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Analysis of the Bioactive Compounds and Antibacterial Test on N-Hexane Extract of Ramie (*Boehmeria nivea*)

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Abstract. Ramie (*Boehmeria nivea*) is one of the Urticaceae family plants with various potentials for pharmacological development. The research aimed to analyze the bioactive compound of n-hexane extracts of B. nivea leaves and flowers. Furthermore, antibacterial tests were carried out on the n-hexane extracts to determine their potency. The metabolites in the leaves and flowers of ramie were analyzed using a Gas Chromatography-Mass Spectrometer (GC-MS). Antibacterial potential analysis was carried out by disc diffusion method using n-hexane extract of ramie leaves and flowers at concentrations of 20%, 40%, 60%, 80%, and 100% in inhibiting *Escherichia coli* and *Klebsiella pneumonia*. The results showed that the metabolome data of the n-hexane extract of ramie leaves and flowers were 40 and 47 compounds, respectively. The crude n-hexane extract of *B. nivea* flower gave antibacterial activity at 80% and 100% concentrations against *E. coli* with an average clear zone of 9.15 ± 0.31 mm and 10.29 ± 0.28 mm. The results of confirmation of the dominant compound by GC-MS showed that the n-hexane extract consisted of tetratetracontane, tetracontane, Stigmast-5-en-3-vol,(3.beta.,24S)-(CAS)Clionasterol, methyl comment D, and 2,6,10,14,18,22-Tetracosahexsaene,2,6,10,15,19,23-hexamethyl-, (all-E). The study showed the antibacterial activity of the n-hexane extracts of ramie flower with moderate inhibition category. The presence of active compounds in ramie leaves and flowers has the potential for various biological activities. Based on the results of this study, the n-hexane extract of *B. nivea* is recommended for other potential biological activities with further tests, such as anti-inflammatory, antioxidant, and analgesic.

Key words: antibacterial, anti-inflammatory, *Boehmeria nivea*, *Escherichia coli*, *Klebsiella pneumoniae*.

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INTRODUCTION

Infectious diseases are the main cause of death in humans (Rossiana *et al*., 2017). Infectious disease is caused by pathogenic microorganisms such as bacteria, viruses, and fungi. One of the microorganisms that cause infectious diseases is *Escherichia coli* and *Klebsiella pneumonia*. *Escherichia coli* is a bacterium that can cause gastrointestinal infections. The presence of fecal bacteria indicates food contamination and can cause food-borne diseases. *Escherichia coli* is the most common cause of diarrhea. According to the Ministry of Health (2021), diarrhea causes 14.5% of deaths in Indonesia, especially in toddlers, and is an endemic disease that can potentially cause Extraordinary Events that can cause death for sufferers. *Klebsiella pneumonia* is a bacterium that can cause urinary tract infections, pneumonia, and sepsis (Dewi *et al*., 2019). *K. pneumoniae* is the largest contributor to the number of causes of pneumonia. According to the Indonesian Health Profile, pneumonia caused 73.9% of deaths. In 2015-2018 pneumonia cases increased, reaching 505,331 patients, with 425 patients dying (Perhimpunan Dokter Paru Indonesia 2020). It indicates that *K. pneumoniae* infection is a serious problem for health.

E. coli is a bacterium that shows relatively high resistance to several antibiotics, including penicillin, amoxicillin, and streptomycin (Normaliska *et al*., 2019). Meanwhile, *K. pneumoniae* is a bacterium that can produce ESBL (Extended Spectrum Beta-Lactamase), causing the bacteria to be resistant to several antibiotics (Harapan et al., 2018), so new antibiotic compounds are needed that can inhibit the two bacteria.

Ramie (*Boehmeria nivea*) is a plant that belongs to the Urticaceae family. *B. nivea* contains active compounds, including alkaloids, lignans, flavonoids, terpenoids, glycosides, carotenoids, and terpenes (Singh *et al*., 2012). It has been reported that ramie has bioactivity such as antibacterial (Jung *et al*., 2020), antioxidant (Chen *et al.*, 2014), and anti-inflammatory (Sung *et al*., 2013) effects.

The parts of the ramie plant, such as leaves and flowers, are considered to have pharmacological activity. Cho *et al.* (2017) reported that *B. nivea* leaves effectively reduce several diseases, including fever, hematemesis, extravasation, and diarrhea. Therefore, flowers have a key role in controlling the stress response and have good antioxidant properties (Tang *et al.*, 2021). The compounds in the leaves and flowers of ramie can be used as an alternative to herbal medicines that can reduce several diseases (Cho *et al.*, 2017). In the previous study, Triani (2021) reported that endophytic fungi were discovered in various organs of the ramie plants. Most of the endophytic fungi were found in the leaves and flowers and indicated antifungal activity against the pathogenic fungi (Triani, 2021). In this study, the leaves and flowers were tested against bacteria to evaluate their potential. Their extracts compounds were also analyzed, which had not been reported in the previous study.

The study conducted Ibrahim (2012) showed that the ramie (*Boehmeria virgata* (first.) Guill) leaves extract was able to inhibit the growth of *Escherichia coli*, *Vibrio cholera*, and *Salmonella typhosa*. A previous study has also been conducted on the n-hexane extract of *Urtica dioica* leaves. The extract showed antibacterial activity against *Escherichia coli* and *Klebsiella pneumoniae* (Dar *et al*., 2012). Herefore, in this study, the leaves and flowers of ramie were extracted using n-hexane. The n-hexane extracts were analyzed for their bioactive compounds to provide information on the compounds that could potentially have biological activity. Furthermore, antibacterial tests were carried out on the n-hexane extract of ramie leaves and flowers against *E. coli* and *K. pneumoniae*. The research aimed to analyze the bioactive compound of n-hexane extracts of B. nivea leaves and flowers.

METHODS

Materials

The young leaves and old flowers of ramie (*Boehmeria nivea*) were obtained from ramie plantations in the Pajagan, Sumedang, West Java. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were obtained from the Microbiology Laboratory, RSP-FK Padjadjaran University. The growth medium used was Mueller-Hinton Agar (MHA). Other materials used were levofloxacin [Oxoid], tween 80 10% [Merck], standard solution of McFarland 0.5, and n-hexane.

Preparation of N-hexane Extract of Ramie Leaves and Flowers

Extracts from the leaves and flowers of ramie (*Boehmeria nivea*) were prepared according to the method by Yudharini *et al*., 2016 with modifications. 100 g each for leaves and flower biomass was used as raw material for the extract. The maceration process was carried out using n-hexane with a ratio of 1: 10 material and solvent. The maceration was conducted for 3 x 24 hours in a closed, dark container and was stored at 25 ºC - 28 ºC. The solution was filtered to separate the wet biomass and the filtrate. The filtration results were then evaporated using a rotary evaporator at a temperature of 40˚C until a concentrated extract was obtained. Then, the sample was stored at 4ºC until the analysis stage

Preparation of Bacteria Suspension

E. coli and K. pneumoniae bacteria from stock were prepared and inoculated into sterile MHA media as much as one ose by streak plate methods. The bacteria were incubated at 37˚C for 24 hours. After incubation, one bacterial colony was taken and inoculated into 10 ml of 0.9% NaCl solution. Then the bacterial suspension was homogenized according to the standard Mc Farland turbidity 0.5 (10^8CFU/mL).

Antibacterial Test of N-hexane Extract of Ramie Leaves and Flowers

Antibacterial testing was carried out using the disc diffusion method. Sterile MHA media was poured into sterile petri dishes. Then the bacteria were scratched on the petri dish using a cotton swab.

Extracts of n-hexane from ramie leaves and flowers were prepared at concentrations of 20%, 40%, 60%, 80%, and 100%, respectively, with negative control of Tween 80 and positive control of levofloxacin. Disc paper (6 mm diameter) was immersed in the extract for ± 1 hour. Then the disc paper was placed on top of the MHA, which had been solidified and planted with bacteria. Observations were carried out by looking at the clear zone formed around the paper discs after an incubation period of 24 hours at 37ºC. The diameter of the clear zone formed was measured using a caliper.

Gas Cromatography-Mass Spectrometry (GC-MS) Analysis of N-hexane Extract of Ramie Leaves and Flowers Contents

The n-hexane extracts of ramie leaves and flowers were analyzed using GC-MS (Gas Chromatography-Mass Spectroscopy) (Shimadzu Co., Japan). The initial column conditions were at 30ºC and heated to 300ºC gradually at 10ºC/5min. High-purity helium gas was used as the carrier gas, which was conditioned to flow at a rate of 1.0 ml/min in split mode. The sample was dissolved in methanol and injected into the GC-MS capillary column as much as 1 µL. The eluted component will be detected in the mass detector. Identification of compounds was carried out based on the retention time and the area of each peak that appeared. The identification of compounds was analyzed based on the Wiley/NIST Library software.

Component Identification

The photo components contained in the n-hexane extract were identified by comparing their spectra with the National Institute of Standards and Technology (NIST) database, which has 62.000 patterns. The name of the compound, retention time, molecular formula, and structure are determined. The percentage area of each component was calculated by comparing the average peak area with the total area. Spectrums of unknown compounds were compared with those of known compounds stored in the NIST library.

RESULTS AND DISCUSSION

Analysis of Bioactive Compounds on N-hexane Extracts

The GC-MS chromatogram for n-hexane extract of ramie leaves and flowers showed the presence of several phytochemical compounds as indicated by the presence of many major and

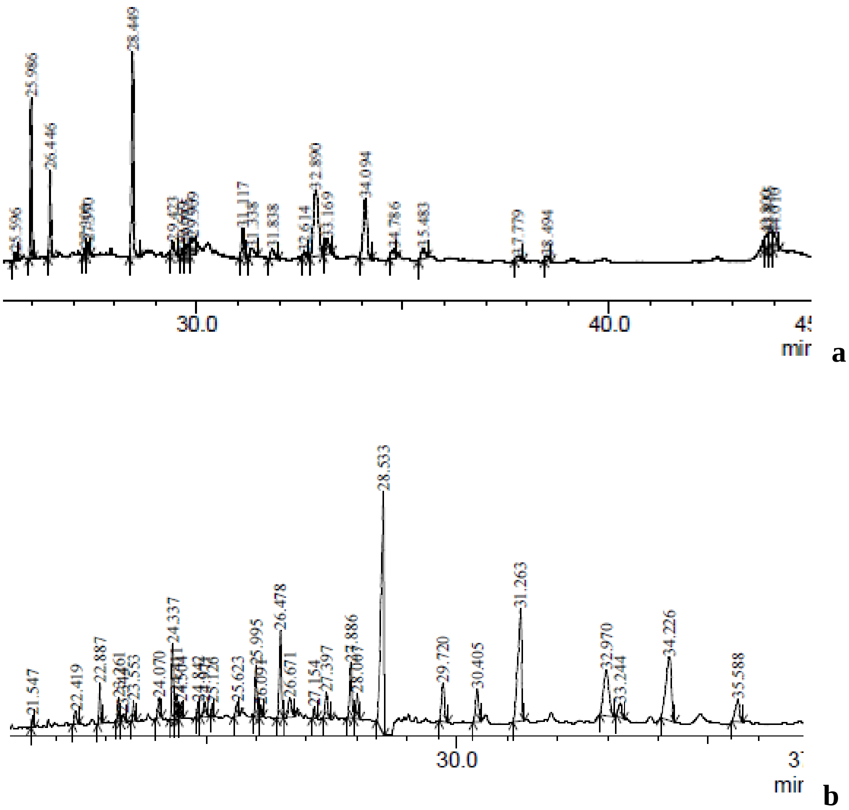

Figure 1. N-hexane extracts of *B.nivea* (a) leaves and (b) flowers chromatogram

Table 1. GC-MS analysis results of phytocomponents compound in n-hexane extracts of *B.nivea* leaves

No	Compounds	Retention time	%Area	Molecular Weight	Compounds Group
1	Tetratetracontane	28.450	16.47	619	Hydrocarbon
2	Stigmast-5-en-3-ol, (3.beta.,24S)- (CAS) Clionasterol	32.890	14.59	414	Steroids
3	Methyl Commate D	34.095	9.74	486	Terpenoids
4	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	25.985	9.03	410	Triterpenoids
5	Hexacosane (CAS) n-Hexacosane	26.445	5.00	366	Hydrocarbon
6	Hexadecanoic acid, octadecyl ester (CAS) Stearyl palmitate	29.910	3.10	508	Fatty acid
7	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS) Phytol	19.990	3.10	296	Hydrocarbon
8	Tetracontane	31.115	2.81	563	Hydrocarbon
9	Hexadecanoic acid, octadecyl ester	43.855	2.66	508	Fatty acid
10	Hexadecanoic acid, octadecyl ester (CAS) Stearyl palmitate	44.010	2.44	508	Fatty acid

minor peak components, as shown in Figure 1.

Identification of dominant photo components based on GC-MS results with several active compounds, retention time, molecular formula, molecular weight, peak area/concentration (%), molecular weight, and a group of compounds in leaves and flowers extracts are shown in Table 1 and Table 2, respectively.

Analysis of compounds in ramie leaves extract with GC-MS detected 40 compounds. Meanwhile, the analysis of ramie flower extract obtained 47 compounds with one unidentified compound. In the n-hexane extract, the major compounds were found with peak areas ranging from 5% consisting of triterpenoids, terpenoids, hydrocarbons, and steroids. The compounds in the minor category consist of fatty acids, fatty alcohols, phenols, terpenoids, steroids, hydrocarbons, and vitamins. Wang *et al*. (2019) have reported that the highest total phenolic content was observed in shoots (4585 ± 320 mg GAE/100 g DW), while roots and petioles had the lowest total phenolic content 442.8 ± 9.8 and 630.9 ± 27.0 mg GAE/100 g DW, respectively. Therefore, the most abundant flavonoids were found in the phloem (2,755 ± 184 mg CE/100 g DW), and the lowest flavonoid content found in the roots and petioles was 636.9 ± 44.2 and 797.4

Table 2. GC-MS analysis results of phytocomponents compound in n-hexane extracts of *B.nivea* flowers

No	Compounds	Retention time	%Area	Molecular Weight	Compounds group
1	Tetratetracontane	28.535	18.86	619	Hydrocarbon
2	Tetracontane	31.260	11.41	563	Hydrocarbon
3	Methyl Commate D	34.225	9.67	486	Terpenoid
4	Stigmast-5-en-3-ol, (3.beta.,24S)- (CAS) Clionasterol	32.970	7.46	414	Steroid
5	Hexacosane (CAS) n-Hexacosane	26.480	4.71	366	Hydrocarbon
6	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	24.335	3.43	354	Fatty acid
7	Isovaleric acid, eicosyl ester	27.885	3.38	382	Fatty acid
8	Tetratetracontane (CAS) n-Tetratetracontane	29.720	3.18	619	Hydrocarbon
9	Stigmast-4-en-3-one (CAS) 4-Stigmasten-3-one	35.590	2.63	412	Steroid
10	Docosa-2,6,10,14,18-Pentaen-22-Al, 2,6,10,15,18-Pentamethyl-, Alltrans	25.995	2.55	384	Fatty acid

± 87.6 mg CE/100 g DW, respectively. Xylem and buds exhibit remarkable antioxidant and antiproliferative activities, which can be explained by their diverse phenolic compositions, especially chlorogenic acid and epicatechin (Wang *et al.,* 2019). The abundant of flavonoid and phenolic compounds in the extracts have an important role in antioxidant ability (Dillak *et al.,* 2019). The plant *Boehmeria nivea* L. may be a valuable source of phenolic compounds used in the food and non-food industries.

Based on GC-MS analysis, the dominant compounds in both flowers and leaves were: tetratetracontane which was found at retention times of 28,450 – 28,535 minutes with peak areas of 16.47%, and 18.86%, stigmatist-5-en-3-ol, (3.beta.,24S)-(CAS) Clionasterol with a retention time of about 32 minutes with a peak area on leaves and flowers of 14.59% and 7.46%, respectively. Methyl commute D was detected by GC-MS at a retention time of about 34 minutes with a peak area of 10%. In addition, the dominant compound found only in flowers was tetracontane with a retention time of 31,260 minutes and a peak area of 11.41%, while the dominant phytochemicals found only in ramie leaves were 2,6,10,14,18,22-Tetracosahexsaene,2,6, 10,15,19,23-hexamethyl-, (all-E)- with a retention time of 25,985 minutes with a peak area of 9.03%. The GC-MS results of the n-hexane extract of ramie flower showed that one compound was unidentified for structure and compound name at the retention time of 25.6 with a peak area of 0.85%. The compound is expected to be a new compound (novel molecule) that needs to be further tested for its characterization.

Tetratetracontane compound is a long chain and unbranched alkane with the molecular formula $C_{44}H_{90}$. These compounds are metabolites for humans, such as antioxidants, and have cytoprotective and antibacterial activities (Gumgumjee *et al.,* 2015; Amudha *et al.,* 2018). Tetracontane is a compound with the formula $CH_3(CH_2)_{38}CH_3$ or $C_{40}H_{82}$. Tetracontane is one of the major compounds which was only detected in flower samples at a retention time of 31.26 minutes with a peak area of 11.41%. Tetracontane is a natural product with anti-inflammatory, analgesic (Arora & Meena, 2017), antibacterial and antioxidant (Belkacemi *et al*., 2020; Indriati *et al*., 2021). Stigmast-5-en-3-ol, (3.beta.,24S)-(CAS) Clionasterol is known to have anti-inflammatory, antipyretic, anti-ulcer, and anti-arthritis (Arora & Meena, 2017). *Ceropegia bulbosa* extract contains tetracontane and stigmast-5-en-3-ol, (3.beta.,24S)- (CAS) clionasterol which has anti-inflammatory, analgesic, antipyretic, anti-ulcer, antiarthritis activity (Arora & Meena, 2017). Meanwhile, Methyl Commate D was also found dominantly in the ethanol extracts of honeycomb waste *Trigona* spp. and is known for its anti-inflammatory (Okoye *et al.,* 2014; Duraisamy & Raja, 2020). 2,6,10,14,18,22-Tetracosahexsaene,2,6,10,15,19,23-hexamethyl-, (all-E)- is a long chain alkane compound with molecular formula $C_{30}H_{50}$ which is known as antioxidant, antitumor, immunostimulant, and chemopreventive (Sudha *et al.,* 2013).

In this study, GC-MS analysis showed the extraction results of different compounds, as reported by Cho *et al*. (2016). The study showed that the results of phytochemical analysis isolated from the leaves of the ramie plant (*Boehmeria nivea*) isolated using HPLC showed the presence of a compound structure identified as -sitosterol, (−)-collide, rutin, and pyrimidinedione by spectroscopic analysis of MS, 1H-, and 13C-NMR. Similar results were also obtained by Chen *et al.* (2014), which showed dominant active compounds such as phenolics and flavonoids.

Several studies have been reported regarding the antioxidant bioactivity of *B. nivea* extract. Lee *et al*. (2014) reported that *B. nivea* extract reduced 80% of free radicals and had a significant antioxidant effect. Arsul et al. (2021) also reported that *B. nivea* extract had an excellent antioxidant effect. The potential of *B. nivea* plant extract has been proven to be effective as an anti-inflammatory on macrophages by inhibiting p38 and JNK. It can be used as a functional compound against inflammation (Sung *et al*., 2013). The ramie extracts could also suppress inflammation mediated by mast cells in cutaneous anaphylaxis (Ji-Ye Lim *et al*., 2020). Therapy using *B. nivea* proved effective in controlling allergic reactions and suppressing the development of histamine and hexosaminidase in mast cells (Sancheti *et al.,* 2011).

Antimicrobial Activity

Antimicrobial tests were carried out using the n-hexane extract of ramie leaves and flower (*B. nivea*) against the two test bacteria, *Escherichia coli*, and *Klebsiella pneumoniae*. The results are shown in Figure 2. The antibacterial test results showed that the n-hexane extract of the leaves and flowers of B. nivea is ineffective against *K. pneumoniae*. In addition, leaves extract with a concentration of 20%; 40%; 60%; 80%; and 100% against *E. coli* bacteria showed no inhibition zone activity. However, the flower extract with a concentration of 80% and 100% showed inhibition zone activity of 9.15 ± 0.31 mm and 10.29 ± 0.28 mm, respectively (with less and moderate inhibition categories) against *E. coli*. Similar results were also obtained from *Averrhoa bilimbi* extracts that showed an inhibition zone in the range of 9 – 10.5 mm against *E.coli* (Prastiyanto *et al*., 2020). It showed that there are compounds in ramie flower extract that have antibacterial activity.

Both leaves and flower extracts have major compounds of tetratetracontane. In previous studies, tetratetracontane was also found in some plant extracts. Tetratetracontane was found in some plant extracts against various microbes, including *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Staphylococcus pneumonia, Streptococcus pyogenes,* and *Pseudomonas aeruginosa* (Agarwal *et al.,* 2017; Albratty *et al.,* 2021). However, in this study, the antibacterial activity was only found in the flowers extract. The major compound which only found in flowers was tetracontane. Tetracontane showed antibacterial against Staphylococcus aureus, Bacillus cereus, and Pseudomonas aeruginosa and antioxidant activity (Belkacemi *et al*., 2020). Therefore, Gollo *et al.* (2020) and Indriati *et al.* (2021) reported that extracts with tetracontane compounds indicated antioxidant activity. The antibacterial activity of flowers might be related to tetracontane in the extracts, as reported by Belkacemi *et al.* (2020).

The study showed that flowers have antibacterial activity with the less to moderate inhibition category. The antibacterial activity might be due to some bioactive compounds, such as tetratetracontane and tetracontane. However, most bioactive compounds are known to have more roles as antioxidants (Amudha *et al.,* 2018), anti-inflammatory, analgesic (Arora & Meena, 2017; Duraisamy & King, 2020), antioxidant (Indriati *et al.,* 2021), immunostimulant, and chemopreventive (Sudha *et al.,* 2013). It can be the basis for carrying out a potential screening process as bioactive prospectives from ramie plants.

CONCLUSION

The n-hexane of ramie (*Boehmeria nivea*) leaves and flowers extract has different secondary metabolite content. This study showed that 40 compounds of n-hexane extract had been successfully analyzed from leaves and 47 compounds from flowers. Analysis of dominant compounds in the extract showed the presence of five main compounds consisting of Tetratetracontane, Tetracontane, Stigmast-5-en-3-ol, (3.beta.,24S)- (CAS) Clionasterol, Methyl Commate D, 2,6 ,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-, (all-E)-. The antibiotic test results of the n-hexane extracts of ramie' flowers showed moderate activity only on *Klebsiella pneumoniae*. These results indicate the potential of ramie flower extract as an antibacterial. It can be the basis for further developing ramie flower extract as an antibacterial by optimation the extraction method and testing it on other types of microbes. Bioactive compounds contained in a plant extract material have potential in pharmacology. Further exploration is needed regarding the potential of bioactive compounds from ramie leaves and flower extracts for other biological activities, such as antioxidants, anticancer, anti-inflammatory, antitumors, immunostimulants, and chemopreventives.

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