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ANTIBACTERIAL POTENTIAL OF *Nicotiana tabacum* LEAVES EXTRACTS AND THEIR ANTIOXIDANT ACTIVITIES

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Abstract

Nicotiana tabacum, recognized as tobacco, has been reported its secondary metabolites and biological activities in the past decade. Madura is one of the islands in Indonesia that produces *N. tabacum* rapidly. In Madura, *N. tabacum* is called as bhekoh. Previously, antioxidant activity of *N. tabacum* leaves extracts has been reported. However, the extracts showed very low antioxidant activity due to low of the stock concentration. Accordingly, this research was aimed at the antibacterial and antioxidant evaluation of *N. tabacum* leaves from Madura with improvement of the concentration. Furthermore, *N. tabacum* leaves were extracted by using different solvents including *n*-hexane, dichloromethane, ethyl acetate, acetone, and methanol. The extracts have been evaluated their antibacterial activities by using colorimetric resazurin microtiter assay (REMA) method. The extracts have been assayed their antibacterial activities against both gram-positive and negative bacteria. While, the antioxidant evaluation has been determined by using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method. The result showed that the extracts have fine antibacterial and antioxidant activities. The methanol extract presented antioxidant activity with IC_{50} of 12.12 ppm compared to gallic acid as a standard. Furthermore, the acetone extract showed the highest antibacterial activity against *Bacillus subtilis*, *Propionibacterium acnes*, and *Salmonella typhi* with MIC of 0.31, 0.63, and 0.63 mg/mL, respectively, compared to ampicillin as a standard. In conclusion, the acetone and methanol extracts have the highest antibacterial and antioxidant activities. Therefore, our result suggested acetone and methanol extracts of *N. tabacum* leaves as antibacterial and antioxidant agents.

INTRODUCTION

Antibacterial is a bioactive substance with characteristics of either bactericidal or bacteriostatic [1]. Bactericidal are biological or chemical substances that can kill the growth of bacteria [2]. Meanwhile, bacteriostatic antibacterial does not literally kill the bacteria. It just inhibits the growth of bacteria. Previously, we have reported some natural products as antibacterial agents including *Ananas comosus* [3] and postpartum herbal medicine [4]. The used bacterial isolates were both gram-positive and negative bacteria such as *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Rhodopseudomonas palustris*, *Ralstonia pickettii*, and *Staphylococcus epidermidis*. Furthermore, both of gram-positive and negative bacteria have a difference of bacteria cell structure or called as the bacteria cell envelope [5]. The gram-positive bacteria have no outer membrane but they have a thick layer of peptidoglycan. While the gram-negative bacteria have an outer membrane cell containing lipopolysaccharide and a thin peptidoglycan. This cell structure affects the ability of antibacterial substances to work against bacteria [3]. Focus on the finding of antibacterial agents from natural products, this research aims at an *in vitro* antibacterial assay on *Nicotiana tabacum*. The previous study reported that *N. tabacum* has many secondary metabolites and also its biological activities belong to antibacterial activity [6]. The antibacterial activity was reported by using an agar well diffusion assay. The result reported that the ethyl acetate extract from *N. tabacum* leaves showed inhibition against *S. aureus* with area inhibition of 159.9±11.31 mm^2. In addition, the methanol extract presented antibacterial activity against *S. dysenteriae* with MIC value of 1.56 mg/mL. Based on the previous research, *N. tabacum* leaf extracts have the potential as antibacterial agent.

Antioxidants is a free radical scavenging substance protecting on human body from dangerous radicals. One of the dangerous radicals is reactive oxygen species (ROS) [7]. ROS is produced by air pollution, a wrong lifestyle, junk food, etc. Moreover, ROS is very reactive to make cell death because the oxygen species has unpaired electron. Hence, this oxygen species attacks reactively various biomolecules in the body such as DNA, proteins, and lipids. In the body, ROS could be found as superoxide anion (SOA). Normally, SOA could be blocked by the body's defense system as known as an enzyme or internal antioxidant. Furthermore, SOA could be converted to unreactive species through superoxide dismutase (SOD) and catalase enzymes [8]. Here is the following step, first is SOD-catalyzed SOA to be less toxic H_2O_2. Before H_2O_2 changes to reactive hydroxyl radicals with the presence of Fe^{2+}, H_2O_2 was decomposed by catalase into harmless substances namely H_2O and O_2. As mentioned before, an internal antioxidant is also not enough to prevent ROS. Therefore, an external antioxidant is needed to support either enzymes or internal antioxidants against ROS. The external antioxidants are obtained from healthy food and supplements with the presence of natural products. Based on our previous research, many natural products have been reported as antioxidants such as *Nicotiana tabacum* [9], *Momordica charantia* [10], *Glycine max* [11], *Chromolaena odorata* [12], *Muntingia calabura* [13], *Ananas comosus* [3], and other Indonesian herbal medicines [4, 14].

N. tabacum (Solanaceae), also known as tobacco, is a cultivated plant in tropical and subtropical regions. In Indonesia, this plant is recognized as *Tembakau*. Because of tropical conditions, Indonesia has been one of the top ten countries in producing tobacco in the world since 1990. In this research, we focus on tobacco sample from Madura. Madura is one of islands in western Indonesia producing tobacco rapidly. In Madura, tobacco was known as *Bekoh*. According to literature studies, there is no report about the potency of pharmacological effects of *N. tabacum* collected from Indonesia. Indeed, this condition gives a chance for Indonesian scientists to reveal its secondary metabolite and biological activity from *N. tabacum*. Based on our reported review, *N. tabacum* has biological activities as antioxidant, antibacterial, antiviral, anti-HIV, anti-inflammatory, anti-poliferative, anti-termite, anti-parasitic, and also cytotoxicity effects [6]. Except nicotine **(1)**, there were other secondary metabolites that have been reported. They are cembranoids, flavones, and sesquiterpenes [6]. Cembranoids and flavonoids are the main secondary metabolite. In addition, cembratine **(2)** has a good bioactivity as anticancer against a HepG2 cell line [15], licoisoflavone **(3)** has the highest anti-tobacco virus mosaic (TMV) [16], and also sesquiterpene **(4)** presented anti-TMV activity [17]. The chemical structures of nicotine **(1)**, cembratine **(2)**, licoisoflavone **(3)**, and sesquiterpene **(4)** are presented in **Figure 1**.

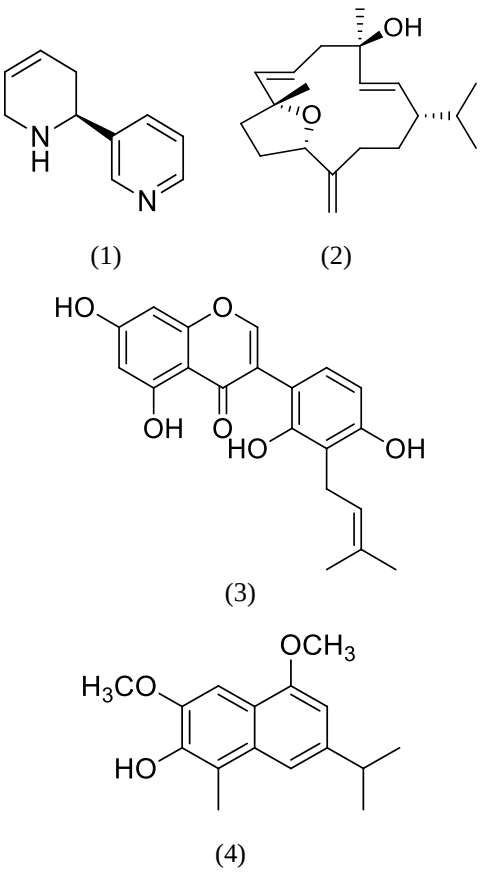

Figure 1. The chemical structures of nicotine **(1)** [6], cembratine **(2)** [15], licoisoflavone **(3)** [16], and sesquiterpene **(4)** [17].

According to our previous reported study, *N. tabacum* has secondary metabolites and their biological activities [6]. There are about 37 isolated compounds that have been reported from a whole part of *N. tabacum* included leaves, stems, roots, and also flowers. For instance, compound of **(2)** was isolated from dichloromethane extract of *N. tabacum* flowers with anticancer activity (IC_{50} 14.38 µM). Furthermore, compounds of **(3)** and **(4)** were isolated from the stems and roots. In addition, the phytochemical profiles and antioxidant activity of *N. tabacum* leaf extract from Madura have been reported previously [9]. The phytochemical screening presented that *N. tabacum* leaf extract

has alkaloid, flavonoid, triterpenoid, phenol, and saponin. Based on the phytochemical screening, a total phenolic content (TPC) was also determined. The result showed that the extracts have a good TPC with a range of 77.70 – 98.09 µg GAE/mg. However, the antioxidant activity has been presented with no significantly inhibitory activity against 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical. Because of the minimum extract concentration, the extracts showed a lower inhibitory activity against DPPH radicals with a range value of 20.92 – 30.87% [9]. Therefore, the extract concentration is recommended to be improved.

In this present study, we focus on antibacterial and antioxidant assay of *N. tabacum* leaves extracts with difference solvents. Various solvents are polar and nonpolar solvents included *n*-hexane, dichloromethane, ethyl acetate, acetone, and methanol. Every extract has been determined a value of IC_{50} with DPPH assay. The difference with the previous research [9] is the stock extracts concentration. The concentration has been improved from 31.95 to be 319.46 µg/mL and also the methanol extract has been evaluated. Meanwhile, antibacterial activity has been evaluated a value of MIC through colorimetric resazurin microtiter assay (REMA) method. In our assay system, REMA method used the isolate bacteria with gram-positive and negative bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Propionibacterium acnes*.

EXPERIMENT

Material

The leaves of *N. tabacum* were collected from Pamekasan, Madura, East Java, Indonesia (7°10’12” S, 113°28’12” E) on December 2022. The plant was identified by PIPOT Faculty of Pharmacy, Surabaya University with a number of 1495/DT/III/2023. The organic solvents in technical grade used for extraction are n-hexane, dichloromethane, ethyl acetate, acetone, and methanol. The used bacteria strain are *Bacillus subtilis* (ATCC-19659), *Staphylococcus aureus* (ATCC-29213), *Salmonella typhimurium* (FNCC-0050), *Propionibacterium acnes* (ATCC-6919), and *Pseudomonas aeruginosa* (ATCC-27853). The bacteria medium are Mueller Hinton Agar (MHA) (Himedia, M173-500G), and Mueller Hinton Broth (MHB) (Himedia, M391-500G). The chemicals for REMA are resazurin (Aldrich, R7017-1G) and McFarland standard by Himedia (R092-1NO). The used chemicals for antioxidant assay are DPPH (Aldrich, 1898-66-4), methanol (Merck, 1.06009.25000), gallic acid (Aldrich, 149-91-7), DMSO (Merck, 1.02952.1000), and aquadest.

Instrumentation

The instrument used for the antioxidant assay is the DLAB SP-UV1000 Spectrophotometer. The instrument for evaporating the extract solvent is a rotary evaporator B-ONE RE-1000 VN.

Procedure

Extraction

The collected *N. tabacum* leaves were dried for 30 days at room temperature. The dried leaves (30 g) ware extracted with various organic solvents. The solvents are n-hexane, dichloromethane, ethyl acetate, acetone, and methanol in 500 mL respectively as reported before [10]. By using maceration technique for 24 hours, *N. tabacum* leaves could be extracted simply.

Antibacterial Assay

Antibacterial assay was conducted by microdilution method namely REMA with resazurin indicator [18]. REMA is based on an ability of cell to reduce a resazurin compound with blue colour to be a resorufin compound in pink colour biologically. The best tool of microdilution method is 96-well plates to save amount of extract. Furthermore, antibacterial assay of *N. tabacum* extracts was done by the following steps [18]:

Extract preparation

Extracts of *n*-hexane, dichloromethane, ethyl acetate, acetone, and methanol were prepared on DMSO with concentration of 100 mg/mL. This stock extract solution was dissolved by using aquadest at concentration of 20 mg/mL. This concentration was used as the highest concentration on the antibacterial activity assay.

Medium preparation

In this assay system, the bacterial medium was MHA for re-culture bacterial and MHB for the tested bacterial suspension. MHA was prepared

with medium of 9.5 g on aquadest of 250 L. While, MHB was prepared with medium of 4,2 g on aquadest of 200 L. In addition, MHB at concentration of 3.3x was also prepared. All prepared medium was sterilized by using autoclave at temperature of 121°C for 15 minutes.

Bacterial culture preparation

The isolated bacteria were included gram-positive and negative bacteria in this antibacterial assay. The isolate bacteria are *B. subtilis*, *S. aureus*, *S. typhimurium*, *P. aeruginosa*, and *P. acnes*. Previously, the tested bacteria were cultured on MHA medium and incubated for 1x24 hours at temperature of 37°C. Before antibacterial assay with REMA method has been started, the bacterial suspension in MHB was prepared. The bacterial suspension was made by using the cultured bacteria in MHA and compared to a standard of McFarland 0.5. This compared bacterial suspension aims to get the tested bacteria at concentration of $5x10^6$ cfu/mL.

Resazurin reagent preparation

Resazurin stock solution was prepared on aquadest at concentration of 0.01 g/mL. Before REMA has been started, the stock solution was diluted to be a working solution with ratio of 1:10.

Colorimetric Resazurin Microtiter Assay (REMA)

As mentioned before, REMA with resazurin as an indicator was used to this antibacterial assay system. First, the prepared extract was added to first hole of 96-well plates with volume of 50 µL at concentration of 20 mg/mL. On the next hole, the extract solution was added with twice dilution by using sterile aquadest until the 8^{th} of hole. After that, the prepared resazurin solution and MHB 3.3x was also added to well hole with volume of 10 µL and 30 µL, respectively. Next, the bacterial suspension ($5x10^6$ cfu/mL) with volume of 10 µL was added on the well hole. Furthermore, the well plates were closed and incubated for 24 hours at temperature of 37°C. Antibacterial activity on the well plates could be identified because of indicator discoloration. If there is the indicator discoloration from blue to pink, it means the bacteria still growth. While, if there is no indicator discoloration, it means the extract could inhibit the bacteria activity. Minimum inhibitory concentration (MIC) value has been evaluated by the lowest concentration of the tested extract. A negative control was prepared with no extract. It was used blanko with an aquadest and the solvents. An ampicillin was used as a positive control.

In vitro DPPH radical scavenging assay

Antioxidant activity of N. tabacum extracts was conducted by in vitro DPPH radical scavenging assay based on the previous reported research with slightly modification [7]. First is preparation of the stock extract solution of every stock extract solution prepared by using methanol with concentration of 10 mg/mL. After that, a working solution of DPPH was dissolved on methanol with concentration of $6x10^{-5}$ M. Furthermore, the test solution was conducted by adding extract and DPPH solution with volume of 49.5 µL and 1.5 mL, respectively. This test solution was mixed and incubated during 20 minutes at room temperature in dark place. The mixed test solution was determined its absorbance value or called as sample absorbance (As) by using UV-Vis spectrophotometer with wavelength of 517 nm. Meanwhile, blank absorbance (Ab) was measured with DPPH solution and methanol only. Gallic acid was used as a standard or positive control. The inhibitory activity was calculated by using equation (1). After that, an IC_{50} value was determined by analysis of either regression equation linear or polynomial.

$$\text{Inhibitory activity (\%)} = \left[\frac{(\text{Ab} - \text{As})}{\text{Ab}}\right] \times 100\% \quad (1)$$

Statistical Analysis

The data were analysed by using a student's t-test and the significant value was set as $p < 0.05$. The graphical representation was design by Microsoft Excel Professional Plus 2016.

RESULT AND DISCUSSION

Extraction

The extracts of N. tabacum leaves from various solvents have been obtained. Among the extracts, a methanol extract of N. tabacum leaves has the highest yield. The yields of the five crude extracts were obtained the n-hexane extract yield of 1.77%, the dichloromethane extract yield of 4.33%, the ethyl acetate extract yield of 4.53%, the acetone extract yield of 2.17%, and the methanol extract yield of 6.78%.

Antibacterial Activity

Antibacterial activity of *N. tabacum* leaves extracts was evaluated through REMA. The antibacterial activities of five crude extracts against gram-positive (*B. subtilis*, *S. aureus*, and *P. acnes*) and negative bacteria (*S. typhimurium* and *P. aeruginosa*) were shown in **Table 1**. Ampicillin was used as a standard. There were eight concentration which are diluted at 10.0, 5.00, 2.50, 1.25, 0.63, 0.32, 0.16, 0.08 mg/mL. The lower inhibitory concentration, the higher antibacterial activity. According to the result, the five extracts have not significantly shown antibacterial activity against *S. aureus* and *P. aeruginosa*. The acetone extract showed the highest antibacterial activity against *B. subtilis*. The acetone and ethyl acetate extracts also showed the higher antibacterial activity against *S. typhimurium* than that of ampicillin as a standard. Furthermore, the ethyl acetate extract also showed the highest inhibitory activity against *P. acnes*. It means the acetone and ethyl acetate extracts have good antibacterial activities against both gram-positive and negative bacteria.

Table 1. Antibacterial activities of *N. tabacum* leaves extracts.

No	Extracts	MIC* (mg/mL)				
		B. subtilis	*S. aureus*	*S. typhimurium*	*P. aeruginosa*	*P. acnes*
1	*n*-Hexane	2.50^{aA}	$>10.0^{bC}$	10.0^{bC}	10.0^{bC}	2.50^{aA}
2	Dichloromethane	1.25^{cB}	$>10.0^{bC}$	10.0^{bC}	10.0^{bC}	0.63^{dG}
3	Ethyl acetate	10.0^{bC}	$>10.0^{bC}$	0.63^{dG}	$>10.0^{bC}$	0.08^{eF}
4	Acetone	0.31^{fD}	$>10.0^{bC}$	0.63^{dG}	10.0^{bC}	0.63^{dG}
5	Methanol	2.50^{aA}	$>10.0^{bC}$	1.25^{cB}	10.0^{bC}	1.25^{cB}
6	Ampicilin	0.31^{fE}	$<0.08^{eF}$	2.50^{aA}	5.00^{gH}	0.63^{dG}

*Each value represents in duplo tests. Data followed by the same minor and capital letters on each row and column respectively are significantly different (p<0.05).

REMA, called as resazurin microtiter assay, is one of *in vitro* antibacterial assay with colorimetric microtiter approach. This assay is simple, rapid, reliable, and sensitive to be used as an antibacterial assay of natural products [18]. In this assay system, resazurin was used as an indicator. Furthermore, resazurin is either an oxidation or reduction indicator used for the evaluation of microbial growth. Moreover, resazurin is a dye indicator, known as Alamar Blue, that could be reduced by aerobic and facultative anaerobic microorganisms [19]. The mechanism reaction of resazurin is the reduction of resazurin (blue color) to be resorufin (pink color) irreversibly as presented in **Figure 2**. The form of resorufin indicates the presence of living microorganisms. In our assay system, the used bacterial strains are aerobic namely *P. aeruginosa* and facultative anaerobic namely *B. subtilis*, *S. aureus*, *P. acnes*, and *S. typhimurium*. However, based on our result, the extracts could not inhibit *P. aeruginosa* significantly. It might be caused an aerobic bacterial is not easy to be inhibited because of the existence of oxygen. An aerobic bacterial means the bacterial strain can growth in an oxygen area only. Based on mechanism reaction in **Figure 2**, the transformation of resazurin to be resorufin is affected on the growth of *P. aeruginosa* massively.

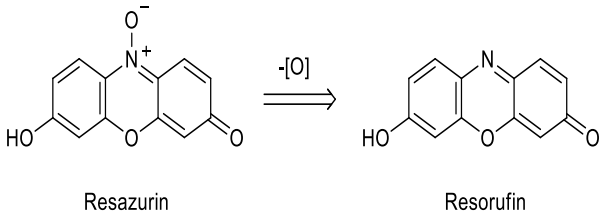

Figure 2. The mechanism reaction of resazurin [19].

Interestingly, the ethyl acetate extract has the higher antibacterial activity against *S. typhimurium* and *P. acnes* than that of ampicillin. Moreover, ampicillin was not enough strong to inhibit gram-negative bacteria (*S. typhimurium* and *P. aeruginosa*). This result is related to the structure of cell envelope. A gram-negative bacterial has an outer membrane to protect peptidoglycan [5]. Therefore, this bacterial is very strong against antibacterial substance. Meanwhile, A gram-positive bacterial has no outer membrane. Hence, the peptidoglycan layer could be destroyed by antibacterial easily. That is also related to ampicillin activity on this result. Ampicillin has strong antibacterial activity against gram-positive bacteria (*B. subtilis*, *S. aureus*, and *P. acnes*). Also, the acetone extract has good antibacterial activities against *B. subtilis*, *S. typhimurium*, and *P. acnes*. However, the previous research reported that the

ethyl acetate extract has an inhibitory activity significantly against *S. aureus* by using different method namely agar well diffusion assay due to difference of plant collected location [20, 21]. Based on this result, both ethyl acetate and acetone extracts of *N. tabacum* leaves from Madura has potential as antibacterial.

In addition, both the ethyl acetate and acetone extracts have good inhibitory activities against *S. typhimurium* and *P. acnes*. *S. typhimurium* or commonly known as *S. typhi* is one of gram-negative bacteria which is characterized as an anaerobic facultative [22]. An anaerobic facultative bacterial means the bacterial can life in a little oxygen place. Moreover, *S. typhi* is also one of the pathogen bacteria which caused typhoid fever [23]. Typhoid disease was commonly found in children fever because of infected foods and drinks. If *S. typhi* is consumed and arrived at small intestine, it makes clinical symptoms such as fever, languid, headache, constipation, bradycardia, and myalgia [24]. In long term, typhoid fever can break intestinal, liver and also brain tissue [25]. While, *P. acnes* is one of gram-positive bacteria which is considered as an aerobic or anaerobic facultative. Furthermore, *P. acnes* is also one of normal floral on human skin which caused acne [26, 27].

Antioxidant Activity

Antioxidant activity of *N. tabacum* leaves extracts was conducted by DPPH radical scavenging assay. DPPH radical scavenging screening of the five extracts and a standard gallic acid was presented as an inhibitory activity (%) in **Figure 3** and summarized at **Table 2**. According to the data, the five extracts have the inhibitory activities with range value of 62.07 – 73.16% compared with gallic acid (88.72%). The result showed that *N. tabacum* leaves extracts have potential against DPPH radical. Among five extracts, the methanol extract has the highest inhibitory activity. Because of the inhibitory activity more than 50%, all of extracts were determined their IC_{50} value. The value of IC_{50} was calculated by analysis regression either linear or polynomial regressions based on graphic in **Figure 4**. Furthermore, an IC_{50} was determined with equation regression when the inhibition of 50%. The IC_{50} data of gallic acid, *n*-hexane, dichloromethane, ethyl acetate, acetone, and methanol extracts are 0.41, 212.22, 205.54, 222.26, 228.60, 12.12 µg/mL, respectively. According to the result, the methanol extract has the highest IC_{50} value with value of 12.12 µg/mL with the very strong category [28]. The smaller IC_{50} value, the better antioxidant activity. Because of no more dose to inhibit free radical.

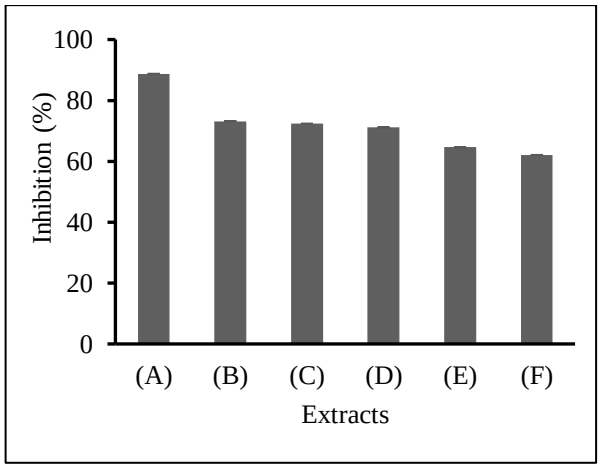

Figure 3. DPPH radical scavenging activity screening of *N. tabacum* leaves extracts; (A) gallic acid, (B) methanol, (C) dichloromethane, (D) ethyl acetate, (E) acetone, and (F) *n*-hexane extracts. Each bar represents the mean ± SD, n=3 at concentration of 319.46 µg/mL.

Table 2. Antioxidant activity of *N. tabacum* leaves extracts.

No	Extracts	DPPH scavenging activity	
		Inhibition (%) ± SD*	IC_{50} (µg/mL)
1	*n*-Hexane	62.07 ± 0.02^{A}	212.22 ± 0.02^{A}
2	Dichloromethane	72.32 ± 0.03^{B}	205.54 ± 0.03^{B}
3	Ethyl acetate	71.11 ± 0.02^{C}	222.26 ± 0.02^{C}
4	Acetone	64.59 ± 0.03^{D}	228.60 ± 0.03^{D}
5	Methanol	73.16 ± 0.04^{E}	12.12 ± 0.01^{E}
6	Gallic acid	88.72 ± 0.01^{F}	0.41 ± 0.04^{F}

*Values represent the mean ± standard deviation (SD) for n=3. Data followed by the same capital letter on each column means significantly different (p<0.05).

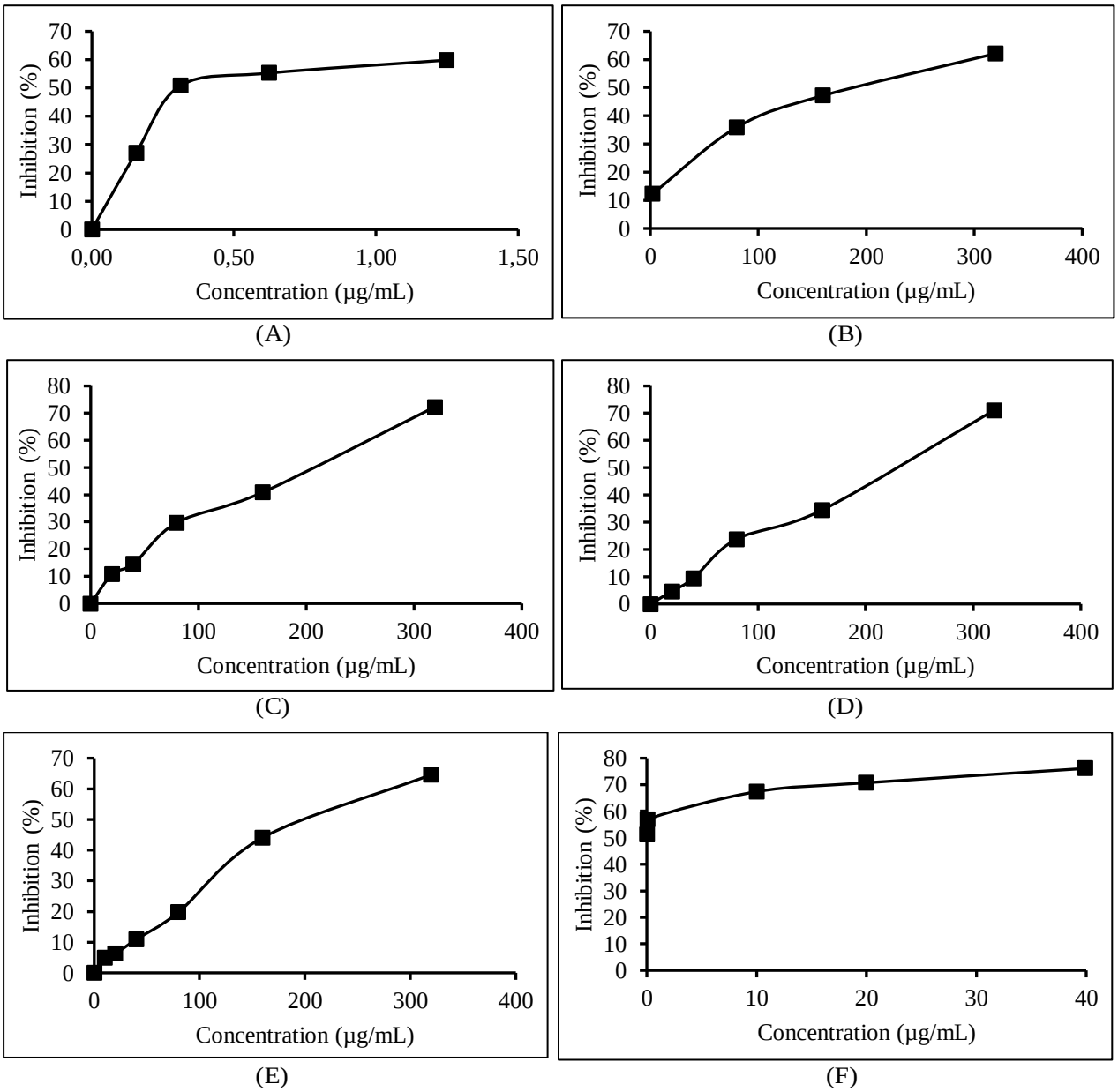

Figure 4. Antioxidant activity of *N. tabacum* leaves extracts; (A) gallic acid, (B) *n*-hexane, (C) dichloromethane, (D) ethyl acetate, (E) acetone, and (F) methanol extracts.

Previously, antioxidant activity of *N. tabacum* leaves extracts has been reported [9]. The result showed that the four extracts, included *n*-hexane, dichloromethane, ethyl acetate, and acetone, have low antioxidant activity. Therefore, the stock extracts concentration was improved from 31.95 to be 319.46 µg/mL in this present study. As a result, the antioxidant activity was also increasing. It means the used stock concentration was very low. On the other hand, there are other previous reported studies about antioxidant activity of *N. tabacum* extracts. The antioxidant activity was evaluated by enzymatic [29] and non-enzymatic assay [30]. A methanol extract of *N. tabacum* stems has shown a high inhibitory activity by enzymatic assay against superoxide dismutase and catalase. While, an aqueous extract of *N. tabacum* stems has a higher inhibitory activity against glutathione-s transferase than that of a methanol extract [29]. Furthermore, the polyphenol extract of *N. tabacum* leaves has been investigated its antioxidant activity against DPPH radicals. The result presented that the polyphenol extract has a higher inhibitory activity against DPPH than that of vitamin C as a standard. In addition, hydroxyl and superoxide anion radicals were also used to evaluate antioxidant activity of the polyphenol extract. The result also showed the antioxidant activity of the polyphenol extract was higher than vitamin C [30]. Based on these data, the methanol,

aqueous, and polyphenol extracts have potential as an antioxidant agent. Interestingly, various extracts of the other *N. tabacum* parts are also recommended to investigate their antioxidant activity.

In this present study, antioxidant activity has been focused on various extracts namely *n*-hexane, dichloromethane, ethyl acetate, acetone, and methanol extracts. The difference solvents were based on increased polarity solvent. Among these organic solvents, methanol is the most polar of all solvents. Additionally, methanol was known as a universal solvent that could extract all polar and non-polar substances. Hence, the methanol extract has shown the highest antioxidant activity against DPPH radicals. This result has the same as the previous research that the methanol extract of *N. tabacum* stems has antioxidant activity [29]. DPPH radical scavenging assay has been chosen in our assay system because of simple and inexpensive methods. The main principal technique of DPPH assay is a discoloration from dark purple to be yellow solution. It could be happened because proton(s) of antioxidant substance are given to DPPH radical. The proposed mechanism showed that an unpaired electron of nitrogen has been stabilized with a proton of antioxidant. Therefore, DPPH radical has been neutralized to be DPPH non-radical.

CONCLUSION

In conclusion, *N. tabacum* leaves, collected from Madura, contributes to antioxidant and antibacterial agents. In the present study, *N. tabacum* leaves extracts were extracted with different organic solvents such as *n*-hexane, dichloromethane, ethyl acetate, acetone and methanol. Among the extracts, the methanol extract showed very strong antioxidant activity (IC_{50} 12.12 ppm) compared with gallic acid. Furthermore, both the ethyl acetate and acetone extracts have the higher antibacterial activity than that of a standard ampicillin against *S. typhimurium* with the same MIC value of 0.63 mg/mL. Further study will focus on other biological activity assay and also identification of bioactive compounds from methanol, acetone, and ethyl acetate extracts.

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