Potency of *Fibraurea tinctora* Lour. extract as anti-bacterial agents towards pathogenic bacteria

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Abstract. This research aimed to explore antibacterial activity of *Fibraurea tinctora* Lour. extracted by ethanol and hot water. The treatments of study consisted of six levels of *F. tinctora* plant extract (0 %, 0.625 %, 1.25 %, 2.50 %, 5.00 % and 10.0 %, respectively) either using ethanol or hot water, resulting in 12 experimental treatments according to 6 × 2 factorial arrangement in a completely randomized design. Each of the treatment was replicated three times. Results of this research showed that either ethanol or hot water *F. tinctora* extract have potency to control farm pathogenic bacteria. In the lowest concentration (0.625 %) both extract significantly inhibited bacteria growth (Minimum Inhibition Concentration). The highest antibacterial activity was in group that had the highest concentration (10 %) of extract in both of the bacteria. *Staphylococcus aureus* were more susceptible to the *F. tinctora* extract than *Escherichia coli*. Result from spectrophotometry UV-vis assessments showed that the total composition of tannin, alkaloid, and saponin from ethanol extract of *F. tinctoria* is higher than its water based extract. Meanwhile, phenol composition of water-based extract from *F. tinctoria* is higher than from ethanol extract.

Keywords: Antibacterial, bioactive compounds, herbal medicine, natural yellow root

1. Introduction

*Fibraurea tinctoria* Lour. is a plant member of the *Menisspermaceae* family known as yellow root or *akar kuning* in Kalimantan, Indonesia. According to Setyawati [1] the plant has been known in the provincial community of Kalimantan, Indonesia. The plant has been used as a cure for various diseases such as headaches, diarrhea, diabetes and dysentery, malaria, lumbago, increase stamina, as an antioxidant and contains secondary metabolites, namely berberine, alkaloids, terpenoids, palmitin and fibleucin. Berberine is an alkaloid isoquinone which has extensive biochemical and pharmacological activities, including anti-diarrhea and anti-cancer. Berberine can also be used to prevent metabolic diseases, heart disorders, anti-inflammatory, and anti-proliferation. Berberine have potency to kill protozoa *Leishmania* sp. [2]. *F. tinctora* which is taken from Vietnam is poisonous to *Plasmodium falcifarum* [3]. In these plants there are furanodi terpenoid compounds. According to Toshihada et al. [4] these compounds can be as antifungal. Meanwhile, according to Su et al. [5], some furanodi terpenoid compounds from *F. tincoria* are anti-inflammatory. Ethnobotany trace, the stem of the plant for the treatment of jaundice. The results from research conducted by Ika [6], the chemical compounds found in the stem of the *F tinctoria* plant are as hepatoprotectors.
There have been no reports about the activity of bioactive compounds from the stem and leaves of *F. tincoria* against bacteria that cause disease in livestock. This research attempts to examine the potency of *F. tinctoria* plant extract as an antibacterial compound. Use of natural resources against pathogenic bacteria is essential to increase the number of livestock free from antibiotic residual and safe for human being.

2. Materials and methods

2.1 *F. tinctoria* preparation

The stems and leaves of *F. tinctoria* were collected from Kecamatan Cigudeg, Kabupaten Bogor, Indonesia. Identification of the plant was done at *Pusat Konversasi Tanaman Kebun Raya* (Center for Plant Conservation Botanic Garden), Bogor, Indonesia. Then stems and roots of *F. tinctoria* were chopped into small pieces and were kept in the room temperature until dry. After that, the small pieces were blended until they reached smoothness. Then, the powder of *F. tinctoria* was extracted by ethanol and water.

The bacteria used were *Staphylococcus aureus* and *Escherichia coli*. They were obtained from Microbiology Laboratory, University of Brawijaya, Malang. Bacteria culture medium was obtained from Biomedical Laboratory of Medicine Faculty of University of Muhammadiyah Malang (Oxoid, USA).

2.2 Extraction of *F. tinctoria*

Extraction of *F. tinctoria* was conducted by using two solvents, 96% ethanol and hot water, respectively. Briefly, a 300 g of *F. tinctoria* powder was macerated in 1 500 mL of 96% ethanol and hot water, respectively for 6 h while being shaken. It was refluxed for 3 h and were filtered using Whatman filter paper no. 42. The filtrate was refluxed again twice by adding ethanol or hot water. The result of filtration was vacuum evaporated in 60 °C until thick ethanol-free or water-free extract obtained.

2.3 Experiment design

The treatments of study consisted of six levels of *F. tinctoria* plant extract (0%, 0.625%, 1.25%, 2.50%, 5.00% and 10.0%, respectively) either using ethanol or hot water, resulting in 12 experimental treatments with three replications in each treatment. Data were subjected to Analysis of Variance following the completely randomized block design in 6 × 2 factorial arrangement. The different solvent and levels of plant extract were applied as fixed factors while replication being a random factor. The differences between treatment means were subjected into Duncan’s Multiple Range Test when *P* < 0.05.

2.4 Bacteria cells observation and counting

Antibacterial activity was measured from the plant extract ability to inhibit bacterial growth. It was measured by using tube dilution method [7]. It was showed from different levels of turbidity of the medium. The clearest medium showed the highest activity of antibacterial to inhibit growth of bacteria. Antibacterial activity was also measured from number of bacteria. The method to count bacterial colony bacteria was done by modified method of droop plate [8].

2.5 Phytochemical screening

Screening of phytochemical was conducted qualitatively and quantitatively. Firstly, phytochemical screening with qualitative methods was tested by using thin layer chromatography test (TLC) in Laboratory of Chemistry, University of Muhammadiyah Malang. Further, quantitative test used spectrophotometry UV-vis conducted at Integrated Research and Testing Laboratory (*Laboratorium Penelitian dan Pengujian Terpadu*), Universitas Gadjah Mada, Yogyakarta. Method used for total phenol and tannin using method from Makkar [9] while for saponins using method according to
Yang et al.[10]. Measurement of alkaloid was conducted using method from Shre evidya and Mehrotra [11].

3. Results and Discussion

3.1 Antibacterial activity of F. tinctora extracts of different solvents and concentrations

The hot water and ethanol extracts of F. tinctora demonstrated almost similar antibacterial activity against S. aureus and E. coli (table 1). Treatment at 2.5 % and 5 % of ethanol extract showed higher inhibitory activity than hot water extract in S. aureus. This condition indicated that F. tinctora extract by ethanol could dissolve more bioactive compounds than extract by hot water, resulting in stronger inhibiting activity to the group of bacteria.

Ethanol extract at 2.5 % concentration in S. aureus showed higher inhibitory activity than in E. coli indicates that gram-positive bacteria (S. aureus) were more susceptible than the Gram-negative (E. coli). Gram-positive bacteria have thicker peptidoglican layer than Gram-negative bacteria, but they don’t have the outer membrane called lipopolysaccharide (LPS). The LPS serves as a barrier passage of most molecules. It excludes many compounds that could damage the cell, including certain antimicrobial medications. This is one reason why Gram-negative bacteria are generally less sensitive to many medications [12]. That result is in agreement with Njaroge et al. [13] when extract from Phylanthus spp. was used. In the lowest concentration (0.625 %), both extract had already inhibited bacterial growth. The greatest activity were in the highest concentration (10 %) for both of the bacteria (table 1).

Table 1. Antibacterial activity of F. tinctora extracts of different solvents and concentrations.

| F. tinctora concentration (%) | S. aureus | E. coli |
|-----------------------------|----------|--------|
|                             | Ethanol  | Hot water | Ethanol | Hot water |
| 0                           | -        | -        | -       | -         |
| 0.625                       | +        | +        | +       | +         |
| 1.25                        | +        | +        | +       | +         |
| 2.5                         | ++       | +        | +       | +         |
| 5                           | ++       | +        | ++      | +         |
| 10                          | +++      | +++      | +++     | +++       |

Note: +++ very active, ++ active, + active enough, - not active

Ethanol extract and hot water significantly decreased the number of both S. aureus and E. coli bacteria ($P < 0.01$; table 2). The higher concentration of F. tinctora extract resulted higher inhibitory effect to the number of S. aureus and E. coli bacteria ($P < 0.01$). In various plant like goldenseal (Hydrastis canadensis L.), berberis/barberry (Berberis vulgaris L.), oregon grape [Mahonia aquifolium (Pursh) Nutt], and gold-thread (Coptis trifolia Salisb), Berberine alkaloids could be found. Berberine from the plants demonstrates effective antimicrobial activity [14]. F. tinctora also contains alkaloids berberin or proto berberin. Antibacterial activity of F. tinctora might be influenced by the alkaloids.

According to table 2, minimum level of F. tinctora extract needed to inhibit S. aureus was 2.5 % for hot water and 5 % for ethanol solvent, indicated that hot water was more effective to extract the plant as antibacterial agent. There was no significant different on 2.5 % or lower of ethanol solvent level and 1.25 % or lower of hot water solvent level on the number of S. aureus colony. Moreover, E. coli was more sensitive to the plant extract as showed on the table 2 that by giving 0.625 % or higher of the extract, the colony were significantly inhibited ($P < 0.01$), observed both in ethanol and hot water solvent.
Table 2. Effect of *F. tinctora* extract to the number of bacteria (Log CFU) in different solvents and concentrations.

| *F. tinctora* concentration (%) | *S. aureus* | *E. coli* |
|--------------------------------|------------|----------|
|                               | Ethanol    | Hot water| Ethanol    | Hot water|
| 0                              | 6.83<sup>ab</sup> | 6.94<sup>a</sup>   | 6.85<sup>c</sup> | 6.99<sup>a</sup>   |
| 0.625                          | 6.80<sup>ab</sup> | 6.84<sup>ab</sup> | 6.71<sup>e</sup> | 6.93<sup>b</sup>   |
| 1.25                           | 6.75<sup>abc</sup> | 6.71<sup>abc</sup> | 6.55<sup>f</sup> | 6.85<sup>c</sup>   |
| 2.50                           | 6.66<sup>abc</sup> | 6.59<sup>bed</sup> | 6.52<sup>fg</sup> | 6.76<sup>d</sup>   |
| 5.00                           | 6.53<sup>bed</sup> | 6.43<sup>de</sup> | 6.41<sup>h</sup> | 6.71<sup>e</sup>   |
| 10.0                           | 6.29<sup>egd</sup> | 6.19<sup>e</sup> | 6.20<sup>i</sup> | 6.50<sup>g</sup>   |

Note: different superscripts in the same column shows highly significant difference (*P* < 0.01)

3.2 Phytochemical of *F. tinctora* (qualitative method)

The result of chemical contents (phytochemical) screening from ethanolic extract and hot water-based extract shown in table 3. From the TLC test, ethanolic extract of *F. tinctora* contains alkaloids, terpenoids, steroid, flavonoids, and anthraquinones. Meanwhile, hot-water based extract of *F. tinctora* showed to have alkaloids, terpenoids, flavonoids, steroids, polyphenol, tannins, and anthraquinones.

Table 3. Phytochemical screening with qualitative methods of *F. tinctora*.

| Phytochemical screening with qualitative methods | Thin Layer Chromatography (TLC) |
|------------------------------------------------|--------------------------------|
|                                                | Ethanol extract | Hot water extract |
| Alkaloids                                       | +                | +                 |
| Saponin Glycoside                               | -                | -                 |
| Terpenoids                                      | +                | +                 |
| Steroids                                       | +                | +                 |
| Flavonoids                                      | +                | +                 |
| Polyphenols & tannins                           | -                | +                 |
| Anthraquinones                                  | +                | +                 |

Chemical compounds found in *F.tinctoria* that causing this plant to have antibacterial abilities are flavonoids and alkaloids. Flavonoids compounds were secreted from plants as a protection towards microorganism infection. So that, flavonoids acquired from plants are effective as antimicrobial agents. Flavonoids is a polyphenol compounds that has several abilities, anti-oxidant, anti-tumor, anti-inflammation, antibacterial and antiviral. Mechanism of alkaloids as antibacterial is by disrupting the peptidoglycan from bacterial cells so that it will make the cell walls become not attach to each other.

3.3 Phytochemical compound of *F. tinctora* (quantitative method)

Result from spectrophotometry UV-vis assessments showed that the total composition of tannin, alcaloid, and saponin from ethanol extract of *F tinctoria* is higher than its water based extract. Meanwhile, phenol composition of water-based extract from *F tinctoria* is higher than from ethanol extract. The total tannin, phenol, alcaloid, and saponin resulted from the water-based extract of *F. tinctoria* were 37.14 %, 15.40 %, 1.96 % and 3.31 % respectively, and from the ethanol extract of *F. tinctoria* were 57.20 %, 17.38 %, 0.54 % and 4.13 % respectively (table 4).
Table 4. Chemical composition of *F. tinctoria* extract.

| No | Parameter test                      | Water extract (% w/w) | Alcohol extract (% w/w) | Methods                  |
|----|--------------------------------------|-----------------------|-------------------------|--------------------------|
| 1  | Total tannin equivalent tannin acid  | 37.14                 | 57.20                   | Spectrophotometry UV-vis |
| 2  | Total alkaloid equivalent quinine    | 15.40                 | 17.38                   | Spectrophotometry UV-vis |
| 3  | Total fenol equivalent gallat acid   | 1.96                  | 0.54                    | Spectrophotometry UV-vis |
| 4  | Saponin from quillaja bark qualitative | 3.31            | 4.13                    | Spectrophotometry UV-vis |

4. Conclusion
Ethanol or hot water *F. tinctora* extract have potency to control farm pathogenic bacteria. In the lowest concentration (0.625 %) both extract had already inhibited bacteria growth (Minimum Inhibition Concentration). The greatest antibacterial activity were in group that had the highest concentration (10 %) in both of the bacteria. Gram-positive bacteria (*S. aureus*) were more susceptible to the *F. tinctora* extract than the Gram-negative (*E. coli*). Result from spectrophotometry UV-vis assessments showed that the total composition of tannin, alcaloid, and saponin from ethanol extract of *F. tinctoria* is higher than its water based extract. Meanwhile, phenol composition of water-based extract from *F. tinctoria* is higher than from ethanol extract.

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References
[1] Setyawati A 2015 *Struktur Histologi Hati, Ginjal Dan Pankreas Mencit (Mus Musculus) Dengan Perlakuan Ekstrak Batang Akar Kuning (Fibraurea tinctoria L.) Selama Organogenesis* [Structure of Histology of Liver, Kidney and Pancreas of Mice (Mus Musculus) with Treatment of Yellow Root Stem Extract (Fibraurea tinctoria L.) During Organogenesis] [Thesis] (Bogor: Graduate School, Bogor Agricultural University) p 34 [in Bahasa Indonesia]
https://repository.ipb.ac.id/bitstream/handle/123456789/79166/2015ase.pdf?sequence=1&isAllowed=y
[2] Mahmoudvand H, Sharififar F, Sharifi I, Ezatpour B, Harandi M F, Makkii M S, Zia-Ali N and Jahanbakhsh S 2014 In Vitro Inhibitory Effect of *Berberis vulgaris* (Berberidaceae) and Its Main Component, Berberine against Different *Leishmania* Species *Iran J. Parasitol.* 9(1) 28–36
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4289877/
[3] Nguyen P-J, Tran H, Tran H, Phan T A, Dolecek C, Farrar J, Tran T H, Caron P, Bodo B and Grellier P 2007 Antimalarial and cytotoxic activities of ethnopharmacologically selected medicinal plants from South Vietnam *J. Ethnopharmacol.* 109 417–27
https://www.researchgate.net/publication/6783436_Antimalarial_and_cytotoxic_activities_of_etnopharmacologically_selected_medicinal_plants_from_South_Vietnam
[4] Toshihada S, Kiyotani T, Maeda M, Katayama T, Yokotani K T, Syafii W and Muladi S 2011 Furanoditerpenes from *Arcangelisia flava* (L.) Merr. and their antifungal activity *Phytochem. Lett.* 4 333–36
https://www.researchgate.net/publication/251708957_Furanoditerpenes_from_Arcangelisia_flava_L_Merr_and_their_antifungal_activity
[5] Su C R, Chen Y F, Liou M J, Tsai H Y, Chang W S and Wu T S 2018 Anti-inflammatory activities of furanoditerpenoids and other constituents from *Fibraurea tinctoria* Bioorganic Med. Chem. 16(21) 9603–09  
https://www.ncbi.nlm.nih.gov/pubmed/18829331

[6] Ika F 2012 Aktivitas hepatoprotektor batang *Fibraurea Tinctoria* Lour secara in vivo [Hepatoprotector activity of *Fibraurea Tinctoria* Lour stem in vivo] J. Trop. Pharm. Chem. 1(4) 293–300 [in Bahasa Indonesia]  
https://ftp.farmasi.unmul.ac.id/index.php/jtpc/article/view/39

[7] Hecht D W, Citron D M, Cox M, Jacobus N, Jenkins S G, Onderdonk A, Roe-Carpenter D, Rosenblatt J E and Wexler H M 2007 Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria: Approved Standard-Seventh Edition (USA: Clinical and Laboratory Standards Institute) p 16–20  
https://pdfs.semanticscholar.org/6dfb/a7b6a53200bf68fffc2dd11df83fcfc7ca9c.pdf

[8] Chen C-Y, Nace G W and Irwin P L 2003 A 6 × 6 drop plate method for simultaneous colony counting and MPN enumeration of Campylobacter jejuni, Listeria monocytogenes, and *Escherichia coli* J. Microbiol. Meth. 55 475–79  
https://pubag.nal.usda.gov/pubag/downloadPDF.xhtml?id=48127&content=PDF

[9] Makkar H P S 2003 Quantification of Tannin in Tree and Shrub Foliage [A laboratory manual] (Dordrecht (Netherlands) : Kluwer Academic Publisher)  
https://www.springer.com/us/book/9781402016325

[10] Yang C Y, Huang Y, Chen and Chang M 2010 Foam properties, detergent Abilities and long-term preservative efficacy of the saponis from *Sapindus mukorossi* J. Food Drug Anal. 18(3) 155–60  
https://www.researchgate.net/publication/228618080_Foam_PROPERTIES_Detergent_ABILITIES_and_Long-term_Preservative_Efficacy_of_the_Saponins_from_Sapindus_mukorossi

[11] Sreedivya N and Mehotra S 2003 Spectrophotometric method for estimation of alkaloids precipitable with Dragendorff’s reagent in plant materials. J AOAC Int. 86(6) 1124–27  
https://pdfs.semanticscholar.org/367a/d7b79e4a41d2e43405ba37ed9ce6ddf31.pdf

[12] Nester E W, Anderson D G and Roberts C E 2012 *Microbiology A Human Perspective* (New York: Mc Graw-Hill International Edition) p 761  
https://www.amazon.com/Microbiology-Perspective-Eugene-W-Nester/dp/0071316132

[13] Njaroge A D, Anyago B and Dossaji S F 2012 Screening of Phyllanthus species for antimicrobial properties Chem. Sci. J. 56 1–11  
https://www.omicsonline.org/peer-reviewed/screening-of-phyllanthus-species-for-antimicrobial-properties-13382.html

[14] Yu H H, Kim K J, Cha J D, Kim H K, Lee Y E, Choi N Y and You Y O 2005 Antimicrobial activity of berberine alone and in combination with ampicillin or oxacillin against methicillin-resistant *Staphylococcus aureus*. J. Med. Food 8(4) 454–61  
https://www.ncbi.nlm.nih.gov/pubmed/16379555