Investigation on the antibacterial activity of the methanol extract of purun tikus root (*Eleocharis dulcis*)

K Rosyidah1,2*, L A P Sari1 and T Rohman1
1 Department of Chemistry, Faculty of Mathematics and Natural Science, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia
2 Laboratory of Organic & Biochemistry, Faculty of Mathematics and Natural Science, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia

*Email: krosyidah@ulm.ac.id

Abstract. One of the vegetations growing in tidal swampland and lebak is purun tikus (*Eleocharis dulcis*) which belongs to the Cyperaceae family. This research studied the antibacterial activity of methanol extract from purun tikus root on *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 bacteria. The chemical compounds of purun tikus root were extracted using the maceration method with methanol solvent, followed by thickening the filtrate using a rotary evaporator. The results of phytochemical screening showed that purun tikus root contains phenolic, tannin, flavonoid, and terpenoid compounds. The result showed that this extract has the activity to inhibit the growth of *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 bacteria at a concentration of 2500 µg/ml and 3000 µg/ml, respectively. The Minimum Inhibitory Concentration (MIC) value of the methanol extract from purun tikus (*E. dulcis*) root for both *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 bacteria was 6.25 µg/ml. Based on the linear regression calculation, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 inhibited at a concentration of 0.96 µg/ml and 1 µg/ml, respectively. The GC-MS spectrum showed that methanol extract of purun tikus contained compounds of the furfural group, namely furfural, 5-methyl furfural, and furfuryl alcohol.

1. Introduction
The southern Kalimantan region consists mostly of swamps. The dominant growth in the tidal and tidal swamps is the purun tikus (*Eleocharis dulcis*). Society in general uses purun tikus leaves as handicrafts in the form of bags and mats [1]. Other benefits of these plants as animal feed, especially for swamp buffaloes such as in Pandak Daun Village, South Kalimantan [2]. Research by Asikin and Thamrin [2] shows that purun tikus can act as a natural controller for white rice stem borer pests. In addition to being a trap plant, purun tikus also acts as a place to live parasitoids and predators. Baehaki et al. [3] reported the antibacterial test of ethanol, ethyl acetate, and n-hexane extracts of purun tikus leaves against the bacteria *Pseudomonas aeruginosa, Bacillus subtilis*, and *Vibrio cholera*. All extracts showed antibacterial activity against all test bacteria. Of the three bacterial species, the highest antibacterial activity is against *B. subtilis* bacteria when compared with *P. aeruginosa* and *V. cholera* bacteria. Zhan et al. [4] also reported the presence of antibacterial activity from methanolic extracts of purun tikus tuber skin against three pathogenic bacteria *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogenes*. The compounds in the tubers of these aquatic plants also have antitumor and antioxidant activity [5].
Based on the description above, further research is carried out on purun tikus especially the root as an antibacterial. The use of these bacteria in research is because *S. aureus* bacteria represent gram-positive while *E. coli* bacteria represent gram-negative. Both of these bacteria are pathogens that cause disease in animals and humans.

This article will discuss the methanol extract of purun tikus root as an antibacterial and analyze its chemical content using phytochemical screening methods and GC-MS analysis.

2. Materials and methods

2.1 Materials

The materials used the root of purun tikus, originating from Purun village South Kalimantan, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922, aqua dest, FeCl₃ 1% (Merck), Mg powder (Merck), concentrated HCl (Merck), 2N HCl (Merck), Mayer reagents, Wagner reagents, Dragendroff reagents, glacial acetic acid (Merck), methanol (pa), NA (nutrient agar), NB (nutrient broth), disc paper, 70% alcohol, sterile cotton, cotton swab, chloramphenicol, DMSO.

2.2 Extraction

The extraction was followed from the research by Rosyidah [18]. The root of purun tikus was washed with running water then cut into pieces and dried. Purun tikus root which had been cleaned, dried at room temperature without direct sunlight exposure. The stages were intended so that the active compound in the sample was not damaged. Also, to reduce water content, stop enzymatic reactions, and prevent mold growth so that it can be stored for a long time [6]. The dried sample was then mashed using a blender to form a powder, aiming to expand the surface of the sample. The smaller the size, the greater the surface area so that the extraction process would be more effective. Purun tikus root powder obtained as much as 109.02 g.

Desiccated deserts were macerated in methanol solvent for 1x24 hours. Purun tikus root powder macerated with 1.5 L methanol solvent for 1 x 24 hours at room temperature, placed in a closed container, and protected from sunlight. Methanol is the most widely used solvent for the extraction process of natural product compounds because it can dissolve all classes of secondary metabolites. This maceration process produces brown Maserati. The filtrate was filtered and the methanol vaporized. The residue was macerated again with a new methanol solvent until clear. The results of Maserati were filtered. The filtrate was evaporated using a pressure rotary vaporizer at 40°C. Methanol extract obtained as much as 5.5g brown with a yield of 5.04%.

2.3 Phytochemical screening

This screening test included metabolite compounds of phenol, flavonoids, tannins, alkaloids, terpenoids and steroids [18].

2.4 Antibacterial test

Zhan [4] shared the antibacterial test. A total of 1-2 ose test bacteria were inoculated into Nutrient Agar slants, then incubated in an incubator at 37°C for 18-24 hours. Antibacterial testing was carried out by the agar diffusion method, using agar nutrient (NA) media. NA media on Petri dishes scratched with test bacterial. A blank disc paper with a diameter of 6 mm was dropped with 50 µl of the sample with concentrations of 3000, 2500, 2000, 1500, and 1000 µg/ml, then placed on NA media that had been inoculated with test bacteria. Incubation was carried out at 37°C for 1 x 24 hours. Observations were made on the formation of inhibition zones around the disc paper. The same procedure was carried out on positive control (chloramphenicol) and negative control (DMSO). The method used for MIC analysis was the agar diffusion method. The extract was then determined by the MIC value using variations in the concentration of 100, 500, 250, 125, and 6.25 µg/ml.
3. Result and discussion

The results of the phytochemical test showed that the extract of the purun tikus root contained several classes of secondary metabolite compounds which can be seen in table 1. Secondary metabolite compounds contained in methanol extracts of purun tikus root are phenols, tannins, flavonoids, and terpenoids. The same results were also shown by others who reported that methanol extracts of purun tikus leaves contain secondary metabolites such as phenolic, tannin, flavonoid, and terpenoids [7].

| Phytochemical test | observed           | result |
|--------------------|--------------------|--------|
| phenol             | green color        | +      |
| flavonoid          | red tomato’s color | +      |
| tanin              | dark blue color    | +      |
| terpenoid          | red color          | +      |
| steroid            | blue or purple color | - |
| alkaloid           | White precipitate (Mayer reagent) | - |
|                    | Brown sediment (Wagner reagent) | - |
|                    | Orange sediment (Dragendorff reagent) | - |

Table 1. Result of phytochemical screening.

The antibacterial activity test in this study used the disc diffusion method (paper disc). The inhibitory activity against bacterial growth was characterized by clear zones formed around the test extract shown in figure 1. The extract used for the activity test was first dissolved with DMSO which also negatively control. A negative control was a solvent that did not affect the activity test [8]. While the positive control used was chloramphenicol. Chloramphenicol was a broad-spectrum bacteriostatic antibiotic that was active against gram-positive and gram-negative organisms [9].

Figure 1. Test of the antibacterial activity of methanol extract of purun tikus root against S. aureus ATCC 25923 and E. coli ATCC 25922.

A: 1000 µg/ml  B: 500 µg/ml  C: 250 µg/ml  D: 125 µg/ml  E: 6.25 µg/ml
(+): chloramphenicol
(-): DMSO

The measurement of inhibition zone diameter showed that the methanol extract of root purun tikus (E. dulcis) against S. aureus and E. coli bacteria showed antibacterial activity. The inhibition zone diameter around 5 mm shows that the inhibitory activity is categorized as weak [10].

The methanol extract of purun tikus root can inhibit the growth of S. aureus (left) and E. coli (right) bacteria, it may be caused by the content of secondary metabolites such as flavonoids, tannins, phenolics, and terpenoids as evidenced by the results of phytochemical tests. Baehaki et al. [3]
reported secondary metabolite compounds such as flavonoids, tannins, and terpenoids able to inhibit the growth of \textit{E. coli} bacteria.

In Table 2, it can be seen that \textit{S. aureus} was the most sensitive bacterium for methanol extract of purun tikus root. The results of research conducted by other reported that the bacterium \textit{S. aureus} has a larger inhibitory diameter than that of the \textit{E. coli} bacteria [4]. Inhibition zones formed are also influenced by factors such as organism sensitivity, culture media, incubation conditions, microorganism concentration, and media composition [11].

| Extract Concentration (µg/ml) | Inhibition zone (mm) |
|-------------------------------|----------------------|
| \textit{S. aureus} ATCC 25923 | \textit{E. coli} ATCC 25922 |
| 3000 µg/ml                    | 12.9                 | 10.2         |
| 2500 µg/ml                    | 13.3                 | 9.8          |
| 2000 µg/ml                    | 11.2                 | 7.1          |
| 1500 µg/ml                    | 10.0                 | 6.7          |
| 1000 µg/ml                    | 7.2                  | 6.3          |
| 500 µg/ml                     | 6.9                  | 4.8          |
| 250 µg/ml                     | 5.2                  | 4.6          |
| 125 µg/ml                     | 3.8                  | 3.5          |
| 62.5 µg/l                     | 2.8                  | 0.7          |
| Chloramphenicol (30 µg)       | 22.7                 | 23.6         |
| Kontrol negatif               | -                    | -            |

The content of secondary metabolites such as flavonoids, phenols, tannins, and terpenoids are known to have the ability to be antibacterial. The phenol group can damage the cell membrane, activate enzymes, and denaturation proteins so that the cell wall is damaged due to decreased permeability [12]. Flavonoid compounds are antibacterial compounds that can denaturation bacterial cell proteins and damage cell membranes [13] function again so that protein damage or denaturation occurs. The denaturation causes protein coagulation and disrupts the metabolism and physiological function of bacteria [14].

![Figure 2. Chromatogram gas chromatography of methanolic extract of purun tikus root.](image)
Tannins have a mechanism of coagulating and denaturing binding proteins with proteins that form H+ ions and cause pH to become acidic so that proteins are denatured. Acidic conditions inactivate enzymes in bacteria and cause impaired metabolism and cell damage and even death [15]. Whereas terpenoids have antibacterial activity associated with damage to cell membranes by lipophilic compounds [14].

Inhibitory zone ability is also influenced by differences in the structure of gram-positive and gram-negative bacterial cell walls. Bacterial cell walls consist of layers of peptidoglycan that differ between gram-positive bacteria and gram-negative bacteria. Gram-positive bacterial cell walls contain 90% and a thin layer of thetic acid while in gram-negative bacteria the cell walls only contain 5-10% peptidoglycan, the rest consists of protein, lipopolysaccharides, and lipoproteins [16].

Based on GC-MS analysis (figure 2), methanol extract of purun tikus root contained three main compounds. They were furfural (15.02%) as peak 1, 5-methyl furfural (11.82%) as peak 2 and furfuryl alcohol (17.64%) as peak 6. The furfuryl alcohol compound was a reduced furfural. The 5-methyl furfural compound was reported to be present in the methanol extract of Cyperus rotundus tubers from the same family as purun tikus [17].

4. Conclusion
The methanol extract of purun tikus root is active antibacterial against gram-positive and gram-negative bacteria. Staphylococcus aureus bacteria which represented gram-positive and E. coli bacteria as gram-negative. The MIC value of S. aureus and E. coli bacteria was 6.25 µg/ml. Phytochemical screening results indicated the presence of phenol compounds, flavonoids, tannins, and steroids. But GC-MS analysis showed that the methanol extract contained the main components of furfuryl alcohol, furfural, and 5-methyl furfural compounds.

Acknowledgments
Thank you to the DIPA of Lambung Mangkurat University 2017 Fiscal Year Number: SP-DIPA-042.01.2.400957/2018 dated December 5, 2017, for funding this research.

References
[1] Sunardi and Istikowati W T 2012 Analisis kandungan kimia dan sifat serat tanaman purun tikus (Eleocharis dulcis) Bioscientiae 9(2) 15–25
[2] Asikin, S and Thamrin M 2012 Manfaat purun tikus (Eleocharis dulcis) pada ekosistem sawah rawa. J. Lit. Pertanian 31(1) 35-42
[3] Baehaki A, Herpandi and Putra A A 2018 Antibacterial activity of extract from swamp plant eleocharis dulcis Orien. J. of Chem. 34(1) 573-75
[4] Zhan G, Pan L Q, Mao S B, Zhang W, Wei Y and Tu K 2014 Study on antibacterial properties and major bioactive constituents of chinese water chestnut (Eleocharis dulcis) peels extracts/ fractions Eur. Food. Res. Tech. 238(5) 789-96
[5] Zhan G, Pan L Q, Tu K and Jiao S 2016 Antitumor, antioxidant and nitrite scavenging effects of chinese water chestnut (Eleocharis dulcis) peel flavonoids J. of Food Sci. 81(10) 2578-86
[6] Lenny S and Zuhra C F 2006 Isolasi dan uji bioaktivitas kandungan kimia utama puding merah (Graptophyllum pictum L. Griff) dengan metode uji brine shrimp J. Komun. Penel. MIPA 17(5) 56-9
[7] Rosyidah K, Rohman T and Fitriani R 2018 Aktivitas antioksidan ekstrak metanol daun purun tikus (Eleocharis dulcis) J. Kimia dan Pend. Kimia 3(3) 135-40
[8] Natheer S E, Sekar C, Amutharaj P, Rahman M S A and Keroz K 2012 Evaluation of antibacterial activity of Morinda cirifolia, Vitex trifolia and Chromolea odorata Afri. J. of Pharm. and Pharm. 6(11) 783-8
[9] Brooks G F, Morse S A, Butel J S, Carroll K C and Mietzner T A 2013 Mikrobiologi Kedokteran 25th edition (Jakarta: EGC)
[10] Davis W W and Stout T R 1971 Disc plate method of microbiological antibiotic essay J. of Microbio. 22(4) 659-65

[11] Srivastava P, Logesh A R, Upreti D K, Dhole T N and Srivastava A 2013 In vitro evaluation of some indian lichens against human pathogenic bacteria Mycosphere 4(4) 734-43

[12] Purwatiningsih I K, Suranindiyah Y Y and Widodo 2014 Aktivitas senyawa fenol dalam buah mengkudu (Morinda citrifolia) sebagai antibakteri alami untuk penghambatan bakteri penyebab mastitis. J. Bulet. Petern. 38(1) 59-64

[13] Ngajow, Mercy, Jemmy A and Vanda S K 2013 Pengaruh antibakteri ekstrak kulit batang mataoa (Pometia pinnata) terhadap bakteri Staphylococcus aureus secara in vitro J. MIPA UNSRAT Online 2(2) 128-32

[14] Heni, Arreneuz S and Zaharah T A 2015 Efektivitas antibakteri ekstrak kulit batang belimbing hutan (Baccaarea angulata merr.) terhadap Staphylococcus aureus dan Escherichia coli J. Kimia Khatulis. 4(1) 84-90

[15] Mayanti T, Julaeha E and Putri Y 2011 Isolasi dan karakterisasi senyawa antibakteri dari fraksi etil asetat kulit batang lansium domesticum cor. CV Kokossan (Bandung: Universitas Padjajaran Fakultas MIPA) pp 10-11

[16] Brooks G F, Carroll K C, Morse S A, Mietzner T A and Butel J S 2013 Medical Microbiology 26th edition (E-book: The McGraw-Hill)

[17] Kusuma A V C, Chozin M A and Guntoro D 2017 Senyawa fenol dari tajuk dan umbi teki (Cyperus rotundus L.) pada berbagai umur pertumbuhan serta pengaruhnya terhadap perkecambahan gulma berdaun lebar J. Agro. Indo. 45(1) 100-7

[18] Rosyidah K, Puspa L A and Rohman T 2018 Aktivitas antioksidan ekstrak metanol daun purun tikus (Eleocharis dulcis) J. Kimia dan Pend. Kimia 3(3) 135-40