bands at the range of 1084-1155 cm⁻¹ conform to C-N, C-O phenol, and C-O ether. In the infrared spectra of **2a** and **2b** were observed the disappearance of the C=O carboxylate peak at 1564-1667 cm⁻¹ indicated the occurrence of decarboxylation. In ¹H-NMR spectra, the only two remaining protons in the aromatic rings

appeared as singlet or doublet peaks with $J = 2-3$ Hz in range of δ 6.46 - 7.56 ppm indicated that the Mannich base group substituted a proton at the ortho position relative to the OH phenolic group of **1**. In $^1\text{H-NMR}$ spectra of **2a** was appeared two singlet peaks equal to 6H of N-CH_3 at 2.16 and 2.26 ppm respectively, and two singlet peaks equivalent to 4H of $\text{Ar-CH}_2\text{-N}$ at 3.24 and 3.56 ppm respectively. While in **2b** was appeared two triplet peaks from 4H of N-CH_2 morpholine at 2.43 and 2.52 ppm respectively, two triplet peaks from a 4H of O-CH_2 morpholine at 3.67 and 3.69 ppm, and two singlet peaks equivalent to 2H of $\text{Ar-CH}_2\text{-N}$ at 3.40 and 3.66 ppm respectively. The data indicated the occurrence of two substitutions of the Mannich base at ortho and para position relative to the hydroxyl group by replacing the H and carboxylic group respectively of **1**.¹⁹ The structures supported by $^{13}\text{C-NMR}$ spectra exhibited the disappearance of carbon of C=O and expected number and types of carbons: aromatic and aliphatic moieties. This result was in line with the result of a Mannich reaction of vanillic acid (**1**) using diethylamine as secondary amine previously reported.²¹ However, our study showed that a Mannich reaction of **1** using diethylamine, 1-methylpiperazine, and pyrrolidine as secondary amine resulted in one Mannich base substitution at the ortho position relative to the hydroxyl group of **1** (compound **2c-e**). The structures supported by $^{13}\text{C-NMR}$ spectra exhibited the appearance of carbon of C=O and expected number and types of carbons: aromatic and aliphatic moieties. All the structures of the synthesized compound were further supported by mass spectra, which provided the molecular masses of the compounds. These values were in complete conformity with the structure expected.

Antioxidant Activity

Figures-1 and 2 presented the relation between concentrations of the title compounds and their ability as DPPH radical scavenger and ferric ion reductant. The activities were concentration-dependent. The IC_{50} or EC_{50} values of their activities were displayed in Table-1. The compounds **2a-e** exhibited moderate to weak antioxidant activity compared to quercetin used as a standard. The IC_{50} values of radical scavenging activity were in the range of 26.55 - 651.16 μM . In this series, the compound 5-(pyrrolidin-1-ylmethyl)vanillic acid (**2e**) showed the best free-radical scavenger activity. However, the activity was lower compared to quercetin ($\text{IC}_{50} = 6.84 \mu\text{M}$). The radical scavenger activities of the Mannich bases were higher than that of the vanillic acid ($\text{IC}_{50} = 11,608.65 \mu\text{M}$). These results were in line with several Mannich bases of phenolic compounds reported earlier.¹¹⁻¹²

Fig-1: The Concentrations and DPPH Radical Scavenging (%) Curve of Synthesized Compounds

The electron-donating groups directly attached to the aromatic ring is one factor that can enhance free radical scavenger.²² The ferric reduction activity of synthesized compounds (**2a-e**) expressed as EC_{50} values were in the range of 17.67 - 60.65 μM . In this series, the compound 2-[(diethylamino)methyl]vanillic acid (**2c**) showed the best ferric reductant activity. However, the activity was lower compared to quercetin used as a standard ($\text{EC}_{50} = 2.71 \mu\text{M}$).

Fig.-2: The Concentrations and Ferrous Equivalent (%) Curve of Synthesized Compounds

Table-1: Antioxidant Activity of Synthesized Compounds (2a-e)

Compound	R ₁	R ₂	DPPH	FRAP
			IC ₅₀ (μM) ⁽¹⁾	EC ₅₀ (μM) ⁽¹⁾
1	C=O(OH)	H	11,608.65	21.40
2a			66.87	45.10
2b			38.33	60.65
2c	C=O(OH)		112.36	17.67
2d	C=O(OH)		651.16	22.51
2e	C=O(OH)		26.55	22.09
Quercetin			6.84	2.71

⁽¹⁾ mean of triplicate determinations

The ferric reduction activity potential of the Mannich bases was relatively the same or lower than that of vanillic acid ($EC_{50} = 21.40 \mu M$). This result was in agreement with the ferric reductant activity of the Mannich base of 1,3,4-oxadiazole reported earlier.²³ The low ferric reducing activity of the compound may be caused by the method was unable to detect slowly-reacting phenolic compound.²⁴ The Mannich

base group was decreasing the reaction rate. The antioxidant characteristics of phenolic compounds supported by the data showed that the compounds were more active as a radical scavenger than as ferric reducing agent.²⁵

CONCLUSION

Five new Mannich base compounds derived from vanillic acid were prepared successfully. The compound 5-(pyrrolidin-1-ylmethyl)vanillic acid (**2e**) showed the best free-radical scavenger activity, while compound 2-[(diethylamino)methyl]vanillic acid (**2c**) showed the best as a ferric reductant. Mannich bases enhance the free-radical scavenger activity but decreasing the ferric reduction potential of the parent compounds. The compounds were found to be moderate antioxidant agents.

ACKNOWLEDGMENT

This work was funded by Universitas Indonesia, Depok, Indonesia (Research Grant, No.: 1768/UN2.R3.1/HKP.05.00/2018). Thanks to the Chemistry Department, Bandung Institute of Technology (ITB), Indonesia, for providing a recording of NMR and mass spectra.

REFERENCES

1. J. Manivannan, T. Silambarasan, J. Shanthakumar, N. Suganya and S. Kanchana, 2016, Role of Antioxidants in Human Health. In: M. Hegde, A. Zanwar, S. Adekar (Eds), Omega-3 Fatty Acids, Springer International Publishing, Switzerland, pp. 501-512, DOI: 10.1007/978-3-319-40458-5
2. Z. Huyut, F. Beydemir and E. Gülçin, *Biochemistry Research International*, ID 7616791, 10 pages, (2017), DOI: 10.1155/2017/7616791
3. K.K. Kattappagari, C.S.R. Teja, R.K. Kommalapati, C. Poosarla, S.R. Gontu and B.V. Reddy, *Journal of Orofacial Sciences*, **7**, 71 (2015), DOI: 10.4103/0975-8844.169745
4. A. Yadav, R. Kumari, A. Yadav, J.P. Mishra, S. Srivatva and S. Prabha, *Research in Environment and Life Sciences*, **9(11)**, 1328 (2016).
5. M.B. Hossain, A.B. Martin-Diana, N.P. Brunton, C. Barry-Ryan, and M. Wilkinson, *Rasayan Journal of Chemistry*, **1(4)**, 751(2008).
6. N. Balasundram, K. Sundram and S. Samman, *Food Chemistry*, **99**, 191(2006), DOI: 10.1016/j.foodchem.2005.07.042
7. R. Manuja, S. Sachdeva, A. Jain and J. Chaudhary, *International Journal of Pharmaceutical Sciences Review and Research*, **22(2)**, 109(2013).
8. P.S.M. Prince, S. Rajakumar and K. Dhanasekar, *European Journal of Pharmacology*, **668**, 233 (2011), DOI: 10.1016/j.ejphar.2011.06.053
9. A. Vinoth and R. Kowsalya, *Journal of Cancer Research and Therapy*, **4(6)**, 1285 (2018), DOI: 10.4103/0973-1482.191057
10. P.S. Misra, P. Shanmugasundaram, R. Chaudhary and M.V. Aanandhi, *Rasayan Journal of Chemistry*, **3(1)**, 51(2010).
11. J. Untung, I. Iskandarsyah and H. Hayun, *Molbank*, 2017, M949(2017), DOI:10.3390/M949
12. G. Roman, *European Journal of Medicinal Chemistry*, **89**, 743(2015), DOI: 10.1016/j.ejmech.2014.10.076
13. B.P. Bandgar, S.A. Patil, R.N. Gacche, B.L. Korbad, B.S. Hote, S.N. Kinkar, S.S. Jalde, *Bioorganic & Medicinal Chemistry Letters*, **20(2)**, 730 (2010), DOI: 10.1016/j.bmcl.2009.11.068
14. I.A. Pearl, *Organic Syntheses*, **30**, 101(2003), DOI: 10.15227/orgsyn.030.0101
15. T. N. Putri, A. Bachtiar and H. Hayun, *Journal of Applied Pharmaceutical Sciences*, **8(5)**, 19(2018), DOI: 10.7324/JAPS.2018.8503
16. H. Hayun, A. Arrahman, E.M. Purwati, A. Yanuar, F. Fortunata, F. Suhargo, D.W. Syafiqah, C. Ignacia, A.R. Novalia, *Journal of Young Pharmacist*, **10(2)**, Suppl s6(2018), DOI: 10.5530/jyp.2018.2s.6
17. O. Atolani, C.B. Adeosun, A.P. Oluyori and J. Olota, *Rasayan Journal of Chemistry*, **12(3)**, 1052 (2019), DOI: 10.31788/RJC.2019.1235122
18. T. M. Siregar, E. Budianto, H. Cahyana and W. Wibowo, *Rasayan Journal of Chemistry*, **11(4)**, 1765 (2018), DOI:10.31788/RJC.2018.1143051

19. R.M. Silverstein, F.X. Webster, D.J. Kiemle, Spectrometric Identification of Organic Compounds, 7th ed., John Wiley & Sons, Inc., New York, NY, USA (2005).
20. C. Dank, S. Felsinger, B. Kirchknopf, M. Mastalir, H. Kählig, A. Roller et al., *Molecules*, **20**, 1686 (2015), DOI:10.3390/molecules20011686
21. H. Mikawa, K. Sato and C. Takasaki, *Bulletin of the Chemical Society of Japan*, **29(2)**, 259 (1956), DOI: 10.1246/bcsj.29.259
22. L.V.B. Hoelz, B.A.C. Horta, J.Q. Araújo, M. Albuquerque, R.B. de Alencastro and J.F.M. da Silva, *Journal of Chemistry and Pharmaceutical Research*, **2(5)**, 291(2010).
23. L. Ma, Y. Xiao, C. Li, Z.L. Xie, D.D. Li, Y.T. Wang, H.T.Ma, H.L. Zhu, M.H. Wang, Y.H. Ye, *Bioorganic & Medicinal Chemistry*, **21(21)**, 6763 (2013), DOI: 10.1016/j.bmc.2013.08.002
24. S. Dontha, *Asian Journal of Pharmaceutical and Clinical Research*, **9 (Suppl. 2)**, 14(2016). DOI: 10.22159/ajpcr.2016.v9s2.13092
25. J.N. Veljković, A.N. Pavlović, S.S. Mitić, S.B. Tošić, G.S. Stojanović, B.M. Kaličanin, D.M. Stanković, M.B. Stojković, M.N. Mitić, J.M. Breanović, *Journal of Food and Nutrition Research*, **52 (1)**, 12(2013).

[RJC-5300/2019]